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Dynamics of the meningococcal carrier state and characteristics of the carrier strains: a longitudinal study within three cohorts of military recruits

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SUMMARY

Three cohorts of Danish male military recruits ($n = 1069$) were studied for pharyngeal meningococcal carriage during 3 months at different seasons: 39–47% of entrants were meningococcal carriers and the carriage rate remained constant over time and season. However, individual changes in the carrier state occurred frequently, and after 3 months 34% had changed carrier state on one or more occasions. Initially, a loss of carriage predominated; on the other hand almost 20% of non-carriers had acquisition of meningococci within the first month. The serological phenotypes of the 670 carrier strains were compared with those of 261 invasive strains recovered concurrently from patients with meningococcal disease country-wide. Both carrier strains and invasive strains were phenotypically heterogeneous. Almost 60% of the invasive strains belonged to three phenotypes: B:15:P1.7, 16, C:2a:P1.2, 5 and C:2b:P1.2, 5. In contrast, these phenotypes only amounted to 3·2% of the carrier strains, among which no phenotype was found with a prevalence above 4·9%. However, 30% of the carrier strains had serological phenotypes identical to those of 80% of the invasive strains. Our results indicated that the transmission rate of potential pathogenic carrier strains did not differ from that of other carrier strains.

INTRODUCTION

Among military recruits [1–3] and also within other semi-closed communities [4], meningococcal carriage rates of 25–70% have been demonstrated. In contrast, carriage of meningococci is unusual in infants and children [5]. During the World Wars, the incidence of meningococcal disease was proven to be much higher among military staff than among civilians and often closely related to the mobilization of military personnel [3, 6]. A recent retrospective study during peace time also demonstrated that meningococcal disease occurred with a fourfold higher incidence among military staff than among civilians [7]. Recruits seem to be at highest risk of disease as these have accounted for above 90% of the cases in military of which 50% occurred within the first 3 months of service [6, 7].

In 1989, a cluster of four cases of serogroup C disease appeared among recruits at Høvelte military camp in Denmark and their relatives [8]. Due to a wide and complex contact network, around 15000 people (1/3 military persons and 2/3 civilians) were offered vaccination. With this background, a high motivation for a study of meningococcal carriage at Høvelte was anticipated.

Most previous longitudinal studies of meningococcal carriage primarily focused on carriage rates [1–3, 9, 10]. The aim of this investigation was to study the changes in the individual meningococcal carrier state in a selected population (military recruits) at risk

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of getting meningococcal disease. Furthermore, we wanted to characterize the carrier strains, and to compare the phenotypic characteristics of these with those of invasive strains collected throughout the country within the same period.

METHODS

Study population

Høvelte military camp is situated 25 km north of Copenhagen and is the educational centre for the recruits of the Royal Life Guard. The military service is 12 months of which the first 3 months are the recruit service. The education is considered to be one of the most exhausting in the Danish military. The daily census at the camp is approximately 1000 persons, including the recruits.

Three cohorts of male military recruits, who came from all parts of Denmark and who entered Høvelte military camp on 1 November 1992 (cohort A), 1 March 1993 (cohort B) and 1 July 1993 (cohort C), respectively, were enrolled.

The carrier investigation

Pharyngeal swabs

All recruits were followed for the first 3 months after their entry to the camp. A pharyngeal swab was taken with a charcoal impregnated cotton-tipped wooden applicator from both tonsils on days 0, 15, 30, 60 and 90. To maintain consistency in the sampling procedure, all swabs were taken by one person (J.A.) and inoculated immediately onto a selective chocolate agar medium (containing the antibiotics: lincomycin 1 μg/ml, amphotericin B 2 μg/ml, polymyxin B sulphate 25 U/ml and trimethoprim lactate 3 μg/ml [11]. Within 3–5 h, all plates were incubated at 36 °C in a humid atmosphere containing 5% CO_2 and observed daily for 3 days.

