

Carriage of *Neisseria meningitidis*: investigations in a military establishment

By J. V. S. PETHER, N. F. LIGHTFOOT, R. J. D. SCOTT, J. MORGAN

*Public Health Laboratory Service, Taunton and Somerset Hospitals,
Musgrove Park Branch, Taunton, Somerset, TA1 5DB*

A. P. STEELE-PERKINS AND S. C. SHEARD

Royal Naval Air Station, Yeovilton, Somerset, BA22 8HT

(Accepted 1 March 1988)

SUMMARY

A prevalence study of personnel on a Royal Naval Air Station revealed that 23.0% of 2479 personnel were carrying a meningococcus. Selected groups of personnel were subsequently swabbed monthly for a year. We have shown that it is only by repeated swabbing and the use of optimal methods including enrichment media that one can have a hope of identifying the 'true' carriage rate. A presumed virulent strain of *Neisseria meningitidis* B15 P1.16 was repeatedly isolated from three personnel who remained well, as did their colleagues both at their work place and socially. The study served to emphasize our lack of knowledge of the virulence factors associated with *N. meningitidis*.

INTRODUCTION

Outbreaks of meningococcal meningitis have a dramatic impact. Disease onset is frequently abrupt and there may be a rapid progression to death within a very few hours. There may also be little or no time to seek medical attention and give appropriate treatment (Abbott *et al.* 1985). The pathogenic mechanisms are poorly understood and strains of *Neisseria meningitidis* that cause disease are indistinguishable from strains that are carried by many people in the general population who remain well.

When a second case of meningococcal meningitis was admitted to hospital from a semi-closed population on a Royal Naval Air Station (RNAS), a point-prevalence study of meningococcal carriage in 2479 personnel was carried out. Following this study a longitudinal survey in small defined sections of the RNAS was carried out for 12 months.

First investigation: history

On 3 December 1985, a 20-year-old female from the Royal Naval Air Station (RNAS), Yeovilton, was admitted to Yeovil District Hospital with meningitis. *N. meningitidis* was isolated from her cerebrospinal fluid but unfortunately the strain was lost on subculture. It had been documented that this strain was sensitive to

sulphonamide, although this had been performed using a 25 μ g disk; the test may therefore have been unreliable. Subsequently, she was shown to have a high level of antibody to group B meningococcus.

A second case occurred on 1 February 1986, when another 20-year-old female was admitted to hospital with meningitis. The meningococcus isolated from her cerebrospinal fluid was a group B type 15 (weak) and was resistant to sulphonamides. On the same day as the second case it was decided, with the full co-operation of the Commanding Officer and medical staff at RNAS, to sample the throats of as many of the military and civilian staff as was possible. During the next 4 days throat swabs were obtained from 2479 personnel. These swabs were directly plated on to New York medium (NYM) (Faur *et al.* 1973) and transferred within 4 h to the Taunton Public Health Laboratory (PHL).

A further female, aged 21, was taken ill with meningitis on her way home on 15 February 1986, and a non-typable group B sulphonamide-resistant strain of *N. meningitidis* was isolated from her cerebrospinal fluid. All three cases recovered and all intimate contacts were offered prophylaxis with rifampicin 600 mg twice a day for 2 days. Happily, no further cases have occurred during the subsequent 26 months.

Background

RNAS Yeovilton is a major Naval Air Station located in the Somerset countryside some seven miles from Yeovil. Approximately 3000 personnel are employed, of whom 2000 are uniformed and 300 are in the WRNS. About 30% of personnel live on site. Living accommodation has changed dramatically from the traditional concept of barracks, and even junior ranks either have individual bedrooms or share with up to three others. Meals are taken in a canteen-style galley.

Some of the Yeovilton personnel are deployed to Norway for winter exercises, and it was initially thought that with the higher carriage-rate of group B type 15 meningococci prevalent in Norway this could have been the origin of the strain in the Yeovilton RNAS population.

Mass swabbing

During the first day when 200–300 swabs were taken, the following routine was established. A tongue depressor was rarely needed. A plain swab was inserted just at the back of one tonsil, was swept up just behind the uvula to the rear of the other tonsil and the movement was then reversed back to the first tonsil. An attempt was made to twist the swab. If the throat was not made uncomfortable it was deemed the swab might have been unsatisfactory. The swab was then applied to one-quarter of a dried NYM plate, which was marked to identify the swabber and the subject. Plates were returned to the Taunton PHL as quickly as possible and incubated in a CO₂ incubator overnight. During the mass swabbing exercise on the second and subsequent days two members of the secretarial staff were employed with five personnel as 'swabbers'. A doctor, either PHLs or RNAS, was always in attendance.

Table 1. Carriage of *N. meningitidis* in 2479 personnel at RNAS, Yeovilton

	Number	Percentage
Group A meningococci	2	0.08
Group B type 15	63	2.5
All group B strains	144	5.8
Group C	51	2.1
Other meningococci including non-groupable strains	298	12.0
Strains of meningococci lost in transit	13	0.5
Total swabs with meningococci	571	23.0
Swabs without meningococci	1908	77.0
Total personnel swabbed	2479	100.0

Table 2. Carriage of *N. meningitidis* in 2486 identifiable naval or civilian personnel

Throat swab result	Civilians	Naval personnel
Meningococci demonstrated	110	483
Meningococci not demonstrated	555	1338
Total	665	1821
Percentage 'positive'	16.5	26.5

Neisseria meningitidis identification and data processing

Likely colonies were Gram-stained after 24 h or 48 h incubation. If Gram-negative diplococci were seen the colony was subcultured, and if this subculture was pure several colonies were inoculated into Gonocheck medium (Brown & Thomas, 1985). All 'positive' Gonocheck colonies were subcultured and sent to Dr D. M. Jones, Director of Manchester PHL, for grouping and subsequent typing and estimation of sulphonamide sensitivity.

