PHENOTYPIC AND GENOTYPIC STUDY OF STREPTOCOCCUS AGALACTIAE IN VAGINA OF PREGNANT WOMEN IN ARGENTINA

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Abstract 
Streptococcus agalactiae (GBS) is a leading cause of serious neonatal infection. In this study we determine the prevalence, serotype distribution and genomic diversity of GBS in vagina of pregnant women. Vaginal swabs of 531 pregnant women were cultured on Columbia Agar Base Blood, GBS Agar Base and Todd Hewitt Broth. GBS were characterized by group and type-specific agglutination. Genomic polymorphism was studied by random amplification of DNA (RAPD). Seventeen patients (3.2%) were positive for GBS, resulting serotype III the most frequent. RAPD detected 16 different RAPD profiles from 21 GBS studied, revealing a good discriminatory power. In this sense, this method showed different genotype from GBS serotype III recovered from successive samples of two patients, suggesting reinfection. In conclusion, the combination of RAPD and serotyping appear promising for epidemiological studies. Finally, findings of reinfection after therapy during pregnancy, led us to suggest performing prenatal GBS screening and intrapartum prophylaxis in order to reduce neonatal risk.

Key words: Streptococcus agalactiae, genotypic study, pregnant women

Group B streptococci (GBS) have been recognized since the late 1960’s as important opportunistic pathogens, representing a common cause of neonatal sepsis and meningitis as well as perinatal maternal infections. The incidence of early-onset neonatal disease and premature rupture of membranes is highly correlated with heavy GBS colonization of the vagina, cervix, and rectum of pregnant women¹. Vertical transmission of GBS from mother to infant is the most common mode of transmission, although other means of transmission have been described, including nosocomial or community acquisition².

Maternal morbidity due to GBS consists of postpartum endometritis, post-cesarean bacteremia and both clinical and subclinical chorioamnionitis³. Morbidity and mortality rates of early-onset GBS sepsis would be significantly reduced if women at high risk to deliver infected infants could be rapidly and accurately identified.

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The current classification of streptococci by serotyping is based on the serologic detection of group and type specific surface antigens. This system can distinguish GBS type Ia, Ib, II, III, IV, V, VI, VII and VIII, some of which may likewise be distinguished by the presence of the proteic antigen c<sup>4,5</sup>. Types I, II and III are equally distributed in early GBS infection, reflecting those found in the maternal genital tract. In late onset neonatal GBS infection almost 40% of cases are caused by type III GBS<sup>6</sup>.

Epidemiological characterization of GBS infections has been limited by the lack of the discriminatory capacity of serotyping. Moreover, the presence of significantly high percentages of nontypeable GBS (10-15 %)<sup>7</sup> and the finding of isolates belonging to the same serotype which may not be epidemiologically related<sup>8-10</sup> constitute severe limitations.

Techniques based on the random amplification of DNA (RAPD) have recently been reported as alternatives to characterize and differentiate GBS isolates<sup>11,12</sup>. The purpose of this study was to study the prevalence of Streptococcus agalactiae in pregnant women, to determine the serotypes distribution between isolates, to discriminate isolates by RAPD and to examine strategies to prevent the transmission of GBS from the mother to neonates.

**Materials and Methods**

**Patients**

GBS isolates were obtained from 531 pregnant women between July 1993 to August 1995 at the Obstetric Service of Hospital Italiano Garibaldi, Rosario, Argentina. A total of 580 vaginal exudates were taken between the 11th to the 43rd week of gestation. Patients studied early in pregnancy were considered at risk, either because of earlier miscarriages and/or a previous history of neonatal infection. The study focused on patients in the last trimester of pregnancy (28-38 weeks of gestation). Deliveries took place between the 36.5th and 41.3th weeks of gestation (Table 1).

**Antibiotic therapy**

All cases with a positive GBS culture, received prophylactic antibiotics. Ampicillin (500 mg) was given every 6 hours for 10 days, while erythromycin was administrated to penicillin allergic patients. A follow up culture from vagina was performed four weeks later. The same treatment was repeated if the follow up culture was positive for GBS.

Intrapartum prophylaxis was given to all patients who had negative cultures after the treatment and those with persistent

