

Sero/subtyping of *Neisseria meningitidis* isolated from patients in Spain

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SUMMARY

To know the types of meningococcal strains in Spain, we serotyped and subtyped 743 *Neisseria meningitidis* isolates recovered between 1990 and 1992 from patients. A great number of serogroup B, serogroup C and non-groupable meningococci reacted with the serotyping reagents while many serogroup C and non-groupable isolates did not react with the serosubtyping reagents (78·2% and 54·8% respectively); only 8·9% of serogroup B meningococci were non-subtypeable (NST). Distribution of serotypes was similar in serogroup C and in non-groupable strains. Isolates showed great variability in antigenic phenotypes (71 in serogroup B, 20 in serogroup C and 25 in non-groupable meningococci). The most frequent antigenic combinations were 4:P1.15 (39·8%) in serogroup B, 2b:NST (55·8%) in serogroup C and 2b:NST (35·6%) in non-groupable meningococci.

INTRODUCTION

Meningococci have been classified into 13 serogroups based on their capsular polysaccharide, further subdivided into serotypes with class 2 or 3 outer membrane protein (OMP) specific monoclonal antibodies (McAbs) and into subtypes with class 1 OMP specific McAbs [1].

Serotyping of *Neisseria meningitidis* is important not only for epidemiological studies but underpins the development of meningococcal vaccines. Most present or proposed vaccines to protect against serogroup B disease include class 1 and class 2 or 3 OMP as vaccine components [2, 3].

Serotype and serosubtype reagents have been used in epidemiological studies and some specific types of strains have been associated with epidemic periods [4–6].

In Spain, in 1979 there was an epidemic period with an incidence of 17·6 per 100000 inhabitants [7]. This high incidence later declined to 3·5 in 1991. Since 1990 we have included sero/subtyping with specific monoclonal antibodies as epidemiologic markers for all meningococcal strains studied in our laboratory, in order to determine if meningococci isolated in Spain are similar to meningococcal strains from other European countries.

MATERIALS AND METHODS

We studied 748 strains of *Neisseria meningitidis* isolated from patients with meningococcal disease during 1990–2 from different areas of Spain. These strains represent about 20% of the meningococcal disease cases reported in Spain at this time.

Isolates were confirmed as *N. meningitidis* in our laboratory as described previously [7]. Serogroups were determined by slide agglutination. The sera used were produced in our laboratory by inoculating rabbits using the strains and inoculation protocol already described [7].

Serotypes and subtypes were determined by a whole cell enzyme-linked immunoassay (EIA). Antigens were prepared as described by Abdillahi and Poolman [1]. Monoclonal antibodies with serotype specificities 1, 2a, 2b, 4, 14, 15 and subtype specificities P1.1, P1.2, P1.3, P1.4, P1.6, P1.7, P1.9, P1.10, P1.12, P1.14, P1.15 and P1.16 were supplied by Dr J. T. Poolman (RIVM, Bilthoven, The Netherlands).

RESULTS

The distribution of serogroups is shown in Table 1. The majority of isolates were of serogroup B or C; only a few strains belonged to other serogroups (A, X, Y). Seventy-three (9.7%) meningococcal strains were non-groupable. A high number of non-groupable strains were found in 1992 (17.8%).

Serotypes and subtypes of all isolates are shown in Tables 2 and 3. There were no significant changes in the relative frequency of serotypes amongst serogroup B strains, serotype 4 being the most prevalent (54.3%) (Table 2); however, we found an increase of serotype 14 and a decrease of serotype 2a in serogroup C isolates from 1.8% and 20% to 8.5% and 10.2% respectively (Table 2). Serotype 2b characterized 67% of all serogroup C isolates. Serotype 2b was the most frequent serotype amongst non-groupable isolates (45.2%), followed by serotype 4 (20.5%) (Table 2). The proportion of non-typable strains was 14.8%, 4.1% and 15.1% in serogroup B, C and non-groupable isolates respectively (Table 2).

Serosubtype P1.15, which was frequently linked to serotype 4 (Table 3), characterized 47.6% of all serogroup B isolates (Table 2). We did not find any strain of serosubtype P1.4 during 1990 while this serosubtype represented 4.5% of all isolates in 1991 and 1992. The proportion of non-subtypable strains was 8.9% amongst serogroup B meningococci.

