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Usefulness of the DNA-fingerprinting pattern and the multilocus enzyme electrophoresis profile in the assessment of outbreaks of meningococcal disease

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SUMMARY

The objective of the study was to assess whether genotypic characterization by means of DNA-fingerprinting pattern (DFP) and multilocus enzyme electrophoresis (MEE) profile as compared to phenotypic characterization would improve the differentiation of *Neisseria meningitidis* strains associated with outbreaks from strains associated with sporadic cases of meningococcal disease. In addition, the differentiation of serogroup C carrier strains from those associated with an outbreak of serogroup C meningococcal disease was investigated.

A total of 118 *N. meningitidis* strains were available for the study: 59 from patients involved in outbreaks of meningococcal disease (2 serogroup B and 2 serogroup C), 37 from patients considered to be sporadic cases and 22 serogroup C carrier strains.

Among the 59 strains from patients involved in outbreaks the 4 strains isolated from the patient registered as the first in each outbreak were designated the index strains. Among the remaining 55 outbreak strains 52 were either DFP-identical or DFP-indistinguishable when compared with the one relevant out of the 4 index strains. This was only the case for 17 of the 37 strains isolated from sporadic cases caused by the same serogroup of meningococci during the outbreak periods, and 5 of the 22 meningococcal strains isolated from healthy carriers. Among the 56 (52+4) DFP-identical or DFP-indistinguishable outbreak strains 5 different electrophoretic types were identified by MEE.

Among 59 assumed outbreak strains a total of 4 were identified as genotypically distinct. Among the 37 mainly DFP-indistinguishable or DFP-different strains from sporadic cases 17 different ETs were identified, and among the 22 mainly DFP-different carrier strains 13 different ETs were identified. Two strains among those selected from sporadic cases were identical to the outbreak strain. None of the local serogroup C carrier strains isolated during the outbreak of serogroup C disease were identical to the outbreak strain.

Both DNA-fingerprinting and MEE improved the differentiation of meningococci when compared with phenotypic characterization. The results indicate that tracing a virulent strain within an open group of contacts is irrelevant.

INTRODUCTION

The Danish surveillance system for meningococcal disease is based upon the collaboration between the Institutions of Medical Officers of Health, the Department of Epidemiology and the Neisseria Department, Statens Seruminstitut [1]. Since 1974 more than 90% of isolates from patients with meningococcal disease in Denmark have been sent to and kept in the Neisseria Department. Approximately 80% of notified cases of meningococcal disease are bacteriologically verified. The meningococcal strains included in the present study are therefore considered to permit a valid analysis of the outbreaks.

Table 1. *Distribution according to serogroup of* N. meningitidis *strains from patients with meningococcal disease in Denmark 1980–9*

Year	Total no. of strains	Percentage of strains belonging to serogroup			
		A	B	C	Others*
1980	144	4	54	37	5
1981	129	5	62	26	8
1982	134	5	61	26	8
1983	134	4	65	31	0
1984	164	2	66	30	2
1985	130	1	76	21	2
1986	225	0	74	22	4
1987	216	1	77	19	3
1988	213	0	78	18	4
1989	181	1	77	19	2

* Serogroups W-135, 29E, X, Y, Z or NG (non-groupable).

In Denmark, the incidence of notified cases of meningococcal disease rose by approximately 60% from 1985 to 1986, i.e. from 3·5 to 5·5/100000/year. There was no change in the serogroup and type pattern. During the period 54–78% of the cases were caused by serogroup B meningococci and 18–31% by serogroup C (Table 1). During the 1980s several outbreaks have been recognized in Denmark, either as outbreaks among teenagers without any known direct contact to each other [2, 3], or as outbreaks where epidemiologic contact between the patients was evident.

The purpose of the present study was to assess whether the use of genotyping (DNA-fingerprinting technique and multilocus enzyme electrophoresis (MEE) typing) would improve the dissociation of strains associated with outbreaks from strains associated with sporadic cases of meningococcal disease. In addition, serogroup C meningococcal strains isolated from healthy carriers during an outbreak of serogroup C meningococcal disease were studied.

MATERIALS AND METHODS

Study population

The study comprised 118 meningococcal strains isolated from 96 patients with meningococcal disease and 22 healthy individuals in whom pharyngeal carriage of meningococci was demonstrated.

Out of the 118 meningococcal strains 59 were isolated from patients who had been involved in outbreaks of meningococcal disease in 4 different geographical areas (Frederiksborg, Ringsted, Holstebro and Randers). Thirty-seven strains were isolated from patients considered to be sporadic cases occurring during the same periods of time and living in the environs of the areas in which the outbreaks were identified. Twenty-two strains were isolated from the throat of healthy individuals.

Information about direct or indirect contact between patients with meningococcal disease was either available from the literature [2, 3] or kindly provided by the Department of Epidemiology, Statens Seruminstitut.

Frederiksborg outbreak

During the period 1986–9 an outbreak of meningococcal disease due to a serogroup B:15:P1.16 strain was identified in Frederiksborg county. The outbreak was characterized by clustering of cases among teenagers and the localization of the clustering changed over time. The present investigation comprised isolates from 31 out of 38 cases of meningococcal disease that occurred within 2 periods of clustering in 1987 and 1 period in 1989, including 3, 5 and 4 cases among teenagers, respectively.

The study was initiated at a time when the outbreak and the first period of clustering had just been recognized. Six serogroup B:non-15 strains and one serogroup B:15:P1.16 strain from sporadic cases of meningococcal disease that occurred in other parts of the country during this period of time were selected for comparison.

Ringsted outbreak

From November 1986 until June 1987 seven cases of meningococcal disease caused by B:15:P1.16 meningococci among pupils attending 2 schools in the Ringsted area were notified. From 6 of these 7 patients an isolate was available.