| TABLE 1.— Women colonized by Streptococcus agalactiae. Study performed in Rosario, Argentina, 1993/5 |
|---------------------------------------------------|--------------------------------------------------|------------------|------------------|
| **Patient** | **Age (years)** | **First Date** | **Second Date** | **Time of screening week** | **Cultures (isolate serial number)** | **Partum (week)** |
| A | 27 | 6-93 / NC | 37 / - | P (6) / - | 39.2 |
| B | 31 | 6-93 / NC | 35.2 / - | P (14) / - | 38 |
| C | 33 | 6-93 / NC | 35.2 / - | P (15) / - | 40.2 |
| D | 22 | 9-93 / NC | 35.6 / - | P (15) / - | 39.2 |
| E | 24 | 9-93 / NC | 34.3 / - | P (78) / - | 39.3 |
| F | 27 | 1-94 / NC | 37 / - | P (157) / - | 41 |
| G | 25 | 2-94 / NC | 36 / - | P (183) / - | 39.2 |
| H | 26 | 3-94 / NC | 37 / - | P (193) / - | 39 |
| I | 23 | 7-94 / 8-94 | 30 / 36 | P (294) / P(325) | 38.5 |
| J | 21 | 8-94 / NC | 27 / - | P (343) / - | 40.5 |
| K | 36 | 8-94 / NC | 37 / - | P (359) / - | 40 |
| L | 27 | 2-95 / NC | 37 / - | P (466) / - | 39.3 |
| M** | 31 | 3-95 / 4-95 | 26 / 28 | P (500) / P(536) | 36.5 |
| N | 25 | 3-95 / 4-95 | 35 / 38 | P (506) / N | 40.5 |
| O | 23 | 3-95 / 5-95 | 34 / 38 | P (515) / P(562) | 39.1 |
| P | 26 | 4-95 / 6-95 | 28 / 35 | P (556) / N | 39.1 |
| Q | 28 | 8-95 / 9-95 | 40 / 41 | P (609) / N | 41.3 |

* NC: not cultured; P: positive GBS culture; N: negative GBS culture; ** This patient presented a third positive GBS isolate (574) at 33 weeks of gestation.
GBS colonization. Prophylaxis consisted of ampicillin 2 grams intravenously (IV) at the beginning of labor, followed by 1 g IV every 4 h until delivery.

In patients I and O, GBS was recovered from two successive vaginal cultures and in patient M, GBS was isolated from three vaginal cultures. All women had received treatment after positive culture (Table 1).

Clinical materials

Two standard culture swabs were collected from vaginal walls. Swabs were inoculated immediately after collection or kept in Stuart transport medium until processed. One swab was cultured on Columbia Agar Base (Oxoid) with 10% blood (CABB) in 10% CO₂ atmosphere for 24-48 h and GBS Agar Base (Oxoid) in anaerobic atmosphere 24-48 h and the other swab was placed in Todd Hewitt Broth supplemented with 15 mg/ml nalidixic acid and 10 µg/ml colistin13. After enrichment for 24-48 h in modified Todd-Hewitt Broth, the specimens were subcultured on CABB and GBS for identification and isolation of GBS. All cultures were incubated at 37°C.

Strains

Beta hemolytic colonies recovered of CABB as well as orange pigmented colonies on GBS Agar Base were identified presumptively by morphology, Gram’s staining reaction, hypurate hydrolysis and CAMP test. GBS were confirmed by specific-group (Streptococcal grouping kit, Oxoid) and specific-type antigen detection (Denka Seiken Co LTD, Japan)4-6.

Minimal Inhibitory Concentration (MIC) to ampicillin was determined by the agar dilution technique with Steers Inoculator14.

Genotypic analysis of GBS isolates

GBS DNA was obtained by treating cells with mutanolysin-proteinase K, followed by phenol extraction. The RAPD reaction was made using 100 ng genomic DNA, 2.5 µM primers, 1.5 mM MgCl₂, 200µM dNTP, 10 mM Tris-HCl pH 8.4, 50 µKCl and 2.5 U of Taq polymerase (Promega) in a final volume of 50 µl. Amplification was performed by using low astringency conditions, as previously described 11. For this reaction, 5 min of denaturation at 95°C was followed by cycles of 1 min at 93°C, 1.5 min at 36°C, and 2 min at 72°C, followed by 10 min at 72°C. Partially degenerate oligonucleotide with the following sequences: 5’- GGTCGACYTTNGYNGGRTC-3’ (N: A, T, C, G; Y: C, T; and R: A, G) was used. The products of amplification were resolved by electrophoresis in 1.2% agarose gel, and detected by coloration with ethidium bromide following conventional procedures11.

GBS isolates were categorized as unrelated strains when their RAPD profiles differed by two or more bands, and differences in band intensity (at a given position) were not taken into account for the differentiation of isolates.

Results

GBS was isolated from the vagina of 17 (3.2%) of the 531 patients between the 26th and 40th weeks of pregnancy (Table 1). A total of 21 GBS isolates were obtained by combining the three mentioned culture media from these 17 patients. As shown in Table 2, supplemented Todd Hewitt broth, increased the sensibility of the solid culture by 20% approximately. The different serotypes found and the corresponding percentages are indicated in Table 3 and Table 4. As shown in Table 4, serotype III was predominant (47.6%) followed by serotypes V and Ib/c (14.3% each).