Identification of N. meningitidis

The presumptive diagnosis of *N. meningitidis* was based on typical colony morphology and a positive oxidase reaction. If two macroscopically distinct meningococcal-like colonies were observed on the same plate, these were handled separately. *N. meningitidis* was identified by the typical appearance seen on microscopic examination of a Gram-stained smear, by the ability of acid production from glucose and maltose but not from saccharose, and by the absence of the enzyme β-galactosidase. Isolates with atypical acid production were further investigated in the Minibact-N assay (Statens Serum Institut, Denmark) for identification of Gram-negative diplococci [12]. All isolates, identified as meningococci were stored in liquid nitrogen until further examination.

Characterization of N. meningitidis

Serological grouping was performed by means of an agglutination test (SLIDEX, Pasteur-Meriéux, France) using latex particles sensitized with polyclonal rabbit antisera against *N. meningitidis* groups A and C and with monoclonal mouse antiserum against *N. meningitidis group B/E. coli K1*. Strains that were recognized as non-groupable (NG) by the SLIDEX assay were further investigated by means of a coagglutination test using polyclonal rabbit anti-sera against *N. meningitidis* groups W-135, 29E, X, Y, Z, coated on protein A-rich *Staphylococcus aureus* cells as carriers [13]. Serological typing and subtyping were performed by means of a whole cell ELISA [14] using monoclonal antibodies (lot number 9301) against outer membrane proteins types 1, 2a, 2b, 4, 14, 15 and subtypes P1.1, P1.2, P1.4, P1.5, P1.6, P1.7, P1.9, P1.10, P1.12, P1.13, P1.14, P1.15 and P1.16, purchased from RIVM, the Netherlands.

The *in vitro* susceptibility to sulphonamide of *N. meningitidis* was expressed as the minimum inhibitory concentration (MIC) of sulphamethoxazole, which was determined by means of the plate dilution method (range 0·031–512 mg/l, fourfold dilution steps). Strains with a MIC $\geqslant$ 8 mg/l were defined as resistant.

Definitions

Phenotype of N. meningitidis

In the present study, the phenotype was defined by the serological group, type, subtype and the susceptibility to sulphonamide. Two isolates, which were recovered on consecutive samplings from the same recruit, were considered as the same strain, if the serological markers were identical or if only a minor change was observed, i.e. a change in one of the serological markers as follows: from groupable to non-groupable (NG), typable to non-typable (NT) or subtypable to non-subtypable (NST) (or *vice versa*). Furthermore, phenotypical identity of two isolates was only accepted, if the MICs of sulphonamide were identical or differed only one fourfold dilution step.

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The carrier state on a single sampling occasion

Carrier. A recruit from whom *N. meningitidis* was isolated from a pharyngeal swab.

Non-carrier. A recruit from whom *N. meningitidis* was not isolated from a pharyngeal swab.

The dynamics of the carrier state

Persistent carrier. A carrier from whom *N. meningitidis* had been isolated on all previous sampling occasions. On day 90, a persistent carrier = a constant carrier.

Persistent non-carrier. A non-carrier from whom *N. meningitidis* had not been isolated on all previous sampling occasions. On day 90, a persistent non-carrier = a constant non-carrier.

Intermittent carrier. A recruit who participated on two or more sampling occasions and who was demonstrated to be non-carrier as well as carrier during the study period.

Acquisition

(*a*) A change from persistent non-carrier to carrier.

(*b*) Carriage of a strain phenotypically distinct from any of those previously isolated from the same person, i.e. new acquisition in persistent or intermittent carriers.

The meningococcal strains

Index strains. All strains isolated on the first sampling occasion.

Acquired strains. See acquisition (above).

Invasive strains of *N. meningitidis*

According to the Danish surveillance programme for meningococcal disease, all meningococci recovered from patients with invasive disease in Denmark are submitted to the Neisseria Department and characterized as described for the carrier strains. Results obtained for strains received during the period 1 November 1992 to 31 December 1993 are included in the present study.

Statistics

The χ^2 test for independence and the McNemars test were employed as statistical analyses of the data of the carrier investigation. The number of persons who either became carriers or non-carriers within a certain time interval were assumed to be Poisson distributed. All estimated rates were based on the assumption that a person as a maximum would change condition once within that time interval and that the rate was constant in the time interval between two consecutive samplings. In the comparison of invasive and carrier strains, the χ^2 test for independence was employed.

Where the word significant is used, it refers to a probability value below 5%.