A member of the computer staff of the Somerset Health Authority documented such personal details as was allowable with the permission of the military authorities. This information was fed into database of an ACT Sirius computer.

Mass swabbing

The results of the mass swabbing of 2479 personnel, naval and civilian, can be seen in Table 1. An overall isolation rate of 23.0% for meningococci was demonstrated. Table 2 shows that within this small and perhaps unrepresentative cohort naval personnel tended to have a higher carriage rate than civilians (26.5–16.5%). A lesser difference was observed when the sex of the subject was taken into consideration (Table 3). Carrier rates by age (Table 4) and service time (Table 5) seemed to show a fall-off in carriage of meningococci associated both with increasing age and therefore, inevitably, with a longer time in the service.

There seemed little difference between military personnel who had been training in Norway and those who had not, and the carriage rate of B 15 strains was similar between the two groups (Table 6). Interestingly, there was some variation in the isolation rate when individual 'swabbers' are taken into account (Table 7).

Table 3. *Carriage of N. meningitidis in 2406 personnel identified according to sex*

Throat swab	Male	Female
Meningococci demonstrated	479	69
Meningococci not demonstrated	1549	309
Total identifiable by sex	2028	378
Percentage 'positive'	23.6	18.3

Table 4. *Carriage of N. meningitidis in 2371 personnel according to age*

Throat swab	Age in years							
	< 20	20-24	25-29	30-34	35-39	40-44	45-49	50+
Meningococci demonstrated	62	178	110	49	59	21	19	39
Meningococci not demonstrated	91	403	364	236	277	152	115	196
Total identifiable by age	153	581	474	285	336	173	134	235
Percentage 'positive'	40.5	30.6	23.2	17.2	17.6	12.1	14.2	16.6

Table 5. *Carriage of N. meningitidis in 1697 military personnel according to service in years*

Throat swab	Service time in years						
	< 1	1-2	2-3	3-4	4-5	5-10	> 10
Meningococci demonstrated	9	46	24	25	19	145	161
Meningococci not demonstrated	7	57	46	61	59	389	648
Total identifiable by service years	16	103	70	86	78	534	810
Percentage 'positive'	56	44.7	34.3	29.1	24.4	27.2	19.9

Table 6. *Carriage of N. meningitidis in 2229 military personnel related to training in Norway*

	Trained in Norway at any time	
	Yes	No
Meningococci demonstrated	152	351
B 15 strains	13	44
Meningococci not demonstrated	439	1287
Total identifiable with respect to training in Norway	591	1638
Percentage 'positive'	25.7	21.4
Percentage B 15 strains	2.2	2.7

Table 7. *Carriage of N. meningitidis in 1901 personnel when related to the swabber*

	Swabber				
	B	C	D	E	F
Meningococci demonstrated	112	119	184	17	8
Meningococci not demonstrated	375	343	634	96	13
Total identified by swabber	487	462	818	113	21
Percentage 'positive'	23.0	25.8	22.5	15.0	38.1

Follow-up

There was little time to warn the reference PHL at Manchester and there were operational difficulties in producing the final results in less than 3 weeks. It was noted that only three persons were carrying a sulphonamide-resistant group B15 meningococcus. These persons had had no immediate contact with the cases of meningitis or with each other. There was considerable debate as to whether these personnel should be given rifampicin in an attempt to eradicate the strain, but since they had not been ill and there was no reported illness in any of their contracts or families it was decided to adopt a policy of 'wait and see'.

All close family and social contacts of the first two cases and the subsequent case had been offered prophylaxis with rifampicin. No further case had occurred by the beginning of March 1986 and there seemed to be an ideal opportunity for a longitudinal study to determine the rate of acquisition, time of carriage and rate of loss of the strains of meningococci identified in the mass swabbing. It was decided to sample a group of persons that included a known carrier of a B15 sulphonamide-resistant meningococcus.

Longitudinal survey

A monthly swabbing exercise to study the acquisition, carriage and loss of Neisseria meningitidis in a semi-closed population of military personnel.

Introduction

From the practical side of the survey several points arose. Four subgroups were originally chosen: (a) females living on the camp (as the three cases had all arisen from this group); (b) a day work group; (c) a shift work group; (d) personnel joining RNAS. Even with close monitoring it rapidly became evident that movement of personnel between groups and those stationed elsewhere resulted in many of the original subjects being lost to the survey. Therefore, following a meeting at RNAS three groups of personnel were identified (R, J and W) that could reasonably be expected to be willing and available to have throat swabs taken once a month for a period of one year. Fortunately, one of the known carriers of a group B15 sulphonamide-resistant meningococcus was in one of the three groups of people. During this year simple experiments were made that were designed to produce an optimum isolation rate for meningococci.

MATERIALS AND METHODS

Initially throats were swabbed using plain cotton-wool swabs. These were plated directly on to NYM supplemented with vancomycin 3.0 mg/l, colistin 7.5 mg/l and trimethoprim 5.0 mg/l. The plates were incubated as quickly as possible in candle jars or, later, jars containing CO₂ generated by sachets. Plates were transferred as rapidly as possible to a CO₂ incubator at the Taunton PHL. All Gram-negative diplococci were subcultured for purity, and after a further 24 h incubation each isolate was tested by Gonocheck. Isolates producing a yellow colour after 30 min were grouped, tested for sulphonamide sensitivity, frozen and stored at -70 °C.

