Pulsed-field gel electrophoresis, virulence determinants and antimicrobial susceptibility profiles of type Ia group B streptococci isolated from humans in Brazil

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Group B streptococci (GBS) infections occur worldwide. Although serotyping has been used for epidemiologic purposes, this does not accurately characterize enough members of a genetically heterogeneous bacterial population. The aims of this work were to evaluate the genetic diversity of 45 type Ia GBS strains isolated in Brazil by pulsed-field gel electrophoresis as well as to evaluate antimicrobial susceptibility profiles and identify virulence genes. Twenty-four strains were assigned to cluster A. All strains under study contained the hylB *and* scpB *genes. The* bca *gene was detected in only 10 strains and none of the streptococci carried the* bac *gene. Thirty-nine strains were resistant to tetracycline.*

Key words: group B streptococci - pulsed-field gel electrophoresis - polymerase chain reaction

Group B streptococci (GBS) are responsible for a large variety of human infections and have been recognized over the last few decades as a leading cause of perinatal disease worldwide (Farley 2001, Weisner et al. 2004). GBS are classified in serotypes (Ia, Ib and II-VIII) that occur in combination with different protein antigens (alpha, beta and rib) (Dmitriev et al. 2001). Currently, serotypes Ia, III and V are the most common in many countries (Duarte et al. 2005, Fluegge et al. 2005, Bergseng et al. 2008, Poyart et al. 2008, von Both et al. 2008).

Pulsed-field gel electrophoresis (PFGE), used to examine GBS strains, is a powerful technique employed for the classification of microorganisms after digestion of the genomic DNA by restriction enzymes (Benson & Ferrieri 2001, Oliveira et al. 2005, 2006, von Both et al. 2008). Using polymerase chain reaction (PCR), Franken et al. (2001) suggested that the *scpB* and *lmb* genes (encoding c5a peptidase and laminin binding protein) must exist in GBS strains that infect humans. The *hylB* gene is frequently detected in these strains (Dore et al. 2003). The *bac* and *bca* genes are present in 23% and 100% of type Ia strains, respectively (Maeland et al. 1997, 2000).

The lack of information on Ia Brazilian strains and the availability of DNA techniques led us to investigate the genetic make-up of type Ia GBS strains isolated in distinct regions of Brazil. Additionally, antimicrobial susceptibility was examined to better characterize these isolates.

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MATERIALS AND METHODS

Bacterial strains - Forty-five human type Ia GBS strains derived from clinical specimens were obtained in Florianopolis, Santa Catarina (n = 3), a city located in the Southern Region of Brazil, in São Paulo, São Paulo (SP) (n = 1), and in Rio de Janeiro, Rio de Janeiro (n = 40), in the Southeast Region of the country. The source of one strain was unknown.

Isolates were obtained from 1981-2002 from public health laboratories, gynaecological clinics, hospitalsand universities. The clinical sources included urine (n = 16), oropharynx (n = 9), vagina (n = 5), anus (n = 2), lung (n = 2), placenta (n = 1), external ear canal (n = 1) and perineum (n = 1). The sources of eight specimens were unknown. A confirmatory identification of serotypes was carried out again by immunoprecipitation in agarose using sera produced in the research facilities of the authors and HCl antigenic extracts from the streptococci (Benchetrit et al. 1982).

PFGE - PFGE was performed as previously described (Oliveira et al. 2005). DNA was digested with the *Sma*I restriction enzyme (Amersham) and submitted to electrophoresis with a program as follows: switch time of 1-30 sec during 23 h, with a 120º angle, at a temperature of 11.3ºC and a voltage gradient of 6 V/cm. The lambda ladder PFGE marker kit (New England Biolabs) was used as a DNA size marker. Gels were stained with ethidium bromide and photographed under UV light. Criteria for analysis of the PFGE patterns were those originally suggested by Tenover et al. (1995) and used in our previous studies (Oliveira et al. 2005, 2006).

