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Prevalence of *Neisseria meningitidis* in family members of patients with meningococcal infection

BY J. A. SAEZ-NIETO

Servicio de Bacteriología, Centro Nacional de Microbiología, Virología e Inmunología Sanitarias, Majadahonda, Madrid, Spain

J. CAMPOS, C. LATORRE, T. JUNCOSA, M. SIERRA, T. GARCIA-TORNELL

Servicio de Microbiología y Unidad de Cuidados Intensivos, Hospital de San Juan de Dios, Barcelona, Spain

B. GARCIA-BARRENO, C. LOPEZ-GALINDEZ AND J. CASAL

Servicio de Bacteriología. Centro Nacional de Microbiología, Virología e Inmunología Sanitarias, Majadahonda, Madrid, Spain

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SUMMARY

The aim of the study was first, to determine the prevalence of *Neisseria meningitidis* among the family members living with patients suffering from meningococcal infections, and second, to ascertain the distribution among these family members of strains epidemiologically related to those isolated from patients.

Forty-two family groups were studied and 135 nasopharyngeal samples were taken from family members living with patients.

Twenty family groups were found to contain meningococcal carriers, and of these 20, 13 contained a carrier of the strain that caused the infection (65%). Among the family members who were carriers, the mother and father most frequently yielded the strain which caused the illness.

The serotypes most frequently encountered both in patients and carriers were 2 and 8, as well as nontypable strains.

The polyacrylamide gel electrophoretic patterns (PAGE) most frequently found were II and IV. A notable feature of the study is the high resistance of the strains to sulphadiazine, since more than 90% of the strains found in patients and more than 75% of those from carriers possessed a minimum inhibitory concentration greater than or equal to 10 μg/ml.

INTRODUCTION

Until the 1960s epidemiological studies of *Neisseria meningitidis* infection were based mainly on the study of serogroups. These studies were fairly limited owing to the low discriminatory capacity of this epidemiological marker since the same serogroup is often predominant in a given region or given period of time.

The description of the serotype antigens, the development of serotyping schemes for serogroup B and C (Frasch & Chapman, 1972*a*; Frasch, McNelis & Gotschlich, 1976; Gold & Wyle, 1970), and the demonstration that these serotype antigens were distributed among the different serogroups and were independent of them (Frasch, 1979), opened up new ways of studying the transmission of infection by these micro-organisms.

When serotyping was first used in epidemiological studies of meningococcal infection, the principal concern of investigators centred on the distribution of serotypes in sporadic cases and outbreaks, as much as on the distribution in asymptomatic carriers. These studies showed the higher incidence of certain serotypes associated with the illness (Frasch & Chapman, 1973; Holten, 1979; Sáez-Nieto *et al.* 1981; Jones & Tobin, 1976; Sáez-Nieto, Fenoll and Casal, 1979), which suggested that these serotypes exhibited a greater virulence.

Recently serotyping techniques are beginning to be used to 'track' meningitis-producing strains in populations with low or high rates of incidence of meningitis (Craven *et al.* 1979; Sáez-Nieto *et al.* manuscript in preparation) or in families of patients with meningococcal meningitis (Marks, Frasch & Shapera, 1979).

In this work, we have studied the serogroups, the serotypes and the SDS-PAGE patterns in meningococcal strains isolated from patients and in the family members living with them, admitted to San Juan de Dios Hospital during the period comprising January 1980 to July 1981.

MATERIALS AND METHODS

Isolation of the strains from patients and their family contacts

A lumbar puncture on admission for biochemical, cytological and microbiological studies, a series of blood cultures (whenever possible) and a pharyngeal swab, were carried out on every child admitted to hospital with suspected meningitis. At the same time nasopharyngeal samples were taken from all the family members.

Spinal fluid cultures

Following the lumbar punctures, one part of the spinal fluid was immediately inoculated at the bedside of the patient into tubes containing heated-blood Mueller–Hinton (Difco) agar and the remainder was collected in a test-tube for immediate examination and cultivation of the sediment which was streaked on heated-blood agar plates and in thioglycollate agar slant tubes (Difco). Both heated blood agar plate and tube were incubated at 35 °C in an atmosphere of 5% CO_2. When growth was observed the usual tests of Gram stain, oxidase and sugars utilization were carried out, according to the method described by Morello & Bonhoff in 1980.

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Blood cultures

In all the patients cultures were carried out in the same way, the number of samples taken varying between 1 and 3 (depending on the clinical state of the patient). The bottles of culture were incubated at 35 °C, and daily readings were taken, making 3 blind cultures, one after 24 h, another after 48 h, and a final one after 7 days. These were always done on plates of heated-blood agar, which were incubated at 35 °C in an atmosphere of 5 % CO_2 for 24–48 h. When growth took place the same procedure was carried out as with the strains isolated from spinal fluid.