In an attempt to produce the optimum isolation rate of meningococci the following systems, which entailed taking two simultaneous throat swabs, were tried.

(1) Plating a plain throat swab on to NYM immediately, at the same time inoculating another plain swab into Stuart's transport medium and plating on to NYM at 5 and 24 h. The results are seen in Table 11.

(2) Plating a charcoal swab on to NYM immediately, at the same time inoculating another charcoal swab into Stuart's transport medium and plating on to NYM at 5 and 24 h (Table 12).

(3) Plating a charcoal swab on to NYM immediately, at the same time inoculating another charcoal swab into Amies' transport medium (Amies, 1967) and plating on to NYM at 5 and 24 h (Table 13).

(4) Plating a plain swab on to NYM immediately, at the same time inoculating an 'enrichment' broth consisting of 10% serum broth with an added supplement of 3.0 mg/l vancomycin, 7.5 mg/l colistin and 5.0 mg/l trimethoprim. The enrichment broth was incubated at 37 °C overnight and then plated on to NYM (Table 14).

Antisera to detect meningococci of groups A, B, C, X, Y, Z, 29E and W135 were obtained from the Manchester PHL. Meningococcal isolates were grown overnight on chocolate agar at 37 °C in 10% CO₂. A suspension was made in 5 ml sterile saline and adjusted to a standard opacity. A drop of each co-agglutination serum was placed in individual wells of an 80-well WHO perspex tray. A drop of the suspension of meningococci was added to appropriate wells, the tray was shaken for up to 2 min and agglutination read by eye. Group B strains were sent to Dr D. M. Jones at the Manchester PHL for subsequent serotyping by co-agglutination, using monoclonal antibodies directed at outer membrane proteins (Frasch, Zollinger & Poolman, 1985).

For sulphonamide testing, an overnight culture in 10% serum broth was diluted 100-fold to produce approximately 1000 c.f.u. per ml. Cultures were spotted on to Oxoid 'Sensitest' sensitivity agar containing 5% lysed horse blood prepared immediately before use and containing doubling dilutions of sulphamethoxazole to produce final concentrations from 0.03 to 64 mg/l. A Denley multi-point inoculator was used to apply the cultures to the plates; control cultures were included. Absence of visible growth was used to determine the end-point, and growth on 8 mg/l interpreted as resistance to sulphonamide.

RESULTS

Tables 8, 9 and 10 detail the monthly swabbing results of the groups of military personnel (R, J and W groups), while experiments to optimize detection of meningococci are to be seen in Tables 11–14.

The total number of isolations of *N. meningitidis* for the year can be seen in Table 15, results of typing these isolates are shown in Table 16, and Table 17 details the number of people who were tested on three or more occasions during the period of the study. Variation in monthly isolation rates may be seen in Table 18.

Table 8. Monthly swabbing results from Group R between March 1986 and March 1987

	1986												1987		
	March (early)	March (late)	April (early)	April (late)	May	June	July	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	
1	BNT	0	—	—	—	BNT	—	—	—	—	—	—	—	—	
2	0	0	0	0	0	0	0	0	—	—	—	0	—	0	
3	0	—	0	0	0	0	0	0	0	0	0	0	0	0	
4	0	0	—	0	0	0	0	—	BP1.3	—	—	0	0	0	
5	—	0	—	—	—	—	—	—	—	—	—	—	—	—	
6	—	—	—	—	0	0	0	0	—	—	—	0	—	0	
7	0	0	—	—	0	0	0	0	—	—	0	0	—	0	
8	0	0	0	0	0	0	—	0	—	—	0	—	—	0	
9	0	B8	0	BNT	—	B8	0	0	BNT	—	—	—	BNT	BNT	
10	NG	B15	B15	B15	0	B15	0	0	B15	0	BNT	B15	—	BNT	
11	0	0	0	0	0	0	B2b	—	—	—	—	0	0	BNT	
12	NG*	0	0	B15*	0	B15 (W)	B15 (W)	—	—	—	—	—	—	—	
13	—	—	0	—	0	0	0	0	0	0	0	0	0	0	
14	BNT	0	—	B15 (W)	0	0	0	—	—	—	0	0	0	0	
15	0	—	—	—	0	0	—	—	—	0	—	—	—	0	
16	0	0	0	0	0	0	—	0	0	0	0	0	0	0	
17	—	0	0	0	—	0	0	0	0	0	0	0	0	0	
18	0	0	—	0	—	—	0	0	0	0	0	0	0	0	
19	—	0	0	0	0	0	—	0	0	0	—	0	—	—	
20	0	0	—	0	0	0	—	0	0	0	0	0	0	0	
22	Y	Y	—	—	—	—	—	—	—	—	—	—	—	—	
23	0	0	0	0	0	—	0	—	—	—	—	0	—	0	
24	Y	—	—	—	—	—	—	—	—	—	—	—	—	—	
25	0	0	0	0	0	—	—	—	—	—	—	—	—	—	
26	0	0	0	0	0	—	—	0	0	0	—	—	—	—	
27	0	0	0	0	0	0	0	0	0	0	—	—	—	—	
28	BNT	0	—	0	—	0	0	0	0	0	—	—	—	—	
30	0	—	—	—	—	—	—	—	—	—	—	—	0	0	
31	0	0	—	—	—	—	—	—	—	—	—	—	—	—	
32	0	0	—	—	—	0	0	0	0	0	—	0	—	0	
33	0	0	—	—	—	—	—	—	—	—	—	—	—	—	

Table 8 (cont.)