Patients I and M were recolonized with the same III serotype GBS in two and three opportunities, respectively, while two different isolates were recovered from patient O (serotype Ia and non-typeable)

RAPD made possible the detection of 16 different amplification profiles from the 21 isolates (Table 3 and Fig. 1). The two isolates obtained from patient I had the same serotype but different RAPD patterns (III₄ and III₆, Table 3). The three isolates from patient M had the same serotype. However, RAPD analysis indicated that one of these isolates was different (III₄ and III₆, Table 3). A non-typeable and la2 serotype isolates were isolated in patient O, which also presented different genotypes (la and NT₃, Table 3).
Table 3). In all three cases of reinfection this methodology suggested reinfection, rather than persistent infection.

MIC for ampicillin ranged from 0.06 µg/ml to 0.12 µg/ml. Both values are interpreted as sensitive according to N.C.C.L.S standards. 

### Discussion

This study provided information on the pattern of GBS colonization in pregnant women from Rosario (Argentina) and the distribution of serotypes and the genomic diversity among GBS isolates.
During a two-year study we observed that the percentage of vaginal colonization by GBS in pregnant women was 3.2%. This value is similar to those reported in India (5.8%), and quite different from those found in Saudi Arabia (13.9%), Nigeria (19.5%), USA (20.4%) and Gambia (22%)15. The low rate of GBS colonization in our pregnant women may reflect not only differences in genetic constitution but also differences in sexual practice and environmental factors such as hygiene and nutrition.

The addition of Todd Hewitt Broth (THB) supplemented with antibiotics to Agar Base Columbia Blood is useful for the routine cultures. The combination of both raised to 100% of the number of detected cases. The THB or other liquid medium had already been considered convenient13.

Serotype distribution among GBS isolates from pregnant women in our study was compared with that reported by others (Table 4). Serotype III was predominant (47.6%) in our pregnant women, similar to that observed in Houston (USA), California (USA) and Ibadan (Africa)16-18, where serotype I occupied the second place in prevalence. On the other hand, in studies performed in Maryland19 and Atlanta20 serotype I (43% and 53%, respectively) was the most frequent recovered from pregnant women. In a recent study in Gambia15 serotype V was the most prevalent, while in our study, it constituted 14.3% of total.

All mothers colonized by GBS were treated intrapartum according to the described scheme. Neither colonization nor infection was detected in neonates at birth through pharyngeal exudate and rectal swabs. The intrapartum treatment in colonized patients was beneficial, as previously found21-23. We observed that treatment during pregnancy for GBS is not effective, as seen as in patients I, M and O (Table 4), who have shown recolonization by GBS.

The genotypic methodology used herein was useful to demonstrate a large prevalence of genetic unrelatedness between GBS isolates from identical serotypes and also provided evidence for persistence or reinfection11. In this study, the observation of reinfection in all cases where more than one isolate was recovered from the same woman, allowed us to verify the success of antibiotic treatment. However, new genotypes were obtained in all cases of recurrent GBS, which supports the concept that antibiotic therapy during pregnancy is not a good strategy for GBS transmission prevention24.

The Centers for Disease Control recently recommended intrapartum treatment to minimize the risk of early onset group B streptococcal sepsis. Two alternative strategies have been suggested, one based on prenatal screening cultures and the other based only on risk factors for GBS24. In our experience25, 26, the first strategy would be more appropriate since neonatal infection and fatal sepsis has been reported in women who did not present risk factors.

Therefore, we suggest implementing a control program for maternal colonization for GBS between the 36th and 38th week of gestation, combining serotyping and RAPD for the epidemiological study of this microorganism and using a scheme of intrapartum treatment in all cases where GBS has been recovered, in order to reduce neonatal morbi-mortality.

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La médicine au XVIII ème siècle

Au XVIII ème siècle, le relèvement de la chirurgie est total: la Compagnie de Saint Côme est rétablie dans ses privileges en 1743.
Les chirurgiens et les couteliers innovent dans l'instrumentation.
Au cours du siècle, se forment les méthodes de raisonnement et d'expériences scientifiques qui resteront intacts malgré l'écroulement des systèmes médicaux - développés sous l'influence des philosophes- qui fut occasionné par la Révolution.
La Société Royale de Médecine est fondée en 1776. Vicq d’Azyr, anatomiste et littérateur distingué prépare la réorganisation de l'enseignement médical qui sera celui du lendemain de la Révolution.

La medicina en el siglo XVIII

En el siglo XVIII, la elevación en jerarquía de la cirugía es total: a la Compañía de San Cosme se le restablecen sus privilegios en 1743.
Los cirujanos y los fabricantes de cuchillas innovan en la instrumentación.
En el curso del siglo, se conforman los métodos de razonamiento y de la experimentación científica que quedarán intactos a pesar de la ruina que abatió los sistemas médicos -que se habían desarrollado bajo la influencia de los filósofos- y que fue ocasionada por la Revolución.
La Sociedad Real de Medicina se funda en 1776. Vicq d’Azyr, anatomista y escritor distinguido, prepara la reorganización de la enseñanza médica, que será el mañana de la Revolución.

Musée d'histoire de la Médecine. Petit guide du visiteur. 
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