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# MENINGOCOCCAL DISEASE CAUSED BY *Neisseria meningitidis* SEROGROUP B SEROTYPE 4 IN SÃO PAULO, BRAZIL, 1990 TO 1996

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## SUMMARY

A large epidemic of serogroup B meningococcal disease (MD), has been occurring in greater São Paulo, Brazil, since 1988.[21] A Cuban-produced vaccine, based on outer-membrane-protein (OMP) from serogroup B: serotype 4: serosubtype P1.15 (B:4:P1.15) *Neisseria meningitidis,* was given to about 2.4 million children aged from 3 months to 6 years during 1989 and 1990. The administration of vaccine had little or no measurable effects on this outbreak. In order to detect clonal changes that could explain the continued increase in the incidence of disease after the vaccination, we serotyped isolates recovered between 1990 and 1996 from 834 patients with systemic disease. Strains B:4:P1.15, which was detected in the area as early as 1977, has been the most prevalent phenotype since 1988. These strains are still prevalent in the area and were responsible for about 68% of 834 serogroup B cases in the last 7 years. We analyzed 438 (52%) of these strains by restriction fragment length polymorphism (RFLPs) of rRNA genes (ribotyping). The most frequent pattern obtained was referred to as Rb1 (68%). We concluded that the same clone of B:4:P1.15-Rb1 strains was the most prevalent strain and responsible for the continued increase of incidence of serogroup B MD cases in greater São Paulo during the last 7 years in spite of the vaccination trial.

**KEYWORDS:** *Neisseria meningitidis:* Ribotyping.

## INTRODUCTION

Meningococcal disease (MD) remains a significant cause of mortality and morbidity throughout the world[1,18]. During the last 10 years different methods have been used to study the epidemiology of MD[9,15,21,23]. *Neisseria meningitidis* can be classified into serogroups, serotypes, and serosubtypes on the basis of antigenic differences in their capsular polysaccharides, class 2 or 3 outer membrane proteins (OMPs), and class 1 OMPs, respectively[12]. Antibodies against both proteins are bactericidal, making serotyping results useful not only for epidemiologic tracing of meningococcal disease, but also for identifying potential vaccine components[5,11].

Another technique used for epidemiological tracing of *N. meningitidis* is multilocus enzyme electrophoresis typing (MEE), or electrophoretic typing (ET typing). ET typing examines electrophoretic differences in enzymes required for growth of the bacteria. ET 5 complex strains, a group of approximately 22 closely related ETs, have become a significant element of meningococcal populations causing outbreaks in different parts of the world[7].

Beginning in 1988, the incidence of MD in greater São Paulo began to surpass the upper 95% confidence limit of an 8-year average incidence (from 1979 to 1986), indicating a new epidemic. This epidemic, which extended to 1997, was different from previous epidemics in that it was caused by *N. meningitidis* serogroup B strains[21].

In a previous study, *N. meningitidis* serogroup B isolates from MD cases recovered from 1989 to 1990 were characterized by serotyping and MEE[8,21]. The increased incidence of MD was paralleled by an increased prevalence of B:4:P1.15 strains. By MEE, the B:4:P1.15 strains belong to ET 5, a member of ET 5 complex. Restriction fragment length polymorphism (RFLPs) of rRNA genes (ribotyping), was adapted to further characterize those *N. meningitidis* ET

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5 epidemic strains[22,25]. The ribotyping analysis, using *Cla*I endonuclease, showed a single rRNA gene restriction pattern (Rb1) for all ET 5 strains, the MEE and ribotyping results were 100% concordant.

In an attempt to control the epidemic of serogroup B disease in greater São Paulo a Cuban produced outer-membrane-protein-based serogroup B meningococcal vaccine (VAMENGOC B/C), was given to about 2.4 million children aged from 3 months to 6 years during 1989 and 1990[17]. The Cuban vaccine is based on OMPs from a Cuban epidemic B:4:P1.15 *N. meningitidis* strain, combined with serogroup C meningococcal capsular polysaccharide[17].

During the period of 1991 to 1996, the incidence of MD in greater São Paulo area did not decrease and most of the serogrouped cultures (61%), were by serogroup B. In 1996 the incidence of MD reached 8.2 per 100,000 inhabitants per year, the highest incidence of meningococcal disease since the large epidemics in the 1970's caused by serogroups A or C.

