Revista Latinoamericana de Microbiología

Volumen *Volume* **47** **Número** *Number* **1-2** **Enero-Junio** *January-June* **2005**

Artículo:

Serotypes of 286 group B streptococci isolated from asymptomatic carriers and invasive disease cases in Mexico

Revista Latinoamericana de MICROBIOLOGÍA

ORIGINAL ARTICLE

Vol. 47, No. 1-2
January - March. 2005
April - June. 2005
pp. 21 - 24

Serotypes of 286 group B streptococci isolated from asymptomatic carriers and invasive disease cases in Mexico

Gerardo C. Palacios,* María N. González,* Magdalena Beltrán,** José L. Arredondo,** Javier Torres,* Fortino Solórzano*

ABSTRACT

Background: Group B *Streptococcus* (GBS) remains as a leading cause of neonatal sepsis and meningitis in developed countries, where type III is the most common serotype. Although GBS is considered an uncommon cause of perinatal pathology in Mexico, a vaginal colonization rate of 14% in pregnant women and a neonatal infection rate of 1/1500 live births have been reported. The aim of this study was to determine the serotype distribution in a collection of 286 GBS strains isolated in Mexico from asymptomatic carriers and in adult and neonatal invasive disease cases.
Methods: The collection included GBS strains isolated between January 1988 and April 1998 at the Instituto Nacional de Perinatologia and Hospital de Pediatria in Mexico City. GBS and serotype were confirmed by latex agglutination. **Results:** Most strains were isolated from asymptomatic carriers (66%). 30% were invasive isolates, and 10% of them were from neonates. 48.6% were type I, 32.9% type III, 14% type II, and 4% were non-typeable. **Conclusion:** Serotype I is predominant in Mexico but participation of serotype III is increasing, and a decrease of non-typeable isolates was detected.

Key words: Group B Streptococci, *Streptococcus agalactiae*, neonatal sepsis, neonatal meningitis, serotypes.

RESUMEN

Antecedentes: El Estreptococo del grupo B (EGB) es un microorganismo predominante en sepsis y meningitis neonatal en países desarrollados, considerándose el serotipo III el más frecuente. En México, se ha detectado hasta en el 14% de las mujeres embarazadas, con una tasa de infección neonatal de 1/1500 recién nacidos vivos. El objetivo fue determinar los serotipos en una colección de 286 cepas de EGB aisladas en México de portadores asintomáticos y enfermos adultos y recién nacidos. **Metodología:** Se incluyeron cepas de EGB aisladas entre enero de 1988 y abril de 1998 en el Instituto Nacional de Perinatología y el Hospital de Pediatría en la Ciudad de México. El género, la especie y el serotipo fueron confirmados por aglutinación de látex. **Resultados:** La mayoría de las cepas fueron aisladas de estado de portador (66%), el 30% procedía de casos de enfermedad, y el 10% de casos neonatales. 48.6% fueron del sertipo I, 32.9% tipo III, 14% tipo II y 4% fueron no tipificables. **Conclusión:** En México predomina el serotipo I, pero parece haber ocurrido un incremento del serotipo III y un descenso en la detección de aislados no tipificables.

Palabras clave: Estreptococo del grupo B, *Streptococcus agalactiae*, sepsis neonatal, meningitis neonatal, serotipos.

INTRODUCTION

Group B *Streptococcus* (*Streptococcus agalactiae*, GBS) remains the most frequent cause of neonatal sepsis and meningitis in the United States and other developed countries, where type III is the most common serotype.[2,18] Although GBS is considered an uncommon cause of perinatal infections in Mexico,[21,25] several studies have found vaginal colonization rates of 10-14% in pregnant women,[8,20] and a neonatal infection rate of 1/1500 live births with a high case-fatality rate (38.5%).[21] Furthermore, in a nation-wide GBS sero-epidemiologic survey a sero-prevalence of 90% demonstrated that the Mexican population has a high rate of exposure to GBS.[17]

Early studies in Mexico demonstrated that serotype I was the predominant serotype (33%), and also showed a low participation of serotype III (3%) with a high prevalence of non-typeable strains (18.2%).[20,21] Therefore, the low frequency of GBS invasive neonatal infections in Mexico was attributed to the low prevalence of type III strains along with increased levels of non-typeable strains.[20,21] More recent studies have confirmed that the predominant serotype in Mexican pregnant women is serotype I (58.8-61.3%) but have found a major participation of serotype III (5.9 -12.8%) with a poorer participation of non-typeable isolates (0 to 5.9%).[8,24] Furthermore, other recent study suggested a major participation of serotype III strains in invasive disease in Mexico.[14] All these data suggest that a change in GBS epidemiology in Mexico has occurred. In the present study, we evaluated the serotype distribution in a collection of GBS strains isolated in Mexico from asymptomatic carriers and adult and neonatal invasive disease cases.

