Prevalence of *Neisseria meningitidis* carriers in the school population of Catalonia, Spain

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SUMMARY

The aim of this study was to determine the prevalence of healthy *Neisseria meningitidis* pharyngeal carriers in a representative sample of the Catalonian school population, as well as its associated factors. The sample was divided into age groups: $\leqslant 5$, 6–7 and 13–14 years old. Parents were given a questionnaire to collect information on sociodemographic and epidemiological variables. Oropharyngeal swabs were collected with a cotton-tipped swab in an Amies transport medium and cultured on Thayer Martin plates at 35 °C in 5% CO_2. The isolates were serogrouped and sero/subtyped. Of the 1406 children studied, 75 (5·34%) meningococcal carriers were detected: 63 B (4·5%), 9 non groupable (0·7%), 2 29E (0·1%) and 1X (0·07%). No serogroup C meningococci were found in this study, probably due to the high A+C vaccination coverage of up to 68·9% in children 6–7 years old. Bivariate analysis identified six statistically significant risk factors for meningococcal carriage: increasing age, recent upper respiratory tract infection, previous antibiotic treatment, number of students in the class, size of the classroom and social class. Multivariate analysis found that only age and previous antibiotic treatment remained statistically significant when the other factors were controlled.

INTRODUCTION

Meningococcal disease (MD) is a serious, life-threatening acute illness caused by *Neisseria meningitidis* in which the bacteria invade the blood stream and often the meninges, causing meningitis. The organism has no known reservoir outside man so asymptomatic carriers have been recognized as the source of transmission of the disease [1].

It has been suggested that environmental factors play a role in the carriage of the bacteria [2, 3]. In some studies, smoking or passive exposure to cigarette smoke has been associated with isolation of *N. meningitidis* from healthy asymptomatic adults and teenagers [2–5]. Virus infection has also been suggested as a predisposing factor for susceptibility to meningococcal disease and to carriage of potentially pathogenic bacteria [6]. Previous antibiotic treatment [7, 8] and age [4, 5, 7, 9–11] have also been reported to be associated with the carriage of meningococci. In most developed countries, meningococcal disease occurs at a low endemic rate of less than five cases per 100 000 inhabitants, increasing by 10–100 times in local outbreaks or epidemic situations [12].

In 1995–6, the incidence of meningococcal disease reached 11·3 cases per 100 000 inhabitants in Galicia, in north-west Spain. During this period, 80% of the

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N. meningitidis isolates were serogroup C, with almost all being of the C:2b:P1.5,2 phenotype [13]. In the same period (1996), in our geographical area (Catalonia, in north-east Spain) serogroup C cases increased to 34% of confirmed *N. meningitidis* cases and to 43% in 1997, when usually serogroup B accounted for 70–80% of cases (with B:4:P1.15 being the most frequent strain found) [14]. Since this represented a change from the usual pattern of the disease in different regions of Spain [15–17], where the predominant serogroup was serogroup B, it was decided to recommend meningococcal A+C polysaccharide vaccination for the population between the ages of 18 months and 19 years in several Spanish regions, including Catalonia, where the campaign of mass vaccination was carried out in October 1997 [18].

With this background, we carried out a study to determine the prevalence of meningococcal carriage and, specifically, of the C:2b:P1.5,2 strain, among a representative sample of the Catalonian school population, and, additionally, to improve our knowledge of the factors that determine carriage.

MATERIAL AND METHODS

The study was carried out between October 1998 and April 1999. Thirty schools and 30 kindergartens (both public and private) in Catalonia (Spain) were selected by means of a random number generating computer programme (15 corresponding to the province of Barcelona, 5 to Tarragona, 5 to Lleida, and 5 to Girona). Three age groups were studied: children under 5 years old from the 30 selected kindergarten and primary schools, children 6–7 years old and children 13–14 years old from the selected schools. The sample size was calculated using a 0·05 α error, a precision of 0·025 and an expected prevalence of 0·03 for the epidemic strain under study (C:2b:P1.5,2). The theoretical sample size was 179 children in each age group. To take into account the variance modification when performing this type of sample, a designed effect of 2·00 was calculated [19], and thus, the size of each age group was 358.

The parents were given a questionnaire to collect information on the following variables: age, sex, place of residence, number of inhabitants in the house, size of the house (m²), social class (based on occupations of the heads of households), parents who smoked, history of underlying disease, previous antibiotic treatment, upper respiratory tract infection during the last month, meningococcal A+C vaccination, pre-vious exposure to a case of meningococcal disease, size of the classroom (m²), and number of students in the class. The questionnaires were accompanied by an informative letter to the parents, asking for their informed consent. All students from each class with written informed consent at the time of the sample collection were included in the study.