For comparison six B:15:P1.16 isolates from patients with meningococcal disease living in the environs of Ringsted and notified during the period December 1986–February 1987 were selected.

Holstebro outbreak

The Holstebro outbreak lasted from February to May 1986 and was notified among students attending a continuation school and their sisters and brothers.

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Five patients were involved in the outbreak; three of them were students and two were siblings of healthy students. All meningococcal isolates were C:2a:P1.2.

For comparison six C:2a:P1.2 isolates from patients with meningococcal disease living in the environs of Holstebro and notified February–May 1986 were included.

Randers outbreak

The following is based upon the material described by Rønne and colleagues [3]. The outbreak in the Randers area took place from November 1983 until April 1984, and was experienced as three periods with clustering of cases. *First cluster*: The first cluster of the outbreak took place in November 1983. Within 10 days 6 cases of meningococcal disease were notified, of which 3 occurred among students attending 2 neighbouring schools (A and B) [3]. *Second cluster*: From January until March another 6 cases of meningococcal disease were notified. Thus, it was not easy to define a target group. *Third cluster*: In the beginning of April 1984, 4 cases of serogroup C meningococcal disease were notified within 1 week. One case occurred at School B.

For comparison 18 serogroup C meningococcal strains from patients living in the environs of Randers and notified November 1983–July 1984 were included.

Carrier strains from Randers and Århus

During the Randers outbreak students at 2 schools in the Randers area (A and B) and at 2 similar schools in Århus (A and B), a city close to Randers, were included in a carriage study [3]. Two cases of serogroup C meningococcal disease had occurred at each of the 2 schools in Randers, while none occurred in Århus. Meningococcal serogroup C strains isolated from the throat of 22 of these students were included in this study [3].

Characterization of *N. meningitidis* strains

Serogrouping and serotyping

Serological grouping was performed by means of a co-agglutination test using rabbit antisera against *N. meningitidis* serogroups A, B, C, X, Y, Z, W-135 and 29-E coated on protein-A rich *S. aureus* cells as carriers [4]. Serological typing and subtyping were performed by a whole-cell ELISA [5] using monoclonal antibodies (serotypes 1; 2a; 2b; 4; 14, 15 and NT (non-typable) and serosubtypes P1.1; P1.2; P1.6; P1.7; P1.9; P1.15, P1.16 and NST (non-subtypable)) kindly provided by J. T. Poolman, RIVM, The Netherlands.

Antimicrobial susceptibility testing

The *in vitro* susceptibility to sulphonamide (sulphamethoxazole) was determined by an agar plate dilution method; strains were designated sulphonamide-susceptible (S) or sulphonamide-resistant (R) (MIC $\geqslant$ 8 mg/l).

DNA-fingerprinting technique

The following enzyme solutions and other materials were used: *Lysozyme*: 10 mg lysozyme (Sigma L 6876) dissolved in 1 ml distilled water. *RNase*: 10 mg RNase (Calbiochem-Behring CORP no. 55674) dissolved in 1 ml distilled water, boiled for 15 min at 100 °C and cooled down to room temperature before storage in small aliquots at −20 °C. *Protease*: 20 mg protease (Sigma P 5147) dissolved in 1 ml distilled water and incubated at 37 °C for 2 h before storage in small aliquots at −20 °C.

Meningococci obtained from lyophilized cultures were inoculated on Danish GC chocolate agar medium (selective for pathogenic Neisseria species). The meningococci were incubated overnight in a humid atmosphere at 37 °C in 5% CO_2, then a single or a few colonies were subcultured and incubated overnight (as under the conditions mentioned above). The bacterial growth was harvested in 1·5 ml of a 50 mM-TRIS buffer at pH 8·0 containing 25% sucrose. The dense suspension was transferred to an Eppendorf Micro test tube, and pelleted by centrifugation for 3 min at 15000 g. The supernatant was discharged and the pellet was resuspended in a mixture of 100 μl of the same TRIS-buffer as before and 100 μl of freshly prepared lysozyme-solution. After 20 min 10 μl of RNase-solution was added. Five minutes later 50 μl of protease-solution was added, and after a further 15 min 150 μl TE-buffer (10 mM-Tris, 1 mM-EDTA, pH 8·0) and 250 μl lysis-mix (93 ml Tris 50 mM, 5 ml 0·2 M-EDTA and 2 ml Triton-x 100) were added. The tubes were then kept continuously on crushed ice for 1–1·5 h.

For DNA-extraction 300 μl of chloroform-isoamylalcohol (24:1), was added and the resulting suspension vigorously shaken until it became homogeneous, whereafter 300 μl phenol was added, and the suspension once again shaken until homogeneity was obtained. After centrifugation for 3 min at 15000 g,

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the supernatant containing the chromosomal DNA was transferred to another tube and the step with chloroform-isoamylalcohol-phenol (24:1:25) repeated, until the supernatant was close to being transparent. After the final centrifugation the supernatant was transferred to dialysis-tubing (9 mm) and dialysed in TE-buffer overnight. (One litre of TE-buffer was used for four DNA-preparations.)

After dialysis the DNA-solutions were again transferred to tubes, and the concentration of DNA present was determined using a spectrophotometer (Carl Zeiss M4QIII). The A_{260} value allowed calculation of the concentration of DNA present, and the A_{260}/A_{280} ratio provided a measure of the purity of the DNA preparation. The concentration ranged from 0·8–2·0 μg/μl. An A_{260}/A_{280} ratio between 1·8 and 2·0 indicated a suitably pure DNA preparation, and it was assumed that 1 OD_{260} was equivalent to 50 μg for double-stranded DNA/ml [6].

