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Carriage of *Neisseria meningitidis* and *Neisseria lactamica* in a school population during an epidemic period in Spain

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SUMMARY

A study was made of the incidence of *Neisseria meningitidis* and *N. lactamica* in a school population; 2470 children aged between 5 and 7 years were studied from four schools in Alcala de Henares (Madrid). Nasopharyngeal swabs were taken in June, November and March, between 1979 and 1983.

In all the surveys except one, the proportion of carriers of *N. lactamica* was higher than that of *N. meningitidis*, reaching a ratio of about 2:1 in the complete study.

The predominant serogroup of meningococcus found was B (41%), with non-groupable strains reaching 43%. A study of serotypes within group B showed a predominance of nontypable strains (48·5%), while those strains considered to be most virulent (types 2 and 1, 8, 15) reached 40%.

Eighteen per cent of *N. lactamica* strains were observed to agglutinate with antimeningococcal sera whilst the remainder of the strains were rough. When these strains were studied with the antiserum-agar technique, using antimeningococcal sera, a high percentage of strains cross-reacted with the meningococci. The susceptibility of strains to sulphadiazine, penicillin, ampicillin, chloramphenicol, rifampicin and spiramycin was determined.

Finally an analysis was made of the effect that an elevated colonization rate of *N. lactamica* might have on colonization by meningococci. The necessity of using fine epidemiological markers in tracing virulent strains in a population at risk is stressed. Selective prophylactic measures are also necessary.

INTRODUCTION

The prevalence of meningococci in asymptomatic carriers has been the subject of numerous studies both in civilian populations, particularly children, and in military personnel (for example Casal & Martin-Bourgon, 1975; Corey *et al.* 1979; Hassan-King *et al.* 1979; Maeyer, Seba & Regnister, 1981; Pateraki *et al.* 1971; Rodriguez-Contreras *et al.* 1981; and Wals *et al.* 1983). However, the relation

between the estimated number of carriers and the number of cases of meningococcal infection in a given population is controversial (Aycock & Mueller, 1950; Wenzel *et al.* 1973). The development of serotyping schemes for the meningococcal serogroups B and C and the association of some serotypes with a higher virulence (Frasch, 1979) has made it possible to improve studies of prevalence by specifically noting the most virulent strains, both in case-free populations (Craven *et al.* 1979) and in populations with high incidence of infection (Wals *et al.* 1983; Coetzee, Frasch & La Mocca, 1983). At the same time the use of serotyping has improved the characterization of outbreaks of meningococcal infection in closed communities (Saez-Nieto *et al.* 1984) and the tracing of strains amongst close contacts of the patient (Frasch & LaMocca, 1982; Saez-Nieto *et al.* 1982).

In this study we sought virulent strains of meningococci in a school population of a town located in an area of medium incidence of meningococcal infection, during a period of maximum incidence of this disease in Spain (Saez-Nieto *et al.* 1981*a*). The proportion of *Neisseria lactamica* in this population was also investigated; this species, closely related to meningococcus, has been isolated in greater proportion in some surveys carried out in school populations (Gold *et al.* 1978; Gelosa, 1981; Blakebrough *et al.* 1982). Finally, the susceptibility of isolates to the principal antimicrobial agents used in the treatment and the prophylaxis of meningococcal infection has been determined.

SUBJECTS AND METHODS

Population

The population studied was 2470 schoolchildren aged from 5 to 7 years, from four schools in Alcala de Henares (Madrid). Samples were taken three times a year in March, June and November, between June 1979 and June 1983. The children studied during these four years were from the same classes.

The number of samples varied in the different surveys from 160 to 300, with an average of 205 children per survey.

Pharyngeal swabs and strains

The nasopharyngeal samples were obtained with sterile swabs and inoculated on Thayer–Martin agar plates. The incubation, isolation and identification conditions have been described previously (Saez-Nieto *et al.* 1981*a*, 1982). The sugar utilization test was made in CTA medium (Difco) and investigation was carried out in the isolated colonies for the existence of beta-galactosidase activity to separate the strains of *N. lactamica*. A total of 465 strains of *N. lactamica* and 251 strains of *N. meningitidis* were isolated during the study.