Microbiological study

The oropharyngeal samples were collected with a cotton-tipped swab which was immediately placed in Amies transport medium (Copan® Ventury Transystem, Italy) and cultured on Thayer Martin agar plates. The plates were incubated at 37 °C in a humid atmosphere containing 5% CO_2 and read on two subsequent days. Presumptive colonies were identified as *N. meningitidis* by standard methods and serogrouping was performed by a latex agglutination test (SLIDEX, Pasteur-Mérieux, France). Isolates were serotyped by the Reference Laboratory for *Neisseria*, Madrid [15].

Statistical analysis of the data

The prevalence of meningococcal carriage was calculated globally and by age groups. The confidence intervals were calculated by the exact binomial method. The questionnaire data were subjected to bivariate and multivariate analyses with the statistical packages Epi-Info and SPSS. The χ^2 test was used for statistical significance. Variables from the univariate analysis were entered into an unconditional logistic regression model, and the adjusted odds ratio and 95% confidence intervals were calculated for the resulting significant variables.

RESULTS

A total of 1406 pharyngeal samples was obtained, 593 corresponding to subjects under 5 years age, obtained from the 30 kindergartens (with a participation rate of 43%, range 29–86%) and from primary schools (participation rate 40%, range 24–84%), 380 to those of 6–7 years (with a participation rate of 44% (25–73%) in public primary schools and 72% (48–96%) in private schools), and 433 to those of 13–14 years (with participation rates of 50 (13–92%) and 61% (23–87%) in private and public secondary schools, respectively).

Table 1. *Prevalence of N. meningitidis carriers and distribution of strains according to serogroup and age*

Age group*	Serogroup B		Serogroup X		Serogroup 29E		Non-serogroupable		Totals	
	Prevalence (%) CI (95%)	Strains (% for age group)	Prevalence (%) CI (95%)	Strains (% for age group)	Prevalence (%) CI (95%)	Strains (% for age group)	Prevalence (%) CI (95%)	Strains (% for age group)	Prevalence (%) CI (95%)	Strains (% for age group)
0–3 years (n = 436)	0·2 (0·006–1·2)	1 (100)	0	0	0	0	0	0	0·2 (0·006–1·2)	1 (100)
4–5 years (n = 157)	1·9 (0·4–5·4)	3 (60)	0	0	0	0	1·3 (0·2–4·5)	2 (40)	3·2 (1·0–7·3)	5 (100)
Total 0–5 years (n = 593)	0·7 (0·2–1·7)	4 (67)	0	0	0	0	0·3 (0·04–1·2)	2 (33)	1·0 (0·3–2·2)	6 (100)
6–7 years (n = 380)	4·5 (2·6–7·1)	17 (85·0)	0·3 (0·007–1·5)	1 (5·0)	0	0	0·5 (0·06–1·9)	2 (10)	5·3 (3·2–8·0)	20 (100)
13–14 years (n = 433)	9·7 (7·0–12·6)	42 (85·7)	0	0	0·5 (0·06–1·7)	2 (4·1)	1·2 (0·4–2·7)	5 (10·2)	11·3 (8·4–14·4)	49 (100)
Total (n = 1406)	4·5 (3·4–5·6)	63 (84·0)	0·07 (0·002–0·4)	1 (1·3)	0·1 (0·02–0·5)	2 (2·7)	0·6 (0·3–1·2)	9 (12·0)	5·3 (4·1–6·5)	75 (100)

* $P < 0.00001$.

The global prevalence of carriers of *N. meningitidis* was 5·3 % (*n* = 75); the prevalence of *N. meningitidis* by age groups and the corresponding 95 % confidence intervals is shown in Table 1. The 75 carriers were obtained from 17 different schools and 2 kindergartens and the distribution of carriers by schools is shown in Table 2.

Most of the meningococcal strains isolated from healthy carriers belonged to serogroup B (63/75, 84 %). Serogroup 29 E accounted for 2·7 % (2/75) and serogroup X 1·3 % (1/75); 9 strains (12 %) were not serogroupable. The distribution of the serogroups according to age groups is shown in Table 1.