Ninety units (U) of the relevant restriction endonuclease, either *Hin*dIII (Boehringer Mannheim 798983, Germany) or *Eco*RI (Boehringer Mannheim 200310, Germany) with the amount of buffer indicated by the manufacturer was added to 100 μg of DNA. The volume was adjusted to maximum 90 μl by the addition of redistilled water. The solutions were incubated in tubes at 37 °C for 2 h. Before electrophoresis was started, one tenth volume tracking dye composed of 500 μl glycerol 87% (Merck Art 4094), 400 μl distilled water and 100 μl bromphenol blue (Merck Art 8122) were added to the digest. The resulting DNA fragments were separated electrophoretically in a 1·5 mm thick gel containing 4% polyacrylamide and 0·16% bis-acrylamide and situated between two glass plates, placed in a vertical slab. The gel was subjected to electrophoresis for 30 min at constant voltage (300 V) and a temperature of 10 °C. As a molecular weight marker was used 10 μl lambda DNA (Boehringer Mannheim 745782) digested with *Pst*I (Boehringer Mannheim 621625).

The gel was stained for 30 min in a solution of 50 μl ethidium bromide (10 μg/μl) in 500 ml TBE-buffer (0·9 M-Tris, 25 mM-EDTA, 0·9 M Borate; pH 8·2) and destained for another 10 min to wash away any superfluous ethidium bromide. Then it was photographed in UV light by using a Polaroid MP3 land camera. The band patterns were studied with the naked eye, and the patterns compared with each other.

When examined with the DNA-fingerprinting technique, the chromosomal DNA from one meningococcal strain usually appeared as approximately 50 bands per strain. The following differentiation between three levels of identity was used:

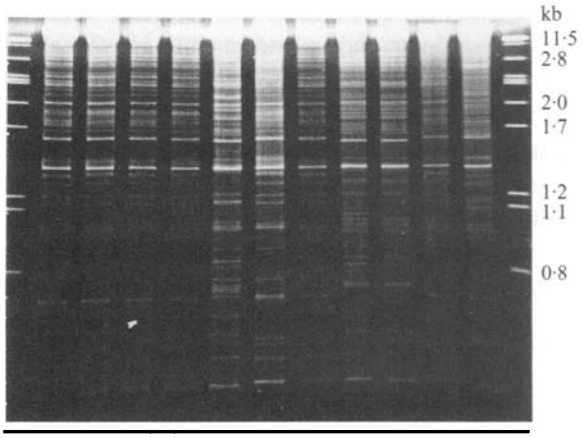

Fig. 1. The DNA-fingerprinting pattern obtained after digestion with *Hin*dIII of the index strain from Randers outbreak (lane 2) and the serogroup C meningococcal strains isolated from sporadic cases occurring during the outbreak period in Randers and environs. When compared to the index strain, strains in lanes 3, 4, 7, 8 and 11 were DFP-identical, in lane 5 DFP-indistinguishable and in lanes 6, 9, 10 and 12 DFP-different. λ-DNA digested with *Pst*I (lane 1 and 13) was used as a molecular weight marker.

When the DFPs of 2 strains were compared the strains were either determinated to be DFP-identical (I), DFP-indistinguishable (~) or DFP-different (D) (Fig. 1). To be DFP-identical the DNA-fingerprinting pattern of the two strains should be completely alike, when the DNA of the strains was cut with the restriction endonuclease *Hin*dIII as well as with the restriction endonuclease *Eco*RI. DFP-indistinguishable strains had five or less different band-positions, and DFP-different strains had more than five bands placed in different positions when compared with each other after digestion with *Hin*dIII and/or *Eco*RI. Examination with the restriction endonuclease *Hin*dIII alone identified the majority of DFP-different strains.

Multilocus enzyme electrophoresis

By multilocus enzyme electrophoresis (MEE) the following eight enzymes were assayed: malic enzyme (ME), isocitric dehydrogenase (IDH), glucose-6-phosphate (G6P), NAD-linked glutamate dehydrogenase (GD1), NAD-phosphate-linked glutamate dehydrogenase (GD2), alkaline phosphate (ALP), adenylate kinase (ADK) and fumarase (FUM). For each enzyme, distinctive migration distances of lysate (electromorphs) was scored in order of decreasing anodal migration.

Table 2. *Explanation to signatures used in Figs 2–5*

	Illustrates one meningococcal strain
Upper row of figures	
	Illustrates serogroup, serotype and serosubtype for a sulphonamide-resistant meningococcal strain. (example: serogroup C: serotype 2a: serosubtype P1.2)
	Illustrates serogroup, serotype and serosubtype for a sulphonamide-susceptible meningococcal strain. (example: serogroup B:non-typable:serosubtype P1.9)
Lower row of figures	
	* Indicates that the strain is an index strain
	Results of the DNA-fingerprinting pattern when compared with the index strain is shown as
	DFP-identical
	DFP-indistinguishable
	DFP-different
	Illustrates results of MEE: The number within the circle designates the ET. (example: ET 7).

For the preparation of enzyme extract meningococci were grown overnight on Danish GC chocolate agar medium at 37 °C in a humid atmosphere containing 5% CO_2 (the same medium as mentioned under the section on DNA-fingerprinting). To obtain sufficient cells one or a few colonies of each strain was subcultured on three plates. The plates were incubated overnight as above. From all three plates the growth was scraped and suspended in three ml, 10 mM-Tris buffer; pH 8·0. The suspensions were constantly kept on crushed ice.

Cells were lysed by sonication (at 9 microns) for 2×30 sec (15 sec break in between). After lysis the suspension was centrifuged at 44400 g for 30 min. The supernatant (lysate) was distributed in five 1·8 ml kryotubes, and stored at −80 °C.