The goal of this paper was to determine if changes have occurred in the B:4:P1.15 epidemic strains isolated since the vaccination trial as measured by serotyping and ribotyping that could explain the continued increase of MD cases by serogroup B.

## MATERIALS AND METHODS

**Meningococcal strains.** All 834 *N. meningitidis* serogroup B strains recovered from blood or cerebrospinal fluid samples from patients with systemic disease in greater São Paulo from 1990 to 1996 received at the National Reference Center were serotyped. These strains represent 51% of all MD cases known to be due to serogroup B in greater São Paulo during this period. We randomly selected 438 strains to be analyzed by ribotyping. Eighty five serogroup B strains previously used for the 1990 direct comparison of MEE and ribotyping were included[25]. Other strains are described in Table 1. Serotyping and MEE, of strains isolated from 1977 to 1990, and the equivalence between ET 5 and Rb1 are described elsewhere[8,21,25]. From 1990, only serotyping and ribotyping were used in order to analyze large numbers of isolates.

**Serogrouping and Serotyping.** All *N. meningitidis* strains were serogrouped as described previously[21]. Serotyping and serosubtyping were done by dot-blotting of whole cell suspensions[28]. MAbs for serotypes 8, 15, 16 and serosubtypes P1.2, P1.3, P1.16 were provided by Dr. W. D. Zollinger. MAbs for serosubtype P1.9 was provided by Dr. J. T. Poolman, and MAbs for serotypes 2a, 2b, 4, 17 and serosubtypes P1.4, P1.7, P1.23, and P1.15 were produced at Adolfo Lutz Institute[20].

**TABLE 1**
Control strains of *N. meningitidis*

| Strain | G:S:Sb[a] | ET/Rb type[b] | Characteristic | Reference |
|---|---|---|---|---|
| H44/76 | B:15:P1.7,16 | ET5/Rb1 | Epidemic in Norway | 13 |
| H355/75 | B:15:P1.15 | ET5/Rb1 | Epidemic in Norway | 12 |
| CU385/83 | B:4:P1.15 | ET5/Rb1 | Epidemic in Cuba | 29 |
| BB393 | B:15:P1.3 | ND/Rb1 | Epidemic in Chile | 25 |
| N.44/89 | B:4:P1.15 | ET5/Rb1 | Epidemic in Brazil | 16 |
| N.150/88 | B:4:P1.15 | ET5/Rb1 | Epidemic in Brazil | 16 |
| N.1002/90 | C:2b:P1.3 | ET49/Rb2 | Epidemic in Brazil | 23 |
| N.610/93 | B:4:P1.15 | ND/Rb1 | Epidemic in Brazil | this work |
| N.300/94 | B:4:P1.9 | ND/Rb3 | Epidemic in Brazil | this work |
| H276 (870227) | B:4:P1.10 | ND/Rb1 | Reference strain for serosubtype P1.10 | 24 |
| Z4008 (882066) | B:4:P1.9 | ND/Rb8 | Reference strain for serosubtype P1.4 | 24 |
| M981 | B:4:- | ND/Rb2 | Reference strain for serotype 4 | 12 |

Not all strains were typed by MEE/ribotyped. In reference 13 it was shown that ET 5 is 100% equivalent to Rb1.
[a] G:S:Sb = serogroup:serotype:serosubtype
[b] ND = Non determinated

**Ribotyping.** Cells were harvested from tryptic soy agar (Difco) containing 1% (v/v) horse serum into 10 ml of 0.1 M NaCl, 0.05 M EDTA, and 0.05 M Tris, pH 8. Cells were lysed with sodium dodecyl-sulphate (SDS), and high molecular weight DNA was extracted as previously described[4]. Approximately 5µg of DNA was digested with *Cla*I endonuclease and blotted[25]. DNA from the pKK3535[2] plasmid was labeled with digoxigenin-11-dUTP and detected with Lumi-Phos 530 (Genius 1 Kit Boehringer Mannheim Biochemical, Indianapolis), *Haemophilus influenzae* biogroup aegyptius strain 3031 *Eco*RI fragments were used as molecular weigh marker in each gel[14]. H.44/76 DNA digested with *Cla*I was also included as a control of Rb1 pattern in each gel.