Four serotypes were represented among the 63 serogroup B strains: serotype 1 (22·2 %), serotype 15 (19 %), serotype 4 (12·7 %) and serotype 14 (1·6 %). Twenty-eight isolates were non-serotypable. Twenty-four sero/subtype combinations were distinguished, with B:15:P1.6 (14·3 %) being dominant. Other antigenic combinations accounted for less than 10 % of the isolates (Table 3) and 15 isolates (23·8 %) could not be subtyped. Similar sero/subtype combinations were found in carriers from the same school.

The bivariate analysis (Table 4) showed that the variables associated with carrier status were age (the greater the age the higher the prevalence), presence of an upper respiratory tract infection, recent use of antibiotics (associated with a lower prevalence), social class (higher prevalence with lower social class), class size and the number of students per class. However, multivariate analysis after adjusting for these variables showed that only age and previous antibiotic treatment remained associated with carrier status. (Table 5).

DISCUSSION

The prevalence of *N. meningitidis* carriers in the sample of the Catalonian school population studied was 5·3 %, with a clear age dependence. In subjects under 3 years the prevalence was only 0·2 % and in those of 13–14 years it reached 11·3 % (Table 1).

In Catalonia, in 1997 and 1998, serogroup B strains accounted for 55 % and 71 % of confirmed cases respectively [14]. Compared with results obtained in other national and international studies where serogroup B was also predominant, the prevalence of meningococcal carriers found in the present study is lower. Nevertheless it is greater than the prevalence of 1·8 % found by Caugant et al. [4] in a random

Table 2. *Distribution of meningococcal carriers by schools*

	School type	Age group (yr)	No.	Carriers
Kindergarten 1 Barcelona	Private	0–5	25	1
Kindergarten 2 Girona	Private	0–5	29	1
School 1 Barcelona	Public primary school	4–5	8	0
		6–7	10	1
School 2 Barcelona	Private	6–7	18	2
		13–14	23	5
School 3 Barcelona	Private	4–5	21	1
		6–7	18	0
		13–14	22	1
School 4 Barcelona	Public secondary school	13–14	14	2
School 5 Barcelona	Private primary school	4–5	11	0
		6–7	19	2
		13–14	44	5
School 6 Barcelona	Private	4–5	4	0
		6–7	23	2
		13–14	5	0
School 7 Barcelona	Private	4–5	14	1
		6–7	21	4
		13–14	37	6
School 8 Barcelona	Public	4–5	13	0
		6–7	8	2
School 9 Barcelona	Public	13–14	43	3
School 10 Barcelona	Private	6–7	45	1
		13–14	47	0
School 11 Barcelona	Private	6–7	24	0
		13–14	27	1
School 12 Girona	Public	4–5	9	1
		6–7	8	0
School 13 Girona	Public	13–14	48	6
School 14 Girona	Private	6–7	32	3
		13–14	66	12
School 15 Lleida	Public	4–5	4	0
		6–7	3	1
School 16 Lleida	Public	13–14	34	5
School 17 Tarragona	Public	4–5	10	1
		6–7	25	0
School 18 Tarragona	Private	4–5	10	0
		6–7	12	2
		13–14	22	3

population sample of subjects aged 0–14 years and is similar to that of 5·5% obtained more recently by Bevanger et al. [20]. Higher prevalence rates were reported by Fontanals et al. [7], in a study carried out in Cerdanyola (Catalonia, Spain) who found 8·9% of subjects under 14 years carried meningococci. Similarly, Cartwright et al. in the United Kingdom [10], and Kriz et al. [21] in the Czech Republic, both in populations under 15 years of age, obtained prevalences of 6·8% and 14·6%, respectively.

In these same studies, the frequency of the serogroup among the carriers varied widely. Thus, serogroup B represented 31% and serogroup C 4·4% in Norway [4], while in the Czech Republic, serogroup B accounted for 41·4% of the total, and serogroup C 37·9% [21]. In contrast, Fontanals et al. [7] found