The recipes for buffers, recommended ingredients and the staining solutions recipes mentioned by Selander and colleagues were followed [7], except for a few modifications: instead of the recommended buffers, buffer D was used for the enzymes GD1, GD2 and ADK, for the enzyme FUM the double amount of malate dehydrogenase was used, and for the enzyme ADK was used 10 times the recommended amount of hexokinase.

In case enzyme activity was absent, a fresh enzyme extract was prepared and electrophoresed to ascertain that the enzyme concentration in the lysate had been adequate, before the enzyme was scored as a null. Each isolate was characterized by its combination of alleles over the number of enzymes assayed, and distinctive profiles of alleles, corresponding to unique multilocus genotypes, were designated electrophoretic types (ET's). Statistical analysis of the data was performed using a computer programme designed by T. S. Whittam and R. K. Selander [7], kindly provided by B. Nørrung, The Royal Veterinary and Agricultural Institute, Copenhagen, Denmark.

Explanation to signatures in Figs. 2–5 is shown in Table 2.

RESULTS

Among the 59 strains from patients involved in the 4 outbreaks 4 strains were designated index strains. Among the remaining 55 outbreak strains 52 were either DFP-identical or DFP-indistinguishable when compared with the one relevant out of the 4 index strains. This was only the case for 17 of the 37 strains isolated from sporadic cases caused by the same

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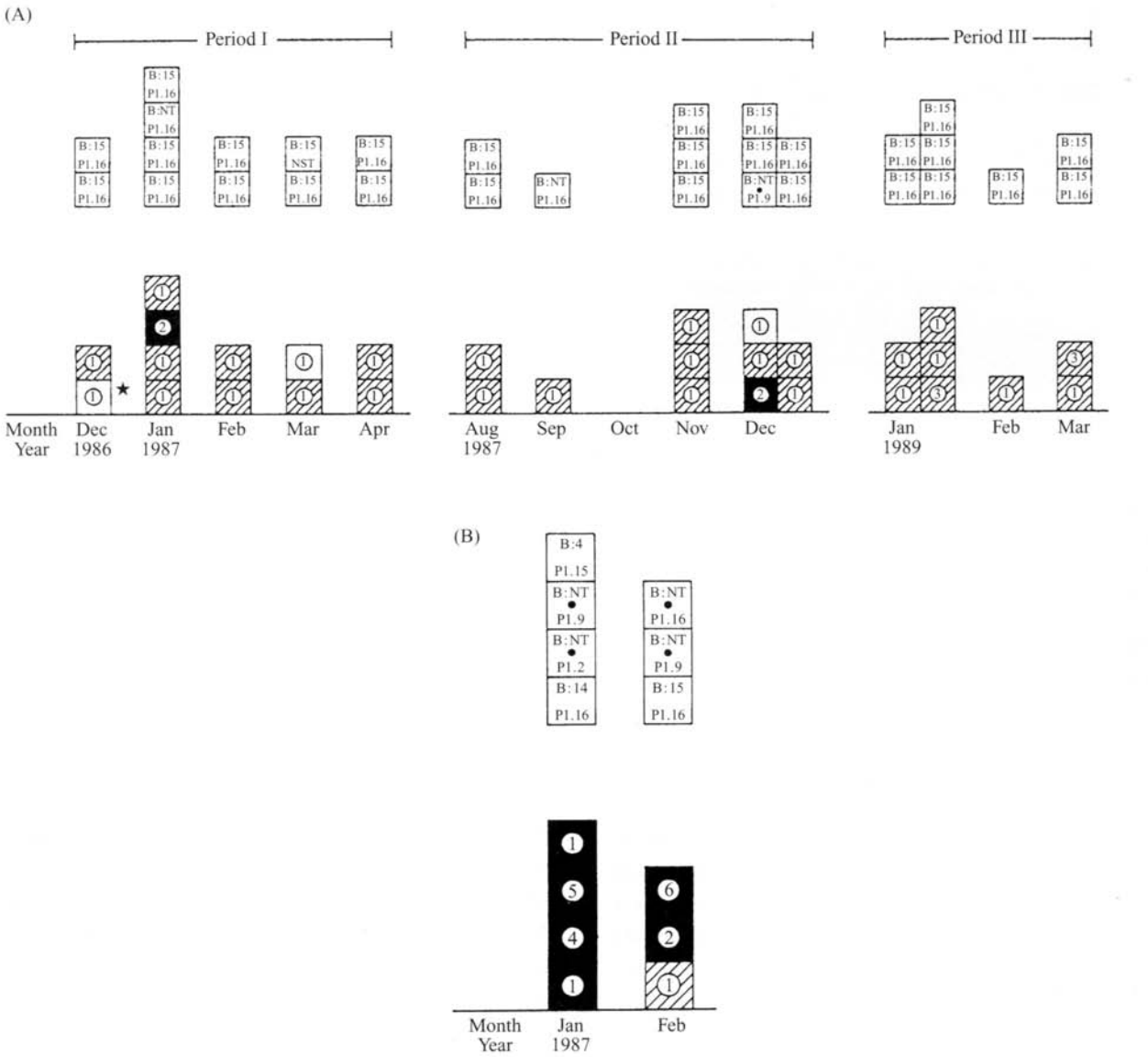

Fig. 2. (A) Outbreak in Frederiksborg: *N. meningitidis* serogroup B strains isolated from 31 cases occurring within three periods characterized by clustering of cases. Upper set of signatures phenotypes. Lower set of signatures genotypes. For explanation to signatures see Table 2. (B) Non-outbreak *N. meningitidis* serogroup B strains from seven sporadic cases occurring in the whole country during the same time as the Frederiksborg outbreak. Upper set of signatures phenotypes. Lower set of signatures genotypes. For explanation to signatures see Table 2.

serogroup of meningococci during the outbreak periods, and 5 of the 22 meningococcal strains isolated from healthy carriers. Among the 56 (52 + 4) DFP-identical or DFP-indistinguishable outbreak strains 5 different ETs were identified. Among 59 assumed outbreak strains a total of 4 were identified as genotypically distinct. Among the 37 mainly DFP-indistinguishable or DFP-different strains from sporadic cases 17 different ETs were identified, and among the 22 mainly DFP-different carrier strains 13 different ETs were identified. The index strains of the 2 serogroup B outbreaks were both ET1, and the index strains of the 2 serogroup C outbreaks were ET7 and ET 28, respectively, thus being closely related with a genetic distance of 0·27 [7].