Meningococcal serogrouping and serotyping

The serogrouping technique (slide agglutination) and serotyping (double immunodiffusion in gel) of *N. meningitidis* isolated have been described previously (Saez-Nieto *et al.* 1981*a*, *b*).

The antiserum agar technique (ASA method) was used in the case of *N. lactamica* to detect the possible cross-reactivity of non-groupable strains with meningococcal sera in slide agglutination tests. The sera for this technique from groups A, B, C,

Y and W 135 were kindly supplied by Dr C. E. Frasch (Bureau of Biologics, FDA, Bethesda, Maryland). This technique was carried out in accordance with the conditions previously established (Craven *et al.* 1978; Saez-Nieto, Vazquez & Casal, 1980).

The electrophoretic patterns of the nontypable strains of *N. meningitidis* were studied by gel electrophoresis (PAGE pattern) (Saez-Nieto *et al.* 1981*b*).

Antimicrobial susceptibility

The antimicrobial susceptibility of *N. lactamica* and *N. meningitidis* strains was studied by determining the minimal inhibitory concentration (MIC), using an automatic multi-inoculator (Microtiter AM 80), with a microbial concentration of 3×10^5 c.f.u./ml. Mueller Hinton agar plates (Difco) containing 5% sheep blood were used for this study. The antimicrobial agents studied were: sulphadiazine, penicillin, ampicillin, chloramphenicol, rifampicin and spiramycin (Saez-Nieto, Vazquez & Casal, 1983).

RESULTS

Distribution of N. meningitidis *and* N. lactamica *in the population*

N. lactamica was found in 18·8% of the 2470 samples studied in the 12 surveys carried out during the period June 1979 to June 1983 (Table 1). In the majority of surveys *N. lactamica* was predominant; in some, the proportion of *N. lactamica* to *N. meningitidis* was greater than 2:1. Only in the survey of March 1982 are the figures for both micro-organisms equal. There was no seasonal effect of any significance in the percentage of meningococcal carriers, though this might have been expected to coincide with the maximum incidence of illness (Fig. 1).

Serogroup of N. meningitidis *and agglutination of strains of* N. lactamica *with antimeningococcal sera*

The distribution of the serogroups of meningococci is shown in Table 2. Serogroup B predominates (41%) followed by serogroups 29E (6%) and Y (5·6%). The remaining serogroups constituted 6·7%. The nongroupable strains (autoagglutinable, polyagglutinable or nonagglutinable) formed 42·7% of the total. A high proportion of rough strains of *N. lactamica* was observed (81·9%), while 10·5% agglutinated with group B serum and 7·6% with some other serotypes (Table 2).

When the antiserum agar method was used to study 130 autoagglutinable strains of *N. lactamica*, it was observed that 90% produced a halo with meningococcal sera. Serum C was notable as reacting with 51% of the strains, 23% of the strains producing a halo with more than one serum (data not given in tables).

Serotypes encountered in the school population

Table 3 shows the serotypes found within serogroup B meningococci. Predominant is the high percentage of nontypable strains (48·5%) of which 85% showed the same electrophoretic (IV) pattern. In second place are strains of types 1, 8, 15 in various combinations (24·5%) and serotype 2 with 14·6%. Other serotypes account for 11·7% of strains. Analysis of the percentages of the most virulent strains in the population studied (Tables 2 and 3), shows that the strains of serogroup B (a

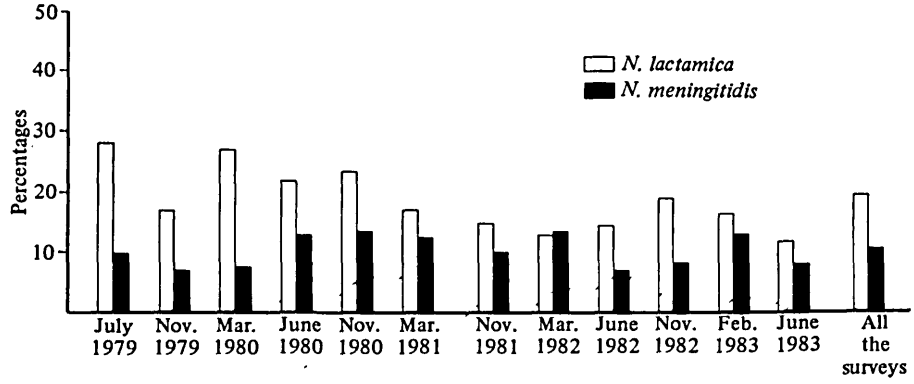

Fig. 1. Distribution of *N. meningitidis* and *N. lactamica* in a school population between 1979 and 1983.