	1986												1987		
	March (early)	March (late)	April (early)	April (late)	May	June	July	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	
35	—	0	0	—	0	0	0	0	0	0	0	—	—	—	
36	0	0	0	—	—	—	—	—	—	—	—	—	—	—	
37	NG	0	0	0	0	NG	0	—	0	0	—	NG	0	—	
38	0	—	—	—	—	—	—	0	0	0	—	0	0	0	
40	0	—	—	—	—	—	—	—	—	—	—	0	—	—	
41	—	0	0	0	0	0	—	—	—	—	—	—	—	—	
42	0	0	—	—	—	—	—	—	—	—	—	—	—	—	
45	—	0	—	—	—	—	—	—	—	—	—	—	—	—	
46	0	0	—	0	0	0	0	0	0	0	0	0	0	0	
47	BNT	—	BP1.3	—	—	—	C2b	BP1.3	—	—	—	0	0	BP1.3	
48	—	29E	29E	0	0	—	—	—	—	—	—	—	—	—	
49	—	—	—	—	—	—	0	—	—	—	—	—	—	0	
50	0	0	0	—	0	0	—	0	—	—	—	0	0	0	
51	0	0	0	0	0	0	0	0	—	—	—	—	—	—	
52	0	—	0	0	0	0	—	—	—	—	—	—	—	—	
53	BP1.2	BP1.2	BNT	BP1.2	—	BP1.2	—	—	—	—	—	—	—	—	
54	0	—	0	0	0	0	0	0	0	0	0	—	—	—	
55	NG*	NG*	NG*	NG*	—	NG*	—	NG*	—	—	—	—	—	—	
56	B15P1.16*	0	0	—	0	0	B15P1.16	0	0	B15P1.16	B15P1.16	B15P1.16	B15P1.16	0	
57	0	0	0	—	—	—	—	—	—	—	—	—	—	—	
58	0	—	—	—	—	—	—	0	0	0	—	—	—	—	
59	0	0	—	0	0	0	0	0	0	0	0	0	0	0	
60	—	—	—	0	0	—	—	—	—	—	—	—	—	—	
61	—	—	—	0	—	—	—	—	—	—	—	—	—	—	
62	0	0	0	0	0	—	—	0	0	0	0	0	0	0	
63	0	0	—	—	—	—	—	—	—	—	—	—	—	—	
64	Y	—	—	—	—	—	—	—	—	—	—	—	—	0	
65	0	0	0	0	—	—	—	0	0	0	0	—	—	—	
66	0	0	0	0	0	—	—	—	—	—	—	—	—	0	
67	B2aP1.2	B2aP1.2	—	B2aP1.2	0	B2aP1.2	0	0	0	B2aP1.2	0	0	NG	0	
68	0	—	0	—	0	0	0	0	—	0	0	—	0	0	

Table 8 (cont.)

	1986												1987		
	March (early)	March (late)	April (early)	April (late)	May	June	July	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	
107	0	—	—	0	—	—	—	—	—	—	—	—	—	—	
108	0	BNT	B8	—	—	—	—	—	—	—	—	—	—	—	
109	0	0	—	—	—	—	—	—	—	—	—	—	—	—	
110	NG	Y	Y	0	—	—	—	—	—	—	—	—	—	—	
111	0	—	0	—	—	NG	—	—	—	—	—	—	—	—	
112	—	—	—	—	—	0	0	—	—	—	—	—	—	—	
113	—	—	—	—	—	0	0	—	—	—	—	—	—	—	
114	—	—	—	—	—	0	0	—	—	—	—	—	—	—	
115	—	—	—	—	—	0	0	—	—	—	—	—	—	—	
116	—	—	—	—	—	—	—	—	—	—	—	0	—	0	
117	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
118	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
119	—	—	—	—	—	—	0	—	—	—	—	—	—	0	
120	—	—	—	—	—	—	0	—	—	—	—	—	—	—	
121	—	—	—	—	—	—	0	—	—	—	—	—	—	—	
122	—	—	—	—	—	—	0	—	—	—	—	—	—	—	
123	—	—	—	—	—	—	0	—	—	—	—	—	—	—	
124	—	—	—	—	—	—	0	—	—	—	—	—	—	—	
125	—	—	—	—	—	—	0	—	—	—	—	—	—	—	
126	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
127	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
128	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
129	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
130	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
131	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
132	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
133	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
134	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
135	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
136	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

Key to Tables 8-10: —, no swab taken; 0, swab taken but meningococci not isolated; NG, non-groupable meningococci isolated; NT, non-typable meningococci isolated; *, sulphamide resistance.

