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).

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.

**Serosubtype 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

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>C</td>
<td>Others</td>
<td>Total</td>
</tr>
<tr>
<td>North</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>10</td>
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<tr>
<td>North east</td>
<td>520</td>
<td>77</td>
<td>26</td>
<td>623</td>
</tr>
<tr>
<td>Central west</td>
<td>99</td>
<td>103</td>
<td>15</td>
<td>217</td>
</tr>
<tr>
<td>South east</td>
<td>1384</td>
<td>712</td>
<td>112</td>
<td>2208</td>
</tr>
<tr>
<td>South</td>
<td>274</td>
<td>275</td>
<td>17</td>
<td>566</td>
</tr>
<tr>
<td>All regions</td>
<td>2285</td>
<td>1169</td>
<td>170</td>
<td>3624</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2 years</td>
<td>413</td>
<td>197</td>
<td>32</td>
<td>642</td>
</tr>
<tr>
<td>3–5 years</td>
<td>320</td>
<td>170</td>
<td>25</td>
<td>515</td>
</tr>
<tr>
<td>6–14 years</td>
<td>378</td>
<td>186</td>
<td>35</td>
<td>599</td>
</tr>
<tr>
<td>≥15 years</td>
<td>313</td>
<td>145</td>
<td>19</td>
<td>477</td>
</tr>
<tr>
<td>All ages</td>
<td>1424</td>
<td>698</td>
<td>111</td>
<td>2333</td>
</tr>
<tr>
<td></td>
<td>638</td>
<td>313</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

*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=8,250).
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 (Wedege 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

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. (%) of isolates by serogroup and period</th>
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<tr>
<td></td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>1 (0-03)</td>
</tr>
<tr>
<td>2a</td>
<td>17 (0-4)</td>
</tr>
<tr>
<td>2b</td>
<td>50 (1-2)</td>
</tr>
<tr>
<td>4</td>
<td>2783 (66-5)</td>
</tr>
<tr>
<td>4,1</td>
<td>1 (0-03)</td>
</tr>
<tr>
<td>4,7</td>
<td>58 (1-4)</td>
</tr>
<tr>
<td>4,10</td>
<td>72 (1-7)</td>
</tr>
<tr>
<td>4,14</td>
<td>18 (0-4)</td>
</tr>
<tr>
<td>4,19</td>
<td>18 (0-4)</td>
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<tr>
<td>4,19,10</td>
<td>2 (0-05)</td>
</tr>
<tr>
<td>4,21</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>1 (0-02)</td>
</tr>
<tr>
<td>10</td>
<td>7 (0-2)</td>
</tr>
<tr>
<td>14</td>
<td>3 (0-1)</td>
</tr>
<tr>
<td>15</td>
<td>169 (4-0)</td>
</tr>
<tr>
<td>17</td>
<td>61 (1-5)</td>
</tr>
<tr>
<td>17,7</td>
<td>—</td>
</tr>
<tr>
<td>17,10</td>
<td>9 (0-2)</td>
</tr>
<tr>
<td>19</td>
<td>81 (1-9)</td>
</tr>
<tr>
<td>19,1</td>
<td>—</td>
</tr>
<tr>
<td>19,7</td>
<td>3 (0-1)</td>
</tr>
<tr>
<td>19,7,1</td>
<td>—</td>
</tr>
<tr>
<td>19,10</td>
<td>51 (1-2)</td>
</tr>
<tr>
<td>19,14</td>
<td>2 (0-05)</td>
</tr>
<tr>
<td>21</td>
<td>—</td>
</tr>
<tr>
<td>NT</td>
<td>496 (11-9)</td>
</tr>
<tr>
<td>ND</td>
<td>300 (7-2)</td>
</tr>
<tr>
<td>All</td>
<td>4183 (100)</td>
</tr>
</tbody>
</table>
Table 3. Serosubtype distribution of invasive *N. meningitidis* isolates by serogroup, Brazil, 1990–2001

<table>
<thead>
<tr>
<th>Serosubtype</th>
<th>No. (%) of isolates by serogroup and period</th>
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<tr>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>P1.1</td>
<td>2 (0.05)</td>
</tr>
<tr>
<td>P1.2</td>
<td>40 (1.0)</td>
</tr>
<tr>
<td>P1.3</td>
<td>76 (1.8)</td>
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<td>P1.3,7</td>
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<td>P1.4</td>
<td>9 (0.2)</td>
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<tr>
<td>P1.5</td>
<td>–</td>
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<tr>
<td>P1.5,2</td>
<td>1 (0.02)</td>
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<tr>
<td>P1.5,10</td>
<td>–</td>
</tr>
<tr>
<td>P1.7</td>
<td>314 (7.5)</td>
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<tr>
<td>P1.7,1</td>
<td>8 (0.2)</td>
</tr>
<tr>
<td>P1.7,2</td>
<td>–</td>
</tr>
<tr>
<td>P1.7,3</td>
<td>–</td>
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<tr>
<td>P1.7,15</td>
<td>1 (0.02)</td>
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<tr>
<td>P1.7,16</td>
<td>5 (0.1)</td>
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<tr>
<td>P1.9</td>
<td>123 (2.9)</td>
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<tr>
<td>P1.10</td>
<td>1 (0.02)</td>
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<tr>
<td>P1.12</td>
<td>–</td>
</tr>
<tr>
<td>P1.14</td>
<td>67 (1.6)</td>
</tr>
<tr>
<td>P1.15</td>
<td>2033 (48.6)</td>
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<tr>
<td>P1.16</td>
<td>158 (3.8)</td>
</tr>
<tr>
<td>P1.19</td>
<td>–</td>
</tr>
<tr>
<td>P1.19,15</td>
<td>30 (0.7)</td>
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<tr>
<td>P1.22-1</td>
<td>–</td>
</tr>
<tr>
<td>P1.22-1,3</td>
<td>–</td>
</tr>
<tr>
<td>P1.22-1,14</td>
<td>2 (0.05)</td>
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<tr>
<td>NST</td>
<td>985 (23.5)</td>
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<tr>
<td>ND</td>
<td>328 (7.8)</td>
</tr>
<tr>
<td>All</td>
<td>4183 (100)</td>
</tr>
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</table>

**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 ± SD, 620 ± 55 strains per year) (Fig. 1). MenC isolates, second in frequency (mean ± SD, 283 ± 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/tabelagrandes_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...
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

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**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.
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%) 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.

Acknowledgements

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References


Invasive meningococcal strains, Brazil, 1990–2001