Table 3. *Distribution of serogroup B meningococci by serotype and subserotype*

Subserotypes	Serogroup/type					
	B:1	B:4	B:14	B:15	NT	Total
P1.1	—	—	—	—	2 (3·2)	2 (3·2)
P1.2,5	—	3 (4·8)	—	—	3 (4·8)	6 (9·6)
P1.3,6	1 (1·6*)	—	—	—	1 (1·6)	2 (3·2)
P1.5	—	—	—	—	2 (3·2)	2 (3·2)
P1.6	3 (4·8)	1 (1·6)	—	9 (14·3)	—	13 (20·7)
P1.7	2 (3·2)	—	1 (1·6)	—	3 (4·8)	6 (9·6)
P1.7,15	—	—	—	—	1 (1·6)	1 (1·6)
P1.9	—	—	—	—	1 (1·6)	1 (1·6)
P1.12	—	—	—	—	2 (3·2)	2 (3·2)
P1.13	3 (4·8)	—	—	—	1 (1·6)	4 (6·4)
P1.14	—	—	—	—	2 (3·2)	2 (3·2)
P1.15	1 (1·6)	3 (4·8)	—	—	—	4 (6·4)
NST	4 (6·3)	1 (1·6)	—	3 (4·8)	10 (15·9)	18 (28·6)
Total	14 (22·3)	8 (12·8)	1 (1·6)	12 (19·1)	28 (44·7)	63 (100)

* Percentages are given within parentheses.

70% of carrier strains were serogroup B and only 6% serogroup C. In our study, serogroup B accounted for 85% of the strains found and no serogroup C strains were recovered which is more in accordance with the results from the United Kingdom [10] and the studies of Bevanger et al. [20], where the frequencies for serogroup C were very low. The absence of serogroup C carriers in the Catalonian population studied coincides with the results of the survey of Smith et al. [22] which found no carriers of serogroup C strains after a campaign of selective vaccination in western Norway.

The campaign of mass meningococcal A+C vaccination carried out 1 year before this study in the 2–19 year old population of Catalonia in order to control the increase in serogroup C cases could explain this finding. The suggestion that vaccination influences carrier status was made by some authors [23–25] over 30 years ago but later studies [26–28] have not supported this association. Indeed, together with the absence of serogroup C carriers, it is noteworthy that cases of the disease due to this serogroup also diminish to a great extent after vaccination.

In other studies carried out in communities where the greatest number of cases were caused by serogroup C, the global prevalences of carriers were very variable. For example, in Galicia, where at the end of 1996 the percentage of cases caused by serogroup C surpassed 80%, the global prevalence of carriers was only 14%. Furthermore, Cartwright et al. in Gloucester (United Kingdom) found a prevalence of 34·1% for serogroup C [10], but after extensive vaccination of the population under 20 years of age, for group C meningococci, the prevalence fell to 2·4% [29]. Rates of 31·2% [8] were reported from Holland and 19·4% in Norway [22]. In almost all studies, the most-frequently found serogroup in carriers was B, ranging between 66% [13], 33% [8], 16% [29] and 5·3% [22] but with the exception of the study of Smith et al. [22] group C meningococcal carriers were also found.

Cheesbourg et al. [30] and Caugant et al. [4] point out that the time of year can influence the results of meningococcal prevalence studies. The study in schoolchildren was carried out during the last quarter of 1998 before the epidemic period in our area, (January–March) and that in pre-school children in the first quarter of 1999, which is the period corresponding to the greatest number of cases in Catalonia, leading us to believe that the time of year alone does not explain the low prevalence obtained.

The distribution of serogroups and serotypes found in our carriers indicates, as in other cited studies, that there is no relationship between the distribution of the groups of circulating strains of meningococci in the community and the distribution of the cases of disease produced. Strain B:15:P1.16 was the dominant strain in this study (14·3%), while, among patients, strain B:4:P1.15 was the most commonly found (25·8%) followed by strain C:2b:P1.5,2 (10·1%) which was not found in this study. Other authors have reached

Table 4. *Distribution of* N. meningitidis *carriers by the studied variables. Bivariate analysis*

Factor	No (%)	OR (95% CI)	P
Underlying disease			
No	70/1300 (5·4)		0·64
Yes	4/94 (4·3)	0·8 (0·24–2·29)	
Previous exposure to a case			
No	74/1396 (5·3)	—	—
Yes	0/7		
A–C meningococcal vaccination			
No	34/709 (4·8)		0·55
Yes	38/690 (5·5)	1·16 (0·7–1·91)	
Upper respiratory tract infection			
No	62/938 (6·6)	2·64 (1·37–5·20)	0·002
Yes	12/460 (2·6)		
Recent use of antibiotics			
No	71/1083 (6·6)	7·37 (2·22–29·4)	0·0001
Yes*	3/318 (0·9)		
Size of dwelling			
<100 m²	50/917 (5·5)	1·10 (0·65–1·86)	0·72
>100 m²	24/480 (5·0)		
Size of the household (no. of persons)			
< = 3	18/422 (4·3)	0·74 (0·41–1·30)	0·27
> = 4	56/980 (5·7)		
Passive smoking			
Non exposed	33/589 (5·6)	1·12 (0·64–1·83)	0·64
Exposed	41/813 (5·0)		
Social class†			
I–III	27/719 (3·8)	0·53 (0·32–0·88)	0·009
IV–V	47/682 (6·9)		
Habitat			
Rural	25/448 (5·6)		
Urban	50/956 (5·2)	0·93 (0·55–1·58)	0·8
Size of the classroom			
0–25 m²	3/220 (1·4)	0·21 (0·05–0·71)	0·004
>25 m²	72/1186 (6·1)		
No of student/class			
0–20	7/592 (1·2)	0·13 (0·05–0·30)	0·000000
>20	68/814 (8·3)		