Table 1. *Distribution of* N. meningitidis *and* N. lactamica *isolates from a school population*

Surveys	*N. lactamica* (%)		*N. meningitidis* (%)		Ratio*	Samples
July 1979	56	(28·3)	19	(9·6)	2·9/1	198
November 1979	42	(16·8)	17	(6·8)	2·5/1	250
March 1980	44	(27·5)	11	(6·9)	4·0/1	160
June 1980	70	(22·0)	40	(12·6)	1·7/1	318
November 1980	47	(23·5)	27	(13·5)	1·7/1	200
March 1981	32	(16·9)	23	(12·2)	1·4/1	189
November 1981	27	(15·3)	18	(10·2)	1·5/1	176
March 1982	26	(13·0)	27	(13·5)	0·9/1	200
June 1982	25	(14·5)	10	(5·8)	2·5/1	173
November 1982	41	(18·8)	18	(8·2)	2·3/1	218
February 1983	32	(16·5)	25	(12·9)	1·3/1	194
June 1983	23	(11·8)	16	(8·2)	1·4/1	194
Total	465	(18·8)	251	(10·2)	1·85/1	2470

* Ratio *N. lactamica*/*N. meningitidis*.

Table 2. N. meningitidis *serogroups isolated from children*

Serogroup	Strains (%)		Samples(%)	*N. lactamica*	
A	5	(2·0)	0·2	—	
B	103	(41·0)	4·2	49	(10·5)
C	8	(3·1)	0·3	11	(2·4)
X	1	(0·4)	0·05	—	
Y	14	(5·6)	0·6	6	(1·3)
Z	1	(0·4)	0·05	—	
29E	15	(6·0)	0·6	12	(2·6)
W135	2	(0·8)	0·1	6	(1·3)
AA*	82	(32·7)	3·3	318	(68·4)
NA†	20	(8·0)	0·8	36	(7·7)
PA‡	—		—	27	(5·8)
Total	251		10·2	465	

* Autoagglutinable. † Nonagglutinable. ‡ Polyagglutinable.

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Table 3. *Prevalence of serotypes into serogroup B isolates in relation to the samples*

Surveys	Samples	*N. meningitidis* (% of samples)	Group B	Serotypes			
				2	1, 8, 15	Others	NT
July 1979	198	19(9·6)	14(7·1)	6(3·0)	—	1(0·5)	7(3·5)
November 1979	250	17(6·8)	12(4·8)	5(2·0)	—	1(0·4)	6(2·4)
March 1980	160	11(6·9)	5(3·1)	—	2(1·25)	1(0·6)	2(1·2)
July 1980	318	40(12·6)	15(4·7)	1(0·3)	5(1·6)	2(0·6)	7(2·2)
November 1980	200	27(13·5)	22(11·0)	—	7(3·5)	5(2·5)	10(5·0)
March 1981	189	23(12·2)	9(4·8)	—	4(2·1)	2(1·05)	3(1·6)
November 1981	176	18(10·2)	9(5·1)	2(1·1)	3(1·7)	1(0·55)	3(1·7)
March 1982	200	27(13·5)	3(1·5)	—	1(0·5)	—	2(1·0)
June 1982	173	10(5·8)	1(0·6)	—	—	—	1(0·6)
November 1982	218	18(8·2)	3(1·4)	—	3(1·4)	3(1·4)	—
February 1983	194	25(12·9)	7(3·6)	1(0·5)	—	—	6(3·1)
June 1983	194	16(8·2)	3(1·5)	—	—	—	3(1·5)
Totals	2470	251	103(4·2)	15(0·6)	25(1·0)	13(0·5)	50(2·0)

Nt, Nontypable.

Table 4. *Serotypes isolated into* N. meningitidis *group B*

Serotypes	Number of strains	Percentage
2	15	14·6
1	10	24·3
8*	6	
15*	9	
4	—	
5	2	1·9
6	—	
9	5	4·8
11	1	1·0
12	4	3·9
14	1	1·0
NT†	50	48·5†
Total	103	

* Including 1, 8; 1, 8, 15 and 8, 15 strains. NT, Nontypable. †PAGE pattern: 85% type IV and 15% others.

