MOLECULAR DIAGNOSIS AND TYPING

Identification of nasopharyngeal carriage of an outbreak strain of Neisseria meningitidis by pulsed-field gel electrophoresis versus phenotypic methods

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The clustering of four cases of meningococcal disease during a 3-month period in a small community with 2233 inhabitants prompted an interventional carrier survey in persons < 19 years old and in family members of the patients. The aims of the survey were to identify the nasopharyngeal carriers and the carriage rate of the outbreak strain, to offer chemoprophylaxis to those carrying the outbreak strain, and to study the discriminatory power of phenotypic methods versus pulsed-field gel electrophoresis (PFGE) on carrier isolates during an outbreak. A high percentage of the population in the age group 0–19 years (73.7%) participated in the study. Among the 469 samples collected in this age group, meningococci were grown from 43 (9.2%). The highest carriage rates were in the age group 18–19 years (36.4%). With a provisional definition of the outbreak strain (group B or non-groupable Neisseria meningitidis with reduced sulphonamide sensitivity), six carriers were identified. All were treated with a single dose of ofloxacin. Four of these persons (0.76% of all tested) were later shown to have harboured the outbreak strain when analysed by PFGE. Three of them were epidemiologically closely related to one of the index cases. Serogrouping alone is not sufficient for the identification of an epidemic strain of N. meningitidis. Complete concordance of type and subtype antigens correctly identified the outbreak strain in this study. PFGE is well suited for the identification of an outbreak strain of N. meningitidis versus non-epidemic strains in tonsillo-pharyngeal specimens.

Introduction

Meningococcal disease usually presents with a highly dramatic clinical picture of meningitis or septicaemia which can be rapidly fatal, i.e., within hours, in fulminant cases. This, in conjunction with the high mortality rate of meningococcal disease, often leads to public fear. Several strategies have been employed to minimise the risk of secondary cases among contacts of index cases. Among these are vaccination (if the causative organism belongs to serogroup A or C), chemoprophylaxis to eradicate nasopharyngeal carriage of meningococci, and preventive treatment. The drugs most widely used for chemoprophylaxis include sulphonamide, rifampicin or a quinolone. Because of widespread sulphonamide resistance, recent guidelines from the USA and England and Wales have recommended the use of rifampicin [1, 2]. Chemoprophylaxis has not been generally recommended in Norway. Instead, a national strategy of penicillin treatment for household members below the age of 15 years has been implemented since 1977 in an attempt to prevent clinical disease [3].

Meningococci may be isolated in c. 10% of the normal population. In a recent survey of the prevalence of meningococcal carriage in Norway, the overall carriage rate was 9.6%, and 8.8% of the isolates represented clones that accounted for the majority of cases of systemic meningococcal disease in Norway during the last two decades [4]. It is generally thought that chemoprophylaxis should be kept to a minimum, both to avoid development of antibiotic resistance and also because of the assumption that carriage of non-pathogenic meningococci may be protective. Thus, it appears logical that
chemoprophylaxis should be restricted to individuals at the highest risk, i.e., household members of cases and, in particular, those harbouring the epidemic pathogenic meningococcal strain [5, 6].

In the period 1 Oct.–31 Dec. 1995, four cases of meningococcal disease, one of them fatal, occurred in the village of Buvika, a small community with 2233 inhabitants. No obvious epidemiological connection between these four cases could be established. The Neisseria meningitidis strain isolated was serogroup B, serotype 15, subtype P1.7,16, sulphonamide resistant, and belonged to a distinct clone of the ET-5 complex [7], not previously identified in Norway. The high incidence of meningococcal disease in this small community, caused by a strain suspected to be a highly virulent clone, was alarming. Therefore, a survey was performed that aimed to identify nasopharyngeal carriers of the outbreak strain, and to offer chemoprophylaxis to those who carried this strain. The applicability and discriminatory power of pulsed-field gel electrophoresis (PFGE) for the identification of the outbreak strain in nasopharyngeal isolates in comparison to phenotypic methods were also studied.