Ethical procedure

The study was performed after permission from the Local Committee of Ethics in Science. Participation was on a voluntary basis and in accordance with the guidelines of the Helsinki II declaration.

RESULTS

Carriage of *N. meningitidis*

A total of 1069 recruits, equally distributed in three cohorts entering military at different seasons, were included in the study; the majority, 71% ($n = 748$), were examined on at least four of the five possible occasions, while only 4% ($n = 44$) were examined once (Table 1). The mean number of samplings per recruit was 3·9 (4168:1069).

Carriage rates

On entry to the military, the carriage rate in the three cohorts ranged between 39 and 47% (Fig. 1). The carriage rate was highest in cohort B recruits (spring) and lowest in cohort C recruits (summer). However, the difference was not significant and in all three cohorts, the carriage rates were almost constant during the 3 months study period.

Fluctuations in the carrier state

Initially (day 0), two groups were identified: carriers and non-carriers. Persistent carriers, persistent non-carriers and intermittent carriers (see definitions) were at the earliest identified on day 15. The dynamics of changes in the carrier state within each cohort were identical and the results are presented for all three cohorts together (Fig. 2). Within the first month, the proportion of intermittent carriers had increased to 22% of the recruits, and on day 90 intermittent carriers amounted to 34%. Among those identified as carriers on day 0, 58% carried meningococci continuously throughout the 3-month study period

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Table 1. *Data on attendance and meningococcal carriage rate in a study including 1069 recruits from three military cohorts. Copenhagen, Denmark, 1992–3*

Sampling	Number of participants	Number of carriers (%)
1 (day 0)	1058	448 (42)
2 (day 15)	970	407 (42)
3 (day 30)	838	343 (41)
4 (day 60)	683	295 (43)
5 (day 90)	619	284 (46)
Total	4168	1777 (43)

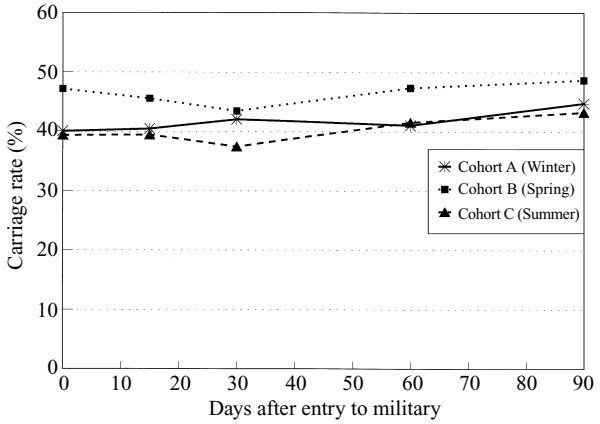

Fig. 1. Seasonal meningococcal carriage rate in three military cohorts. The cohorts were followed at different seasons during 3 months after entry to military. Copenhagen, Denmark, 1992–3.

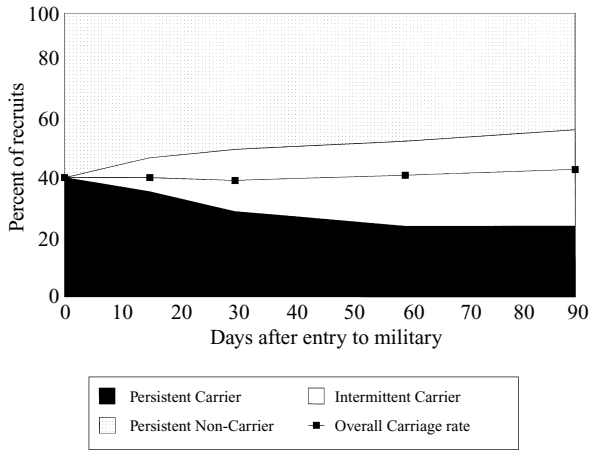

Fig. 2. Dynamics of the meningococcal carrier state. Pooled data for three military cohorts. Distribution of persistent carriers, persistent non-carriers and intermittent carriers. Copenhagen, Denmark, 1992–3.

(constant carriers) and 71 % among those identified as non-carriers on day 0 were constant non-carriers. Overall, 11–16 % of the recruits changed carrier state between two consecutive sampling occasions. Within the first month after entry to military, a loss of carriage predominated, while at the end of the 3-month study period carriers and non-carriers changed carrier state with the same frequency.

