Phenotypic characterization of *Neisseria meningitidis* strains isolated from invasive disease in Brazil from 1990 to 2001

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Phenotype characterization of 11 181 invasive *Neisseria meningitidis* isolates collected in Brazil from 1990 to 2001 was performed. Based on laboratory data, there were 7436 (67 %) serogroup B isolates, 3391 (30 %) C, 236 W135, 51 Y, four 29E, three X, one Z, and 59 of unknown serogroup. Phenotype B:4,7:P1.19,15 (54 %) remained the most common during the whole of the 12-year period. Two waves were observed within the serogroup C population: the most frequent phenotype C:2b:P1.3 (47 %) was replaced after 1998 by non-typable isolates (C:NT:NST) (16 %).

INTRODUCTION

The severity, permanent sequelae, and the fulminant and contagious nature of meningococcal disease (MD) make it a feared public health problem worldwide (Brandtzaeg, 1996; Tzeng & Stephens, 2000). Twelve serogroups of *Neisseria meningitidis* (Men) can be differentiated based on the chemical and immunological properties of their capsular polysaccharides. A Men isolate can also be further classified into serotype and serosubtype based on structural differences of PorB (class 2 or 3) and PorA (class 1) outer-membrane proteins (OMPs), respectively. Standard nomenclature lists serogroup:serotype:serosubtype (Popovic *et al.*, 1998; Tzeng & Stephens, 2000).

Most MD cases in the world are caused by serogroups A, B, C, W135 and Y (MenA, MenB, MenC, MenW and MenY, respectively) with geographical and temporal variation (Tzeng & Stephens, 2000). In Brazil, 68 332 MD cases were notified from 1990 to 2001, and the annual incidence rate was estimated to be around 1–3 cases per 100 000. For 35 % of the cases, the serogroup was determined, of which about 46 % were diagnosed by isolation of the causative strain (Márcia L. Carvalho, personal communication; http://portal.saude.gov.br/portal/arquivos/pdf/doencas_meningo.pdf).

Abbreviations: CRNM, Centro de Referência Nacional para Meningites (National Reference Centre for Meningitis Brazil); ET, electrophoretic type; MD, meningococcal disease; Men, *Neisseria meningitidis*; MenA, -B, -C, -29E, -W, -X, -Y, -Z, *N. meningitidis* serogroup A, B, C, 29E, W135, X, Y, Z, respectively; NST, non-serosubtypable; NT, non-serotypable; OMP, outer-membrane protein.

Free or conjugate serogroup-specific capsular polysaccharide vaccines are available against MenA, MenC, MenW and MenY. Since MenB polysaccharide is poorly immunogenic (Finne *et al.*, 1983), research on vaccine candidates has targeted non-capsular antigens, mainly the PorA OMP, which is expected to provide serosubtype-specific protection (de Moraes *et al.*, 1992; Peeters *et al.*, 1996; Tzeng & Stephens, 2000). A bivalent MenB vaccine, consisting mainly of OMP-containing vesicles expressing the two serosubtypes most frequent in the country, is being studied in Brazil (Jessouroun *et al.*, 2004; Sacchi *et al.*, 2001).

As the immunogenicity and epidemic features of MD may differ according to the serogroup, characterization of the strain from an invasive case is essential for the control of an epidemic. Surveillance of serosubtype distribution over time is also crucial if OMP vaccines are to be used to prevent MenB disease. This report describes the serogroup, serotype and serosubtype distribution of invasive Men isolates collected in Brazil during the 12-year period 1990–2001.

METHODS

Meningococcal isolates. The data about the isolates were obtained from the Bacteriology Section of Instituto Adolfo Lutz, National Reference Centre for Meningitis (Centro de Referência Nacional para Meningites, CRNM), a public health laboratory of the state of São Paulo. Strains were voluntarily forwarded to the CRNM mainly by regional public health laboratories throughout Brazil.