Materials and methods

Population studied

The village of Buvika is situated in the municipality of Skan in the county of Sør-Trøndelag located in the central part of Norway. The total population in Skan is 5800, with 2233 persons living in Buvika. Inhabitants in the age group 0–19 years were selected for testing. In addition, nasopharyngeal samples were obtained from family members of the patients and from school-teachers. Parents and school-teachers were contacted by letter. The inhabitants were informed by the local press. Written consent was obtained from the parents or guardians of children under the age of 12 years.

Collection of throat culture samples

Tonsillo-pharyngeal samples were collected by three physicians from the Department of Microbiology. Swabs were rubbed against the tonsillar regions on both sides and the posterior pharyngeal wall and placed in modified Amies Transport Medium (Transwab, Medical Wire and Equipment, Corsham, Wiltshire). Most samples from the school children were collected within 1 day, and the remainder were taken 3 days later. College students and pre-school children were sampled at the local physicians’ office during the same week. Samples from individuals who were given chemoprophylaxis were obtained 3 weeks later.

Culture and identification

Most of the throat swabs were plated on the day of collection or the day after, on modified New York City Medium, made from Gonococcal Medium Base (Difco) with saponin-lysed defibrinated ox blood 10%, yeast extract 1% and glucose 0.1%. The medium was made selective by adding (mg/L) lincomycin 1, colistin sulphate 6, amphotericin B 1 and trimethoprim lactate 6.5. Plates were incubated at 35°C in air with CO2 5% for 2 days. Colonies of Neisseria spp. were subcultured and identified by API NH (bioMérieux SA, Lyon, Marcy-l’Etoile, France).

SEROGROUPING AND SEROTYPING

SEROGROUPING was performed by slide agglutination of bacterial suspensions in saline heated to 100°C for 5 min. Rabbit antisera against group A and group C and a monoclonal anti-group B antibody (Murex Diagnostics, Dartford) were used. Restricted serotyping and subtyping were performed with monoclonal antibodies (MAbs) against serotype antigen P15 and subtype epitopes P1.7 and P1.16, by a dot-blot method as described by Wedege et al. [8] to enable rapid identification of the outbreak strain. All strains were subsequently tested with the complete set of MAbs.

Susceptibility testing

Minimum inhibitory concentration (MIC) of sulpha-diazine (a sulphonamide) was determined on Mueller Hinton Sensitivity Test Medium (Difco) with defibri-nated horse blood 5% by the E-test method (AB Biodisk, Solna, Sweden).

Multilocus enzyme electrophoresis

The isolates were characterised by their combination of alleles at 14 enzyme loci as described previously [4, 7].

Pulsed-field gel electrophoresis

Genomic DNA was prepared by minor modifications of the method described by Poh and Lau [9]. Briefly, meningococci were subcultured overnight on blood agar and harvested in TN buffer (0.1 M Tris-HCl, pH 7.5; 1 M NaCl). After centrifugation, the pellet was resuspended in 0.3 ml of TN buffer and mixed with 0.3 ml of low-melting point agarose 2% at 58°C, pipetted into a plug mould and allowed to solidify. The agarose plugs were transferred into 4 ml of EC lysis solution (6 mM Tris-HCl, 100 mM EDTA, 1 M NaCl, Brij-58 0.5%, deoxycholate 0.2%, lysozyme 0.5 mg/ml and RNAase 0.025 mg/ml) and incubated overnight at 37°C with gentle shaking. The plugs were then further incubated in ESP solution (0.5 M EDTA, Sarcosyl 1% and proteinate K 0.5 mg/ml) for 24 h at 50°C with gentle shaking followed by washing four times (each 1 h) with TE buffer (10 mM Tris-HCl, 0.1 M EDTA) at 22°C. After washing, plugs were digested with restriction endonucleases SfiI and SpeI (4 h at 37°C) before PFGE (CHEF DR-II, BioRad). The electrophoresis conditions were: agarose 1% gel, 6 V/cm, 14°C, 28 h.
switch time 10–45 s (after $SfiI$ digestion) or 0.2–30 s (after $SpeI$ digestion). The gel was stained with ethidium bromide.

The criteria used for interpreting the PFGE patterns were in accordance with those proposed by Tenover et al. [10]. A strain was considered identical to the outbreak strain if the two patterns were indistinguishable, or closely related if a single genetic event could explain the difference between the patterns.