**Source of epidemiological data.** The data pertaining to meningococcal disease among people residing in the greater São Paulo were obtained from the System for Epidemiological Vigilance Alexandre Vranjac, State of São Paulo where they were consolidated and analyzed.

## RESULTS

**Incidence and serotype prevalence.** The incidence of MD, including meningitis and septicemia, in greater São Paulo has increased since 1988 and reached its highest level in 1996 with 8.2 cases per 100,000 inhabitants (Table 2). The incidence of serogroup B MD also reached its highest level at the same year with 1.8 cases per 100,000 inhabitants. Table 3 shows the number of strains of the predominant serogroup B serotypes and serosubtypes found in the greater São Paulo area since 1990. Serotype B:4 strains have been prevalent since 1987 and have accounted over 80% of all cases since 1990 (Fig. 1). The relative frequency of serotype 4 increased from 20% during 1977-1981 to 80% in 1988, just before the 1989 epidemic. During 1990 to 1996 the frequency of serosubtype P1.15 increased from 68% to 85%, and the combination of serotype 4 and serosubtype P1.15 (B:4:P1.15) was the most frequent. After 1990 the proportion of B:4:P1.15 strains increased gradually from 60% to 75% of all group B isolates in 1996. Over the years (1990 to 1996), the proportion of NT strains was almost constant at 10%, and that of nt strains decrease from 30% to 7% in 1996.

**Ribotyping and Serotyping.** The results of 438 *N. meningitidis* isolates analyzed by ribotyping are presented in Figure 1. Because of the equivalency of the methods, ET 5/ Rb1 is used to describe strains originally defined as ET 5 by MEE[7,8], and Rb1 by ribotyping[25]. In a previous study, we demonstrated that the increased incidence of MD, during the period between 1978 to 1990, was paralleled by an increased prevalence of a single group B clone, B:4:P1.15, of the ET 5 complex.

**TABLE 2**
Incidence of meningococcal disease in greater São Paulo from 1990 to 1996, and distribution by year of serogroup B isolates used in this study

| Year | MD* | | Serogroup B | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | CI | Cases | CI | Cases | Laboratory collection (%) Studied by Serotyping | Number of strains studied by Ribotyping | % of studied strains related to Cases | % of studied strains related to Collection | % of ET 5 strains | % of Rb 1 strains | % of B:4:P1:15 strains |
| 1996 | 8.2 | 1337 | 1.8 | 290 | 87(30) | 81 | 28 | 93 | ND | 81 | 75 |
| 1995 | 8.0 | 1278 | 1.7 | 272 | 130(48) | 62 | 23 | 48 | ND | 77 | 74 |
| 1994 | 6.8 | 1088 | 1.5 | 245 | 100(41) | 50 | 20 | 50 | ND | 76 | 62 |
| 1993 | 5.3 | 842 | 1.4 | 224 | 126(56) | 58 | 26 | 46 | ND | 79 | 70 |
| 1992 | 5.3 | 833 | 1.3 | 215 | 99(47) | 50 | 23 | 50 | ND | 86 | 76 |
| 1991 | 6.0 | 926 | 1.1 | 167 | 104(62) | 52 | 31 | 50 | ND | 65 | 52 |
| 1990 | 6.2 | 941 | 1.4 | 215 | 188(87) | 85 | 39 | 45 | 80 | 79 | 66 |
| Total | | 7245 | | 1628 | 834(51) | 438 | 27 | 52 | | 78 | 68 |

* Abreviations: MD, Meningococcal disease; CI, Coefficient of incidence by 100,000/inhabitants; ET 5 strains and Rb1 strains, groups of genetic related strains previously defined[7,8,17,22,25]; ND, Non determinated.

**TABLE 3**

Relative distribution of serotypes and serosubtypes among serogroup B meningococci recovered from patients with meningococcal disease in greater São Paulo between 1990 and 1996 ($n$ = 834)

