

Short Communication

Prevalence of Group B *Streptococcus* serotypes III and V in pregnant women of Rio de Janeiro, Brazil

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Abstract

GBS serotypes III and V were the most prevalent in pregnant women and exhibited resistance to tetracycline, clindamycin and sulfamethoxazole/trimethoprim. Serotype III showed high sialic acid content and PFGE analysis discerned 33 heterogeneous profiles. Phenotypic and genotypic characterization could be relevant to control GBS infections unaffected by intra-partum chemoprophylaxis.

Key words: Group B *Streptococcus*, antimicrobial susceptibility, sialic acid, PFGE.

Streptococcus agalactiae (Group B *Streptococcus*, GBS) are the most common cause of serious bacterial infections in newborn and adults with underlying medical disorders. At the time of birth, GBS is vertically transmitted to the neonate, and it is estimated that 50% or more of newborns exposed to GBS will become colonized if the mother is a carrier (Trappe *et al.*, 2011)

The first-line agent against GBS infection is penicillin, and penicillin resistance among GBS strains has not been reported so far. However, for patients allergic to penicillin, macrolides (*e.g.*, erythromycin) and lincosamides (*e.g.*, clindamycin) are the alternative choices for the treatment of GBS infections. In Brazil, previous studies showed resistance to tetracycline (21.36%), erythromycin (5.83%) and clindamycin (1.94%) in cities located in the Southern and Southeast regions (Oliveira *et al.*, 2005).

Although the physical structure of the capsular polysaccharide (CPS) of each GBS serotype is unique, all ten different GBS serotypes (Ia, Ib, II-IX) invariably share a terminal α -2,3-linked *N*-acetylneuraminic acid (sialic acid), which is identical to the predominant sialic acid found on human cells. GBS sialic acid enhances their virulence by interfering specifically with opsonic components

of the complement system and, represent a key step toward understanding the mechanisms of Sia-dependent virulence at the host-pathogen interface (Weiman *et al.*, 2010). CPS serotyping is the classic method for typing GBS in epidemiological studies. However, phenotypic methods have limited accuracy and often result in some non-typeable isolates. Earlier studies have demonstrated the discriminatory ability and reliability of pulsed-field gel electrophoresis (PFGE) in further differentiation of GBS strains of the same serotype (Rolland *et al.*, 1999).

The purpose of this study was to characterize GBS isolates recovered from pregnant healthy women attended in a public hospital located in the metropolitan area of Rio de Janeiro, Brazil, analyzing genotypic (PFGE) and phenotypic profiles related to serotype, antibiotic susceptibility and sialic acid content.

Thirty-three isolates consecutively isolated from pregnant (> 25 weeks of gestation) women were mostly obtained with a swab cultured from the lower vagina. No one had any signs or symptoms of infection at the moment of sampling (unpublished data). Microorganisms were formerly identified as group B by means of a latex-agglutination grouping kit (Streptococcal Grouping Kit, Oxoid,

São Paulo, Brazil) and serotyped by immunoprecipitation in agarose using new sera produced locally and HCl antigen extracts from 20-mL broth cultures (Lancefield, 1933). GBS were stored after lyophilisation and recovered in Brain Heart Infusion broth (BHI; Difco). Six Brazilian strains 85147 and 90222 (serotype Ia; oropharynx and urine, respectively), 80340 and 90356 (serotype III; vagina and cerebrospinal fluid), 88641 and 90186 (serotype V; vagina and blood) partially evaluated in some virulence features (Costa *et al.*, 2011) were also included in all assays.

GBS isolates were tested for susceptibility to the following antibiotics by the disk diffusion method considering breakpoints established in M100-S21 CLSI standards (2011): ampicillin (10 µg), cefazolin (30 µg), clindamycin (2 µg), erythromycin (15 µg), imipenem (10 µg), norfloxacin (5 µg), penicillin (10 IU), sulfamethoxazole/trimethoprim (23.75 µg/1.25 µg), tetracycline (30 µg), and vancomycin (30 µg) (Baltimore Biological Laboratories [BBL], Sparks, MD).