The majority of serogroup C meningococci were non-subtypable with this serosubtype reagent panel (78.2%) (Table 2), although the proportion fell from 89.1% in 1990 to 62.7% in 1992. Serosubtype P1.1, 2 was most commonly found amongst serogroup C isolates (10.6%) (Table 2). We did not find this serosubtype in 1990 but its proportion increased to 8.4% in 1991 and to 23.7% in 1992. Serosubtype P1.2 increased from 1.8% to 6.8%.

Forty non-groupable strains (54.8%) were non-subtypable (Table 2). Serosubtypes P1.1, 2 (10.9%) and P1.15 (9.6%) were the most common amongst this serogroup.

We found 116 different antigenic phenotypes (71 amongst serogroup B, 20 amongst serogroup C and 25 amongst non-groupable strains) (Table 3). The most

Table 1. *Meningococcal disease in Spain (1990–2). Distribution of serogroups by year*

Year/ serogroup	1990	1991	1992	Total
A	1 (0.4)	—	—	1 (0.1)
B	166 (72.5)	151 (59.2)	156 (59.1)	473 (63.2)
C	55 (24.1)	83 (32.5)	59 (22.4)	197 (26.4)
X	—	—	2 (0.7)	2 (0.3)
Y	—	2 (0.8)	—	2 (0.3)
NG*	7 (3.0)	19 (7.5)	47 (17.8)	73 (9.7)
Total	229	255	264	748

* Non-groupables.

frequent serotype of serogroup B meningococci was the 4:P1.15 (39.7%) followed by the 15:P1.7, 16, the 15:P1.16 and the NT:P1.15 with 5.7%, 5.3% and 4.4% respectively, while 15 strains (3.2%) were non-typable and non-subtypable in this serogroup. The most frequent phenotypes amongst serogroup C isolates were 2b:NST (55.8%) and 2a:NST (15.2%), while five isolates (2.5%) did not react with any of the serotype or serosubtype antibodies. The most common serotype-serosubtype combinations were 2b:NST (35.6%), 2b:P1.1, 2 (9.6%) and 4:P1.15 (8.2%) in non-groupable meningococci. Six strains (8.2%) were both non-typable and non-subtypable in this group.

DISCUSSION

Serogroup B strains predominated in causing meningococcal disease in Spain during epidemic periods in the 1970s and 1980s [8, 9]; serogroup C strains, about 3% in the 1970s [8] and 12–14% in the 1980s [9], made up about 25% of all meningococcal strains during the last 3 years. The great number of non-groupable isolates (17.8%) found in 1992 was surprising (Table 1); the majority of these strains were non-groupable because they showed autoagglutination in saline. When we analysed the serotype distribution (Table 2) within the three serogroups (B, C and non-groupable), we found that it was similar between serogroup C and non-groupable strains. Therefore, we think that perhaps most of these non-groupable meningococci could be serogroup C isolates which have lost totally or partially their capsular polysaccharide. Serogroup A strains which made up about 15% of the total in the 1970s [8], were identified only sporadically in the early 1990s.

It is very interesting to note that in this study the great majority of meningococcal isolates reacted with serotype antibodies regardless of whether they were serogroup B, C or non-groupable isolates (85.2%, 95.9% and 84.9% respectively). Only 89 strains (12%) were non-serotypable, this proportion being lower than that found by other authors in different European countries [5, 6, 10]. However, the number of non-subtypable strains was very different in the three meningococcal serogroups: 42 (8.9%) in serogroup B, 154 (78.2%) in serogroup C and 40 (54.8%) in non-groupable strains. We think that the serotype reagents used in this study provide useful data in epidemiological studies in Spain. Although most serogroup B meningococcal strains were subtyped, this was not the

Table 2. *Relative distribution of serotypes and subtypes expressed as percentage among serogroup B, serogroup C and non-groupable meningococci isolated from patients in Spain (1990-2)*