* Departamento de Infectología y Unidad de Investigación en Enfermedades Infecciosas y Parasitarias, Hospital de Pediatría, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social.
** Departamento de Infectología, Instituto Nacional de Perinatología, Secretaría de Salud.

Received November 19, 2004; received in revised form April 5, 2005; accepted May 11, 2005.

Rev Latinoam Microbiol 2005; 47 (1-2): 21-24

MATERIALS AND METHODS

A collection of 286 GBS strains isolated from asymptomatic carriers and invasive disease adult and infant cases between January 1988 and April 1998 at the Instituto Nacional de Perinatologia and Hospital de Pediatria in Mexico City was evaluated. Information about the clinical source of isolates was obtained from the microbiology lab registers. Isolates obtained from blood, urine, cerebrospinal fluid (CSF), abscesses, bronchial specimens or vascular catheter tip were considered as invasive disease isolates. Those obtained from cervicovaginal secretion, semen, or pharyngeal cultures were considered as carrier isolates.

Every isolate was stocked in Todd-Hewitt broth (Difco) and stored at -70ºC. Thawed cultures were routinely streaked onto 5% sheep blood agar plates and incubated at 37ºC overnight before each experiment. An isolate was confirmed as GBS when was gram positive, catalase negative, sodium hyppurate-hydrolysis positive with a CAMP reaction positive, and a group polysaccharide detection positive by latex agglutination (Pastorex Strepto B, Diagnostic Pasteur, Marnes La Coquette, France). Serotyping was carried out by the latex agglutination assay (Pastorex Strepto B, Diagnostic Pasteur, Marnes La Coquette, France).

RESULTS

Serotype I was the most frequent (139, 48.6%) followed by serotype III (94, 32.9%) and serotype II (14.3%). Twelve strains (4%) were non-typeable (Table 1).

Most strains were isolated from asymptomatic carriers (66.4%) and most of them from cervicovaginal swabs (59%). Thirty percent of the sample was constituted of invasive isolates, most of them from pregnant adult urine samples (20%), neonatal blood (6.6%) and CSF (4.2%) cultures (Table 1).

Most invasive isolates were serotype I (51/96, 53.1%) and type III (36/96, 37.5%). On the other side, serotype II (34/41, 83%) and non-typeable (10/12, 83%) isolates were more frequently identified among carrier samples.

DISCUSSION

Although GBS is responsible for much of the perinatal morbidity and mortality in developed countries, in Mexico is considered an uncommon cause of perinatal infections,[2,21,25] which was attributed to the low vaginal colonization rate (1.5%).[3] Early studies in Mexico demonstrated that serotype I was the predominant serotype (33%), and also showed a low participation of serotype III (3%) with a high prevalence of non-typeable strains (18.2%).[20,21] Thus, the low frequency of invasive neonatal infections by GBS in Mexico was attributed to the low prevalence of type III strains along with increased levels of non-typeable strains.

Recent studies from Villaseñor et al,[24] González-Pedraza et al,[8] and the findings of the present paper suggest that serotype I is still predominant in Mexico (58.8%, 61.3%, and 48.6%, respectively). Data from these studies however show an increase of serotype III isolates (5.9% and 12.8%),[8,24] when compared with early information reported by Solorzano et al (3%).[20] We found a higher participation of serotype III isolates (32.9%), although 30% of our sample were invasive isolates and 10% of them were obtained from neonatal invasive disease cases.

Other important difference is related to the poorer participation of non-typeable isolates in the two more recently published studies (0 and 5.9%)[8,24] and in the present one (4.2%), compared with the earliest report by Solorzano et al.[20] These differences could be attributed to differences in

Table 1. Clinical source and serotype determined by the latex agglutination assay of 286 Mexican group B *Streptococcus* isolates.

Clinical origin	Serotype				
	I	II	III	NT	Total
Cervicovaginal	75	34	51	9	169 (59.1%)
Urine	33	4	21	1	59 (20.6%)
Blood	10	2	6	1	19 (6.6%)
Semen	11	-	6	1	18 (6.3%)
CSF	6	1	5	-	12 (4.2%)
Abscesses	-	-	3	-	3 (1.0%)
Pharyngeal	2	-	1	-	3 (1.0%)
Vascular catheter tip	1	-	1	-	2 (0.7%)
Bronchial specimens	1	-	-	-	1 (0.3%)
Total	139 (48.6%)	41 (14.3%)	94 (32.9%)	12 (4.2%)	286

Rev Latinoam Microbiol 2005; 47 (1-2): 21-24

sampling methods, sample size and probably to actual changes in serotype distribution occurred in Mexico through the time. Nevertheless, it is important to observe that the frequency of non-typeable isolates that we found (4.2%) is similar to other recent studies in Mexico[8,24] Although other serotypes have been identified among non-typeable isolates using different methods, we did not look for other more recently identified serotypes such as IV to VIII in our sample because some of them cause invasive disease more frequently in elderly individuals and non-pregnant adults with preexisting illnesses.[1,2,6,14,23]