For sialic acid analyses, GBS isolates grown in 100 mL of BHI for 18 h at 37 °C were recovered by centrifugation, washed once in 0.01 M PBS and lyophilised. After determination of the weight of lyophilised cells, the extraction procedures were carried out based on methods previously described by Kamerling and colleagues (Kamerling *et al.*, 1982).

For clonality analyses using PFGE, plugs containing genomic DNA were prepared as described by Rolland and colleagues (Rolland *et al.*, 1999). DNA was digested by *Sma*I restriction enzyme (Amersham Biosciences do Brasil Ltda, São Paulo, SP) and submitted to PFGE with a program as follows: switch time of 1 to 30 s, 23 h, 120° angle, 11.3 °C and a voltage gradient of 6 V/cm. Lambda phage concatemers were used as DNA size standards with size range 50-1,000 kb.

The criteria used for definition of the clonality were those suggested by Tenover *et al.* (1995). DNA banding patterns were analysed for similarity as follows: [i] Genetically *indistinguishable* if their restriction patterns had the same number of bands and the same apparent size (they were assigned the same capital letter and numerical index); [ii] *Closely related* to the predominant strain (assigned here as A₁ pattern) if the PFGE profile differed from the profile A₁ by changes that resulted in two or three band differences; [iii] *Possibly related* to the predominant strain if the PFGE profile differed from the predominant A₁ profile by changes with four to six band differences; [iv] *Unrelated* to the predominant PFGE pattern if the PFGE pattern differed from the profile A₁ by changes consistent with seven or more band differences. An *unrelated* isolate was considered to belong to a different clone and was ascribed a different capital letter. GBS isolates belonging to a specific serotype and displaying identical, closely related, or possibly related PFGE profiles were assigned to the same pattern (Tenover *et al.*, 1995).

In attempt of classifying minor typing pattern variations a supplementary analysis was performed as suggested by Tenover *et al.* (1997). Therefore, quantitative pairwise comparison of pulsotypes was accomplished by using the Dice coefficient of similarity. A dendrogram was constructed by using the software Bionumerics (Version 2.0 Applied Maths, Kortrijk, Belgium) to provide an image of the genetic relationship between isolates with arithmetic averages with a tolerance coefficient of 1.0% and optimization of 1.0%. PFGE patterns were defined grouping isolates that showed > 70% dendrogram identity, as previously used in some GBS strains (Savoia *et al.*, 2008).

The presence of GBS vaginal or rectal carriage was detected in 24.3% of parturient women observed in our community. The carriage rate found by us was higher when compared with the results described in Santa Catarina (21.6%) (Pogere *et al.*, 2005), Ribeirão Preto (17.9%) (Zusman *et al.*, 2006) and São Luis (20.4%) (Costa *et al.*, 2008) (Fisher's Exact Test, $p = 0.0192$, $p = 0.005$, $p = 0.0143$, respectively). However, the present data were similar to those described by other authors in Brazil: Rio de Janeiro (26%) (Benchetrit *et al.*, 1982) and Campinas (27.6%) (Nomura *et al.*, 2006).

Among the 39 GBS isolates studied, the serotypes III and V were the most prevalent and the most resistant to antibiotics in agreement with the results of other authors (Oliveira *et al.*, 2005). Epidemiological analysis, showed that serotype III was the most prevalent with 15 (38.46%) isolates, followed by 10 (25.64%) isolates of serotype V, 3 (7.69%) of serotype II, 4 (10.26%) of serotype Ia, 2 (5.13%) of serotype Ib, and 5 (12.82%) non-typeable isolates (Table 1).