PCR - DNA extraction was performed according to Sambrook et al. (1989). DNA fragments of the different GBS genes were amplified at a temperature of 53ºC. PCR-amplified products were run on agarose gels

and stained with ethidium bromide. The 123-bp lambda ladder kit (Invitrogen) was used as a DNA size marker. A metabolic gene encoding the glucose 6-phosphate-isomerase-1 was employed as a positive control in all reactions. Primers were designed by the authors and prepared by Promicro (SP) as follows: 5' -GTACCTTG-GTGCAAAAGCAG (forward) and 5' -GAGAAGTTT-GCTGATGTAGG (reverse; gene encoding the glucose 6-phosphate-isomerase-1); 5' -CTACAATTCCAGG-GAGTGCA (forward) and 5' -ACTTTCTTCCGTC-CACTTAG (reverse; *bca*, encodes alpha protein); 5' -AAGCAACTAGAAGAGGAAGC (forward) and 5' -TTCTGCTCTGGTGTTTTAGG (reverse; *bac*, encodes beta protein); 5' -CCTGCTAAGACTGCTGATAC (forward) and 5' -CATAAGCATAGTCGTAAGCC (reverse; *scpB*, encodes C5a peptidase); 5' -CACCAATCCCCA-CTCTACTA (forward) and 5' -TGTGTCAAACCATC-TATCAG (reverse; *hylB*, encodes hyaluronate lyase). One strain of *Streptococcus pyogenes* was used as a negative control. Two GBS strains obtained from humans, provided by Dr. L. M. Teixeira (Universidade Federal do Rio de Janeiro), were used as positive controls.

Antimicrobial susceptibility testing - Susceptibility to seven antimicrobial agents was examined by using the single-disk diffusion method and Clinical Laboratory Standards Institute guidelines (CLSI 2006).

RESULTS

After digestion with *Sma*I, 34 electrophoretic profiles belonging to eight clusters (A - H) were observed for the 45 strains (Fig. 1). Twenty-four of the strains belonged to the A cluster (A1-A16 profiles). Five isolates belonged to the B cluster (B1-B3 profiles), three belonged to the C cluster (C1 and C2 profiles), nine belonged to the D cluster (D1-D9 profiles) and each of the remaining four strains belonged to clusters E, F, G and H, respectively. The A1 profile was identified in a strain isolated in 1984 in Florianopolis and 18 years later in Rio de Janeiro. There is a distance of 1,150 km between these two locations. All strains were *bac* negative, *hylB* and *scpB* positive and 35 were *bca* negative. As expected, virulence genes were detected in control strains and the metabolic gene was detected in all reactions (Fig. 2). In addition, all strains were sensitive to penicillin, vancomycin, clindamycin, erythromycin and chloramphenicol. Resistance to tetracycline was seen in 39 isolates and sensitivity was observed for five strains. Forty-three GBS strains were sensitive to rifampin. Intermediate susceptibilities to tetracycline and rifampin were observed for one and two strains, respectively. PFGE and virulence profiles, antimicrobial susceptibilities and the clinical sources of the strains are shown in Table.

DISCUSSION

Our data describing the molecular epidemiology of 45 Ia GBS strains isolated in Brazil show a remarkable clustering, with 24 isolates in cluster A. It is important to note that the strains were collected over a period of 21 years. There was no specific local or time clustering, as clusters were spread over the 21-year time period and were from all sources. Other researchers found a more enhanced genetic heterogeneity in strains of serotype Ia, which were isolated during a shorter period of time. Skjaervold et al. (2004) identified 10 different electrophoretic profiles among 12 strains isolated in 1999 and