Pharyngeal swabs

The nasopharyngeal samples were obtained using sterile swabs, in modified Stuart medium, and the samples immediately streaked on Thayer–Martin agar plates. The plates were incubated at 35 °C for 24 h in an atmosphere of 5 % CO_2.

Colonies grown under these conditions were tested as indicated; in addition a study of beta-galactosidase was made.

Strains

Forty-two meningococcal strains obtained either from the spinal fluid or the blood of patients, 20 strains isolated from nasopharynx of these same patients and 29 strains obtained from the nasopharynx of family members living with the patient were studied.

These strains were confirmed as being meningococci by means of Gram stain, oxidase and sugar utilization in Mueller–Hinton agar with bromothymol blue as a pH indicator and a final concentration of 1 % of the following sugars; lactose, dextrose, maltose and sucrose. Afterwards their serogroup, serotype, PAGE-pattern and resistance to sulphadiazine were also investigated.

Serogrouping and serotyping

The serogrouping and serotyping, as well as the production of serogroup and serotype specific antisera, have been described previously (Sáez-Nieto *et al.* 1981). The extraction of serotype antigens was carried out following the Frasch & Friedman method, (1977).

PAGE-patterns

The PAGE-patterns were studied by means of the Weber & Osborn technique (1969) modified by Frasch & LaMocca (1978), using slabs, with slight modifications of our own. An acrylamide concentrating gel consisting of 3 % acrylamide and 0·25 % bisacrylamide was added.

After 2 h fixation in 5 % tricloroacetic acid, the slabs were stained with 0·25 % Coomassie Brilliant Blue in a 46:8 methanol:acetic acid solution. Decolorization was then carried out by using a 20:8 methanol:acetic acid solution.

Resistance to sulphadiazine

The minimum inhibitory concentrations were determined by the dilution method in agar according to the conditions described previously (Sáez-Nieto *et al.* 1981).

Table 1. *Meningococcal carrier-rates in families with a case of meningitis*

Family groups*	Totals	(%)
No. studied	42	(100)
With meningococcal carrier (s)	20	(47·6)
With carriers of the same serogroup as patient strain	17	(40·5)
With carriers showing strains epidemiologically related to that of the patient†	13	(30·9)
In which the father is the only carrier of the patient strain	6	(14·3)
In which the mother is the only carrier of the patients strain	4	(9·5)
In which both the father and the mother carry the patients strain	1	(2·4)
In which another member of the family is the only carrier of the patient strain	2	(4·8)

* Each family group consists of one patient and his family members.

† By 'epidemiologically related' we mean those strains which possess the same serogroup, together with the same serotype and/or PAGE-pattern.

RESULTS

Epidemiological relationship between the meningococci of patients and those of the family members

Forty-two family groups were studied (42 patients and 135 family members) (Tables 1 and 2). Of these family members 29 (21·5%) proved to be meningococcal carriers, and of these, 14 carried a strain similar to that of the patient in the same family group.

Twenty (48%) of the family groups studied contained a family member with meningococcus in the nasopharynx, 17 (40·5%) contained strains of the same serogroup as the patient, and of these, 13 (31%) also showed the same serotype and/or the same PAGE-pattern as the strain which caused the infection. The strains in this last group were considered to be epidemiologically related to that of the patient.

Tables 1 and 2 show the frequency with which strains similar to that causing the infection occur in the family members. It can be seen that the father and mother are the most common carriers, followed by siblings. In other family members, children as well as adults, the patient's strain was not found, although there were strains of the same serogroup. We also found, in a small proportion of cases, serogroups different from that of the causative strain (Table 3). This table shows the 20 family groups where meningococcal strains in the family members of the patient were found, as well as the serogroup, serotype, PAGE-pattern and their resistance to sulphadiazine. The minimum inhibitory concentration of sulphadiazine

Table 2. *Distribution of strains from carriers, according to their relationship with the patient, and their equality of serogroup, serotype with the patient strain*

	Number of		Strains with the same	
Relationship	Samples	Carriers	Serogroup	Serotype
Fathers	38	9	9	7
Mothers	38	8	6	5
Siblings	35	5	4	2
Uncles/Aunts	10	4	1	—
Cousins	8	1	1	—
Grandparents	3	—	—	—
Others	3	—	—	—
Totals	135	27	21	14
%	100	20·0	15·6	10·4

also coincided in the epidemiologically related strains (Table 3). From this table we conclude that the strains isolated from the patients occur more frequently in their respective family members than would happen simply by chance.