### Criteria for chemoprophylaxis

Before the start of the study it was decided that persons carrying meningococci with defined phenotypic characteristics — i.e., serogroup B or non-groupable strains that showed decreased susceptibility to sulphadiazine (MIC $\geq 8$ mg/L) — should be offered eradication therapy with rifampicin 10 mg/kg twice daily for 2 days (for those < 16 years old) or a single dose of ofloxacin 400 mg ($\geq 16$ years old).

### Results

#### Participation

A total of 523 persons from the Buvika population participated in the study. Of these, 469 were <19 years old, correlating with a participation rate of 73.7% of the population in this age group. The proportion of inhabitants in the different age groups that were sampled varied from 12% in the age group 1–2 years to 100% in age group 6–15 years (elementary school and junior high school) (Table 1). Numbers above 100% are explained by the fact that population statistics were from the previous year.

#### Carriage rate

From a total of 49 carriers (24 females and 25 males), 51 isolates of $N$. meningitidis were obtained, giving an overall carriage rate of 9.4%, varying from zero in the age group 6–7 years to 36.4% in the age group 18–19 years (Table 1). $N$. lactamica was isolated from 76 samples (14.5%), 52 (25%) in the age group 0–9 years. The highest carrier rate, 41.5%, was in the age group 4–5 years.

#### Phenotypic characteristics of the strains

A total of 51 isolates of $N$. meningitidis, from 49 persons, was characterised by serogroup, serotype, subtype and sulphonamide sensitivity. Twenty-two isolates were serogroup B, one was serogroup C, none was serogroup A and 28 were non-groupable (NG). The resolution with respect to restricted type/subtype is given in Table 2. The strains were subsequently tested with the complete set of MAbS (not shown). Fig. 1 shows the distribution of the 51 isolates according to their MIC of sulphadiazine as determined by the E-test. Seven isolates displayed decreased susceptibility to sulphadiazine, of which four were serogroup B (MIC $\geq 256$, 256, 64 and 8 mg/L), two were NG (MIC 96 and 64 mg/L) and one was serogroup C (MIC 24 mg/L).

### Table 1. Carriage of $N$. meningitidis in different age groups in Buvika village during an outbreak of meningococcal disease in the period 1 Oct.–31 Dec. 1995

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of inhabitants*</th>
<th>Number tested (%)</th>
<th>Number of carriers (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>66</td>
<td>8 (12)</td>
<td>0</td>
</tr>
<tr>
<td>2–3</td>
<td>65</td>
<td>31 (47.7)</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>4–5</td>
<td>70</td>
<td>53 (75.7)</td>
<td>2 (3.8)</td>
</tr>
<tr>
<td>6–7</td>
<td>56</td>
<td>57 (101.8)</td>
<td>0</td>
</tr>
<tr>
<td>8–9</td>
<td>56</td>
<td>56 (100)</td>
<td>3 (5.3)</td>
</tr>
<tr>
<td>10–11</td>
<td>63</td>
<td>67 (106.3)</td>
<td>4 (6.0)</td>
</tr>
<tr>
<td>12–13</td>
<td>60</td>
<td>60 (100)</td>
<td>3 (5.0)</td>
</tr>
<tr>
<td>14–15</td>
<td>66</td>
<td>66 (100)</td>
<td>9 (13.6)$^1$</td>
</tr>
<tr>
<td>16–17</td>
<td>74</td>
<td>49 (66.2)</td>
<td>13 (26.5)$^2$</td>
</tr>
<tr>
<td>18–19</td>
<td>60</td>
<td>22 (36.7)</td>
<td>8 (36.4)</td>
</tr>
<tr>
<td>$\geq 20$</td>
<td>1597</td>
<td>54 (3.4)</td>
<td>6 (11.1)$^3$</td>
</tr>
<tr>
<td>Total</td>
<td>2233</td>
<td>523 (23.4)</td>
<td>49 (9.4)</td>
</tr>
</tbody>
</table>

$^1$One carrier of the outbreak strain.
$^2$Two carriers of the outbreak strain.