Serogroup identification. All Men isolates had the serogroup confirmed by slide agglutination (Popovic *et al.*, 1998) with polyclonal

Table 1. Distribution of *N. meningitidis* serogroups by the major administrative regions of Brazil and by age group, from 1990 to 2001

—, Number of isolates was too small to calculate a percentage.

No. and percentage of meningococcal isolates by serogroup and period																			
1990–1993					1994–1996				1997–2001				Entire period, 1990–2001						
	B	C	Others	Total	B	C	Others	Total	B	C	Others	Total	B	C	Others	All	%*	Cum%*	
Major administrative region																			
North	No.	8	2	0	10	3	0	0	3	25	6	0	31	36	8	0	44		
	%	—	—	—	—	—	—	—	—	—	—	—	—	81·8	18·2	0	100	0·4	
North east	No.	520	77	26	623	364	57	2	423	1033	137	9	1179	1917	271	37	2225		
	%	83·5	12·4	4·2	100	86·1	13·5	0·5	100	87·6	11·6	0·8	100	86·2	12·2	1·7	100	19·9	
Central west	No.	99	103	15	217	113	66	7	186	254	84	6	344	466	253	28	747		
	%	45·6	47·5	6·9	100	60·8	35·5	3·8	100	73·8	24·4	1·7	100	62·4	33·9	3·7	100	6·7	
South east	No.	1384	712	112	2208	1217	793	32	2042	1476	739	88	2303	4077	2244	232	6553		
	%	62·7	32·2	5·1	100	59·6	38·8	1·6	100	64·1	32·1	3·8	100	62·2	34·2	3·5	100	58·6	
South	No.	274	275	17	566	201	226	10	437	465	114	30	609	940	615	57	1612		
	%	48·4	48·6	3	100	46	51·7	2·3	100	76·4	18·7	4·9	100	58·3	38·2	3·5	100	14·4	
All regions	No.	2285	1169	170	3624	1898	1142	51	3091	3253	1080	133	4466	7436	3391	354	11181		
	%	63·1	32·3	4·7	100	61·4	36·9	1·6	100	72·8	24·2	3	100	66·5	30·3	3·2	100	100	
Age group																			
≤2 years	No.	413	197	32	642	489	324	15	828	953	307	34	1294	1855	828	81	2764		
	%	64·3	30·7	5·0	100	59·1	39·1	1·8	100	73·6	23·7	2·6	100	67·1	30·0	2·9	100	33·5	33·5
3–5 years	No.	320	170	25	515	259	188	2	449	475	172	18	665	1054	530	45	1629		
	%	62·1	33·0	4·9	100	57·7	41·9	0·4	100	71·4	25·9	2·7	100	64·7	32·5	2·8	100	19·7	53·2
6–14 years	No.	378	186	35	599	365	174	6	545	635	223	11	869	1378	583	52	2013		
	%	63·1	31·1	5·8	100	67·0	31·9	1·1	100	73·1	25·7	1·3	100	68·5	29·0	2·6	100	24·4	77·6
≥15 years	No.	313	145	19	477	318	224	14	556	596	165	50	811	1227	534	83	1844		
	%	65·6	30·4	4·0	100	57·2	40·3	2·5	100	73·5	20·3	6·2	100	66·5	29·0	4·5	100	22·4	100
All ages	No.	1424	698	111	2233	1431	910	37	2378	2659	867	113	3639	5514	2475	261	8250		
	%	63·8	31·3	5·0	100	60·2	38·3	1·6	100	73·1	23·8	3·1	100	66·8	30·0	3·2	100		

*Percentage (%) and cumulative percentage (Cum%) of the total number of isolates from all regions ($n=11\,181$) or of the total number of patients of known age ($n=8250$).

Fig. 1. Yearly numbers of *N. meningitidis* isolates from invasive disease in Brazil, by serogroup, 1990–2001. The total number of isolates for each year is shown above the respective data points. †The unknown serogroup (NK) includes 45 polyagglutinable, 10 autoagglutinable, and four non-serogroupable strains; *data available at http://portal.saude.gov.br/portal/arquivos/pdf/doencas_meningo.pdf

goat or horse antisera prepared at CRNM against MenA, MenB, MenC, Men29E, MenW, MenX, MenY and MenZ, as described previously (Alkmin *et al.*, 1994).