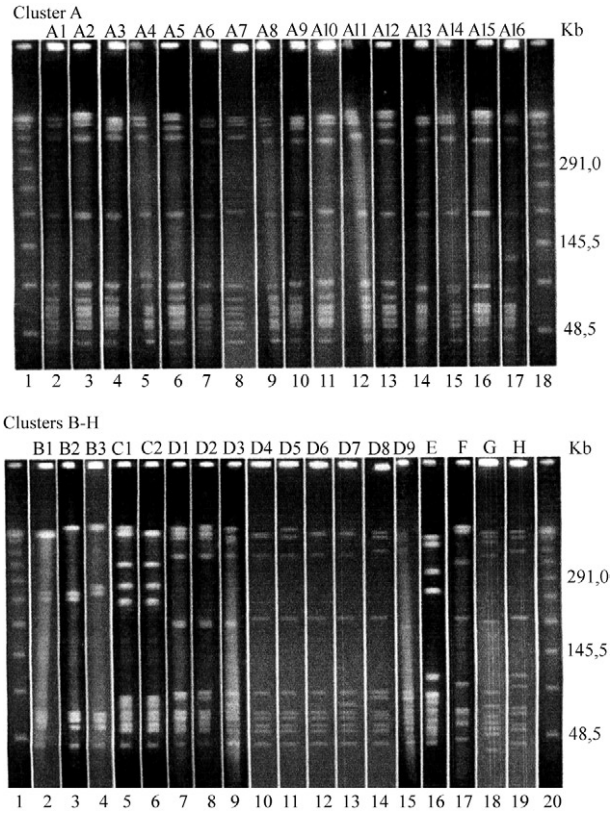

Fig. 1: pulsed-field gel electrophoresis (PFGE) profiles displayed by the Ia group B streptococci strains. Cluster A: Lanes 1,18: lambda ladder PFGE marker; 2-17: A1-A16 profiles. Clusters B-H: Lanes 1, 20: lambda ladder marker; 2-4: B1-B3 profiles; 5, 6: C1 and C2 profiles; 7-15: D1-D9 profiles; 16-19: E-H profiles.

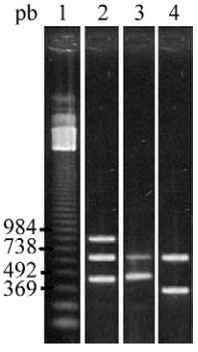

Fig. 2: polymerase chain reaction amplified products of a group B streptococci positive control strain in an agarose gel. 1: 123-bp marker; 2: *scpB* (853-bp), housekeeping gene (669-bp) and *hylB* (444-bp); 3: housekeeping gene and *bac* (479-bp); 4: housekeeping gene and *bca* (376-bp).

TABLE

Pulsed-field gel electrophoresis (PFGE), virulence profiles, antimicrobial susceptibilities and clinical sources of the Ia group B streptococci strains

PFGE profile	Strain	Year	Body site	State	*bca*	Tetracycline	Rifampin
A1	01019	2001	urine	RJ	+	R	S
A1	02022	2002	urine	RJ	-	R	S
A1	01015	2001	urine	RJ	-	R	S
A1	84127	1984	ear canal	SC	+	R	S
A2	89311	1989	vagina	RJ	-	R	S
A3	85418	1985	pharynx	unknown	-	R	S
A3	83154	1983	anus	SC	-	R	I
A4	01020	2001	urine	RJ	-	R	S
A4	01016	2001	unknown	RJ	-	R	S
A5	88606	1988	vagina	RJ	-	R	S
A5	90351	1990	lung	RJ	+	R	S
A6	02057	1002	urine	RJ	+	R	S
A7	02029	2002	unknown	RJ	-	R	S
A8	01022	2001	urine	RJ	+	R	S
A9	81779	1981	pharynx	SP	-	S	S
A10	90177	1990	vagina	RJ	-	R	S
A11	01008	2001	unknown	RJ	-	R	S
A12	88686	1988	vagina	RJ	-	R	S
A13	02053	2002	urine	RJ	-	R	S
A14	01009	2001	urine	RJ	-	R	S
A14	01028	2001	vagina	RJ	-	R	S
A14	01029	2001	urine	RJ	-	R	I
A15	96027	1996	urine	RJ	-	R	S
A16	02068	2002	unknown	RJ	-	R	S
B1	90218	1990	lung	RJ	-	S	S
B1	90220	1990	perineum	RJ	-	R	S
B2	90222	1990	urine	RJ	-	R	S
B3	85427	1985	pharynx	RJ	-	I	S
B3	85685	1985	pharynx	RJ	-	R	S
C1	91239	1991	pharynx	RJ	+	R	S
C1	91402	1991	pharynx	RJ	+	S	S
C2	91199	1991	pharynx	RJ	+	R	S
D1	90096	1990	pharynx	RJ	+	R	S
D2	02044	2002	urine	RJ	-	R	S
D3	90352	1990	placenta	RJ	-	S	S
D4	02008	2002	unknown	RJ	-	R	S
D5	02016	2002	unknown	RJ	-	R	S
D6	02035	2002	unknown	RJ	+	R	S
D7	01017	2001	urine	RJ	-	S	S
D8	02067	2002	urine	RJ	-	R	S
D9	02023	2002	urine	RJ	-	R	S
E	89496	1989	pharynx	RJ	-	R	S
F	90194	1990	urine	RJ	-	R	S
G	83093	1983	anus	SC	-	S	S
H	96028	1996	unknown	RJ	-	R	S

