Background & objectives: There is paucity of information on vaginal and rectal colonization with multiple serotypes of group B streptococci (GBS). As part of an ongoing cohort study evaluating the natural history of vaginal and rectal colonization by GBS, the colonization with multiple serotypes was studied in 102 non-pregnant women aged 18-30 yr.

Methods: Up to ten separate colony picks of beta-haemolytic streptococci (total 1515 isolates) were selected from vaginal and rectal primary culture plates. The colonies were identified as GBS, and their capsular polysaccharides (CPS) serotypes determined using monospecific rabbit antisera for types Ia-VIII by double immunodiffusion in agarose (DID). A colony dot immunoblot (DB) assay, using monospecific rabbit antiserum to purified type polysaccharides conjugated to tetanus toxoid, was developed to serotype efficiently the multiple colony picks of GBS.

Results: The CPS serotype distribution, examining only the 177 “a” or first colony picks from the 102 patients, was 30.5 per cent for Ia; 28.2 per cent for type III; 15.3 per cent for type II; and 13.6 per cent for type V. Only 2.8 per cent were nontypeable. Eighty of the 102 patients (78.4%) were colonized with only one serotype; 20 (19.6%) had two serotypes and two patients (2%) had three serotypes in their vaginal and/or rectal paired cultures. Overall, 91.9 per cent of the culture sites colonized with one to three CPS types (from the total number of colonies picked) were identified with a minimum of three colony picks. In 75 patients with vaginal/rectal pairs the GBS serotype concordance of only the “a” colony was 89.3 per cent and concordance decreased to 80 per cent when the serotype concordance of the total colony picks was analyzed.

Interpretation & conclusion: In conclusion, there was a relatively high prevalence of serotype non-concordance in this population, and 21.6 per cent of patients had multiple GBS serotypes.

Key words GBS colonization - multiple colony picks - serotype concordance

There are many unanswered questions regarding the epidemiology of vaginal and rectal colonization with group B streptococci (GBS) in women1-2. Among these is the issue of whether women may be colonized with more than one GBS serotype when assessed at a specific time. It has been documented that multiple serotypes of group A streptococci (GAS) may simultaneously spread within a family demonstrating positive skin or skin lesion cultures3. Although in that study three GAS colonies were picked from each culture, analysis of multiple serotypes isolated simultaneously from the same culture sites in patients was not done4. In epidemiological studies of an Australian aboriginal community, multiple Vir types of GAS were isolated at the same time from pyoderma lesions in children4. However, vaginal and/or rectal colonization with multiple serotypes of GBS has been rarely documented in women. The present study was designed to test the hypothesis that nonpregnant women
may be colonized with more than one serotype simultaneously.

Material & Methods

Study population and specimen collection: The 102 nonpregnant women (18-30 yr) were part of a cohort study evaluating the natural history of vaginal (V) and rectal (R) colonization by GBS. They were seen at a primary health care center (n=20), a student health clinic (n=19) and a sexually transmitted disease clinic (n=63). All were unmarried, 92 per cent currently sexually active, and 46 per cent nonwhite. Separate sterile Dacron-tipped swabs were used to sample the vagina and rectum. Each swab was placed in a separate Amies transport tube and transported to the laboratory within 12 h.

Recovery and identification of GBS: The vaginal swab was inoculated to a Columbia agar plate with 5 per cent sheep blood and a selectively broth; the rectal swab, to a colistin-nalidixic acid agar (CNA) plate and selective broth. Plates and broths (Prepared Media Laboratories, Mississauga, ON) were incubated for 18-24 h at 35°C in 5-7 per cent CO₂. Plates were examined visually for beta haemolytic colonies; broths were subcultured onto 5 per cent sheep blood agar plates (BAP).

To obtain a broad sample of GBS serotypes present, 10 separate colony picks (labeled “a-j”) were picked for identification from each V and R culture. Colonies were selected from the original plate, but if it had < 10 cfu (colony forming units) per plate, the remaining picks were made from the subculture plate of the selective broth medium. Some V and/or R culture sites were colonized very lightly with only one or two cfu/ml of GBS.