Characterization of the carrier strains

Growth of *N. meningitidis* was obtained from 1777 of the 4168 pharyngeal swabs (43 %) (Table 1). Based on phenotypical characterization (see definitions), one isolate of *N. meningitidis* was recovered from 1762 of the swabs, whereas two phenotypically distinct isolates (two strains) were recovered simultaneously from 15 swabs: Thus, in total 1792 isolates of *N. meningitidis* were identified.

By the phenotypical characterization, 670 meningococcal strains were identified among the 1792 isolates; 451 meningococcal isolates were isolated from the recruits on day 0, when they entered military (= 451 index strains), while among the remaining 1341 isolates recovered on sampling occasions 2–5, 219 (16 %) were determined to be acquired (see definitions). Thus, 1122 (84 %) of the isolates recovered on sampling occasions 2–5 belonged to the same phenotypes as the index strains. Out of the total of 670 meningococcal strains recovered in this study, around one third ($n = 219$) were acquired strains.

Serogrouping

Group B and C accounted for 31·8 % and 6·5 % of the strains, respectively; 44·2 % were NG (Table 2*a*). No difference between the distribution according to serogroups of the 451 index strains and that of the 219 acquired strains was observed.

Serotyping

Type 4 (28·4 %), 15 (12·2 %) and 14 (10·0 %) were predominant among the carrier strains (Table 2*b*); 40·0 % were NT. A significant difference in distribution according to types of the index strains and of the acquired strains was observed. Thus, type 2a and NT were found more frequently and type 4 less frequently among the acquired strains than among the index strains, respectively.

Serosubtyping

Subtype P1.6 (15·4 %) and subtypes P1.2, 5 (19·1 %) were found with the highest frequency (Table 2*c*). Only 14·8 % were NST. No difference in the dis-

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Table 2. *Percentage distribution according to* (*a*) *serogroups*, (*b*) *serotypes and* (*c*) *serosubtypes of 670 meningococcal carrier strains and of 261 invasive strains isolated during the study period. Copenhagen, Denmark, 1992–3*

	Carrier strains			
	Index strains ($n = 451$)	Acquired strains ($n = 219$)	Total ($n = 670$)	Invasive strains* ($n = 261$)
(*a*) Serogroups				
A	0	0	0	0
B	33·3	28·8	31·8	73·1
C	6·0	7·8	6·5	24·9
X	0·9	0·9	0·9	0
Y	4·0	3·2	3·7	0·4
Z	3·3	6·8	4·5	0
W-135	3·5	6·4	4·5	0·8
29E	4·0	3·7	3·9	0
NG†	45·0	42·4	44·2	0·8
(*b*) Serotypes				
1	4·7	4·1	4·5	0·4
4	31·7	21·5	28·4	12·3
14	9·8	10·5	10·0	3·1
15	13·7	9·1	12·2	45·7
16	n.d.‡	n.d.	n.d.	4·2
2a	2·2	6·3	3·6	15·0
2b	1·1	1·8	1·3	8·1
NT†	36·8	46·7	40·0	11·2
(*c*) Serosubtype				
P1.1,(7)	9·5	9·1	9·4	4·6
P1.2,(5)	19·7	17·9	19·1	19·2
P1.6	12·0	22·3	15·4	2·7
P1.9	8·2	5·5	7·3	6·2
P1.15	8·4	8·7	8·5	3·5
P1.16	4·4	3·7	4·2	4·6
P1.(7),16	4·4	3·2	4·0	43·8
Others	18·0	16·0	17·3	3·1
NST†	15·3	13·6	14·8	12·3

* Typing and subtyping not done in one strain.
† NG, non-groupable; NT, non-typable; NST, non-subtypable.
‡ n.d., not done.

tribution according to subtypes of the index strains and the acquired strains was observed, although subtype P1.6 was found almost twice as frequently among the acquired strains as among the index strains.

Determination of the susceptibility to sulphonamide

One third of the carrier strains were resistant to sulphonamide. No difference in the proportion of resistant strains among the index strains and the acquired strains was observed.