* 53% penicillins, 22% cephalosporins, 18% macrolides.
† I–III: professional, intermediate and skilled non-manual workers; IV–V: skilled and non skilled manual workers.

the same conclusion [4, 31] when studying the phenotypes of strains found in carriers in a representative sample of the general population in Norway. However, the finding of several strains with the same phenotype in the same school suggests the possible existence of certain evolutionary lines or population clusters limited to very specific geographical areas. At the same time it confirms the utility of phenotyping as an epidemiological tool for evaluating the dissemination of strains in a semi-closed community, as well as for the detection of their acquisition in persistent and intermittent carriers [32].

One aspect that should be noted is the high proportion of carriers of non-serogroupable strains which are usually non-capsulate. Cartwright et al. [10], in 1987, highlighted the greater facility of these strains isolated from the blood or CSF of patients to agglutinate compared with those isolated from pharyngeal swabs of the same patients, suggesting that microorganisms with capsular polysaccharides are more pathogenic. Molecular markers in both capsulated and uncapsulated isolates might clarify this finding. In the present study, the non-serogroupable strains accounted for 12% of the total, a percentage

Table 5. *Association of the studied variables with carrier status. Multivariate analysis*

Variable	ORa*	95% CI	P
Age	1·13	1·04–1·23	0·004
Antibiotics	0·25	0·07–0·94	0·04
Respiratory infection	0·98	0·47–2·03	0·95
Classroom size			
12–20 m²	1·27	0·16–2·32	0·82
21–40 m²	2·71	0·34–7·81	0·34
Children/class			
20–30	2·07	0·78–5·45	0·14
>30	1·71	0·50–5·85	0·38
Social class	1·62	0·98–2·68	0·05

* ORa, Odds ratio adjusted by an unconditional logistic regression model.

somewhat below that obtained by other authors. In studies carried out in populations with a predominance of serogroup B cases, this percentage is very variable, ranging between 17% [21], 22·5% [7], 38% [10], 44% [4] and 55% [20]. As Reller et al. have pointed out [33], colonization with non-serogroupable meningococci may also be an immunizing process, since carriers of these strains show an increase of antibodies against capsular polysaccharides (A, B, C, Y). In studies where serogroup C caused the greatest number of cases in the community, the percentage of non-serogroupable strains among carriers is also very variable, with reported frequencies from 19·7% [13] to 52% [22].

Age has been repeatedly associated with the prevalence of carriers [4, 7, 8, 11, 28, 34, 35] and in some studies [4, 7, 8] this association was quantified following multivariate analysis. We found it to be the factor most closely associated with carriage and there was an increasing prevalence with age (Table 1). Although the 8–12 years age group was not studied, the values which we found were clearly increasing, so it would be expected that the prevalence in the 8–12 years age group would be lower than that of the 13–14 years group and higher than that of the 6–7 years group. This supports the premise that carrier status is an immunizing process and that with the passage of time an immune response is generated which would avoid the development of the disease [36, 37].

Although other studies have shown gender [2, 4, 7, 11, 35], passive smoking [2, 4, 38], overcrowding and social class to be related with carrier status, we found no association for any of these variables.

The use of antibiotics in the month previous to the taking of the sample was associated with a lower prevalence of carriers [7, 8], although the magnitude of the association was less than that for age. This coincides with the results of Imrey et al. [39] and others [7, 8] and suggests that carrier prevalence may be related to the patterns of prescription and consumption of antibiotics in the community.

We conclude that circulation of meningococcal strains in the healthy school population of Catalonia is low but increases with age. The absence of serogroup C carriage, a situation only described by one other prevalence study carried out after a vaccination campaign [22], is probably explained by the mass A+C vaccination campaign carried out 1 year before the study was made.

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