Type and subtype determination. Isolates were serotyped and serosubtyped by a whole-cell dot-blotting assay (Wedegge *et al.*, 1990). Some mAbs were introduced during the study period, and in 2001, the full sets consisted of mAbs for serotypes 1, 2a, 2b, 2c, 4, 5, 7, 9, 10, 11, 14, 15, 17, 19, 21 and 22, and to serosubtypes P1.1, P1.2, P1.3, P1.4, P1.5, P1.7, P1.9, P1.10, P1.12, P1.14, P1.15, P1.16, P1.19 and P1.22-1. Many of the partially, non-serotypable (NT), and

non-serosubtypable (NST) isolates were re-examined after the inclusion of new mAbs into the typing panel. Several strains still remaining NT or NST were further submitted to *porA* and *porB* gene typing, and the data have been reported previously (Sacchi *et al.*, 1998b, 2001).

Electrophoretic type (ET) determination. Several epidemic strains isolated in different regions of Brazil from 1990 to 2001 were analysed by multilocus enzyme electrophoresis (MLEE) to determine their clonal characteristics. The methods and results have been reported previously (Barroso *et al.*, 1996; Sacchi *et al.*, 1992a, b, 1994, 1995).

Table 2. Serotype distribution of invasive *N. meningitidis* isolates by serogroup, Brazil, 1990–2001

–, No isolate; NT, non-serotypable; ND, not submitted to typing.

Serotype	No. (%) of isolates by serogroup and period											
	B						C					
	1990–1996		1997–2001		1990–2001		1990–1996		1997–2001		1990–2001	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
1	–		1	(0·03)	1	(0·01)	–		–		–	
2a	17	(0·4)	23	(0·7)	40	(0·5)	419	(18·1)	89	(8·2)	508	(15·0)
2b	50	(1·2)	26	(0·8)	76	(1·0)	1529	(66·2)	353	(32·7)	1882	(55·5)
4	2783	(66·5)	51	(1·6)	2834	(38·1)	77	(3·3)	6	(0·6)	83	(2·4)
4,1	–		1	(0·03)	1	(0·01)	–		2	(0·2)	2	(0·1)
4,7	58	(1·4)	2494	(76·7)	2552	(34·3)	1	(0·04)	96	(8·9)	97	(2·9)
4,10	72	(1·7)	71	(2·2)	143	(1·9)	2	(0·1)	–		2	(0·1)
4,14	–		5	(0·2)	5	(0·1)	–		–		–	
4,19	18	(0·4)	–		18	(0·2)	1	(0·04)	–		1	(0·03)
4,19,10	2	(0·05)	–		2	(0·03)	–		–		–	
4,21	–		3	(0·1)	3	(0·04)	1	(0·04)	5	(0·5)	6	(0·2)
7	1	(0·02)	30	(0·9)	31	(0·4)	–		1	(0·1)	1	(0·03)
10	7	(0·2)	7	(0·2)	14	(0·2)	1	(0·04)	–		1	(0·03)
14	3	(0·1)	38	(1·2)	41	(0·6)	–		2	(0·2)	2	(0·1)
15	169	(4·0)	146	(4·5)	315	(4·2)	4	(0·2)	5	(0·5)	9	(0·3)
17	61	(1·5)	11	(0·3)	72	(1·0)	6	(0·3)	–		6	(0·2)
17,7	–		28	(0·9)	28	(0·4)	–		3	(0·3)	3	(0·1)
17,10	9	(0·2)	6	(0·2)	15	(0·2)	1	(0·04)	–		1	(0·03)
19	81	(1·9)	38	(1·2)	119	(1·6)	8	(0·3)	2	(0·2)	10	(0·3)
19,1	–		36	(1·1)	36	(0·5)	–		1	(0·1)	1	(0·03)
19,7	3	(0·1)	25	(0·8)	28	(0·4)	–		1	(0·1)	1	(0·03)
19,7,1	–		1	(0·03)	1	(0·01)	–		–		–	
19,10	51	(1·2)	49	(1·5)	100	(1·3)	3	(0·1)	4	(0·4)	7	(0·2)
19,14	2	(0·05)	61	(1·9)	63	(0·8)	2	(0·1)	4	(0·4)	6	(0·2)
21	–		5	(0·2)	5	(0·1)	1	(0·04)	5	(0·5)	6	(0·2)
NT	496	(11·9)	97	(3·0)	593	(8·0)	158	(6·8)	382	(35·4)	540	(15·9)
ND	300	(7·2)	–		300	(4·0)	97	(4·2)	119	(11·0)	216	(6·4)
All	4183	(100)	3253	(100)	7436	(100)	2311	(100)	1080	(100)	3391	(100)