Serogroup B	Year			Total (%)
	1990	1991	1992	
Serotypes				
1	6	4.6	8.3	30 (6.3)
2a	—	—	0.6	1 (0.2)
2b	1.2	2	2.6	9 (1.9)
4	50.6	55	57.7	257 (54.3)
14	3.6	4	3.2	17 (3.6)
15	21.7	17.2	17.3	89 (18.8)
Non-serotypable (NT)	16.9	17.2	10.3	70 (14.8)
Subtypes (%)				
P1.1	3	—	1.3	7 (1.5)
P1.1, 2	0.6	2.6	0.6	6 (1.3)
P1.1, 6	—	—	0.6	1 (0.2)
P1.1, 7	3.6	0.6	1.3	9 (1.9)
P1.1, 10	0.6	—	—	1 (0.2)
P1.1, 16	2.4	—	—	4 (0.8)
P1.2	2.4	0.6	0.6	6 (1.3)
P1.4	—	4.6	4.5	14 (2.9)
P1.4, 16	0.6	—	—	1 (0.2)
P1.6	1.8	3.3	5.1	16 (3.4)
P1.7	4.8	2.6	1.3	14 (2.9)
P1.7, 9	—	—	1.3	2 (0.4)
P1.7, 14	—	—	0.6	1 (0.2)
P1.7, 15	0.6	—	0.6	2 (0.4)
P1.7, 16	3.6	7.9	6.4	28 (5.9)
P1.9	4.2	3.3	1.3	14 (2.9)
P1.10	4.8	1.3	1.9	13 (2.7)
P1, 10, 15	—	0.6	—	1 (0.2)
P1.12	1.8	2	2.6	10 (2.1)
P1.14	0.6	2	4.5	11 (2.3)
P1.15	44.6	53	45.5	225 (47.6)
P1.15, 16	2.4	—	—	4 (0.8)
P1.16	10.8	6.6	8.3	41 (8.7)
Non-subtypable (NST)	6.6	8.6	11.5	42 (8.9)
Total of isolates tested	166	151	156	473
Serogroup C				
Serotypes				
1	5.4	2.4	6.8	9 (4.6)
2a	20	22.9	10.2	36 (18.3)
2b	67.3	65.1	69.5	132 (67.0)
4	1.8	1.2	1.7	3 (1.5)
14	1.8	2.4	8.5	8 (4.1)
15	—	1.2	—	1 (0.5)
NT	3.7	4.8	3.4	8 (4.1)
Subtypes				
P1.1	3.6	—	—	2 (1.0)
P1.1, 2	—	8.4	23.7	21 (10.6)
P1.1, 15	1.8	—	—	1 (0.5)
P1.2	1.8	4.8	6.8	9 (4.6)
P1.6	1.8	1.2	1.7	3 (1.5)

Table 2. (cont.)

Serogroup C	Year			Total (%)
	1990	1991	1992	
P1.7. 16	—	—	1.7	1 (0.5)
P1.9	—	1.2	3.4	3 (1.5)
P1.10	—	1.2	—	1 (0.5)
P1.15	1.8	1.2	—	2 (1.0)
NST	89.1	81.9	62.7	154 (78.2)
Total of isolates tested	55	83	59	197
Non-groupable				
Serotypes				
1	—	—	2.1	1 (1.4)
2a	—	—	14.9	7 (9.6)
2b	42.8	52.6	42.6	33 (45.2)
4	14.3	31.6	17.0	15 (20.5)
14	14.3	5.3	2.1	3 (4.1)
15	—	5.3	4.2	3 (4.1)
NT	28.6	5.3	17.0	11 (15.1)
Subtypes				
P1.1, 2	—	—	17.0	8 (10.9)
P1.1, 7	—	5.3	—	1 (1.4)
P1.2	—	—	4.2	2 (2.8)
P1.4	—	5.3	—	1 (1.4)
P1.6	14.3	—	4.2	3 (4.1)
P1.7	—	—	2.1	1 (1.4)
P1.9	—	—	6.4	3 (4.1)
P1.10	—	—	2.1	1 (1.4)
P1.12	14.3	5.3	4.2	4 (5.5)
P1.14	—	—	2.1	1 (1.4)
P1.15	14.3	15.8	6.4	7 (9.6)
P1.16	—	5.3	—	1 (1.4)
NST	57.1	63.1	25.5	40 (54.8)
Total of isolates tested	7	19	47	73

case for serogroup C isolates, so new subtype reagents are required for this group of meningococci.

More than 50% of serogroup B meningococci were serotype 4 and almost 50% of these serotype 4 strains reacted with the P1.15 monoclonal subtype reagent. In fact, the most common combination of serotype and serosubtype in serogroup B strains was 4:P1.15; however, the phenotype 15:P1.16 which has been associated with outbreaks in northern Europe [11, 12] only represented 5.3% of all serogroup B meningococci in this study; Sáez-Nieto and colleagues [13] found that serotype 2 was the most common amongst serogroup B meningococci during 1978–80 (years of higher incidence of meningococcal disease in Spain) followed by serotype 15 strains; they did not find any serotype 4 isolates. These proportions did not change until 1987, when serotype 15 became the most common amongst serogroup B meningococci in Spain [14], the proportion of serotype 4 strains diminishing. These studies were performed using double immunodiffusion in gel [13]. Roman and colleagues [14] studied the serosubtype of 215 serogroup B serotype 15 meningococcal strains isolated in Spain from 1978–87, and found about 50% were P1.16 and 50% were P1.15 (linked frequently to serotype 4). We found the