Phenotyping

Based on the serological markers, 158 phenotypes were identified among the 670 meningococcal strains: 194 of these strains were fully classifiable, representing 53 serologically distinct phenotypes. Among the acquired strains, 66–74% belonged to phenotypes that had been found among the index strains.

Overall, the most common serological phenotype was NG:NT:P1.7 (4·9%), whereas among fully classifiable phenotypes B:4:P1.1, 7 (4·3%) was observed most frequently (Table 3). Only 3·7% could

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Table 3. *Prevalence (per cent) of selected serological phenotypes among 670 carrier strains and 261 invasive strains. Copenhagen, Denmark, 1992–3*

	Carrier strains			
Serogroup: Serotype: Subtype	Index strains ($n = 451$)	Acquired strains ($n = 219$)	Total ($n = 670$)	Invasive strains ($n = 260$*)
B:4:P1.1,7	4·4	4·1	4·3	0·4
B:4:P1.6	2·0	2·3	2·1	1·2
B:4:P1.15	1·1	0	0·7	1·5
B:4:NST†	2·2	0·5	1·6	2·7
B:14:P1.7,16	0·7	0·5	0·6	2·3
B:15:P1.7,16	2·7	1·4	2·2	40·0
B:NT:P1.9‡	3·3	4·1	3·6	4·2
C:2a:P1.2,5	0·7	1·8	1·0	10·0
C:2a:NST	0·4	0·8	0·6	4·2
C:2b:P1.2,5	0	0	0	6·5
W135:NT:P1.6	2·2	4·6	3·0	0·4
Y:14:P1.2,5	3·5	0·9	2·7	0·4
Z:NT:P1.6	0·7	4·1	1·8	0
NG:2a:P1.2,5§	0·7	2·3	1·2	0
NG:15:P1.7,16	0·9	0·9	0·9	0
NG:NT:P1.7	5·8	3·2	4·9	0
NG:NT:NST	3·5	4·1	3·7	0

* Typing and subtyping not done in one strain.
† NST, non-subtypable.
‡ NT, non-typable.
§ NG, non-groupable.

neither be classified by grouping, typing nor by subtyping (NG:NT:NST). In total, known pathogenic strains of B:15:P1.7, 16, C:2a:P1.2, 5 and B:4:P1.15 [15–17], constituted less than 4% of the carrier strains.

Resistance to sulphonamide was closely correlated with certain serological phenotypes: B:14:P1.7, 16, B:15:P1.7, 16, Z:NT:P1.6, Z:NT:P1.10, NG:15:P1.7, 16 and NG:NT:P1.7 strains were all resistant to sulphonamide.

Strains that belonged to Z:NT:P1.6 were observed almost six times as frequently among the acquired than among the index strains, and thus were disseminated to a higher degree than other strains within the community (Table 3). In contrast, no significant spread of B:15:P1.7, 16 or C:2a:P1.2, 5 could be demonstrated.

Acquisition rate

In carriers and intermittent carriers possible new acquisition between two consecutive samplings was assessed by phenotyping of the meningococcal isolates.

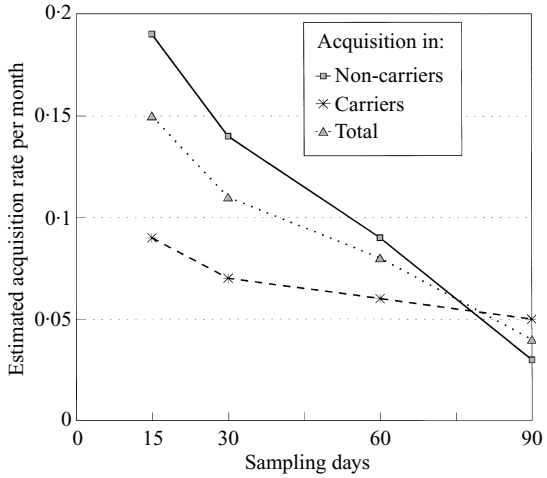

Fig. 3. Estimated acquisition rate per month in non-carriers and carriers. Copenhagen, Denmark, 1992–3.