Table 3. Serosubtype distribution of invasive *N. meningitidis* isolates by serogroup, Brazil, 1990–2001

–, No isolate; NST, non-serosubtypable; ND, not submitted to typing.

Serosubtype	No. (%) of isolates by serogroup and period											
	B						C					
	1990–1996		1997–2001		1990–2001		1990–1996		1997–2001		1990–2001	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
P1.1	2	(0.05)	5	(0.2)	7	(0.1)	1	(0.04)	1	(0.1)	2	(0.1)
P1.2	40	(1.0)	7	(0.2)	47	(0.6)	352	(15.2)	20	(1.9)	372	(11.0)
P1.3	76	(1.8)	83	(2.6)	159	(2.1)	834	(36.1)	113	(10.5)	947	(27.9)
P1.3,7	–	–	–	–	–	–	4	(0.2)	–	–	4	(0.1)
P1.4	9	(0.2)	10	(0.3)	19	(0.3)	–	–	–	–	–	–
P1.5	–	–	31	(1.0)	31	(0.4)	18	(0.8)	46	(4.3)	64	(1.9)
P1.5,2	1	(0.02)	28	(0.9)	29	(0.4)	2	(0.1)	88	(8.1)	90	(2.7)
P1.5,10	–	–	4	(0.1)	4	(0.1)	–	–	6	(0.6)	6	(0.2)
P1.7	314	(7.5)	20	(0.6)	334	(4.5)	3	(0.1)	1	(0.1)	4	(0.1)
P1.7,1	8	(0.2)	317	(9.7)	325	(4.4)	–	–	28	(2.6)	28	(0.8)
P1.7,2	–	–	1	(0.03)	1	(0.01)	–	–	–	–	–	–
P1.7,3	–	–	–	–	–	–	–	–	1	(0.1)	1	(0.03)
P1.7,15	1	(0.02)	1	(0.03)	2	(0.03)	–	–	–	–	–	–
P1.7,16	5	(0.1)	126	(3.9)	131	(1.8)	–	–	2	(0.2)	2	(0.1)
P1.9	123	(2.9)	86	(2.6)	209	(2.8)	6	(0.3)	6	(0.6)	12	(0.4)
P1.10	1	(0.02)	8	(0.2)	9	(0.1)	143	(6.2)	64	(5.9)	207	(6.1)
P1.12	–	–	25	(0.8)	25	(0.3)	–	–	2	(0.2)	2	(0.1)
P1.14	67	(1.6)	22	(0.7)	89	(1.2)	6	(0.3)	3	(0.3)	9	(0.3)
P1.15	2033	(48.6)	1208	(37.1)	3241	(43.6)	56	(2.4)	23	(2.1)	79	(2.3)
P1.16	158	(3.8)	31	(1.0)	189	(2.5)	15	(0.6)	–	–	15	(0.4)
P1.19	–	–	8	(0.2)	8	(0.1)	–	–	–	–	–	–
P1.19,15	30	(0.7)	972	(29.9)	1002	(13.5)	–	–	54	(5.0)	54	(1.6)
P1.22-1	–	–	1	(0.03)	1	(0.01)	–	–	–	–	–	–
P1.22-1,3	–	–	3	(0.1)	3	(0.04)	–	–	–	–	–	–
P1.22-1,14	2	(0.05)	62	(1.9)	64	(0.9)	–	–	9	(0.8)	9	(0.3)
NST	985	(23.5)	191	(5.9)	1176	(15.8)	771	(33.4)	492	(45.6)	1263	(37.2)
ND	328	(7.8)	3	(0.1)	331	(4.5)	100	(4.3)	121	(11.2)	221	(6.5)
All	4183	(100)	3253	(100)	7436	(100)	2311	(100)	1080	(100)	3391	(100)