I: intermediate susceptibility; R: resistant; RJ: Rio de Janeiro; S: sensitive; SC: Santa Catarina; SP: São Paulo. The genes *hylB* and *scpB* were detected in all strains. All isolates were sensitive to penicillin, vancomycin, clindamycin, erythromycin and chloramphenicol.

2000 in Norway. Savoia et al. (2008) identified seven clusters among 16 strains isolated in 2005 and 2006 in Italy and six of these strains were allocated to one single cluster. Martins et al. (2007), however, reported that 59 of 60 serotype Ia strains isolated between 2000-2004 in Portugal belonged to a single cluster.

There was previously no data about the genetic diversity among serotype Ia Brazilian strains. A similar study was conducted by our research group to analyse strains of type II, III and V isolated in humans in the same geographic areas and period of time (Oliveira et al. 2005). A predominant profile was clearly defined for each of these serotypes but was not identified in our Ia isolates, which instead displayed a slightly higher genetic heterogeneity than others.

The *hylB* and *scpB* genes were detected in all isolates. According to the literature, the *hylB* gene is uncommon in bovine GBS strains but is often present in human strains. Some authors even suggest that the presence of the *scpB* gene in human isolates is mandatory (Dmitriev et al. 2001, 2004, Franken et al. 2001). However, Duarte et al. (2005) detected *scpB* in 97% of human isolates and Dore et al. (2003) described one *hylB* negative human strain. Ten of our strains carried the *bca* gene. According to the literature, between 30-55% of serotype Ia GBS human strains carry this *bca* gene (Dore et al. 2003, Duarte et al. 2005).

In the present study, none of the 45 streptococci carried the *bac* gene. This was also observed in the Brazilian isolates studied by Duarte et al. (2005).

Variable susceptibilities of GBS to erythromycin and clindamycin (from 4-40%) are described in the literature (Hsueh et al. 2001, Uh et al. 2001, Díaz et al. 2008). Simões et al. (2007) did not detect resistance to erythromycin and clindamycin among Ia isolates collected between 2003-2004 in São Paulo. We also did not detect resistance to these two antibiotics. Duarte et al. (2005) described some resistance to erythromycin (5%) among Ia strains collected between 2000-2001 in Rio de Janeiro. In the present report, susceptibility to other antimicrobial agents is in agreement with other data in the literature (Hsueh et al. 2001, Uh et al. 2001, Simões et al. 2007).

Our data may suggest a relevant perpetuation and dissemination of cluster A throughout Brazil and may suggest the direction of further studies on this GBS serotype in geographical areas of the Southern Hemisphere, especially in developing countries.

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