The analysis of the antimicrobial susceptibility profiles of 39 GBS isolates revealed colonizing isolates, universally susceptible to ampicillin, cefazolin, erythromycin, imipenem, norfloxacin, penicillin, and vancomycin (Table 1). Conversely, 69.23% and 7.69% isolates were resistant to tetracycline and clindamycin, respectively. In another study from Rio de Janeiro, a high prevalence of tetracycline resistance was also observed among clinical isolates from human sources (90.9%) (Duarte *et al.*, 2005).

A significant number of currently assessed GBS isolates were resistant to sulfamethoxazole/trimethoprim (17.95%). Frequent resistance to sulfamethoxazole/trimethoprim (53.85%) was previously reported among GBS isolates in Southern Croatia (Barisić *et al.*, 2003), but not in any region of Brazil.

As reported in a previous study from Kuwait, GBS was the third most common cause of urinary tract infection, and the recommended therapy for acute uncomplicated cystitis in adults is sulfamethoxazole/trimethoprim (Sweih *et al.*, 2005). Therefore, local epidemiological data are necessary in order to choose appropriate antimicrobial therapy. High level antimicrobial resistance among colonized, pregnant women is likely driven by antibiotic use, coupled with

Table 1 - Characteristics of the multiple-strain clonal clusters of 39 GBS isolates in Rio de Janeiro

Serotype	Total	PFGE patterns	Number of isolates					
			Resistance phenotypes			Sialic acid content ^a		
			SMX/TMP	TET	CLIN	< 2	2-4	≥ 4
Ia	4	A ₁ (2), B ₁ (1), B ₂ (1)	R (1)	R (2)	R (1)	3	0	1
Ib	2	A ₂ (1), C ₁ (1)	R (1)	R (1)	R (0)	0	1	1
II	3	D ₁ (1), D ₂ (1), E (1)	R (1)	R (2)	R (0)	1	2	0
III	15	F ₁ (2), F ₂ (1), G ₁ (1), H ₁ (2), I ₁ (1), J ₁ (1), K ₁ (2), L ₁ (1), M ₁ (1), N ₁ (1), N ₂ (1), O ₁ (1)	R (2)	R (12)	R (1)	3	8	4
V	10	P ₁ (2), P ₂ (1), Q ₁ (1), R ₁ (1), S ₁ (2), T ₁ (1), U ₁ (1), V ₁ (1)	R (2)	R (7)	R (1)	4	5	1
NT	5	W ₁ (1), W ₂ (1), X ₁ (1), Y ₁ (1), Z ₁ (1)	R (0)	R (3)	R (0)	2	3	0

SMX/TMP, trimethoprim-sulphamethoxazole; TET, tetracycline; CLIN, clindamycin; NT, non-typeable. ^aµg/mg of cells dry weight.

high transmission probabilities. Our current findings highlight the need for routine susceptibility testing of GBS, particularly in individuals with penicillin allergy, to ensure proper therapy. In Rio de Janeiro, penicillin remains the first choice among drugs for prophylaxis and treatment of early-onset GBS infections in our community.

The distribution of sialic acid levels in the serotypes of GBS tested was classified into three categories: (i) Low sialic acid content of less than 2.0 µg/mg of cell dry weight; (ii) Moderate sialic acid content of more than 2.0 but less than 4.0 µg/mg; (iii) High sialic acid content of equal to or more than 4.0 µg/mg. All GBS strains exhibited sialic acid content at different levels. Higher (≥ 4 µg/mg) contents were observed among some strains of serotypes Ia, Ib, III and V.

DNA fingerprinting by PFGE revealed identical genomic profiles demonstrating clonality amongst a few number of GBS isolates (Figure 1). Analysis of band patterns of GBS isolates by using both criteria established by Tenover *et al.* (1995) and Dice coefficient of similarity (Savoia *et al.*, 2008) showed distinct clonal origin for the majority of isolates. However, different results were observed by both methods for some samples.