Carriage of meningococci

Table 9. Monthly swabbing results from Group J between March 1986 and March 1987

	1986												1987		
	March (early)	March (late)	April (early)	April (late)	May	June	July	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	
1	0	0	—	—	—	—	—	—	—	0	—	—	—	—	
2	0	—	—	—	—	—	—	—	—	—	—	—	0	0	
3	NG	—	—	—	—	—	—	—	NG	—	—	—	0	NG	
4	0	0	—	—	—	—	—	—	—	—	—	—	—	—	
5	NG	NG	—	—	—	—	0	—	NG	—	—	—	—	—	
6	B16	B16	—	—	—	—	—	—	—	—	—	—	—	—	
7	B16	0	0	—	—	—	0	—	—	—	—	—	—	—	
8	0	—	—	—	—	—	—	—	—	—	—	—	—	—	
9	0	—	—	—	—	—	—	—	0	—	—	—	—	0	
10	0	—	—	—	—	—	—	—	—	—	—	—	—	—	
11	0	—	—	—	—	—	—	0	—	—	—	—	—	0	
12	—	—	0	—	—	—	—	—	—	—	—	—	—	—	
13	—	—	—	—	0	—	—	—	—	—	—	—	—	—	
14	—	—	—	—	B15*	—	—	—	0	NG*	—	—	—	0	
15	—	—	—	—	0	—	—	—	—	—	—	—	—	—	
16	—	—	—	—	0	—	—	—	—	—	—	—	—	0	
17	—	—	—	—	0	—	—	—	—	—	—	—	—	—	
18	—	—	—	—	0	—	—	—	—	—	—	—	—	—	
19	—	—	—	—	0	—	—	—	—	0	—	—	0	0	
20	—	—	—	—	0	—	—	—	—	—	—	—	B15P1.16	—	
21	—	—	—	—	0	—	—	—	—	0	—	—	—	0	
22	—	—	—	—	0	—	—	—	—	—	—	—	0	0	
23	—	—	—	—	0	—	—	—	—	0	—	—	0	0	
24	—	—	—	—	0	—	—	0	—	—	—	—	0	0	
25	—	—	—	—	0	—	—	C	—	C	—	—	—	—	
26	—	—	—	—	0	—	—	—	—	0	—	—	—	—	
27	—	—	—	—	C	—	—	—	—	—	—	C	—	—	
28	—	—	—	—	0	—	—	—	—	—	—	—	—	BP1.3	

Table 9 (cont.)

	1986												1987		
	March (early)	March (late)	April (early)	April (late)	May	June	July	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	
29	—	—	—	—	0	—	—	—	—	—	—	—	—	—	
30	—	—	—	—	0	0	—	—	—	—	—	—	—	—	
31	—	—	—	—	0	—	—	—	—	—	—	—	—	—	
32	—	—	—	—	0	—	—	—	—	0	—	—	0	0	
33	—	—	—	—	C	—	—	—	0	0	—	NG	BNT	—	
34	—	—	—	—	NG	NG	—	—	—	—	—	NG	NG	NG	
35	—	—	—	—	—	0	0	—	—	—	—	—	0	—	
36	—	—	—	—	—	0	—	—	—	—	—	0	—	—	
37	—	—	—	—	—	0	—	—	0	0	—	0	0	0	
38	—	—	—	—	—	0	—	—	—	—	—	—	—	—	
39	—	—	—	—	—	0	—	—	—	—	—	—	—	—	
40	—	—	—	—	—	0	—	—	—	—	0	0	0	0	
41	—	—	—	—	—	C	—	—	—	C	0	0	C	C	
42	—	—	—	—	—	0	—	—	—	—	—	0	0	—	
43	—	—	—	—	—	0	—	—	—	—	—	—	—	—	
44	—	—	—	—	—	C	—	—	—	—	—	—	—	NG	
45	—	—	—	—	—	0	0	—	NG	—	—	0	0	NG	

Table 10. Monthly swabbing results from Group W between March 1986 and March 1987

	1986												1987		
	March (early)	March (late)	April (early)	April (late)	May	June	July	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	
1	0														
2	0														
3	0				0										
4	0		0	0	0	0									
5	0		0	0	0	BPI.3*									
6	0		0	0	0					0					
7	NG		NG		NG					NG*					
8	0			0											
9	C														
10	0		0	0											
11	29E*	Z/29E	0	0	0				29E*	0	NG				
12	Y	0	0	0	0	Y									
13	0		0	0	0										
14	0		0	0	0										
15	C	C		C	C				B15	NG			0	0	
16	0				0					BNT					
17	0														
18	BNT												BNT		
19	0														
20	0			0	0										
21	0		NG*	0	0				0				NG		
22	0		0	0	0	0									
23	0														
24	NG		NG	BNT					NG	0					
25	BNT*	BNT*													
26	0	0			0				0						
27	0		0	0											
28	0		0	0	0	0									
29	0														
30	B15														
31	0	0	0	0											

Table 10 (cont.)

	1986												1987		
	March (<i>early</i>)	March (<i>late</i>)	April (<i>early</i>)	April (<i>late</i>)	May	June	July	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	
32	NG	0	C	C	C	—	—	—	—	—	—	—	—	—	
33	0	—	—	—	0	—	—	—	—	—	—	—	—	—	
34	0	0	—	—	0	—	—	0	—	—	—	0	—	0	
35	0	—	—	—	—	—	—	—	—	—	—	—	—	—	
36	0	0	—	0	—	—	—	—	—	—	—	—	—	—	
37	0	0	—	NG	—	—	—	—	—	—	—	—	—	—	
38	0	—	—	0	0	—	—	0	—	—	—	—	—	—	
39	NG	NG	—	—	—	—	—	—	—	—	—	—	—	—	
40	0	—	0	0	—	—	—	—	—	—	—	0	—	0	
41	0	0	0	0	—	—	—	—	—	—	—	—	—	—	
42	0	0	—	0	0	—	—	—	—	—	—	—	—	—	
43	—	0	—	—	0	—	—	—	—	—	—	—	—	—	
44	—	0	—	—	—	—	—	—	—	—	—	—	—	—	
45	—	0	—	—	0	—	—	—	—	—	—	—	—	—	
46	—	0	—	—	—	—	—	—	—	—	—	—	—	—	
47	—	0	—	—	—	—	—	—	—	—	—	—	—	—	
48	—	0	—	—	0	—	—	—	—	—	—	—	—	—	
49	—	0	—	0	—	—	—	—	—	—	—	—	—	—	
50	—	Y	—	Y	0	—	—	—	—	—	—	—	—	—	
51	—	0	0	0	0	0	Y	0	0	—	—	—	—	—	
52	—	0	0	0	0	0	0	0	0	—	—	—	—	0	
53	—	0	0	NG	—	—	—	—	NG	—	—	—	—	—	
54	—	0	0	—	—	—	—	—	—	—	—	—	—	—	
55	—	0	—	—	—	—	—	—	—	—	—	—	—	—	
56	—	0	0	0	—	—	—	—	—	—	—	—	—	—	
57	—	0	0	NG	NG	—	—	—	—	—	—	—	—	—	
58	—	0	—	0	0	—	—	0	—	—	—	—	0	—	
59	—	0	0	0	—	—	—	—	—	—	—	—	—	—	
60	—	Y	0	0	—	—	—	—	—	—	—	—	—	—	
61	—	0	—	0	0	—	—	—	—	—	—	—	—	—	
62	—	0	—	0	0	—	—	—	—	—	—	—	—	—	

Table 10 (cont.)