Frederiksborg outbreak (Figs. 2A, 2B)

When compared with the index strain 28 of the 30 other strains isolated from patients involved in the Frederiksborg outbreak were DFP-identical or DFP-indistinguishable. These 28 strains belonged to the ET1/ET3-clone: 26 were ET1 and 2 were ET3. Two strains were DFP-different from the index strain; both

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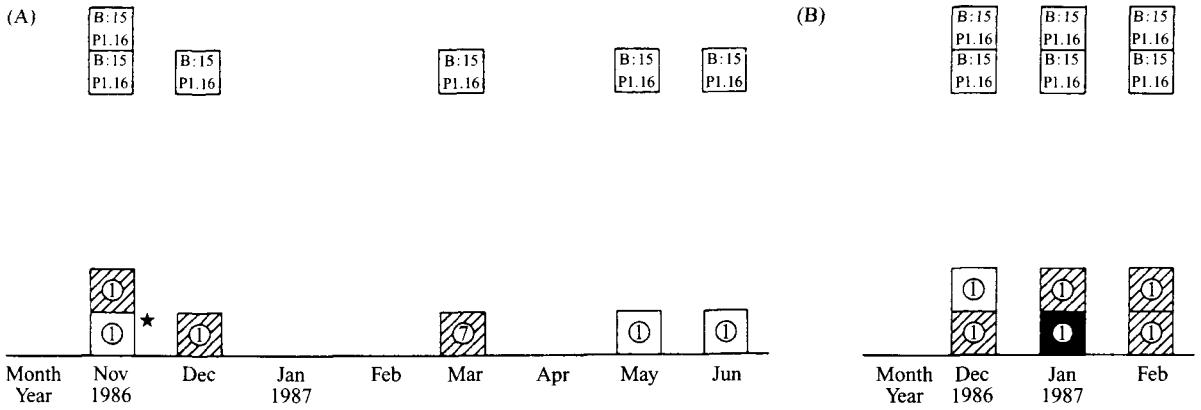

Fig. 3. (A) Ringsted outbreak: *N. meningitidis* serogroup B strains isolated from six patients involved in the outbreak. Upper set of signatures phenotypes. Lower set of signatures genotypes. For explanation to signatures see Table 2. (B) Ringsted: *N. meningitidis* serogroup B non-outbreak strains, i.e. six strains from sporadic cases occurring during the same period of time in Ringsted and environs. Upper set of signatures phenotypes. Lower set of signatures genotypes. For explanation to signatures see Table 2.

were ET2 (genetic distance 0·6). The results obtained from examination with DNA-fingerprinting and MEE were in accordance with the serological results, and further revealed differences between serologically identical or indistinguishable strains. One sulphonamide-resistant B:NT:P1.16 strain was DFP-indistinguishable and ET1, supporting that this strain probably belonged to the clone of outbreak strains. Another two DFP-different strains were ET2. They did not share identical DNA-fingerprinting pattern. One was sulphonamide-resistant B:NT:P1.16 and the other sulphonamide-susceptible B:NT:P1.9. It is therefore unlikely that these two strains belonged to the clone of outbreak strains.

For the strains selected from sporadic cases results obtained with DNA-fingerprinting emphasized those of the serological characterization: The one strain being DFP-indistinguishable and ET1 was the only B:15:P1.16. A B:NT:P1.16 strain was sulphonamide-susceptible, DFP-different and ET6. The two DFP-different strains that were ET1 and sulphonamide-resistant were B:4:P1.15 and B:14:P1.16, respectively.

Ringsted outbreak (Figs. 3A, 3B)

As all outbreak strains were B:15:P1.16 and DFP-identical or DFP-indistinguishable, the results obtained with DNA-fingerprinting emphasized those of the serological characterization. The outbreak strains were divided into 2 groups of 3 strains sharing identical DNA-fingerprinting pattern. When examined with MEE, one DFP-indistinguishable strain was ET7, with a genetic distance of 0·6 from ET1, while the index strain and the remaining four strains were ET1.

Since all strains from sporadic cases within the area were B:15:P1.16 strains and ET1, examination with MEE emphasized the serological results. However, one of these was DFP-different.

Holstebro outbreak (Figs. 4A, 4B)

Serologically all outbreak strains and strains from selected sporadic cases were sulphonamide-resistant C:2a:P1.2, but examination with DNA-fingerprinting and MEE revealed that 1 strain among the assumed outbreak strains and 2 strains among strains from sporadic cases did not belong to the ET28/ET29-clone (the outbreak strains).

Randers outbreak (Figs. 5A, 5B)

The C:2a:NST strains were all identical to the C:2a:P1.2 strains when examined with DNA-fingerprinting and ET7. Among the outbreak strains the non-typable strain (C:NT:P1.2) was DFP-indistinguishable and ET8. ET7 and ET8 belong to the same clone (genetic distance 0·133).