Table 5. *Minimal inhibitory concentration (MIC) of sulphadiazine of* N. meningitidis *and* N. lactamica *isolates*

		MICs (μg/ml)*							
Serogroups	Strains	⩽1	5	10	25	50	⩾100	MIC50	MIC90
A	5	—	1	—	2	2	—	21·25	40·6
B	103	9	34	18	23	13	6	7·4	41·7
C	8	—	—	—	1	7	—	35·7	47·1
X	1	—	—	1	—	—	—	10·0	10·0
Y	14	2	9	1	2	—	—	3·2	14·5
Z	1	1	—	—	—	1	—	50·0	50·0
29E	15	1	9	3	1	—	1	3·9	22·5
W135	2	1	1	—	—	—	—	1·0	4·2
AA†	82	5	28	13	21	9	6	8·1	43·9
NA‡	20	2	13	1	4	—	—	3·5	40·0
Total	251	20	95	37	54	32	13	7·9	40·5
%		(8·0)	(52·6)		(39·4)				
N. lactamica	100	8	13	19	32	9	19	14·7	64·1
%		(8·0)	(32·0)		(60·0)				

* MIC ⩽ 1 μg/ml, sensitive; MIC (5–10 μg/ml), moderately resistant; MIC ⩾ 25 μg/ml, resistant.
† Autoagglutinable.
‡ Nonagglutinable.

majority of the epidemic wave) only constituted 4·2% of the total samples, and within these the virulent serotypes (2 and 1, 8, 15) were recovered from only 1·6% of the children studied.

Antimicrobial susceptibility of N. meningitidis *and* N. lactamica

Tables 5 and 6 show the MICs of the different antimicrobial agents studied. Only 8% of both *N. meningitidis* and *N. lactamica* were sensitive to sulphadiazine

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Table 6. *MICs of antibiotics of* N. meningitidis *and* N. lactamica *isolates*

Serogroups (number of strains)	Antibiotics	Range (μg/ml)	MIC50	MIC90
B (103)	Penicillin	0·006–0·4	0·020	0·08
	Ampicillin	0·012–0·4	0·034	0·15
	Rifampicin	0·006–0·8	0·020	0·24
	Chloramphenicol	0·2–1·6	0·57	0·78
	Spiramycin	0·2–6·4	0·64	2·08
Others* (46)	Penicillin	0·006–0·2	0·019	0·045
	Ampicillin	0·025–0·2	0·027	0·068
	Rifampicin	0·006–0·8	0·020	0·26
	Chloramphenicol	0·4–0·8	0·57	0·76
	Spiramycin	0·4–6·4	0·71	1·57
NG (102)†	Penicillin	0·006–0·4	0·026	0·14
	Ampicillin	0·006–0·4	0·075	0·24
	Rifampicin	0·012–0·8	0·080	0·33
	Chloramphenicol	0·1–0·8	0·38	0·71
	Spiramycin	0·1–6·4	1·02	2·92
N. lactamica (100)	Penicillin	0·025–0·8	0·12	0·19
	Ampicillin	0·025–0·8	0·15	0·35
	Rifampicin	0·1–1·6	0·36	0·74
	Chloramphenicol	0·2–3·2	0·30	0·40
	Spiramycin	0·2–6·4	2·27	4·62

* See Table 2.

† Including nonagglutinable and autoagglutinable strains.

(MIC < 1 μg/ml). The moderately resistant strains (MICs between 5 and 10 μg/ml) comprised 52·6 % and 32 % respectively, and the resistant strains 39·4 % and 60 %.

No important difference was encountered in the MICs of antibiotics within the meningococci (Table 6), with the exception that the majority of nongroupable strains had higher MICs. This was also seen in the *N. lactamica* strains, where the MICs to rifampicin and to spiramycin particularly are greater than in the meningococci.