	1986												1987		
	March (early)	March (late)	April (early)	April (late)	May	June	July	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	
100	—	—	—	—	—	0	—	C	C	0	0	0	0	0	
101	—	—	—	—	—	0	0	0	—	—	—	—	—	—	
102	—	—	—	—	—	0	0	0	0	0	0	0	0	—	
103	—	—	—	—	—	0	—	—	—	—	—	—	—	—	
104	—	—	—	—	—	0	0	0	0	0	0	0	—	—	
105	—	—	—	—	—	0	—	—	—	—	—	—	—	—	
106	—	—	—	—	—	—	0	0	0	0	0	0	0	0	
107	—	—	—	—	—	—	0	—	—	—	—	NG	NG	Y	
108	—	—	—	—	—	—	0	—	—	—	—	—	—	—	
109	—	—	—	—	—	—	0	NG	NG	NG	—	NG	NG	NG	
110	—	—	—	—	—	—	—	—	—	0	—	—	0	—	
111	—	—	—	—	—	—	—	—	—	0	—	—	—	—	

Table 11. *Comparison of direct 5 and 24 h plating of plain swabs stored in Stuart's transport medium on the isolation of meningococci from throat swabs*

Total swabs tested at 0, 5 and 24 h	73
Meningococci isolated direct	13 (17.8%)
at 5 h	6 (8.2%)
at 24 h	1 (1.4%)
Meningococci isolated at 5 h or 24 h but not direct	1 (1.4%)
	(1 colony)
Swabs with meningococci detected by both methods	14 (19.2%)

Table 12. *Comparison of direct, 5 h and 24 h plating of charcoal swabs stored in Stuart's transport medium on the isolation of meningococci from throat swabs*

Total swabs tested at 0, 5 and 24 h	90
Meningococci isolated direct	10 (11.1%)
at 5 h	9 (10.0%)
at 24 h	6 (6.7%)
Meningococci isolated at 5 h or 24 h but not direct	1 (1.1%)
Swabs with meningococci by both methods	11 (12.2%)

Table 13. *Comparison of direct 5 h or 24 h plating of charcoal swabs in Amies' transport medium on the isolation of meningococci from throat swabs*

Total swabs tested at 0, 5 and 24 h	68
Meningococci isolated direct	7 (10.3%)
at 5 h	10 (14.7%)
at 24 h	7 (10.3%)
Meningococci isolated at 5 h or 24 h but not direct	4 (5.9%)
Swabs with meningococci by both methods	11 (16.2%)

Table 14. *Comparison of direct plating and enrichment broth on the isolation of meningococci from throat swabs*

Total swabs direct-plated and then placed in enrichment broth	77
Meningococci isolated	
direct	15 (19.9%)
through broth	16 (20.8%)
direct but not through broth	3 (3.9%)
through broth but not direct	4 (5.2%)
Total positives by any method	19 (24.7%)

Table 15. *Results of monthly swabbing*

Personnel swabbed	283
Negative personnel	190 (67.1%)
Positive personnel	93 (32.9%)
Specimens obtained	1374
<i>N. meningitidis</i> isolations	267 (19.4%)

Table 16. *Serological types of N. meningitidis isolated from 283 personnel throughout the year*

Number of personnel carrying group	
Group A	0
Group B	37 (13.1%)
Group C	11 (3.9%)
Group X	0
Group Y	9 (3.2%)
Group Z } 29 E }	3 (1.1%)
Group NG	30 (10.6%)
Mixed groups	3 (1.1%)
Group B 15	11 (3.9%)
Group B 15*	4 (1.4%)

Many patients had a groupable strain on one occasion and a non-groupable strain on another; these patients were included in the grouped category.

* Sulphonamide-resistant.

Table 17. *Isolations of Neisseria meningitidis from patients tested three or more times*

Completely negative	127 (62.9%)
Positive on one or more test	75 (37.1%)*
Positive on every test	7 (3.5%)
Total	202

* Amongst these 75 positive personnel a total of 506 swabs were taken and meningococci were isolated 245 times, i.e. 48.4%.

DISCUSSION

The carriage rate of all serotypes of meningococci (23.0% of 2479 personnel, and 19.4% of 1374 swabs over a year) equates with previous observations. No undue significance should be placed on the variation in carriage rates over the months. Variations undoubtedly occurred due to aggressiveness of the swabber, logistics of delivery of specimens or subsidiary experiments (note Table 7 and 11-14). There has been extensive discussion about the relative importance of high or low carriage rates (Fraser *et al.* 1973; Wenzel *et al.* 1973). Dudley & Brennan (1934) showed that high and persistent carriage rates of meningococci were not associated with cases of meningococcal meningitis, and numerous papers have shown that the carrier rate bears no direct relationship to the incidence of clinical disease (Greenwood, Hassan-King & Whittle, 1978).