RESULTS AND DISCUSSION

For the period 1990 to 2001 there were 11 181 Men isolates from invasive MD in the CRNM database (8250 of known age, ranging from birth to 88 years; 5898 male, 503 of unspecified gender; male:female ratio, 1.23). Isolates were recovered from cerebrospinal fluid (91.9%), blood (8%), and synovial, pleural or pericardial fluids (0.1%). No further information about the site of infection was available.

Submissions of MenB isolates remained predominant and relatively constant over time (mean $\pm$ SD, 620 $\pm$ 55 strains per year) (Fig. 1). MenC isolates, second in frequency (mean $\pm$ SD, 283 $\pm$ 82 strains per year), reached a peak in 1995, falling gradually over the next 3 years, and increasing slightly after 1999. MenB and MenC comprised 67 and 30 % of all isolates, respectively (MenB:MenC ratio, 2.19). This ratio is in agreement with the numbers of MD cases by serogroup reported in Brazil for the same period, of which

64 % were MenB, 34 % MenC, and the remaining 2 %, MenW, MenX, MenZ and Men29E (Márcia L. Carvalho, personal communication). A statistically significant positive correlation was observed between the annual number of MD cases (http://portal.saude.gov.br/portal/arquivos/pdf/doencas_meningo.pdf) and isolates referred to CRNM (Pearson's correlation coefficient = 0.59; $P = 0.0432$), despite the differences in the magnitude of their values (cases:strains ratio, 6.1) (Fig. 1).

The distribution pattern of isolates correlated with the population density and income across the Brazilian regions (http://www.ibge.gov.br/home/estatistica/populacao/censo2000/tabelasgrandes_regioes211.shtm). Thus, the southeast, which is the highest-income, most populated region, forwarded most of the isolates ($n = 6553$; 58.6 %), whereas the north ranked last in population density, income and isolation ($n = 44$, 0.4 %) (Table 1). As estimates of disease and

Fig. 2. Proportions of the most frequent PorB and PorA *N. meningitidis* phenotypes of serogroup B (a) and serogroup C (b) isolated from invasive disease in Brazil, 1990–2001. The number of isolates for each year is shown above the respective data points. *The sum (%) of B:4:P1.15, B:4,7:P1.15 and B:4,7:P1.19,15; †the sum (%) of C:2b:NST and C:2b:P1.3.

isolates are likely to be less accurate in lower-income areas due to more limited access to health services and laboratory facilities, this may help explain the scarce number of isolates in northern Brazil. The predominant serogroups differed with place and time. In northern and northeastern regions, MenB accounted for more than 80 % of all isolates. In the south (1990–1996), central west (1990–1993) and southeast (44 % in 1995), the serogroup distribution differed due to emerging MenC strains, which contributed about 50 % of all isolates.