Serogroups

The meningococci isolated from the patients (Table 4) fall within serogroup B (92·8 %) and C (4·8 %). One autoagglutinable strain was found, which was considered similar to one of the strains of serogroup B isolated in one of the family contacts of this patient, since the remaining 3 displayed identical epidemiological markers (Family 9).

In addition, meningococci were isolated from the nasopharynx in 20 patients and all coincided in serogroup, serotype, PAGE-type and sulphadiazine resistance with the strain isolated from blood or spinal fluid.

Table 4 shows the serogroups of meningococci found in family members. These have the same distribution as the patient strains with the exception of 4 strains from serogroup Y (all of them found in the same family group) and of one strain from serogroup A. Also two strains of *Neisseria lactamica* were isolated from two children (Families 18 and 20).

Serotypes and PAGE-patterns

The meningococci found in patients could be grouped in serotype 2 (45 %) and the complex 1, 8, 15 (7 %); the remainder of the strains were nontypable. The proportion is similar among the carriers although one other serotype or PAGE-type not found in the patient also appeared (Table 4).

The PAGE-patterns of patient strains are distributed fundamentally between pattern IV (40·5 %) and II (35·7 %). This distribution is very similar to that of the carriers.

Table 3. *Distribution in family groups of the serogroups, serotypes, PAGE-patterns and sulphadiazine resistance of the strains isolated from patients and their family members*

Family	Relationship	Source	Group	Type	PAGE	MIC of sulphadiazine*
1	Patient	SF	B	NT‡	IV	25
	Patient	NP	B	NT	IV	25
	Mother	NP	B	2	II	5
	Father	NP	B	NT	IV	25
2	Patient	SF	B	NT	IV	5
	Mother	NP	B	NT	IV	5
3	Patient	Blood	B	2	II	25
	Patient	NP	B	2	II	25
	Aunt	NP	B	B	IV	25
4	Patient	SF	B	2	II	10
	Patient	NP	B	2	II	10
	Father	NP	B	2	II	10
	Mother	NP	B	2	II	10
	Sister	NP	B	NT	X	25
5	Patient	SF	B	2	I	10
	Patient	NP	B	2	I	10
	Mother	NP	B	2	I	10
6	Patient	SF	B	NT	IV	1
	Patient	NP	B	NT	IV	1
	Father	NP	B	NT	IV	1
7	Patient	SF	B	NT	IV	25
	Father	NP	B	1, 8, 15	IV	25
8	Patient	SF	B	NT	IV	25
	Father	NP	B	8	IV	25
9	Patient	SF	AA†	2	II	100
	Mother	NP	B	2	II	100
10	Patient	Blood	B	NT	IV	10
	Brother	NP	Y	2	II	100
	Uncle	NP	Y	2	II	100
	Aunt	NP	Y	2	II	100
	Aunt	NP	Y	2	II	100
11	Patient	Blood	B	2	I	25
	Mother	NP	B	NT	III	50
12	Patient	SF	B	1	IV	25
	Patient	NP	B	1	IV	25
	Father	NP	B	NT	IV	25
13	Patient	SF	B	NT	IV	100
	Patient	NP	B	NT	IV	100
	Father	NP	B	NT	IV	100
14	Patient	SF	B	NT	II	25
	Father	NP	B	1, 8	IV	10
15	Patient	SF	B	NT	II	25
	Sister	NP	B	NT	II	50
	Cousin	NP	B	1, 8	IV	5
16	Patient	SF	B	2	II	50
	Brother	NP	B	6	III	25

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Table 3. (*cont.*)

Family	Relationship	Source	Group	Type	PAGE	MIC sulphadiazine*
17	Patient	SF	B	2	II	50
	Mother	NP	A	—	—	100
18	Patient	SF	B	2	II	50
	Brother	NP	*Neisseria lactamica*			
19	Patient	SF	B	2	II	25
	Sister	NP	B	2	II	25
20	Patient	SF	B	2	II	25
	Patient	NP	B	2	II	25
	Father	NP	B	NT	IV	1
	Mother	NP	B	2	II	25
	Brother	NP	*Neisseria lactamica*			

* Minimum inhibitory concentration in μg/ml.
† Autoagglutinable strain.
‡ Nontypable strains.
SF, Spinal fluid; NP, Nasopharynx.

Resistance to sulphadiazine

This was very high, 92·8% of the patient strains had MICs greater or equal to 10 μg/ml of sulphadiazine while in the strains of family members this proportion was 75% (Table 5).