The estimated acquisition rate per month in non-carriers and carriers is illustrated in Figure 3. The overall acquisition rate per month declined significantly through time from 0·15 between day 0–15 to 0·04 between day 60–90. However, the significant ($P < 0·001$) decrease in acquisition rate was only seen in

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non-carriers, whereas that in carriers was rather constant over time. Initially, the acquisition rate in non-carriers was significantly ($P = 0{\cdot}005$) higher (0·19) than that in carriers (0·09), but between day 60 and 90 the rates were almost the same.

Estimation of the sensitivity of the sampling procedure for the detection of pharyngeal carriage

For the diagnosis of meningococcal carriage the sensitivity of the test (taking of specimen and laboratory procedure) could be estimated as follows: 106 initial carriers, who participated on all five sampling occasions and who also carried the index strain on at least day 90, were presumed to be constant carriers of the index strain. During the study only the index strain was isolated from each of the recruits. The mean sensitivity of the sampling procedure by taking pharyngeal swabs on one, two or three inter-current occasions was found to be 86, 95 and 97%, respectively.

Characterization of invasive strains

In total 261 strains of meningococci were recovered from 261 patients with invasive disease throughout Denmark within the study period. Groups B and C were predominant, whereas non-encapsulated (NG) strains were infrequent (Table 2*a*). The prevalence of groups B, C and NG among the invasive strains differed significantly from that of the carrier strains.

Among the invasive strains, types 15, 2a and 4 predominated (Table 2*b*). Types 15 and 2a were found significantly more frequently among the invasive than among the carrier strains, whereas type 4 was more prevalent among the carrier strains than among the invasive strains. Subtype(s) P1.7, 16 was more frequently found among the invasive strains than among the carrier strains (Table 2*c*), whereas subtype P1.6 was more frequently observed among the carrier strains than among the invasive strains.

Fifty-one serological phenotypes were found among the 260 invasive strains. Phenotypes B:15:P1.7, 16 and C:2a:(P1.2, 5/NST) were found much more frequently among the invasive than among the carrier strains, 40% and 10% versus 2% and 1%, respectively (Table 3). C:2b:P1.2, 5, a rather frequent invasive phenotype (6·5%), was not found among the carrier strains. Almost 60% of the invasive strains belonged to the 3 phenotypes, B:15:P1.7, 16, C:2a:P1.2, 5 and C:2b:P1.2, 5 and these only accounted for 3·2% of the carrier strains. In general, the invasive strains were serologically more homogeneous than the carrier strains. None of the invasive strains was NG:NT:NST.

Two thirds of the invasive strains were resistant to sulphonamide.

DISCUSSION

Carriage rates of meningococci from studies conducted before 1969 are less reliable due to the belief that *N. lactamica* was a lactose fermenting variant of *N. meningitidis*. Thus, carriage rates may have been overestimated [5]. Choice of culture media, the number of swabs taken, the site swabbed, the number of different investigators and the handling of the sample are additional variables that affect the sensitivity of the test procedure for detection of meningococcal carriage [9, 10, 18]. Both an early [19] and recent study [9] estimated a sensitivity for detection of meningococcal carriage by taking one sample of approximately 50%. The interpretation of a single negative culture has been questioned previously [19, 20] and Cartwright has proposed that meningococcal carriage is excluded by taking three independent samples simultaneously [5]. We found sensitivities of 86% and 95% by taking one and two pharyngeal swab(s), respectively. Thus, our estimates for carriage rates and changes in the carrier state are based upon a relatively sensitive test procedure.

In comparison with other longitudinal carrier studies among military recruits [1–3], carriage rates found in our three cohorts investigated were surprisingly constant. A consistent finding in these previous studies has been an increase in carriage rate during the first 3 months after entry to the military. Although the carriage rate was slightly higher on all five samplings during the spring season than during winter and summer, no significant difference in the seasonal carriage rates was demonstrated. A lack of seasonal fluctuation in carriage rates was also found by other investigators [1, 3] and is in contrast to the seasonal fluctuations in incidence of meningococcal disease [21, 22]. The initial carriage rate of 39–47% was higher than we would expect in Denmark, although carriage rates > 25% in civilians aged 15–24 years have been a consistent finding during both epidemic and endemic conditions, even in recent studies in Northern Europe [23–25]. Our data indicate a carriage rate of 40–50% among civilian males aged

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18–22 years in Denmark as the recruits came from all parts of the country.