| Variable | Data for year of recovery | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1990 | | 1991 | | 1992 | | 1993 | | 1994 | | 1995 | | 1996 | |
| | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Serotypes | | | | | | | | | | | | | | |
| 2a | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 2b | 5 | 2 | 5 | 5 | 1 | 1 | 1 | 1 | 0 | 0 | 6 | 4 | 1 | 1 |
| 4 | 150 | 80 | 77 | 74 | 84 | 85 | 105 | 83 | 84 | 84 | 110 | 84 | 75 | 86 |
| 8 | 6 | 3 | 2 | 2 | 3 | 3 | 4 | 3 | 2 | 2 | 2 | 2 | 1 | 1 |
| 15 | 6 | 3 | 2 | 2 | 3 | 3 | 1 | 1 | 0 | 0 | 1 | 1 | 2 | 2 |
| 17 | 0 | 0 | 0 | 0 | 3 | 3 | 1 | 1 | 3 | 3 | 2 | 2 | 0 | 0 |
| NT | 20 | 11 | 17 | 16 | 5 | 5 | 14 | 11 | 11 | 11 | 8 | 6 | 8 | 10 |
| Total | 188 | 100 | 104 | 100 | 99 | 100 | 126 | 100 | 100 | 100 | 130 | 100 | 87 | 100 |
| Serosubtypes | | | | | | | | | | | | | | |
| P1.2 | 3 | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| P1.3 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 1 | 1 | 7 | 5 | 1 | 1 |
| P1.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 5 | 6 | 4 | 2 | 2 |
| P1.9 | 0 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 5 | 5 | 6 | 4 | 1 | 1 |
| P1.15 | 127 | 68 | 67 | 64 | 78 | 79 | 97 | 76 | 80 | 80 | 93 | 73 | 73 | 85 |
| P1.16 | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 3 | 1 | 1 | 0 | 0 | 1 | 1 |
| P1.23 | 0 | 0 | 0 | 0 | 3 | 3 | 3 | 3 | 0 | 0 | 4 | 3 | 2 | 2 |
| nt | 58 | 30 | 35 | 34 | 12 | 12 | 19 | 14 | 7 | 7 | 13 | 10 | 6 | 7 |
| Total | 188 | 100 | 104 | 100 | 99 | 100 | 126 | 100 | 100 | 100 | 130 | 100 | 87 | 100 |

## DISCUSSION

One possible explanation for a rise in the number of meningococcal infections is the introduction of a new strain of *N. meningitidis* in a susceptible population, as occurred in the Netherlands (B:2b:P1.2) in 1966, and Norway (B:15:P1.7,16) in 1974[6,13,19,24].

In greater São Paulo during 1988 to 1989, about 78% and 56% of the strains were B:4 and B:4:P1.15 respectively. The increased spread of the phenotype B:4:P1.15 could account for the increase of MD in those years[21]. After the 1989-90 vaccination trial, B:4 and B:4:P1.15 strains continued to be the most frequent recovered phenotype. The incidence of MD by serogroup B strains during 1990 to 1995 was around 1.4 per 100,000 inhabitants, and during 1996, this coefficient rose to 1.8 per 100,000 inhabitants, the highest rate since the 1970's epidemics (Fig. 1).

The case control study designed to estimate the protective efficacy of the Cuban vaccine in the campaign in greater São Paulo, indicated the vaccine may be effective in preventing serogroup B meningococcal disease in older children and adults[17]. The bactericidal assay on sera from Brazilian children who received two doses of vaccine also revealed age-dependent differences in serological response[16].

Problems faced in development of an improved group B meningococcal vaccine are the diversity of group B serotypes and serosubtypes, and how to choose representative strains for evaluation in experimental vaccines. Among the major OMPs, the porins, classes 1, 2 and 3 proteins, are important targets for bactericidal antibodies, therefore considered vaccine candidates[11]. OMP vaccines are prepared from one particular strain; since many of the major OMP show a high degree of variation among different strains, large proportion of the protective antibodies induced are expected to be strain-specific.

During the vaccination period, (1989-1990), most isolates were the same serotype and serosubtype (54%) as the vaccine strain (B:4:P1.15). An additional 3% were P1.15 and 15% were serotype 4. In Cuba, over 95% of serogroup B strains were 4:P1.15 what could explain why the efficacy of the same vaccine in Cuba was higher[17].

