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# Serotypes and antimicrobial susceptibility of group B *Streptococcus* isolated from pregnant women in Mexico.

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**ABSTRACT. Title:** Serotypes and antimicrobial susceptibility of group B *Streptococcus* isolated from pregnant women in México

**Background:** Group B streptococci (SGB) are a leading cause of neonatal sepsis and meningitis. Women colonized might also develop pregnancy-associated infections including urinary tract infection, bacteremia and postpartum endometritis. There are nine different capsular types, and with the possibility of a GBS vaccine in the future; it is essential to understand which of these types are circulating. We determined the serotype distribution of isolated rectovaginal and urine GBS obtained from an obstetric population, and also their antibiotic sensitivity patterns.

**Methods:** A total of 101 strains were studied: SGB were confirmed by latex agglutination (Slidex Strepto-Kit bioMérieux), and the serotype was confirmed by latex agglutination (Pastorex *Streptococcus* B. Becton Dickinson, EUA).

Antimicrobial susceptibility testing was performed by a Kirby-Bauer disk diffusion.

**Results:** All SGB were serologically typeable; 61.3% were type I, 25.7% were type II and only 12.8 were type III. All of them were 100% susceptible to beta lactam agents. Only 5.9% strains were resistant to erythromycin.

**Conclusion:** In the last years, no major changes have been observed in the distribution of serotypes of SGB in México, serotype I is still the most common. This makes us think of the possibility of a vaccine that includes serotypes I and II that represent close to 90% of the isolations.

**Key words:** *Streptococcus agalactiae*, serotypes, neonatal sepsis, susceptibility, pregnant women.

**RESUMEN. Título:** Serotipos y susceptibilidad antimicrobiana de cepas de *Streptococo* del grupo B aislado de mujeres embarazadas en México.

**Objetivo:** El *Streptococo* del grupo B (SGB) es la causa principal de sepsis neonatal y meningitis. Las mujeres colonizadas también pueden desarrollar infecciones asociadas al embarazo que incluyen infección del tracto urinario, bacteremia y endometritis posparto. Hay 9 tipos capsulares diferentes, y con la posibilidad de una vacuna en el horizonte, es esencial entender cual de estos serotipos está circulando. Por lo anterior, determinamos la distribución de serotipos de SGB aislados de cultivo rectal, vaginal y de orina obtenidos de una población obstétrica, así como sus patrones de sensibilidad antimicrobiana.

**Metodología:** Un total de 101 cepas fueron estudiadas: SGB fue confirmado por aglutinación en látex (Slidex Strepto-kit bioMérieux), y serotificados por aglutinación en látex (*Streptococo* Pastorex B. Becton Dickson, EUA).

La susceptibilidad antimicrobiana se realizó por difusión con disco de Bauer Kirby.

**Resultados:** Todos los SGB fueron tipificables por serología, 61.3% fueron tipo I, 25.7% tipo II y sólo 12.8 tipo III. Todos fueron 100% susceptibles a agentes beta láctámicos. Sólo 5.9% cepas fueron resistentes a eritromicina.

**Conclusión:** En los últimos años no se han presentado cambios en la distribución de serotipos de SGB en México, siendo todavía el serotipo I el más frecuente. Esto nos hace pensar en la posibilidad de una vacuna que incluya los serotipos I y II que representan casi 90% de los aislamientos.

**Palabras clave:** *Streptococcus agalactiae*, serotipos, sensibilidad antimicrobiana, mujeres embarazadas, sepsis neonatal.

## INTRODUCTION

Group B *Streptococcus* (GBS) remains the main cause of neonatal sepsis with an incidence of 2 to 3 per 1000 births and a case mortality ratio of 15 %.<sup>8</sup>

GBS are differentiated from other beta hemolytic streptococci by Lancefield serological typing.<sup>7</sup> *S. agalactiae* subgroups are determined by serotyping based on cell wall polysaccharides. In contrast to developed countries, where

type III accounts for more than two-thirds of all GBS related neonatal disease cases and is a predominant serotype, the most frequently identified serotypes in México are Ia and Ia/c, with serotype III present at a low frequency.<sup>12</sup> Thus, the low frequency of GBS invasive neonatal infections in México has been attributed to the low prevalence of type III strains.<sup>13,19</sup>

The emerging of resistant strains of GBS to the commonly used antimicrobials has caused concern over the adequacy of the standard prophylactic regimens.<sup>4,15</sup> Some investigators have advocated the need for routine susceptibility testing of isolated GBS obtained from penicillin-allergic patients because of the increasing resistance of GBS strains to the alternative antibiotic regimen.<sup>8,16</sup>

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In this study, we determine the serotype distribution and the antibiotic sensitivity patterns of isolated rectovaginal and urine GBS obtained from an obstetric population in the late third trimester.

## MATERIAL AND METHODS

We performed a prospective transversal study of serotype distribution and antibiotic susceptibilities of isolated rectovaginal and urine GBS from an obstetric population between March 1999 and December 2001. The protocol for this study was approved by the Commission for Research, Bioethics and Biosafety of the Family Medicine Department, School of Medicine, UNAM. Written informed consent to join the study was obtained from each patient before enrollment. SGB-positive patients were referred to an attending physician for follow-up and treatment of both the patient and the neonate.

GBS were isolated from vaginal, rectal and urine cultures obtained during medical visits in the late third trimester from 691 pregnant women attending a Primary Health Center in Tlalpan, México. 101 GBS isolates were cultured from 97 (14%) of 691 women. The clinical sources were as follows: vagina 77 strains, urine 5 strains and rectal 19 strains.