### Table 2. Phenotypic characteristics of 51 $N$. meningitidis carrier isolates in the Buvika population

<table>
<thead>
<tr>
<th>Number of isolates</th>
<th>MIC of sulphadiazine (mg/L)</th>
<th>Serogroup*</th>
<th>Serotype Subtype$^1$</th>
<th>Outbreak strain$^1$ (yes/no)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3/0</td>
<td>B</td>
<td>15:P1.7,16</td>
<td>3/0</td>
</tr>
<tr>
<td>1</td>
<td>1/0</td>
<td>NG</td>
<td>15:P1.7,16</td>
<td>1/0</td>
</tr>
<tr>
<td>1</td>
<td>1/0</td>
<td>NG</td>
<td>15:N</td>
<td>0/1</td>
</tr>
<tr>
<td>17</td>
<td>1/16</td>
<td>B</td>
<td>N:N</td>
<td>0/17</td>
</tr>
<tr>
<td>2</td>
<td>0/2</td>
<td>NG</td>
<td>N:P1.7</td>
<td>0/1</td>
</tr>
<tr>
<td>2</td>
<td>0/2</td>
<td>B</td>
<td>N:P1.7</td>
<td>0/1</td>
</tr>
<tr>
<td>1</td>
<td>0/1</td>
<td>NG</td>
<td>N:P1.16</td>
<td>0/1</td>
</tr>
<tr>
<td>23</td>
<td>0/23</td>
<td>NG</td>
<td>N:N</td>
<td>0/23</td>
</tr>
<tr>
<td>1</td>
<td>1/0</td>
<td>C</td>
<td>N:N</td>
<td>0/1</td>
</tr>
<tr>
<td>51</td>
<td>7/44</td>
<td></td>
<td></td>
<td>4/47</td>
</tr>
</tbody>
</table>

*NG, non-group A, B or C.
$^1$N, type/subtypes other than 15:P1.7,16.
$^2$Outbreak strain according to PFGE pattern.
Fig. 1. Distribution of 51 N. meningitidis carrier isolates according to MIC (mg/L) of sulphadiazine as determined by the E-test.

PFGE patterns
Reproducible results were obtained by DNA digestion with SfiI and SpeI. Fewer bands were obtained with SfiI (7–13 bands) than with SpeI (10–18 bands). All 51 isolates were typable and could be divided into ≥25 PFGE patterns. After digestion with SfiI or SpeI, four isolates and the outbreak strain had identical PFGE patterns. These four isolates were consequently considered to belong to the same clone as the outbreak strain. The remaining 47 carrier isolates displayed PFGE patterns that were easily distinguished from the outbreak strain. Fig. 2 illustrates the patterns observed with the Norwegian epidemic strain 44/76, the outbreak strain isolated in blood culture from one of the index cases, and the six isolates that fulfilled the provisional phenotypic definition of the outbreak strain. Two representative patterns observed with six and five isolates, respectively, are shown for comparison.

Carriage of the outbreak strain
Six persons carried a N. meningitidis strain that fulfilled the criteria for chemoprophylaxis (i.e., N. meningitidis serogroup B or NG strains with decreased sulphonamide sensitivity). All were ≥16 years old and were treated with a single dose of ofloxacin 400 mg. Meningococci were absent in throat samples collected 3 weeks later. Three of the six isolates were serogroup B, type 15:P1.7,16; one was NG, type 15:P1.7, 16; one was NG, type 15, and one was serogroup B, non-typable. The first four isolates were genotypically identical to the outbreak strain when examined by PFGE. Two of the four persons carrying the outbreak strain lived in the same household as one of the patients (mother and brother), one was a close friend of this family. One carrier of the outbreak strain had no known contact with the patients or the other carriers. With multilocus enzyme electrophoresis, only these four isolates were found to belong to the ET-5 complex. All four isolates represented the new variant that was responsible for the outbreak.

Discussion
The extremely high incidence of meningococcal disease in a small community prompted this interventional carrier survey. Norwegian guidelines do not recommend chemoprophylaxis for nasopharyngeal carriage eradication; instead, penicillin treatment is given orally to household members below the age of 15 years. Penicillin treatment does not eradicate meningococcal carriage efficiently, while short-term rifampicin or ofloxacin treatment is effective [11, 12]. In an attempt to try to prevent secondary cases, and at the same time reduce anxiety among the inhabitants, we decided to perform this study and to offer chemoprophylaxis to those carrying the outbreak strain. Ideally, chemoprophylaxis should be restricted to those carrying the outbreak strain and should be initiated as soon as possible after the index case. In meningococcal disease clusters, the majority of new clinical cases appear within the first week [13]. A restrictive use of antibiotics should reduce the risks of development of antimicrobial resistance, and the potential for interference with the development of cross-protective antibodies caused by colonisation with low-pathogenic Neisseria spp. [14].