The strains selected from sporadic cases from the environs of the Randers area represented a variety of serotypes and subtypes. The 7 sulphonamide-resistant strains were C:2a:P1.2; 5 of these belonged to the ET7/ET8 clone of outbreak strains, and 2 were ETs with a genetic distance of less than 0·3 from the clone of outbreak strains. Five strains were sulphonamide-susceptible C:NT:P1.1 and ET10 (genetic distance 0·6). They were mutually DFP-

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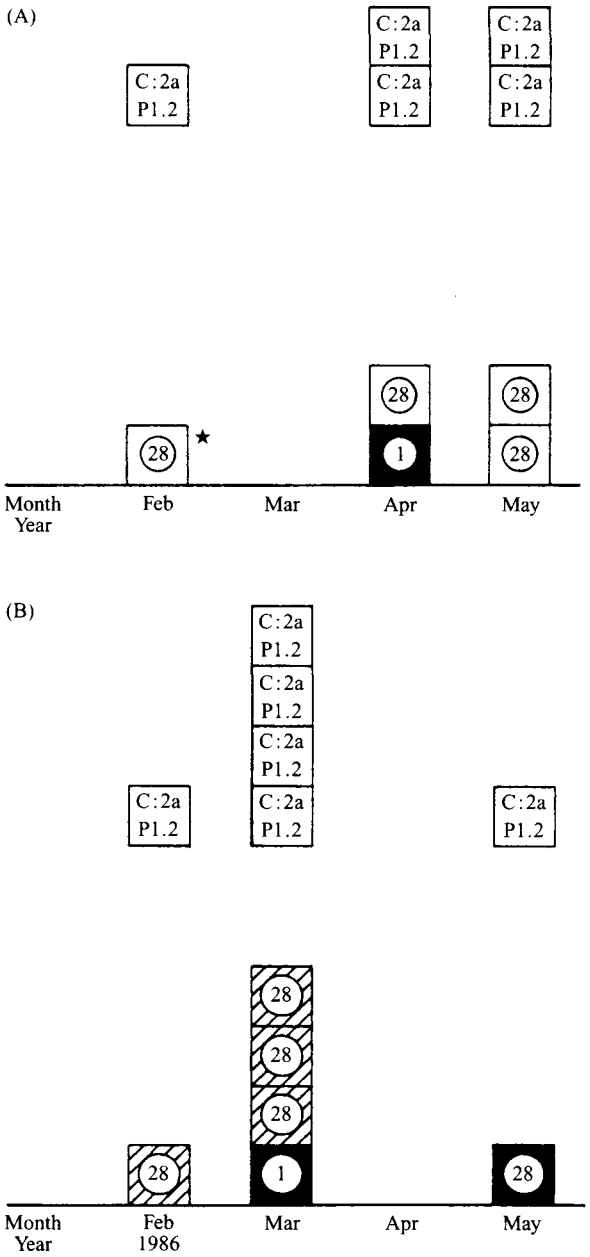

Fig. 4. (A) Holstebro outbreak: *N. meningitidis* serogroup C strains isolated from five patients involved in the outbreak. Upper set of signatures phenotypes. Lower set of signatures genotypes. For explanation to signatures see Table 2. (B) Holstebro: *N. meningitidis* serogroup C non-outbreak strains, i.e. isolates from six sporadic cases occurring during the outbreak period in Holstebro and environs. Upper set of signatures phenotypes. Lower set of signatures genotypes. For explanation to signatures see Table 2.

identical although DFP-different from the outbreak strain. The only two ET16 strains (genetic distance 0·6) were sulphonamide-susceptible C:2a:P1.6 and mutually DFP-indistinguishable. The remaining four strains were DFP-different and represented various ETs not related to the ET7/ET8 clone.

Carrier strains from Randers and Århus (Fig. 5C)

When characterized with DNA-fingerprinting and MEE none of the 12 serogroup C carrier strains from Randers was identical to the outbreak strain. Of the 10 carrier strains from Århus only one was DFP-identical and the same ET as the outbreak strain, while two strains were DFP-identical to the index strain of the outbreak, but did not belong to the clone of outbreak strains.

DISCUSSION

Until recently the major tools used in bacterial typing were serological tests, relying on polyclonal and monoclonal sera raised in animals, and biochemical tests. However, over the last 10 years developments in molecular immunology and genetics have provided a wealth of new tools for bacterial typing [8]. Meningococcal isolates have been characterized genetically by DNA-based approaches such as restriction fragment length polymorphism analyses and pulsed field gel electrophoresis fingerprinting [9,10], and by multilocus enzyme electrophoresis (MEE), which indexes the genotype [7]. Each method has its advantages and disadvantages. Many of the newer molecular typing techniques generate bands in a gel or on a membrane that are then compared for similarity. The methods are comparative and do not usually define a type against a reference band profile [11]. We have chosen to use one of these methods, a DNA fingerprinting technique with a high resolution capacity, and the MEE which has been widely used to study genetic relationships among meningococci [12].

The discriminating capacity of the DFP as used experimentally in this study and that of the MEE turned out to be suitable for epidemiological purposes. Minor differences between results of DNA fingerprinting and MEE typing as those obtained in one serogroup B and one serogroup C outbreak (Figs. 2A, 5A) might be expected, because small mutations or base rearrangements giving changes in the DFP may not result in amino acid substitutions affecting the enzyme electrophoretic mobility [13]. It was demonstrated that 3 strains judged to be outbreak strains based on serology and epidemiological data were genetically different, belonging to ET-clones with a genetic distance of 0·6 from the outbreak strains (Figs. 2A, 4A). Thus, investigations with DNA-fingerprinting and MEE revealed some strains to be of another clonal origin than the true outbreak