DISCUSSION

Although numerous studies have been reported in which the carrier state was analysed in all types of populations and in different degrees of epidemicity of meningococcal infection, discrepancies still exist regarding the part played by asymptomatic carriers of meningococcus in the spread of the disease. In 1950 Aycock & Mueller established that the incidence of illness did not appear to be a function of the percentage of carriers. This observation was corroborated by Wenzel *et al.* (1973), who cast doubts on the usefulness of determining the percentage of carriers when only the serogroup of the isolated strains was studied. These authors observed that what influenced the occurrence of outbreaks or epidemics was not so much the proportion of carriers as the prevalence of specific strains.

This observation was confirmed by the association of certain serotypes with a greater frequency of case occurrence (Frasch, 1979; Saez-Nieto *et al.* 1981*a*; Holten, 1979). Accordingly, tracing the most virulent antigens (B2, C2 or B15) in population at risk could result in the prediction of cases or outbreaks.

Craven *et al.* (1979) reported that, during studies on case-free military personnel, no virulent serotype could be found amongst the meningococci isolated from the population.

Despite the development of techniques for tracing virulent strains in populations, there have been few studies in which these techniques have been used. However, serotyping has been used in studies of close contacts of patients (Frasch & LaMocca, 1982; Saez-Nieto *et al.* 1982) and in institutional populations where there has been an outbreak of meningococcal infection (Saez-Nieto *et al.* 1984).

In our study, we sought virulent strains in a school population during a period of maximum illness incidence in Spain (1979–83) (Saez-Nieto *et al.* 1981*a*).

It is clear from the results that there was a predominance of carriers of group B meningococci; this was expected, because this serogroup was predominant in Spain during the study period. In common with the findings of others (Gold *et al.* 1978; Corey *et al.* 1979), a high number of non-capsulated isolates was also found.

The greater proportion of carriers of *N. lactamica* in this age group confirms the report of Gold and co-workers (1978).

The most frequent antigenic combinations of group B meningococci in carriers were similar to those found in patients (Saez-Nieto *et al.* 1981*b*); although the percentages in relation to the total samples studied (Table 3) were very small, this contrasts with the high levels of endemic types recorded in this period.

These results lead us to conclude that factors other than the existence of virulent serotypes in a population are responsible for the appearance of cases of meningococcal infection. In this context we might include the possible protective role of *N. lactamica* in preventing colonization by meningococci. Gold *et al.* (1978) observed that colonization of the nasopharynx by *N. lactamica* was associated with the formation of bactericidal antibodies against meningococci; in this study we have found that a high percentage of the strains studied exhibited cross-reactivity with antimeningococcal sera.

Although no resistance was encountered to the principal antibiotics used in treatment and prophylaxis of meningococcal infection, there was a similarity in the MIC of strains to sulphadiazine to those encountered in patients (Saez-Nieto *et al.* 1983). Although strains from carriers were slightly more sensitivie, this probably reflects the fact that uncommon serogroups are usually sensitive to sulphonamide. In the case of rifampicin, few strains showed MICs $> 3{\cdot}2\ \mu g/ml$.

These facts lead us to emphasize, once more, the importance of not introducing any generalized chemoprophylaxis in a population unless such prophylaxis is directed towards the selective elimination of those strains considered virulent. Selective chemoprophylaxis was successfully used in the eradication of an outbreak of meningococcal infection in a nursery (Saez-Nieto *et al.* 1984).

Finally we propose the following. (1) The greater use of serotyping in studies of the carrier state. This would lead to a better characterization of the strains and therefore facilitate more selective control measures in carriers of virulent strains; carriers of strains that can play a protective role would not be treated. (2) In the studies on carriers in infants it should be borne in mind that *N. lactamica* is predominant. However, Riou *et al.* (1983) have recently proposed a new *Neisseria* taxon for strains isolated from carriers and previously identified as noncapsular meningococci. This new species, provisionally named *N. polysaccharea*e, possesses

phenotypic characteristics similar to *N. meningitidis* except in its nutritional requirements in Catlin medium, the production of polysaccharide with sucrose (a test not habitually used to identify meningococci isolated from carriers) and in the absence of aminopeptidase activity. If this new species of *Neisseria* cannot be easily identified it may, like *N. lactamica*, throw doubt on the figures obtained in carrier studies. We have studied 30 non-capsulated strains of meningococci; 11 showed the characteristics of the new taxon, and it is therefore necessary to carry out further studies in order to establish the incidence of this neisseria in carriers.

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