DISCUSSION

The first studies of serotypes that were carried out were directed at determining the prevalent serotypes in patients and asymptomatic carriers. Through these studies we know that the majority of the meningococci of serogroup B and C isolated from cases of meningitis belong to a small number of serotypes. Thus Frasch & Chapman in 1973 established that serotype 2 was responsible for 50% of cases caused by serogroup B in the U.S.A. during the years 1963–1971. In 1976 Jones & Tobin found 60% of strains in sporadic cases of meningitis and septicaemia in England were serotype 2. Holten (1979) found a very high proportion of strains of serotype 15 in the outbreak recorded in Norway in 1978; and Sáez-Nieto *et al.* (1981) established that serotype 2 and the complex 1, 8, 15 were those most commonly found in cases occurring in Spain in 1980.

A second phase of epidemiological studies was directed at the transmission of these predominant serotypes from healthy carriers to potential patients. Studies were carried out in population groups with a high or low incidence of meningitis cases (Craven *et al.* 1979). In addition, to evaluate the transmission of virulent strains, serotyping studies were carried out in groups of family members. Frasch & LaMocca (1981) in a study of 25 families, discovered that 70% of these families contained a carrier with the same strain as that which had caused the infection in the patient and that the remaining 30% exhibited strains that were different from

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Table 4. *Distribution of serogroups, serotypes and PAGE-types of* 89 *strains of* N. meningitidis *isolated from patients and their family members*

		Serogroups					Serotypes					PAGE-types			
Source	Total	A	B	C	Y	AA*	1	2	6	8	NT†	I	II	IV	Others
SF or blood from patients	42	—	39	2	—	1	2	19	—	1	20	6	15	17	4
NP from patients	20	—	18	2	—	—	2	8	—	—	10	3	6	7	4
NP from family members	27	1	22	—	4	—	—	11	1	5‡	10	1	11	12	3
Totals	89	1	79	4	4	1	4	38	1	6	40	10	32	36	11

* Autoagglutinable strains.
† Nontypable strains.
‡ Includes 3 strains 8(1). 1 strain 8 and 1 strain 1, 8, 15.
SF, Spinal fluid; NP, Nasopharynx.

Table 5. *Resistance to sulphadiazine in strains isolated from patients and family members*

Source	Minimal Inhibitory Concentration (μg/ml)					
	1	5	10	25	50	100
SF or Blood from patients	2	1	5	23	7	4
NP from patients	2	—	2	11	3	2
NP from family	2	3	4	9	4	7
Totals	6	4	11	43	14	13

SF, Spinal fluid; NP, Nasopharynx.

that of the patient. In other studies the same authors established that the mother was the most frequent carrier of the patient's strain, finding 8/24 cases where the mother was the only carrier, as opposed to 1/24 in which the father was the only carrier.

In our own study we have found proportions similar to those of Frasch with regard to family carriers of the strain epidemiologically related to that of the patient, since 13 of 20 family groups with meningococcal carriers exhibited the same strain as the patient, and 7 strains differing from the causal strain. The father is the family member in which the same strain as the patient appears most frequently (7/20), followed by the mother (5/20). These figures concur with those of Farriers *et al.* (1975) which showed that it was adult family members who most frequently yielded strains with characteristics similar to that of the patient.

We studied 42 families of patients suffering from meningitis, 20 (48 %) of which contained a carrier among the family members. This number drops to 17 (40·5 %) if we include only the same serogroups as those of the patients, and the number becomes even smaller, 13 (31 %), if we focus on the serotypes and the PAGE-types. From these figures we can conclude that it is essential to use accurate epidemiological markers if we wish to improve our knowledge of the transmission of the disease and the variation of the pathogenicity of the different strains of *Neisseria meningitidis*. The collection of these epidemiological markers has been enriched in recent years with schemes to determine the lipopolysaccharide of the outer membrane of the wall (Zollinger & Mandrell, 1977) and of the protein antigens of serogroup A (Zollinger & Mandrell, 1980). These schemes are bound to play a part in future epidemiological studies of these micro-organisms.

Finally, as regards the serotypes encountered in patient strains, the clear predominance of serotype 2 (45·2 %) is notable. This fact is consistent with the situation to be found in the Catalan region where the hospital studied is situated. We must also highlight the high percentage of strains resistant to sulphadiazine (MIC 25 μg/ml or more) which constitute 81 % of the strains isolated in the patient, a figure very similar to that found in Spain during the same period of the study (Sáez-Nieto *et al.* 1981).

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