Isolates were identified as GBS by agglutination technique using the PathoDx™ typing system (Diagnostic Products Corporation, Los Angeles, CA). They were stored in litmus milk at -70°C until sent by overnight courier on blood agar slants (Gibson Laboratories Inc., Lexington, KY) to the GBS Molecular Reference Laboratory at the University of Minnesota.

Initial processing: All isolates (colony picks “a-j”) were subcultured on BAP to assess purity. Individual colonies were picked to Todd-Hewitt broth (THB) and after overnight incubation, were frozen at -30°C until they were processed for typing.

Serotyping:

Immunoprecipitation in agarose by double immunodiffusion (DID) – The “a” colony from each patient was grown in THB overnight, and the cell pellet was extracted with hot HCl. These extracts were tested by immunoprecipitation in agarose (DID) with monospecific rabbit antisera to GBS polysaccharide serotypes Ia, Ib-VIII, the alpha and beta components of the c protein, as well as the R1, R3, R4 and R5 group B streptococcal protective surface antigen (BPS) species of R antigen.

Dot blot (DB) – A small aliquot from the THB cultures of all “a-j” isolates was spotted on a polyvinylidene fluoride (PVDF) membrane (Immobilon™, Millipore, Bedford, MA) marked with a grid pattern. Spotted on each membrane also were appropriate positive and negative controls, including uninoculated THB. The membrane was washed, blocked and blotted for capsular polysaccharide type (CPS) only, using monospecific rabbit antisera to tetanus toxoid-conjugated purified GBS capsular polysaccharides (Channing Laboratory, Boston). For each individual patient, the membrane with its “a-j” samples was blotted with antiserum to the serotype of its own “a” pick as determined by DID. The membrane was then incubated with alkaline phosphatase-conjugated F(ab’)2 goat anti-rabbit IgG, F(ab’)2 fragment (Jackson ImmunoResearch Laboratories, West Grove, PA) and developed with a nitroblue tetrazolium-3-bromo-4-chloroindolyl phosphate (NBT-BCIP) substrate. Results were read visually on a scale of 1+ to 4+, with readings of ≥ 2+ considered positive.

The DB result of the “a” isolate was read for concordance with the serotype as determined by DID; results of the “b-j” isolates, for concordance with the CPS type of the “a”. The “b-j” isolates not blotted with antiserum to the same serotype as the “a”, or giving weak reactions (≤ 1+) were tested with antisera to the heterologous types. Those still not blotted by any antiserum or with weak reactions, were tested by immunoprecipitation in agarose by double immunodiffusion.
Results

The pie diagrams (Fig.) depicted the distribution of serotypes for 177 “a” colony picks, the first colony or predominant colony pick, and the 1515 “a-j” colony picks. In this comparison, the predominant serotypes for either set of data were Ia, III, and II. The fourth most prevalent CPS type was V. The nontypeable isolates were very few. The cell-surface protein expression profiles of the 177 “a” colony picks were: alpha only, 40.7 per cent; alpha plus beta, 10.2 per cent; R1 only, 1.1 per cent; R4 only, 30.5 per cent; R1 plus R4, 13.6 per cent; and 4 per cent of the isolates had none of these commonly studied proteins (P Ferrieri and AE Flores, unpublished data).

Analysis of the 1515 GBS isolates from 102 patients revealed that 86 per cent of the vaginal and/or rectal sites sampled had six or more colony picks and 69.5 per cent of culture sites had ten colony picks of GBS. Of the culture sites sampled, there were 14.1 per cent with four or fewer colony picks. The GBS serotype concordance in 75 women who had vaginal and rectal paired cultures obtained at the same visit was analysed. In analyzing these data, if only the “a” colony was studied, the serotype concordance between the two culture sites in each woman was 89.3 per cent. However, when multiple GBS colonies were picked and serotyped, this concordance decreased to 80 per cent (Table I).