Transmission from carrier to carrier is probably by the oral-respiratory route, and it is generally thought that crowding significantly increases the rate of spread and acquisition, a crucial factor being crowded sleeping quarters. Accommodation is not crowded at RNAS Yeovilton, and this may be reflected in the relatively static carriage rates throughout the year.

Since, happily, there was no further case of meningitis, we had no opportunity to identify factors that might influence susceptibility to meningococcal disease. Whilst immunological defects (Whittle *et al.* 1976) or inherited deficiencies of

Table 18. Monthly swabbing results from groups R, J and W between March 1986 and March 1987*

Group	1986												1987		
	March early	March late	April early	April late	May	June	July	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	
R	84 (22)	72 (17)	58 (12)	55 (12)	49 (1)	54 (10)	40 (10)	61 (5)	59 (9)	58 (4)	49 (10)	49 (9)	46 (7)	52 (13)	
J	11 (4)	5 (2)	4 (2)	0	22 (4)	13 (3)	2 (0)	2 (0)	11 (5)	13 (3)	17 (2)	21 (6)	22 (5)	19 (6)	
W	42 (11)	44 (7)	46 (6)	42 (8)	60 (8)	36 (4)	19 (8)	12 (1)	27 (9)	21 (4)	15 (4)	16 (4)	26 (6)	20 (4)	
Total tested	137	121	108	97	131	103	61	75	97	92	81	86	94	91	
Total positive	37	26	20	20	13	17	18	6	23	11	16	19	18	23	
Percentage positive	27.0	21.5	18.5	20.6	9.9	16.5	29.5	8.0	23.7	12.0	19.8	22.1	19.1	25.3	

* Isolations of meningococci in parentheses. Total number of swabs taken was 1374 (267) = 19.4%.

various complement proteins (Densen *et al.* 1987) have been suggested as predisposing to meningococcal disease, Greenwood *et al.* (1987) failed to find any explanation for the distribution of cases between villages during an outbreak of meningococcal disease in the Gambia, West Africa. There is a suggestion that an incidental virus infection such as influenza, perhaps combined with a genetically determined susceptibility, may predispose to meningococcal disease (Leading Article, 1982; Pether, 1982; Young *et al.* 1972).

The most noteworthy feature of this extensive study was that there were a few people who throughout the period of investigation carried a strain of *N. meningitidis* that had been previously thought would be likely to cause disease. There was no illness either in the carriers or in their families. Thus prolonged carriage of a sulphonamide-resistant Group B15 P1.16 strain of *N. meningitidis* is possible without the presence of overt disease. This has also been observed in Gloucestershire (Cartwright, Stuart & Noah, 1986). Likewise, other people were seen to be long-term carriers of other strains of meningococci. Identification of the subtype of group B meningococcus may be influenced by the amount of polysaccharide covering the proteins used in the typing system. It is likely that strains of meningococci isolated successively from person R 82, for instance, are all B P1.2, although the strain isolated in September was identified as 'non-typable' (D. M. Jones, personal communication).

The use of Amies' transport medium seemed to be better at preserving meningococci than Stuart's (Tables 11, 12 and 13), and subsequent plating at 5 and 24 h increased the isolation rate of these organisms. It has to be recognized, however, that isolations of meningococci from swabs that have been in transport medium for 24 h will inevitably be poor, since plating at 5 h will have removed a proportion of meningococci from the swab. However, towards the end of the study the authors had the feeling that they were using methods that were unsatisfactory in detecting small numbers of meningococci, and that an even harsher technique allied with the use of a more nutrient transport medium would have revealed a higher carriage rate. Table 14 indicates that the isolation rate was increased by a further 5.2% (19.5–24.7), when the isolates of meningococci from an enrichment broth were added to those from direct plating. Intermittent isolations of the same serotype from some subjects would seem to reinforce this view. It can be seen from Table 15 that of the 283 personnel who were swabbed over the period of the study, 202 were swabbed three or more times. Of these latter personnel 75 were positive on one or more occasion but only 7 were positive every time they were swabbed.

From these 75 personnel a total of 506 swabs were taken, and meningococci were isolated from just under half of the swabs (245). This suggests that a single swabbing exercise, even under the ideal conditions of direct plating and prompt incubation, will only reveal half the carriers of meningococci. When carriage rates are examined for the 202 patients who were tested on three or more occasions the 75 'positive' personnel represent a carriage rate (37.1%) which is perhaps a truer reflection of the carriage rate in the population under study than the rate obtained when only a single month's swabbing results are taken into account (average 19.5%; see Table 18). The authors suggest this rate of 37.1% would have been even higher had an enrichment medium been used throughout the study.

It is possible that some people were carrying two or more different serotypes. Our techniques would not have detected two strains being carried at the same time, since only one colony was picked for subculture. Case R 47, for instance, appeared to carry strain B:P:1.3, then C2b and apparently reverted to carrying B:P:1.3 strain. It seems unlikely that R 47 lost his original strain of B:P:1.3 and then acquired a strain of C2b which he subsequently lost, only to regain the original B:P:1.3 all within a period of a few months; results of this study suggest that loss and acquisition of meningococci are unusual events. It therefore seems more likely that he was carrying the two strains throughout the study. The authors suggest that in future studies an attempt should be made to sample a minimum of at least two colonies. At the same time, enrichment medium and direct plating on to pre-warmed plates that were immediately incubated in an atmosphere high in carbon dioxide should be used. Broome (1986) quotes Schoenback & Phair (1948), who suggest that since they found that 5% of carriers had more than one serogroup in their throats at least three colonies per plate should be sampled. A strain of B15 P1.16 SR appeared to lose its sulphonamide resistance (see Table 8, R 56). It is recognized that the pattern of meningococcal carriage in any one population is constantly changing (Poolman *et al.* 1986), and there is very little information that can be used to predict outbreaks.