The distribution of isolates differed with the age group, as shown in Table 1. The meningococcal strains were isolated mainly from children under 5 years of age, which is in

agreement with the age distribution of MD. According to Tzeng & Stephens (2000), the attack rate and case-fatality ratio among children can be twenty times that of adults, and in epidemic outbreaks there is a shift in disease to older children, adolescents and adults. Over the 12-year period, 77.6 % of the 8250 cases of known age occurred in individuals <15 years old, mainly in children aged ≤5 years (53.2 %). MenB was the most frequent isolate at all ages, with about twice as many cases as MenC. However, there was an increase in MenC isolation in those aged ≤5 years in the south and southeastern states of São Paulo and Rio de Janeiro (1994–1996), as well as in those 15–24 years old, mainly from Rio de Janeiro (1995). These increases and the age shift to adolescents reflect MenC outbreaks reported in

those geographic regions during the 1990s (Barroso *et al.*, 1996; Kupek *et al.*, 2001; Sacchi *et al.*, 1994, 1995).

A large diversity of serotypes and serosubtypes was observed within both the serogroup B and the serogroup C population, although few of them predominated (Table 2 and Table 3). Isolates were mainly of serotypes 4 (38.1%), 4,7 (34.3%) and NT (8%) in serogroup B; and of serotypes 2b (55.5%), NT (15.9%) and 2a (15%) in serogroup C (Table 2). The most frequent serosubtypes were P1.15 (43.6%), NST (15.8%), P1.19,15 (13.5%), P1.7 and P1.7,1 (about 4% each) within the MenB population; and NST (37.2%) and serosubtypes P1.3 (27.9%), P1.2 (11%) and P1.10 (6.1%) within the MenC population (Table 3). MenW strains belonged to serotypes 2a (113 strains), NT (57), 19,10 (22), 2b and 4 (three each), 17 and 19 (two each), 4, 7, 14 and 15 (one each), and 30 isolates were not serotyped; MenW serosubtypes were P1.2 (89), NST (52), P1.16 (39), P1.5,2 (22), P1.3 and P1.15 (one each), and 32 strains were not serosubtyped (data not shown).

The year-by-year circulation of the prevalent MenB phenotypes was relatively stable and widespread (Fig. 2a), while the predominant MenC phenotypes were less persistent and less disseminated (Fig. 2b). This is in agreement with the classic features of Men outbreaks, according to which MenB epidemics and hyperendemic levels may persist for longer than 10 years, while MenC waves usually resolve in 1–3 years (Wenger, 1999).

Phenotype B:4,7:P1.19,15 (formerly serotyped as B:4:P1.15 and B:4,7:P1.15) was predominant, accounting for 54% of 7105 MenB submitted to typing (Fig. 2a). This phenotype, responsible for an epidemic emerging in 1988 in São Paulo (Sacchi *et al.*, 1998a), still represented 74% of MenB isolates by 2000–2001. Strains B:NT:NST and B:4,7:NST (earlier B:4:NST), more frequent during the 1980s (Sacchi *et al.*, 1992b, 1998a), dropped to 3% in 1994, probably as the result of a more comprehensive typing panel. At that time, phenotype B:4:P1.7 (at present B:4,7:P1.7,1) emerged, remaining second (8%) in frequency thereafter. It was referred mainly by the northeastern (147 strains; 50%) and some southeastern areas (124 strains; 42%), whereas B:4,7:P1.19,15 strains were more disseminated throughout Brazil. The 'Norwegian' epidemic phenotype B:15:P1.7,16 was almost confined to the south, which referred 82 (83%) of 99 B:15:P1.7,16 isolates. Despite the differences in the immunogenicity of their OMPs, the prevailing phenotypes (B:4,7:P1.19,15; B:4,7:P1.7,1; B:15:P1.7,16) belonged to the same electrophoretic type, the ET-5 complex (Sacchi *et al.*, 1992b; A. P. S. Lemos, unpublished data), thus sharing genetic relatedness.