Serological methods have been used extensively for routine epidemiological studies and played an important part in the development of meningococcal vaccines. The current

**FIGURE 1**

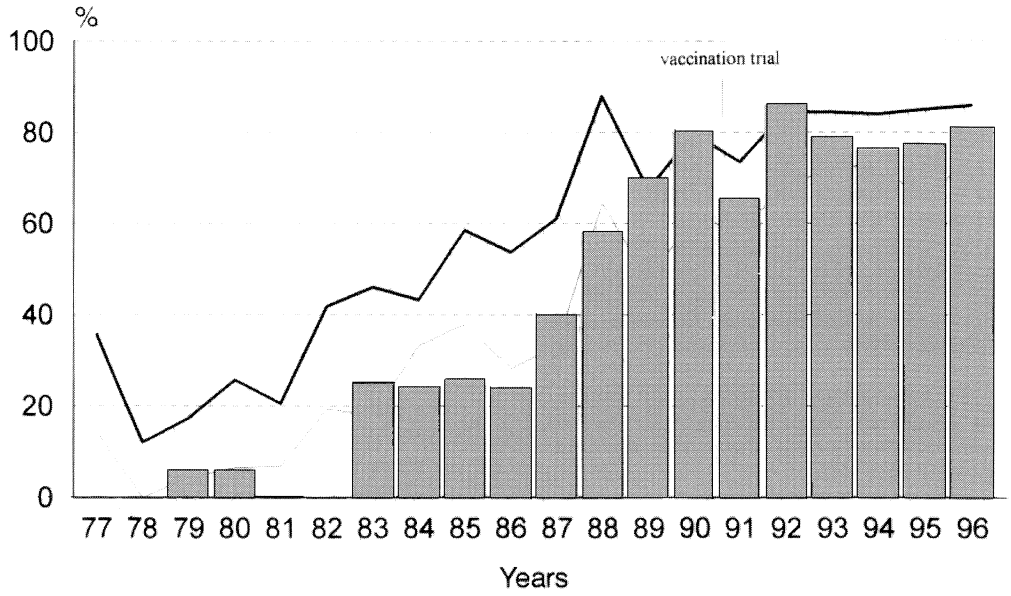


Figure 1 - Prevalence of *Neisseria meningitidis* B:4 (thick line), B:4:P1.15 (thin line), and Rb1 (column) in greater São Paulo during the period of 1977 to 1996.

reagent panel of MAbs does not completely identify all the diversity found in the porins. Many isolates remain non - or only partially serotyped or serosubtyped, consequently many of the antigenic characteristics of the meningococcal porins are still not known. More detailed information on exposure of other epitopes on *N. meningitidis* would allow better assessment of vaccine potential protective immunogenicity.

The total effects on the *N. meningitidis* circulating in the greater São Paulo population after the administration of the Cuban vaccine to 2.4 million persons in 1989-90 are not understood. Although, we do not know what would have occurred if the vaccine had not been administered, the proportion of isolates that were B:4:P1.15 ET5/Rb1 has continued to increase since 1990. We conclude that the same meningococcal clone (B:4:P1.15 ET5/Rb1) is still responsible for the high incidence of MD in São Paulo, in spite of the vaccination trial.

## RESUMO

**Doença meningocócica causada por *Neisseria meningitidis* sorogrupo B sorotipo 4 em São Paulo, Brasil, 1990 a 1996**

Epidemia de doença meningocócica por sorogrupo B tem ocorrido na Grande São Paulo desde 1988. Uma vacina cubana, baseada em proteína de membrana externa de uma cepa de *Neisseria meningitidis* sorogrupo B: sorotipo 4: sorosubtipo P1.15 (B:4:P1.15), foi administrada em cerca de 2.4 milhões de crianças entre 3 meses a 6 anos de idade durante 1989 e 1990. A administração da vacina teve pouco ou nenhum efeito na evolução da epidemia. Para detectar mudança clonal que poderia explicar o contínuo aumento da doença depois da vacinação, foram sorotipadas 834 cepas isoladas entre 1990 e 1996 de pacientes com doença sistêmica. Cepas B:4:P1.15 isoladas desde 1977, têm sido o fenótipo mais prevalente desde 1988. Essas cepas ainda são prevalentes na região e foram responsáveis por cerca de 68% dos 834 casos por sorogrupo B nos últimos 7 anos. Foram analisados 438 (52%) dessas cepas por RFLP do rRNA gene (ribotipagem), e o perfil mais frequente foi o Rbl (68%). Concluímos que o clone B:4:P1.15 - Rbl foi o mais prevalente e responsável pelo contínuo aumento da doença meningocócica na Grande São Paulo durante os últimos 7 anos apesar da campanha de vacinação.

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