Discovery of an acquisition or loss is rendered almost impossible because of the technical difficulties detailed above. It could be suggested that patient W 70 acquired a meningococcus (BNT) (Table 10) after 3 negative results which were followed by 5/6 positives. Similarly R 80 (Table 8), whose results revealed no positive isolation until he had been sampled 4 times, when 3 isolates of group B *N. meningitidis* were identified. One is left with the suggestion that acquisition or loss of a meningococcus is an unusual event whose predictability is low with current sampling methods.

The present study emphasizes, perhaps, that failure to grow meningococci from a throat swab should not be taken as evidence to confirm the absence of these bacteria. Thus the accuracy of carriage rates is questionable. Likewise, if carriage of more than one serotype is a frequent occurrence we will usually detect merely the predominant serotype in any one throat.

The authors acknowledge the help and co-operation of all the personnel at RNAS Yeovilton, the nursing staff and especially Chief Petty Officer Medical Assistant P. Manley. They also acknowledge help given by the Somerset Health Authority computer staff and the enormous amount of work undertaken by the staff of the Taunton Public Health Laboratory, especially Georgina Broom, who typed this manuscript many times. Dr D. M. Jones and the staff of the Manchester PHL are also thanked for examining many strains of meningococci.

REFERENCES

- ABBOTT, J. D., JONES, D. M., PAINTER, M. J. & YOUNG, S. E. J. (1985). The epidemiology of meningococcal infections in England and Wales, 1912–1983. *Journal of Infection* **11**, 241–257.

- AMIES, C. R. (1967). A modified formula for the preparation of Stuart's transport medium. *Canadian Journal of Public Health* **58**, 296-300.
- BROOME, C. V. (1986). The carrier state: *Neisseria meningitidis*. *Journal of Antimicrobial Chemotherapy* **18**, Supplement A, 25-34.
- BROWN, J. D. & THOMAS, K. R. (1985). Rapid enzyme system for the identification of pathogenic *Neisseria* spp. *Journal of Clinical Microbiology* **21**, 857-858.
- CARTWRIGHT, K. A. V., STUART, J. M. & NOAH, N. D. (1986). An outbreak of meningococcal disease in Gloucestershire. *Lancet* *ii*, 558-561.
- DENSEN, P., WEILER, J. M., GRIFFISS, J. M. & HOFFMANN, L. G. (1987). Familial properdin deficiency and fatal meningococcaemia. *New England Journal of Medicine* **316**, 922-926.
- DUDLEY, S. F. & BRENNAN, J. R. (1934). High and persistent carrier rates of *Neisseria meningitidis*, unaccompanied by cases of meningitis. *Journal of Hygiene* **34**, 525-541.
- FAUR, Y. C., WEISBURD, M. H., WILSON, M. E. & MAY, P. S. (1973). A new medium for the isolation of pathogenic *Neisseria* (NYC medium). 1. Formulation and comparison with standard media. *Health Laboratory Science* **10**, 44-54.
- FRASCH, C. E., ZOLLINGER, W. D. & POOLMAN, J. T. (1985). Serotype antigens of *Neisseria meningitidis* and a proposed scheme for designation of serotypes. *Reviews of Infectious Diseases* **7**, 504-509.
- FRASER, P. K., BAILEY, G. K., ABBOTT, J. D., GILL, J. B. & WALKER, D. J. C. (1973). The meningococcal carrier-rates. *Lancet* *i*, 1235-1237.
- GREENWOOD, B. M., GREENWOOD, A. M., BRADLEY, A. K., WILLIAMS, K., HASSAN-KING, M., SHENTON, F. C., WALL, R. A. & HAYES, R. J. (1987). Factors influencing susceptibility to meningococcal disease during an epidemic in The Gambia, West Africa. *Journal of Infection* **14**, 167-184.
- GREENWOOD, B. M., HASSAN-KING, M. & WHITTLE, H. C. (1978). Prevention of secondary cases of meningococcal disease in household contacts by vaccination. *British Medical Journal* *i*, 1317-1319.
- LEADING ARTICLE (1982). How does influenza virus pave the way for bacteria? *Lancet* *i*, 485-486.
- PETHER, J. V. S. (1982). Bacterial meningitis after influenza. *Lancet* *i*, 804.
- POOLMAN, J. T., LIND, I., JÓNSDÓTTIR, K., FRØHOLM, L. O., JONES, D. M. & ZANEN, H. C. (1986). Meningococcal serotypes and serogroup B disease in North West Europe. *Lancet* *ii*, 555-558.
- SCHOENBACH, E. B. & PHAIR, J. J. (1948). Appraisal of the techniques employed for the detection of sub-clinical (inapparent) meningococcal infections. *American Journal of Hygiene* **47**, 271-281.
- WENZEL, R. P., DAVIES, J. A., MITZEL, J. R. & BEAM, W. E. (1973). Non-usefulness of meningococcal carriage-rates. *Lancet* *ii*, 205.
- WHITTLE, H. C., ODULOJU, A., EVANS-JONES, G. & GREENWOOD, B. M. (1976). Evidence for familial immune defect in meningococcal meningitis. *British Medical Journal* *i*, 1247-1250.
- YOUNG, L. S., LAFORCE, F. M., HEAD, J. J., TEELEY, J. C. & BENNETT, J. V. (1972). A simultaneous outbreak of meningococcal and influenza infections. *New England Journal of Medicine* **287**, 5-9.