BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-979020222e20633

Article

Prevalence of *Streptococcus agalactiae* capsular types among pregnant women in Rio de Janeiro and the impact of a capsular based vaccine

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Streptococcus agalactiae (group B *Streptococcus*, GBS) remains one major neonatal pathogen, being maternal colonization a risk factor for infection development. Despite effective, the usage of antibiotics to prevent neonatal infections has limitations. The bacterial polysaccharide capsule is a virulence determinant, a target for vaccine directed to pregnant women, and also the most useful epidemiological marker of GBS infections. Capsular polysaccharides are diverse and disease severity varies according to the expressed type. Here, capsular typing of 124 GBS isolates recovered from pregnant women was determined by a multiplex PCR-based method. The most frequent types were Ia (33.0%), II (25.8%) and V (21.8%). Other types found were Ib (8.9%), III (8.9%) and IV (1.6%). While type Ia was prevalent during the whole period (2002-2018), fluctuations in distribution of other types, specially V, were observed over time. Capsular type III, traditionally associated with severe neonatal infections, was poorly detected. Distribution of maternal GBS capsular types in the metropolitan area of Rio de Janeiro, with prevalence of Ia and II, is quite different from other parts of the world. The knowledge about GBS capsular type distribution is essential to predict the theoretical impact of developing capsule-based vaccines in the local population.

Keywords: Antibiotic. Molecular epidemiology. Pregnancy. *Streptococcus agalactiae*. Vaccine.

INTRODUCTION

Streptococcus agalactiae (Group B *Streptococcus*, GBS) is a major neonatal pathogen, causing life-threatening diseases, such as pneumonia, bacteremia and meningitis. Besides being a cause of neonatal death, GBS infections can lead to adverse outcome in survivors, with neurological and psychomotor impairments in these children (Verani, Schrag, 2010; Madhi, Dangor, 2017). Neonatal infections are classified as early onset disease (EOD), when they occur in the first week of life, and late onset disease (LOD), occurring after one week to three months of life (Verani, Schrag, 2010). The major risk factor for EOD is maternal vaginal colonization and the most effective preventive measure available is intrapartum antibiotic prophylaxis (IAP), with beta-lactams as recommended therapy and clindamycin or vancomycin as alternative drugs. Implementation of IAP is based on defined risk factors and GBS positive culture at 36-37 weeks of pregnancy (ACOG, 2020). However, several limitations concerning IAP can be addressed, as the lack of GBS screening in late pregnancy, preterm delivery labor and reduced penicillin susceptibility (Madhi, Dangor, 2017). Despite IAP limitations, regions that adopted these guidelines have experienced reduction in EOD rates (CDC, 2010). In Brazil, public health authorities do not recommend IAP (Brasil, 2012). In addition, no national data about prevalence of GBS maternal colonization, neonatal infection or serotypes is available, being data limited to local investigations in few regions.

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Regarding GBS pathogenesis, the polysaccharide capsule plays an important role, preventing phagocytosis (Patras, Nizet, 2018). Capsule presents antigenic variability, due to its carbohydrate composition. Ten types are described until now (Ia, Ib, II-IX). Whereas types Ia, Ib, II, III and V account for 98% of colonization isolates globally, type III has been associated with severe neonatal infections (Madrid *et al*., 2017; Russel *et al*., 2017). Therefore, capsular typing of circulating isolates represents a useful epidemiological strategy to trace the pathogen.

The GBS capsule has been in the center of several efforts to the development of a vaccine directed to pregnant women. Maternal immunization against GBS is a promising alternative to prevent neonatal infections, since high antibody levels to individual capsular types, as well as to a trivalent conjugated vaccine (Ia, Ib and III) have been detected in previous clinical trials (Madhi *et al*., 2016; Hillier *et al*., 2019). Other approach to GBS vaccine development relies on immunogenic surface proteins, as alpha-like proteins (Maeland *et al*., 2015). However, an important limitation is their variable distribution among the GBS population.

This is a retrospective study that aimed to evaluate the distribution of capsular types of *S. agalactiae* recovered from pregnant women resident in the metropolitan area of Rio de Janeiro, Brazil, over a period of 16 years and to predict the impact of capsular polysaccharide-based vaccines in such population.

MATERIAL AND METHODS

Subjects and bacterial isolates

One hundred twenty-four GBS isolates were available for the study. They were recovered from 124 pregnant women, assisted in antenatal care services (private and public) located in the metropolitan area of Rio de Janeiro, from March 2002 to March 2018. Records about subjects were limited to specimen, date of specimen collection and colony count of urine culture. Bacterial isolates were recovered from vaginal secretion (61) and urine (63). Significant bacteriuria (CFU > 10^5/mL) was observed in 88.9% of urine cultures. Species identification was performed after blood agar growth, using standard phenotypic tests (hemolysis, catalase, CAMP and hippurate hydrolysis).