Findings from a recent study suggest a major participation of serotype III in invasive disease in Mexico.[14] Moreover, the high rate of exposure of Mexican women to GBS[17] suggests that GBS epidemiology in Mexico is changing and probably serotype distribution too. Serotyping methods used could be contributing to these differences too. Solórzano et al[20] obtained their results by the capillary precipitin method using hot-HCL antigen extracts. In contrast, in more recent studies,[8,24] including the present one, isolates were serotyped by the latex agglutination assay that works with whole cells and is highly specific and sensitive.[5] Although it has been demonstrated that exists variability in levels of type-specific antigen production in GBS isolates and that the method of type-specific antigen extraction can markedly influence the results of serotyping, serotyping is even the basic method to classify clinical GBS isolates.[2,4,5,14] Molecular methods such as pulsed-field-gel-electrophoresis (PFGE), PCR, sequencing, and multilocus enzyme electrophoresis (MEE) are valuable approaches for subtyping GBS isolates, to compare isolate relatedness, and for monitoring both typeable and non-typeable GBS isolates for potential clonal divergences.[1,2,7,10,19]

This and other recent published studies suggest that there is a greater interest than before on GBS in Mexico.[8,24] This interest can explain that GBS is detected more frequently, not only in colonization but also in disease cases, and therefore that isolates of more virulent serotypes like serotype III are reported more frequently. It is important to know serotype distribution because some specific serotypes have been associated with a higher virulence. A number of other studies have suggested that isolates of a single clone or a limited number of clones could account for much of the morbidity and mortality caused by GBS.[7,9,13,19,22] Hence, a high-virulence clone (HVC) was proposed to cause much of the morbidity and mortality when a collection of GBS isolates was examined by multi-locus enzyme electrophoresis.[13] HVC isolates could be further distinguished by their inability to grow at 40ºC.[11,12] Some studies in Mexico found the serotype III HVC among clinical isolates.[15,16] These results demonstrated that a highly virulent clonal type of GBS is circulating also in Mexico.

Results from the present study demonstrate that the predominant serotype in Mexico is the serotype I but suggest that serotype III is increasing, not only in colonization state but also in invasive disease. A continuous survey is needed to know the variability of serotype distribution in any given area, including Mexico.

REFERENCES

1. Amundson, N.R., A.E. Flores, S.L. Hillier, C.L. Baker, & P. Ferrieri. 2005. DNA macrorestriction analysis of nontypeable group B streptococcal isolates: clonal evolution of nontypeable and type V isolates. J Clin Microbiol. 43:572-576.
2. Baker, C.J. & M.S. Edwards. 1995. Group B streptococcal infections. pp. 980-1054 In: J.S. Remington and J.O. Klein (Eds) Infectious diseases of the fetus and newborn infant, 4th ed. W.B Saunders, Philadelphia.
3. Collado, M.L., R.R. Kretschmer, I. Becker, A. Guzmán, L. Gallardo, & C.M. Lepe. 1981. Colonization of Mexican pregnant women with group B *Streptococcus*. J Infect Dis. 143:134.
4. Doran, T.I., D.C. Straus & S.J. Mattingly. 1981. Factors influencing release of type III antigens by group B streptococci. Infect Immun. 31:615-623.
5. Elliott, J.A., T.A. Thompson, & R.R. Facklam. 2004. Increased sensitivity of a latex agglutination method for serotyping group B *Streptococcus*. J Clin Microbiol. 42:3907.
6. Farley, M.M. Group B streptococcal disease in nonpregnant adults. Clin Infect Dis. 33:556-561.
7. Fleming, K.E., J.F. Bohnsack, G.C. Palacios, S. Takahashi, & E.E. Adderson. 2004. Equivalence of high-virulence clonotypes of serotype III group B *Streptococcus agalactiae* (GBS). J Med Microbiol. 53:1-4.
8. González-Pedraza, A.A., M.C. Ortiz-Zaragoza & R. Mota-Vázquez. 2002. Serotypes and antimicrobial susceptibility of group B *Streptococcus* isolated from pregnant women in Mexico. Rev Latinoamer Microbiol. 44:133-136.
9. Helmig, R., N. Uldbjerg, J. Boris, & M. Kilian. 1993. Clonal analysis of *Streptococcus agalactiae* isolated from infants with neonatal sepsis or meningitis and their mothers and from healthy pregnant women. J Infect Dis. 168:904-909.
10. Kong, F., S. Gowan, D. Martin, G. James & G.L. Gilbert. 2002. Serotype identification of group B streptococci by PCR and sequencing. J Clin Microbiol. 40:216-226.
11. Mattingly, S.J., J.J. Maurer, E.K. Eskew & F. Cox. 1990. Identification of a high-virulence clone of serotype III *Streptococcus agalactiae* by growth characteristics at 40ºC. J Clin Microbiol. 28:1676-1677.
12. Mattingly, S.J. & E.K. Eskew. 1993. Temperature sensivity of fructuose-1,6-biphosphate aldolase accounts for the inability of the high virulence clone of *Streptococcus agalactiae* to grow at 40ºC. Curr Microbiol. 26:147-150.
13. Musser, J.M., S.J. Mattingly, R. Quentin, A. Goudeau & R.K. Selander. 1989. Identification of a high-virulence clone of type III *Streptococcus agalactiae* (group B *Streptococcus*) causing invasive neonatal disease. Proc Natl Acad Sci USA. 86:4731-4735.
14. Palacios, G.C., E.K. Eskew, F. Solorzano & S.J. Mattingly. 1997. Decreased capacity for type-specific-antigen synthesis accounts for high prevalence of nontypeable strains of group B streptococci in Mexico. J Clin Microbiol. 35:2923-2926.
15. Palacios, G.C., E.K. Eskew, F. Solorzano & S.J. Mattingly. 1999. Identification of the high-virulence clone of group B streptococci in mexican isolates by growth characteristics at 40°C. Curr Microbiol. 38:126-131.
16. Palacios, G.C., B.C. Timmons, E.K. Eskew, F. Solorzano & S.J. Mattingly. 2003. Identification of the high-virulence clone of group