The present survey started 2 weeks after the last case was diagnosed. To shorten the time between sampling and chemoprophylaxis, simple phenotypic criteria were chosen to identify potential carriers of the outbreak strain. The provisional definition of the outbreak strain

Fig. 2. N. meningitidis PFGE patterns produced with restriction endonuclease SfiI. Lane 1, mol. wt standard; 2, Norwegian reference strain 44/76; 3, index patient; 4–9, meningococci identified by provisional phenotypic criteria defined for implementing ofloxacin treatment; 10–11, representative carrier strains of which more than three isolates produced the same PFGE pattern.
included both group B and NG strains with reduced sulphonamide susceptibility, as expression of capsular polysaccharide may vary in nasopharyngeal isolates [15]. Reduced sulphonamide susceptibility as the only criterion identified all (four) outbreak strain isolates; three other isolates also had reduced sulphonamide sensitivity. The combined criteria, reduced sulphonamide susceptibility and group B or NG strains, included the outbreak strain isolates and two additional isolates. Thus, two individuals who received chemoprophylaxis did not carry the outbreak strain. A complete agreement of type and subtype antigens between the outbreak strain and the carrier isolates correctly identified the outbreak strain.

PFGE is well suited for the identification of an epidemic strain versus non-epidemic strains in tonsillo-pharyngeal specimens during an outbreak of meningococcal disease. The main drawback of this method is that it takes 4–5 days for completion, and the technique is available only in a few well-equipped laboratories.

Nearly 75% of the inhabitants in the age group 0–19 years participated in the survey. This high rate was achieved in spite of the very short time available for planning; only one short information letter was sent to the families who had children at school. Close cooperation with the school administrators and teachers was essential in organising the large-scale collection of samples. Among the 523 individuals tested, 24 females and 25 males carried meningococci, giving an overall carriage rate of 9.4%, with the highest rate (36.4%) in the age group 18–19 years, figures that are similar to those found in studies in non-epidemic situations in Norway [4]. The present study found equal numbers of male and female carriers; a male excess of carriers has been reported in other studies [4, 15]. With genotypic identification methods, four (0.76%) of 523 individuals were carriers of the outbreak strain; three of them were in the age group 14–17 years and one was >20 years old. Three of the isolates were found among individuals who were epidemiologically related to one of the patients. One carrier had no known contact with any of the index patients or with the other carriers of the outbreak strain. Using phenotypic identification methods, the Stonehouse survey identified 1.4% as carriers of an outbreak strain [15]. The present finding of a similarly low carriage rate of the outbreak strain supports the hypothesis that such strains may be less transmissible and more virulent than other meningococci [14]. It also accords with results from other studies that have shown a restricted pattern of spread of outbreak strains as judged from the occurrence of co-primary and secondary cases of meningococcal disease [13]. A high N. lactamica carriage rate was found compared with the rates reported in other studies [14, 15]. It is thought that colonisation with N. lactamica is beneficial, as it may induce protective antibodies cross-reacting with N. meningitidis [14]. No new cases of meningococcal disease have occurred in this community in the succeeding 2 years.

Complete concordance of type and subtype antigens correctly identified the outbreak strain in this study. Thus, a very restricted number of serotype/subtype reagents was necessary for identification of the outbreak strain. Decreased sensitivity to sulphonamides, a phenotypic trait common in group B strains isolated in Norway in recent years, can be used for provisional definition of an outbreak strain in order to start treatment of carriers as early as possible during an outbreak. PFGE is well suited for the identification of an outbreak strain among tonsillo-pharyngeal meningococcal isolates. If a future strategy should be based upon giving selective chemoprophylaxis to those carrying an outbreak strain, methods that could identify an outbreak strain within a shorter period of time, and with as good discriminatory power as PFGE, are highly desirable.

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References