Capsular typing

GBS isolates were submitted to capsular typing by a multiplex Polymerase Chain Reaction (PCR)-based method, according to previously described protocols (Poyart *et al*., 2007; Murayama *et al.,* 2009). DNA was obtained by thermal lysis. Briefly, McFarland 3 suspensions were prepared in 300 µL of TE buffer and boiled for 5 min. PCR reagents and agarose were from Thermo Scientific (Waltham, MA, USA). PCR occurred in the thermal cycler Veriti Thermal Cycler (Applied Byosistems, Foster City, CA, USA). Amplification products were subjected to electrophoresis on 1% agarose gel and visualized under UV light.

Statistical analysis

Fischer exact test was used to verify any association between capsular type distribution and isolate source (https://www.graphpad.com/quickcalcs/contingency1. cfm).

RESULTS AND DISCUSSION

In this study, the predominant GBS capsular type was Ia (33.0%), followed by II (25.8%), V (21.8%), Ib (8.9%), III (8.9%) and IV (1.6%). Neither association was observed between capsular type distribution and isolate source nor between capsular types and occurrence of significant bacteriuria in urine specimen. While maternal colonization rates vary slightly around the world, the distribution of capsular types among circulating isolates is diverse, considering clinical conditions (colonization x infection), geographical and temporal differences (Shabayek, Spellerberg, 2018). The prevalence of capsular types has varied locally, being Ia, Ib and III prevalent in North America, Europe and Oceania, types Ia, Ib, III and V prevalent in Africa and other types (VI-IX) significantly found in South-eastern Asia (Russel *et al*., 2017; Shabayek, Spellerberg, 2018).

In order to better visualize the distribution of capsular types during the whole period, sampling was divided into four-time intervals: 2002-2005, 2006-2009, 2010-2013 and 2014-2018 (Table I). Type Ia was the most frequent in all time intervals, followed by type II. South America, particularly Brazil, seems to have a peculiar predominance of type Ia in both colonization and infection isolates (Palmeiro *et al*., 2010; Souza *et al*., 2013; Botelho *et al*., 2018). Type II was found consistently in all periods, being the second most prevalent, as previously observed in the region (Botelho *et al*., 2018). Type V was the third predominant, being among the most common in 2002-05 and 2010-13 and among the least detected in 2006-09 and 2014-18. Capsular type III has not been frequently detected in any period of time, being one of the least detected during 2010-13. Type IV, detected here only in two isolates recovered from distinct subjects in 2008, has recently emerged associated with the hypervirulent clone CC-17, probably due to capsular switch (Shabayek, Spellerberg, 2018). CC-17 emerged as the most invasive genetic lineage to newborns, regardless the capsular type (Jones *et al*., 2016). However, strains belonging to CC-17, expressing capsular type III, are far the most related to LOD, especially meningitis (Shabayek, Spellerberg, 2018). As observed to capsular type IV, type III had a limited spread in this area.

TABLE I - Distribution of GBS capsular types over time

Capsular type	Time intervals				Total
	2002-2005	2006-2009	2010-2013	2014-2018	
Ia	4 (26.7%)	10 (33.3%)	19 (35.8%)	8 (30.8%)	41 (33%)
Ib	1 (6.7%)	1 (3.3%)	4 (7.5%)	5 (19.2%)	11 (8.9%)
II	4 (26.7%)	9 (30%)	13 (24.5%)	6 (23.1%)	32 (25.8)
III	2 (13.3%)	4 (13.3%)	2 (3.8%)	3 (11.5%)	11 (8.9%)
IV	0	2 (6.6%)	0	0	2 (1.6%)
V	4 (26.7%)	4 (13.3%)	15 (28.3%)	4 (15.4%)	27 (21.8%)
Total	15 (12.1%)	30 (24.2%)	53 (42.7%)	26 (21%)	124 (100%)

The knowledge of GBS capsular type distribution among pregnant women is important not only to understand the pathogen epidemiology in a given region, but it is also essential to guide the development of capsular polysaccharide-based vaccines directed to this population. In this view, the capsular type distribution can predict the impact of such formulation in each local population. Our data and other studies conducted in Brazil suggest that a trivalent formulation with types Ia, Ib and III, that had undergone to phase II clinical trial, despite being suitable to several other geographic regions (Madhi *et al*., 2016) would not be effective to pregnant women living in Brazil. The theoretic impact of such vaccine would be 51% in this population, as shown here. Data of serotype distribution prior to vaccine implementation is also essential to detect changes in disease incidence due to non-vaccine serotypes, as shown to other pathogens such as *Streptococccus pneumoniae,* after pneumococcal conjugated vaccines (Waight *et al*., 2005).

In conclusion, this study highlights the consistent predominance of GBS capsular type Ia isolates, as well as the low rate of type III isolates, among pregnant women living in the second most populated region in Brazil. Such data is essential to trace the epidemiological behavior of GBS and to evaluate the impact of a vaccine based on capsular polysaccharides, especially in low and middle incoming countries, where studies are scarce.

ACKNOWLEDGEMENTS

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES, Cod 001).

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Received for publication on 23rd July 2020
Accepted for publication on 04th October 2020