Table 3. *Major antigenic phenotypes of Neisseria meningitidis isolated from patients in Spain (1990-2)*

Serogroup B	Serotype						
	1	2a	2b	4	14	15	NT
Subtype							
P1.1	2	—	—	—	1	2	2
P1.1, 2	—	—	1	4	—	1	—
P1.1, 6	1	—	—	—	—	—	—
P1.1, 7	—	—	—	3	4	—	2
P1.1, 10	—	—	—	—	—	1	—
P1.1, 16	—	—	—	1	—	2	1
P1.2	—	—	—	2	—	3	1
P1.4	—	—	—	9	—	1	4
P1.4, 16	—	—	—	—	—	1	—
P1.6	4	—	—	6	3	1	2
P1.7	1	—	—	1	2	9	1
P1.7, 9	—	—	—	—	—	1	—
P1.7, 14	—	—	—	—	1	—	—
P1.7, 15	—	—	—	1	—	1	—
P1.7, 16	—	—	—	1	—	27	—
P1.9	—	—	—	2	1	4	7
P1.10	1	—	3	4	1	—	4
P1.10, 15	—	—	—	1	—	—	—
P1.12	—	—	—	3	—	2	5
P1.14	2	—	—	8	—	—	1
P1.15	7	—	1	188	4	4	21
P1.15, 16	—	—	—	4	—	—	—
P1.16	1	—	—	10	—	26	4
NST	11	1	4	8	—	3	15
Serogroup C							
P1.1	—	1	1	—	—	—	—
P1.1, 2	—	3	13	—	3	—	2
P1.1, 15	—	—	1	—	—	—	—
P1.2	—	2	7	—	—	—	—
P1.6	2	—	—	—	—	—	1
P1.7, 16	1	—	—	—	—	—	—
P1.9	2	—	—	—	—	1	—
P1.10	1	—	—	—	—	—	—
P1.15	—	—	—	—	—	—	—
NST	3	30	110	—	5	—	5
Non-groupable							
P1.1, 2	—	1	7	—	—	—	—
P1.1, 7	—	—	—	1	—	—	—
P1.2	—	—	—	—	—	—	2
P1.4	—	—	—	1	—	—	—
P1.6	—	1	—	1	—	—	1
P1.7	—	—	—	—	—	1	—
P1.9	—	—	—	2	—	—	1
P1.10	—	—	—	1	—	—	—
P1.12	1	—	—	1	1	1	—
P1.14	—	—	—	1	—	—	—
P1.15	—	—	—	4	—	—	1
P1.16	—	—	—	1	—	—	—
NST	—	5	26	—	2	1	6

phenotype B:4:P1.15 the most common in a previous study [9] with a selection of strains from the 1986–90 period. It would be interesting to know if some serotype 15 meningococci as classified by Román and colleagues [14] are in fact serotype 4 meningococcal strains or alternatively whether there has been an important epidemiological shift in the frequency of serotypes amongst Spanish serogroup B isolates. We think that many meningococci classified as serotype 15 by immunodiffusion may be serotype 4 strains with non-specific reactivity with serotype 15; further studies with these strains should be performed. The phenotype B:2, very frequent during the previous epidemic period [13], was found very rarely (1.9%) in this study.

The most common serotype amongst serogroup C meningococci was 2b. A previous Spanish study showed a similar proportion of 2a and 2b serotype strains amongst serogroup C meningococci from 1978–87 [14]. However, serotype 2a has been isolated less frequently in the 1990s.

Some experimental vaccines against serogroup B meningococcal disease have been tested in Norway, Cuba and Chile [2, 3, 15]. The Cuban vaccine was prepared using the B:4:P1.15 epidemic strain, which is the most frequent phenotype found in this study amongst serogroup B meningococci (39.7%). It would be interesting to carry out an evaluation of the efficacy of this vaccine in our country. Also, a polyvalent serogroup B class 1 OMP vaccine, active against all detectable serosubtypes, is under investigation; as we found that only 8.9% of the serogroup B strains in Spain were non-subtypeable, such a polyvalent vaccine could in theory give protection against more than 90% of meningococcal disease in Spain.

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