Rev Latinoam Microbiol 2005; 47 (1-2): 21-24

B streptocci by using a probe containing a putative aldolase gene. Curr Microbiol. 47:319-322.
17. Palacios-Saucedo, G., R. Caltenco-Serrano, J. Torres-Lopez, R. Tapia-Conyer, O. Muñoz-Hernandez & F. Solorzano-Santos. 2002. Exposición a Estreptococo del grupo B en mujeres mexicanas en edad reproductiva. Salud Pub Mex. 44:50-56.
18. Payne, R.N., A.B. Burke, L.D. Day, D.P. Christenson, R.T Thompson & P. Ferrieri. 1988. Correlation of clinical and pathologic findings in early onset neonatal group B *Streptococcal* infection with disease severity and prediction of outcome. Pediatr Infect Dis J. 7:836-47.
19. Quentin, R., H. Huet, F. Wang, P. Geslin, A. Goideau & R. Selander. 1995. Characterization of *Streptococcus agalactiae* strains by multilocus enzyme genotype and serotype: Identification of multiple virulent clone families that cause invasive neonatal disease. J Clin Microbiol 33:2576-2581.
20. Solorzano-Santos, F., G. Echaniz-Aviles, C.J. Conde-Gonzalez, E. Calderon-Jaimes, J.L. Arredondo-Garcia & M. Beltran-Zuñiga. 1989. Cervicovaginal infection with group B Streptococci among pregnant Mexican women. J Infect Dis. 159:1003-1004.
21. Solorzano-Santos, F., R.D. Diaz-Ramos & J.L. Arredondo-García. 1990. Diseases caused by group B *Streptococcus* in Mexico. Pediatr Infect Dis J. 9:66.
22. Takahashi, S., E.E. Adderson, Y. Nagano, N. Nagano, M.R. Briesacher & J.F. Bohnsack. 1998. Identification of a highly encapsulated, genetically related group of invasive type III group B streptocci. J Infect Dis. 177:1116-1119.
23. Tirrell, G.J., L.D. Senzilet, J.S. Spika, D.A. Kertesz, M. Alagaratnam, M. Lovgren & J.A. Talbot. 2000. Invasive disease due to group B streptococcal infection in adults: results from a Canadian, population-based, active laboratory surveillance study-1996. Sentinel Health Unit Surveillance System Site Coordinators. J Infect Dis. 182:168-173.
24. Villaseñor-Sierra, A., P. Morales-Velázquez, G. Palacios-Saucedo & F. Solórzano-Santos. 2004. Prevalencia de *Streptococcus agalactiae* del serotipo III en embarazadas. Ginecol Obstet Mex. 72:103-108.
25. Walsh, J.A. & S. Hutchins. 1989. Group B streptococcal disease: its importance in the developing world and prospect for prevention with vaccines. Pediatr Infect Dis J. 8:271-277.

Correspondence to:

Gerardo C. Palacios.
Unidad de Investigación Biomédica del Noreste
y Unidad Médica de Alta Especialidad
Hospital Regional de Especialidades No. 25
Calle 2 de Abril y San Luis Potosí S/N.
Colonia Independencia.
64720 Monterrey, Nuevo León, México
Tel./Fax: +52 (81) 8190-4035
E-mail: palsaugc@att.net.mx