Among 3170 serotyped MenC isolates, phenotype C:2b:P1.3 (earlier C:2b:NST) predominated until 1997, being then replaced by non-typable strains. Outbreaks of these A4 cluster strains (Caugant, 1998; Sacchi *et al.*, 1992a) occurred in some southern and southeastern states (Barroso *et al.*, 1996; Sacchi *et al.*, 1994, 1995), which forwarded about 80%

of them. The C:2b:P1.10 strains responsible for an upsurge in Rio de Janeiro, which referred 137 (97%) out of 142 strains during 1994–1996, also belonged to the A4 cluster (Barroso *et al.*, 1996). The emergent C:NT:NST phenotype, the most frequent since 1998, steadily increased from 2% in 1990 to 57% in 2001, being almost limited to São Paulo. Preliminary results for the identification of these strains have recently shown that they belong to a new phenotype. A detailed study of their PorA and PorB OMPs and of their clonal group is under way, and the results will soon be available (A. P. S. Lemos, unpublished data).

The data presented herein may not reflect the full picture of the circulation of Men in Brazil, since this is not a population-based study, nor were the strains collected systematically from the whole of the country. Nevertheless, the magnitude of the numbers achieved is high enough to help make decisions concerning the prevention and control of MD, at least in some geographic regions. The laboratory data were obtained from the most comprehensive Brazilian collection of Men isolates, so far the best source for studying their characteristics throughout the country. It yielded information on the most frequent MenB phenotypes for the selection of two strains (B:4,7:P1.19,15 and B:4,7:P1.7,1) with appropriate OMPs for the vaccine formulation under study in Brazil. Besides the efforts that are now being made to perform population-based studies, isolation and referral of circulating strains must be continued and encouraged in order to enhance the knowledge of their phenotypic and genetic features, essential to design cost-effective strategies for MD control and prevention.

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REFERENCES

- Alkmin, M. G. A., Shimizu, S. H., Landgraf, I. M., Gaspari, E. N. & Melles, C. E. A. (1994). Production and immunochemical characterization of *Neisseria meningitidis* group B antiserum for the diagnosis of purulent meningitis. *Braz J Med Biol Res* 27, 1627–1634.
- Barroso, D. E., Carvalho, D. M., Nogueira, S. A. & 8 other authors (1996). Epidemiology and molecular analysis of epidemic meningococcal disease related to group C *Neisseria meningitidis* in a Brazilian metropolis: Rio de Janeiro, 1993–1995. In *Abstracts of the Tenth International Pathogenic Neisseria Conference, Baltimore, MD, 1996*, poster 150, pp. 436–437. Edited by W. D. Zollinger, C. E. Frasch & C. D. Deal. Bethesda, MD: National Institutes of Health. Available online at <http://neisseria.org/ipnc/1996/Neis1996-chap7.pdf>