Cultures were inoculated onto 5% sheep blood agar (Bioxon) and streaked for isolation. After overnight incubation at 35°C, the blood agar plates supplemented with colistin (10 mg/l) and nalidixic acid (15 mg/l) were inspected for beta-hemolytic colonies.

Strains were considered likely to be GBS if they were gram positive, catalase negative, sodium hyppurate-hydrolysis positive, and had a positive CAMP reaction. Serologic confirmation was made by detection of group polysaccharide with latex particles coated with group-specific antibodies (Slidex Strepto-Kit ABCDFG bioMérieux, France).

Strains were serotyped by latex agglutination (Pastorex *Streptococcus* B, Becton Dickinson, Cockeysville, Maryland, EUA). These reagents detected serotypes I to V and any of the three above recently described; however 100% of our isolations corresponded to the first three serotypes.

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method according to the guidelines of the National Committee for Clinical Laboratory Standards<sup>11</sup> used with Bio Rad paper disk screened for susceptibility to ampicillin, penicillin, cefotaxime, amoxicillin and erythromycin.

## RESULTS

A total of 101 clinical *S. agalactiae* were serologically typeable. 61.3% were type I, 25.7% were type II and only

**Table 1.** Serotypes of strains of *S. agalactiae*, classified by source of isolation

| Serotype | No of isolates |        |       | Total %   |
|----------|----------------|--------|-------|-----------|
|          | Vagina         | Rectum | Urine |           |
| I        | 44             | 15     | 3     | 62 (61.4) |
| II       | 23             | 3      | 0     | 26 (25.7) |
| III      | 10             | 1      | 2     | 13 (12.9) |
| Total    | 77             | 19     | 5     | 101       |

12.87% were type III. The serotype distribution, classified by source of isolation, is shown on table 1.

All of the 101 clinical isolates of group B streptococci were susceptible to penicillinG, ampicillin, amoxicillin and cefotaxime. Six strains (5.9%) were resistant to erythromycin; by source of isolation we obtained 6.5%, 5.3% and 0% (vaginal, rectal and urine respectively) strains resistant to erythromycin.

## DISCUSSION

GBS sepsis is a devastating disease for the newborn with a 20% mortality rate and a 30% risk of long-term neurologic sequelae for survivors.<sup>4</sup> Why some infants and mothers develop disease and others remain asymptomatic has not yet been answered. Although the immune status of colonized mothers has been suggested to play a role of protection provided to their infants,<sup>2</sup> some authors have suggested that differences in virulence of GBS isolates may also contribute to neonatal infection.<sup>2,14</sup>

Studies evaluating the role of GBS in perinatal diseases in México are limited, but available data indicate that in contrast to what is found in the United States, only 10% of pregnant Mexican women are colonized with GBS.<sup>(18)</sup> Also in contrast to developed countries where type III is a predominant serotype, the most frequently identified serotypes in México are Ia and Ia/c, with serotype III present at a low frequency. This serotype is most frequently associated with invasive diseases.<sup>20</sup>

In this work, the most common serotype of the 101 strains isolated from the genital tract of pregnant women was type I (61.4%). In Chiapas, México, Ocampo et al<sup>12</sup> examined the serotypes of 78 SGB strains isolated from pregnant women; 67.9% were type I, 19.2% were type II and 12.8% were type III, data similar to ours. Collins et al,<sup>5</sup> in Lima, Peru, also reported serotype I as the most common, but with 53%. As opposed to this, Berg in Sweden,<sup>3</sup> Aitmhah in Casablanca<sup>1</sup> and Ko in Taiwan,<sup>20</sup> found that serotype III was the most common. Although all the serotypes of SGB appear to have a worldwide distribution, the few reports in Latin America refer to serotype I as the most frequently isolated in pregnant women.

The results of the susceptibility studies showed that the beta lactam agents tested displayed excellent activity "in vitro"; all isolates were susceptible to ampicillin, penicillin, amoxicillin and cefotaxime, Aitmhand et al,<sup>1</sup> Moyo et al<sup>(9)</sup> and Pearlman et al<sup>15</sup> also reported 100% sensitivity for all beta lactam antibiotics probed *in vitro*.

The rate of resistance of erythromycin found in our strains (5.9%) was lower than that of other reports like those of Silverman et al,<sup>17</sup> Murdoch et al<sup>10</sup> and Bland et al<sup>4</sup> who refer 9.6, 16 and 19% respectively.

The increase in penicillin allergic patients and the possible development of serotype-specific conjugate vaccine have increased the need for knowledge of the antimicrobial susceptibility and the capsular serotypes of group B streptococci.<sup>1</sup>

Based on our data, we conclude that changes have not been presented in the distribution of serotypes of SGB in Mexico, being the most common still serotype I, not only in our country but apparently also in Latin America. That makes us think of the possibility of a vaccine that includes serotypes I and II that represent approximately near 90% of the isolations according to the few reports in our country; however, due to the infrequent isolation of SGB with serotype III, the complications associated don't seem to be a health problem in México as in other countries.

In contrast, in the United States and other developed countries, invasive infection due to SGB is a major health public problem on both infants and adults, Harrison et al<sup>6</sup> refers that a tetravalent I, II, III, V vaccine would provide coverage against 99%, 94% and 82% of serotypes causing invasive infection for early-onset, late-onset, and nonpregnant adult cases in Maryland EUA, respectively.

Nevertheless, 6% resistance to erythromycin no important change has been presented in the sensitivity of the SGB *in vitro* to the different established alternatives for their treatment, and at least in our population, penicillin is still the drug of choice.

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