The criteria proposed by Tenover *et al.* (1995) that defined genetic events and categories of genetic and epidemiological relatedness allowed the differentiation of GBS isolates into 33 pulsotypes as follows: type Ia and Ib isolates, 3 and 2 patterns, respectively; type II strains, 3 patterns; type III and V, 12 and 8 patterns; non-typeable strains, 5 patterns. No predominant patterns were detected among GBS isolates.

Figure 1 also illustrates the results of analysis of computer-generated dendrogram using the Dice coefficient. Data showed minor typing pattern variations for some strains. Dice coefficient analysis was unable to show various genetic events that occurred in each isolated, which leads to different patterns of bands detected in the Tenover method. A particular pulsotype (B1 and B2) showed Dice

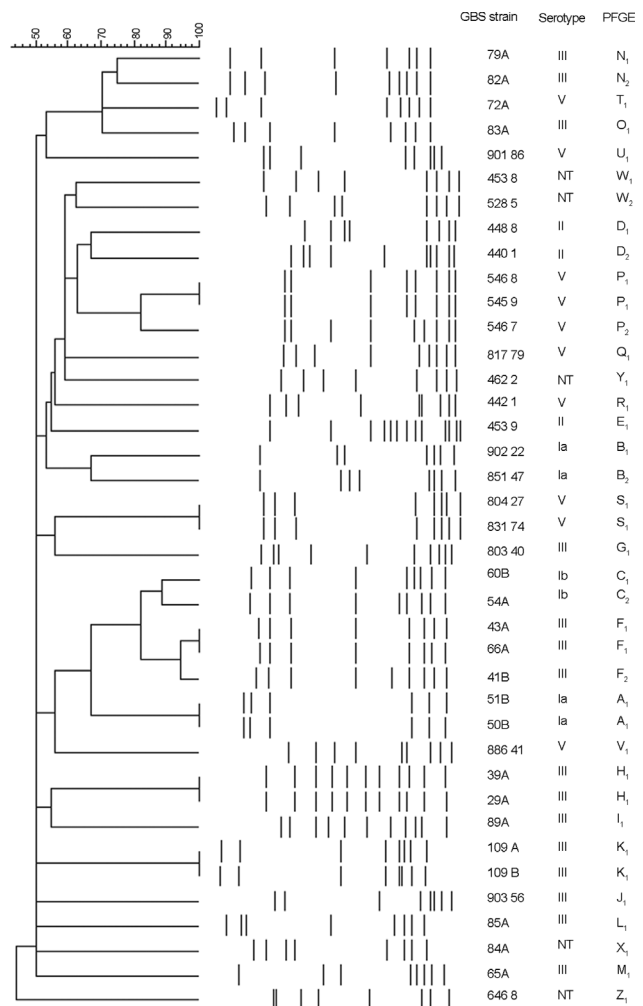


Figure 1 - Phylogenetic analysis of PFGE profiles for GBS isolates. The dendrogram was constructed with PFGE profiles by similarity and clustering analyses using the unweighted-pair group method with arithmetic averages and Dice's coefficient. The levels of genetic similarity in percentages are shown on the left. The strain serotype and codes PFGE subtype are indicated on the right.

coefficient less than 70% of similarity. Conversely, different pulsotypes (F1, F2, C1 and C2) showed Dice coefficient with higher than 80% similarity. Data showed the relevance of using both methods of analysis of PFGE patterns of GBS endemic isolates.

In conclusion, the high degree of genetic heterogeneity of the GBS strains not only among the different polysaccharide-specific serotypes, but within each serotype as well, indicates the endemic nature of GBS infection in our community. However, PFGE-type clonal groups were observed for GBS III, V and Ia serotypes. Moreover, an increased resistance to tetracycline, clindamycin and sulfamethoxazole/trimethoprim was observed among GBS strains isolated from asymptomatic carriers from Rio de Janeiro. Data also emphasize that a simple combined strategy of intrapartum prophylaxis with universal neonatal screening for GBS and an assessment of risk factors for perinatal GBS disease could significantly reduce the rate of late-onset GBS infections in our community.

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