- Brandtzaeg, P. (1996).** Systemic meningococcal disease: clinical pictures and pathophysiological background. *Rev Med Microbiol* 7, 63–72.
- Caugant, D. A. (1998).** Population genetics and molecular epidemiology of *Neisseria meningitidis*. *Acta Pathol Microbiol Immunol Scand* 106, 505–525.
- de Moraes, J. C., Perkins, B. A., Camargo, M. C. & 10 other authors (1992).** Protective efficacy of a serogroup B meningococcal vaccine in São Paulo, Brazil. *Lancet* 340, 1074–1078. Erratum in *Lancet* 340, 1554.
- Finne, J., Leinonen, M. & Makela, P. H. (1983).** Antigenic similarities between brain components and bacteria causing meningitis. Implications for vaccine development and pathogenesis. *Lancet* 2, 355–357.
- Jessouroun, E., Silveira, I. F. B., Lorangeira, A. P., Pereira, S., Fernandes, S. A., Rabinovitch, L., Frasch, C. E., Castro-Faria-Neto, H. C. & Bozza, P. T. (2004).** Outer membrane vesicles (OMVs) and detoxified lipooligosaccharide (dLOS) obtained from Brazilian prevalent *N. meningitidis* serogroup B strains protect mice against homologous and heterologous meningococcal infection and septic shock. *Vaccine* 22, 2617–2625.
- Kupek, E., Puricelli, R. C. B. & Westrupp, M. H. B. (2001).** Effectiveness of a mass immunization campaign against serogroup C meningococci in children in the federal state of Santa Catarina, Brazil. *Braz J Infect Dis* 5, 324–331.
- Peeters, C. C. A. M., Rümke, H. C., Sundermann, L. C., Rouppe van der Voort, E. M., Meulenbelt, J., Schuller, M., Kuipers, A. J., van der Ley, P. & Poolman, J. T. (1996).** Phase I clinical trial with a hexavalent PorA containing meningococcal outer membrane vesicle vaccine. *Vaccine* 14, 1009–1015.
- Popovic, T., Ajello, G. W. & Facklam, R. R. (1998).** *Laboratory Methods for the Diagnosis of Meningitis caused by Neisseria meningitidis, Streptococcus pneumoniae, and Haemophilus influenzae*. Atlanta, GA: Centers for Disease Control, World Health Organization.
- Sacchi, C. T., Zanella, R. C., Caugant, D. A. & 7 other authors (1992a).** Emergence of a new clone of serogroup C *Neisseria meningitidis* in São Paulo, Brazil. *J Clin Microbiol* 30, 1282–1286.
- Sacchi, C. T., Pessoa, L. L., Ramos, S. R., Milagres, L. G., Camargo, M. C. C., Hidalgo, N. T. R., Melles, C. E. A., Caugant, D. A. & Frasch, C. E. (1992b).** Ongoing group B *Neisseria meningitidis* epidemic in São Paulo, Brazil, due to increased prevalence of a single clone of the ET-5 complex. *J Clin Microbiol* 30, 1734–1738.
- Sacchi, C. T., Tondella, M. L. C., Lemos, A. P. S., Gorla, M. C. O., Berto, D. B., Kumiochi, N. H. & Melles, C. E. A. (1994).** Characterization of epidemic *Neisseria meningitidis* serogroup C strains in several Brazilian states. *J Clin Microbiol* 32, 1783–1787.
- Sacchi, C. T., Tondella, M. L., Gorla, M. C. & 7 other authors (1995).** Genetic structure of *Neisseria meningitidis* serogroup C epidemic strains in south Brazil. *Rev Inst Med Trop Sao Paulo* 37, 281–289.
- Sacchi, C. T., Lemos, A. P. S., Camargo, M. C. C., Whitney, A. M., Melles, C. E. A., Solari, C. A., Frasch, C. E. & Mayer, L. W. (1998a).** Meningococcal disease caused by *Neisseria meningitidis* serogroup B serotype 4 in São Paulo, Brazil, 1990 to 1996. *Rev Inst Med Trop Sao Paulo* 40, 65–70.
- Sacchi, C. T., Lemos, A. P. S., Whitney, A. M., Solari, C. A., Brandt, M. E., Melles, C. E. A., Frasch, C. E. & Mayer, L. W. (1998b).** Correlation between serological and sequencing analyses of the PorB outer-membrane protein in the *Neisseria meningitidis* serotyping system. *Clin Diag Lab Immunol* 5, 348–354.
- Sacchi, C. T., Lemos, A. P. S., Popovic, T. & 8 other authors (2001).** Serosubtypes and PorA types of *Neisseria meningitidis* serogroup B isolated in Brazil during 1997–1998: overview and implications for vaccine development. *J Clin Microbiol* 39, 2897–2903.
- Tzeng, Y. L. & Stephens, D. S. (2000).** Epidemiology and pathogenesis of *Neisseria meningitidis*. *Microbes Infect* 2, 687–700.
- Wedegé, E., Hoiby, E. A., Rosenqvist, E. & Froholm, L. O. (1990).** Serotyping and subtyping of *Neisseria meningitidis* isolates by co-agglutination, dot-blotting and ELISA. *J Med Microbiol* 31, 195–201.
- Wenger, J. D. (1999).** Serogroup B meningococcal disease: new outbreaks, new strategies. *JAMA (J Am Med Assoc)* 281, 1541–1543.