<table>
<thead>
<tr>
<th>No. of V/R pairs (%)</th>
<th>“a” colony</th>
<th>“a-j” colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant**</td>
<td>67 (89.3)</td>
<td>60 (80)</td>
</tr>
<tr>
<td>Non-concordant</td>
<td>8 (10.7)</td>
<td>15 (20)</td>
</tr>
</tbody>
</table>

* 27 unpaired culture sites (19 rectal and 8 vaginal) were excluded from the analyses
** All the same serotype or same combination of serotypes

Table II. Distribution of patients with one or more serotypes in all “a-j” picks from vaginal and/or rectal culture sites

<table>
<thead>
<tr>
<th>No. of serotypes</th>
<th>Culture category</th>
<th>No. (%) patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>with V/R paired cultures</td>
<td>57</td>
</tr>
<tr>
<td>1</td>
<td>R only</td>
<td>17</td>
</tr>
<tr>
<td>1</td>
<td>V only</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>with V/R paired cultures</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>R only</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>V only</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>with V/R paired cultures</td>
<td>2</td>
</tr>
</tbody>
</table>

N = 102
Of great interest was the frequency of colonization with more than one GBS serotype among the total 102 patients. As seen in Table II, the commonest distribution pattern was colonization with only one serotype, seen in 78.4 per cent. However, 20 patients (19.6%) were colonized with two serotypes and two patients were colonized with three serotypes.

In the 22 patients colonized with more than one serotype, there were ten different serotype combinations recovered (data not shown). The commonest serotype combinations were Ia/Ib, Ia/III, Ia/NT, and III/V. The two patients colonized with three serotypes had Ia/II/III or II/III/V. Serotype Ia was seen in 14 of the 22 (63.6%) patients.

Of interest was the total number of colony picks that were required to detect the total number of GBS serotypes in a culture site. As seen in Table III, cumulatively, 91.3 per cent of culture sites with one to two serotypes or 91.9 per cent of culture sites with up to three different serotypes were identified with a minimum of three colony picks.

Table III. Minimum number of colony picks required to detect all serotypes in a culture site

<table>
<thead>
<tr>
<th>No. of serotypes found</th>
<th>No. of colony picks</th>
<th>No. (%) of culture sites with no. of serotypes identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1**</td>
<td>1</td>
<td>147 (85.0)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>8 (4.6)</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>2 (1.2)</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>1 (0.6)</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>4 (2.3)</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>3 (1.7)</td>
</tr>
</tbody>
</table>

N=173†

* To detect all serotypes/culture site
** Based on serotypes of b - j, “a” colony was concordant
† Does not include four culture sites with only one isolate

Discussion

In this study on GBS colonization among non-pregnant women, it was astounding to discover that with evaluation of up to ten different GBS colonies per culture site serotype concordance between the vaginal and rectal paired cultures was only 80 per cent. There were only minimal differences in the distribution of the various serotypes when comparing the single colony “a” picks versus the “a-j” multiple colony picks. Further, the predominant types were the same, and, of note, was the predominance of serotype Ia followed by serotype III. Further support for the prominent role of serotype Ia was that it was also the commonest serotype found in combination with one or more other serotypes.

The finding of simultaneous colonization of women with multiple GBS serotypes has been reported rarely. In one report of pregnant women cultured at multiple sites during pregnancy and/or delivery, 23 per cent of the GBS positive women had more than one serotype at the same time10. Among the 102 non pregnant women in the present study, multiple serotypes were detected in 21.6 per cent. It is curious that co-colonization with more than one GBS serotype was not uncommon in this sexually active group of women. The majority, 61.8 per cent of this cohort of relatively young, non pregnant women were seen at a sexually transmitted disease clinic. It is possible that the acquisition and colonization with multiple GBS serotypes may be related to the number of sexual partners. It has been demonstrated that frequent sexual intercourse and/or multiple sex partners in this group of women was associated with vaginal acquisition of a new serotype of GBS9.

It is not practical in routine microbiology laboratories to pick more than one GBS colony to pursue identification and antimicrobial susceptibility testing. Although in special epidemiological studies there may be a role for studying more than one bacterial colony based on morphology, degree of beta haemolysis or no haemolysis, or colony size, it is impractical to recommend selecting as many colonies as were done in the current study. Noteworthy was the finding that a minimum of three colony picks identified one to three GBS serotypes in 91.9 per cent of the vaginal or rectal culture sites sampled.

Detection of vaginal/rectal colonization with multiple GBS serotypes may have relevance to the design of clinical trials of specific GBS CPS vaccines and in...
evaluating their efficacy in preventing homologous serotype colonization and infection in women and infants. In summary, the findings of the present study demonstrated that colonization with more than one GBS serotype is not uncommon in women.

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References


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