Approximately two thirds of the recruits remained either constant carriers or constant non-carriers through the 3 months. Thus, one third changed carrier state (became intermittent carriers) and this group is interesting as they may be at increased risk of contracting disease. As suggested by Cartwright circulation of meningococci is probably facilitated by shared living and sleeping accommodations whether overcrowding is present or not [5]. The high acquisition rate observed in non-carriers within the first month is consistent with the observation that the peak in incidence of disease in recruits was found after approximately 4 weeks in service [7, 26]. A high acquisition rate rather than a high carriage rate may be of importance for the emergence of sporadic cases and outbreaks of disease as previously suggested by Wenzel and colleagues [27].

In the present study, meningococcal strains were characterized and distinguished phenotypically by the serological group, type, subtype and the determination of the susceptibility to sulphonamide: Approximately half (44%) of the carrier strains were NG and one third belonged to group B, proportions which are identical to those found in other recent carrier studies [23, 25]. In Denmark, recruits entering military are not vaccinated against meningococcal disease. Although, a reduced group C carriage rate upon vaccination has been demonstrated, it is still questionable whether vaccination would have any impact on carriage of meningococci [5, 10]. In the present study, the group B/group C ratio was 4·9 among the 670 carrier strains and 3·0 among the 261 invasive strains; this suggests that group C strains might have been slightly more virulent than group B strains. As in other studies type 4 was the most frequent among the carrier strains, whereas the most frequent subtype, P1.6, was not previously found to have a similar prevalence [23, 28]. Type 4 has been frequent among invasive strains in the Netherlands [29], but the high frequency of type 4 among the carrier strains in our study (1992/93) was not reflected in a shift towards a higher prevalence of type 4 among the invasive strains in the following years (unpublished results: prevalence approximately 10% annually, 1991–5).

Based on serological phenotyping, the carrier strains were very heterogeneous. The invasive strains were more homogeneous than the carrier strains; B:15:P1.7, 16 accounted for 40% of these; only 3·1% of the carrier strains belonged to this phenotype (B/NG:15:P1.7, 16), which is in accordance with previous European studies [23–25]. Overall, 30% of the carrier strains had phenotypes identical to 80% of those of the invasive strains. The phonotype B:NT:P1.9 was found with the same frequency among both the carrier (3·6%) and the invasive strains (4·2%); the other more prevalent carrier phenotypes were rarely found among the invasive strains (Table 3).

The discriminatory capacity using a combination of these serological markers was high. Phenotyping may be considered an appropriate epidemiological tool for the evaluation of the dissemination of strains within a semi-closed community and for detection of acquisition in persistent and intermittent carriers. The proportions of carrier strains and invasive strains characterized as NT and/or NST emphasize the limitations of the present panel of monoclonal antibodies in typing of carrier strains, whereas this was only observed to a limited extent in invasive strains. The recent introduction of new DNA-based approaches for typing and subtyping may have implications for the future characterization of meningococci [30]. However, some investigators found that phenotypical characterization correlated well with genotyping methods as MEE [31] and RFLP [32].

By RFLP and MEE, carrier strains were found to be very heterogeneous [33, 34], with major differences between carrier strains and invasive strain as assessed by MEE [35]. However, Caugant and colleagues also found that all B/NG:15 (resistant to sulphonamide) carrier strains belonged to the same complex (ET-5) as the predominant invasive clone (B:15:P1.16) [23, 33]. In our study 27 (4%) out of 670 carrier strains belonged to B/NG:15 (resistant to sulphonamide) and these might theoretically belong to the potentially pathogenic ET-5 complex. Conclusions regarding the prevalence of other potential pathogenic clones/phenotypes (e.g. C:2a:-) among the carrier strains must be considered with caution as emphasized by others [36].

Around two thirds of the phenotypes among the acquired carrier strains were identical to those of the index carrier strains. However, only one strain, the presumed non-pathogenic Z:NT:P1.6, was found significantly more frequently among the acquired than among the index strains. B/NG:15:P1.7, 16 had the same transmission rate as other strains and the hypothesis that this strain is less transmissible than other potentially pathogenic strains as suggested by

Cartwright and colleagues [25] was not supported by our study.

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