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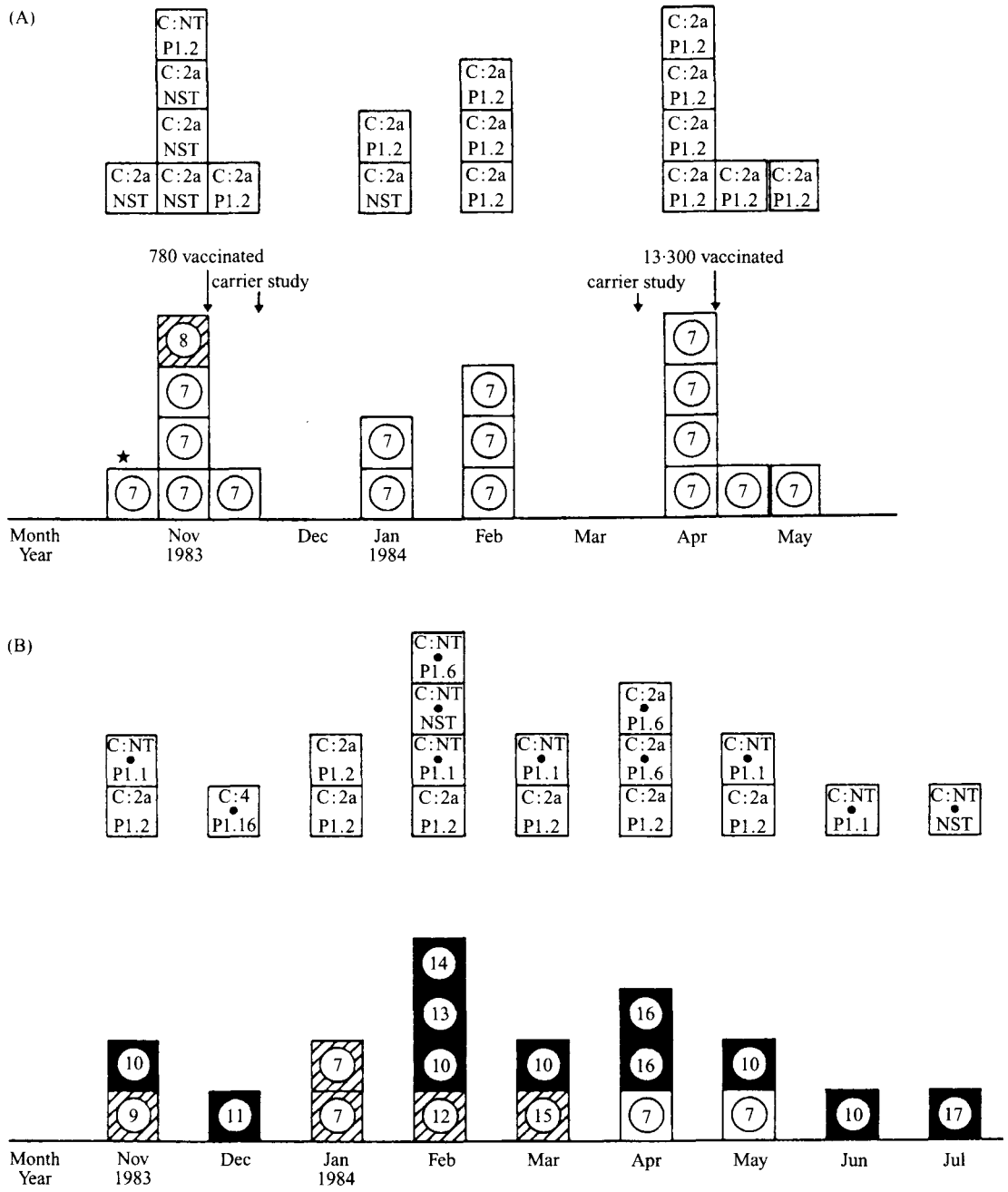

Fig. 5(A, B). For legend see facing page.

strains. These cases must be sporadic cases that occurred by chance within an outbreak due to one particular clone of meningococcal strains. On the other hand, for one serogroup B outbreak (Fig. 3A) and one serogroup C outbreak (Fig. 4A) it was demonstrated that the majority of strains from sporadic cases were genetically identical to the outbreak strains (Figs. 3B, 4B). These results indicate that the outbreak strains might have been more widespread than primarily thought.

In the present study, the strain designated as the index strain was the strain isolated from a patient registered as the first in an outbreak, i.e. the choice of index strain was based on knowledge about contact between patients involved in an identified outbreak. As observed by analysis of strains isolated during e.g. the Frederiksborg outbreak (Fig. 2A), sporadic cases can occur within an outbreak due to a well-defined clone of meningococci. If such a case occurs very early in the outbreak, one might be so unlucky primarily to choose this as the index strain. However, such a mistake would be revealed by the rapidly increasing

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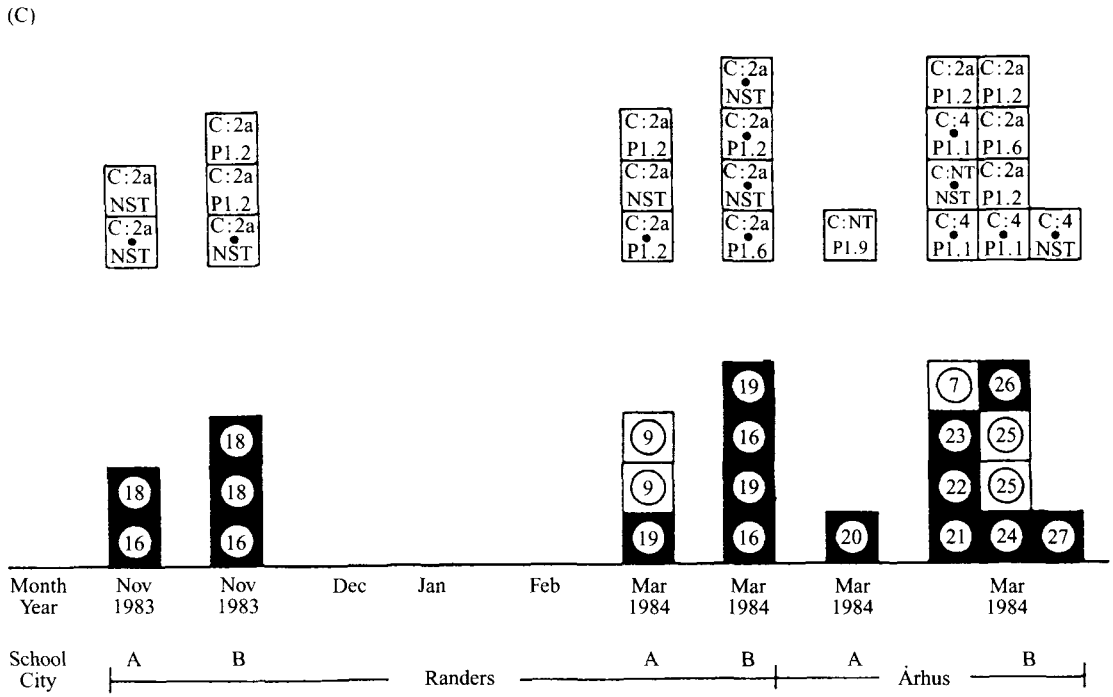

Fig. 5. (A) Randers outbreak: *N. meningitidis* serogroup C strains isolated from 17 patients involved in the outbreak. Upper set of signatures phenotypes. Lower set of signatures genotypes. For explanation to signatures see Table 2. (B) Randers: *N. meningitidis* serogroup C non-outbreak strains, i.e. isolates from 18 sporadic cases occurring during the outbreak period in Randers and environs. Upper set of signatures phenotypes. Lower set of signatures genotypes. For explanation to signatures see Table 2. (C) Carrier study: 12 serogroup C meningococcal strains isolated from healthy carriers in Randers and 10 serogroup C meningococcal strains isolated from healthy carriers in Århus during the same period of time as the outbreak in Randers took place. Upper set of signatures phenotypes. Lower set of signatures genotypes. For explanation to signatures see Table 2.

number of isolated strains being mutually similar and different from the 'index' strain.

During the present study it became evident that the majority of B:15 outbreak strains were DFP-indistinguishable from, and not DFP-identical to, the index strain. This finding corresponds to that of Kristiansen and colleagues, who found that B:15 meningococcal strains that caused invasive disease were similar, but not identical [14] and Renaud and colleagues, who investigated the use of DNA fingerprinting as an epidemiological marker on *Staphylococcus epidermidis* [15]. The pattern is in accordance with the widely accepted concept that phenotypically well-characterized bacteria causing a clear-cut outbreak are of the same clone [16].

This concept was also supported by the results obtained with MEE. Examined with MEE the majority of B:15 outbreak strains were ET1. Two strains (both ET3) (Fig. 2A), isolated from 2 patients with direct contact, were closely related to the outbreak strains; with a genetic distance less than 0·15 they belonged to the same clone. One strain was ET7 (Fig. 3A) with a genetic distance of 0·6 and did not belong to the clone of the outbreak strains. Probably this strain was isolated from a sporadic case occurring within the outbreak period. Based on serology and DNA-fingerprinting this strain could be considered part of the outbreak; in this case the value of serological characterization was limited compared with that of MEE.

The strains isolated from teenagers involved in three clusters in the Frederiksborg county [2] were DFP-identical and ET1 or ET3 in accordance with contact between the patients. Interestingly the strains isolated from the patients attending the same school but involved in the first and the third cluster 2 years apart were DFP-identical and ET1, supporting the observations of Cartwright and colleagues and Ashton and colleagues that meningococcal disease caused by B:15 meningococcal strains shows a relatively high incidence persisting in the same community over several years [17, 18].

When examined with DNA-fingerprinting and MEE all serogroup C outbreak strains, except one for the Holstebro outbreak, were DFP-identical and ET 28 (Holstebro outbreak) or ET7 (Randers outbreak).

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Within the Holstebro outbreak one strain was DFP-different and ET1 (genetic distance 0·6). These results obtained for serogroup C outbreaks show that DFP and MEE-profile are useful markers, and hereby also support the concept that phenotypically well-characterized bacteria causing a clear-cut outbreak are of the same clone.

Facinelli and colleagues obtained a slight difference between five serogroup C strains isolated during an outbreak period and another strain isolated 1 year later [19]. Mastrantonio and colleagues showed 62 serogroup C strains isolated from patients with meningococcal meningitis, living in different Italian towns, to be divided into two groups of DFP-indistinguishable strains [20]. They did not find any correlation among the DNA fingerprinting patterns and place or period of isolation of the strains.

The present study included 22 serogroup C meningococcal strains from healthy carriers, isolated in Randers from students attending the 2 schools at which 4 cases occurred (12 strains) and in the neighbouring city Århus (10 strains) during the same period of time as the Randers outbreak was identified. When characterized by serology 11 of the 12 carrier strains isolated from contacts to cases in Randers were indistinguishable from the virulent C:2a:P1.2 outbreak strain [3]. However, none of the 12 serogroup C carrier strains from Randers was genotypically identical to the outbreak strains when examined with DNA-fingerprinting and MEE. The present study thus indicated that tracing a virulent strain among contacts to index cases is irrelevant.

These results are in agreement with those of Mastrantonio and colleagues, who found the DFP of serogroup C carrier strains to be remarkably heterogenous [20], and with those of Caugant and colleagues, who identified only 7% and 9%, respectively, of the virulent strains of the ET-5-complex and the ET-37-complex among 109 healthy carriers in Norway, during a period of time in which strains of the two clones caused 91% of systemic disease in Norway [21]. In general swabbing of contacts is not recommended because of a pronounced lack of correlation between organisms causing meningococcal disease and those carried by close contacts [22].

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