Epidemiology of meningococcal disease in Denmark 1974–1999: contribution of the laboratory surveillance system

I. LIND* AND L. BERTHELSEN

Neisseria Unit, Department of Respiratory Infections, Meningitis and Sexually Transmitted Infections, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen, Denmark

SUMMARY

The Danish meningococcal disease laboratory surveillance system was established in 1974, based on close collaboration between local Departments of Clinical Microbiology and the Reference Laboratory at Statens Serum Institut. The completeness of the clinical notification system integrated with the laboratory surveillance system has been estimated to be more than 95%. Overall 4257 (79%) of 5356 cases of meningococcal disease notified during 1974–1999 were confirmed by culture of *Neisseria meningitidis*. The proportion of culture-confirmed cases ranged from 70% in 1989 to 89% in 1980. Only 26 patients (0·6%) with culture-confirmed meningococcal disease were not notified. Serological phenotype and susceptibility to penicillin and sulphonamide were determined for all isolates. Multilocus enzyme electrophoresis and/or DNA-based analyses were used for the assessment of clusters and outbreaks. Meningococcal antibody tests and counter-immunoelectrophoresis were used for the ascertainment of suspected cases. These combined systems allowed timely and reliable management of outbreaks and identification of clusters.

INTRODUCTION

In Denmark, surveillance and prevention of communicable diseases have been regulated by royal prescripts and governmental circulars for more than 200 years. The notification system for meningococcal disease (epidemic meningitis) was established in 1867. Figure 1 illustrates changes in incidences of meningococcal disease during the nineteenth century. As in some other European countries [1, 2] and the U.S. Army [3] epidemics occurred during World War I and World War II. Notifications were not available from 1963 to 1967.

In 1974 the compulsory clinical notification system was supplemented with a voluntary laboratory surveillance system based on close collaboration

between local Departments of Clinical Microbiology and the National Neisseria Reference Laboratory at the Statens Serum Institut. At that time vaccines protecting against serogroup C meningococcal disease had become available [4] and in 1974 the newly developed serogroup A vaccine [5] was found to be effective during an epidemic caused by sulphonamide-resistant serogroup A meningococci in Finland [6]. It was, therefore, considered important to monitor the proportions of invasive meningococcal strains by serogroup, and to evaluate laboratory methods that could be useful in the ascertainment of clinically suspected, but culture-negative cases of meningococcal disease. Further methods for determination of epidemiological markers were subsequently established and found to be useful tools in the detection and investigation of clusters of cases or outbreaks of meningococcal disease.

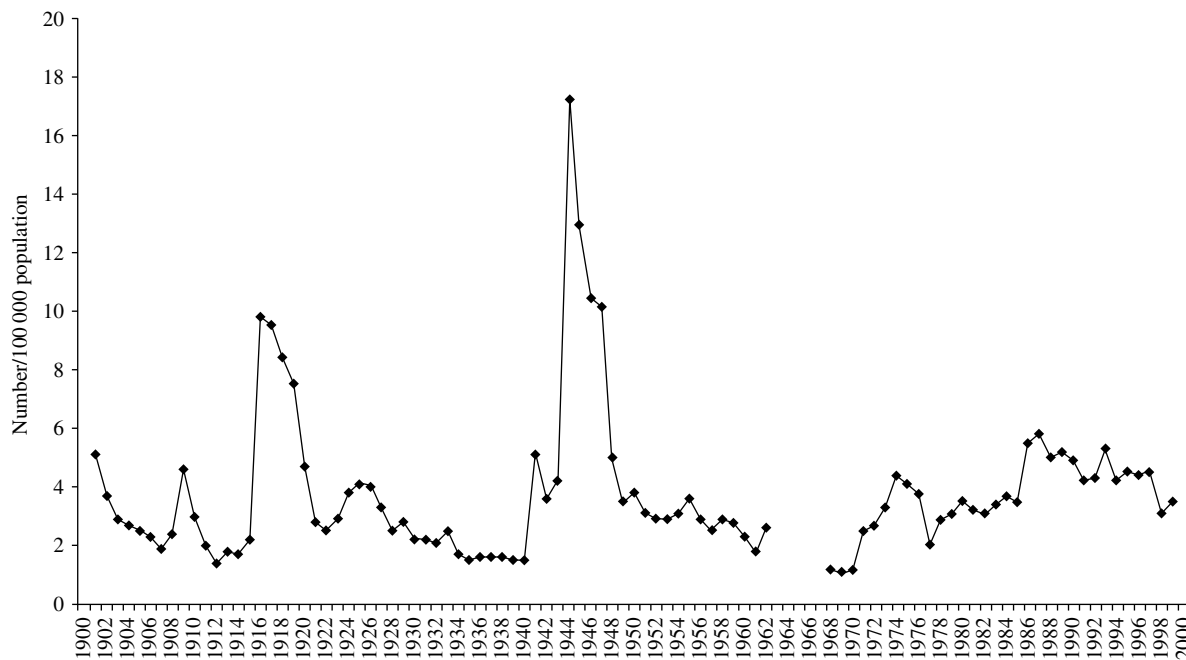* Author for correspondence.
(Email: il@ssi.dk)

Fig. 1. Meningococcal disease in Denmark 1901–1999: numbers of notified cases per 100 000 per year.

In this paper we describe the development of services at the Danish Reference Laboratory and how laboratory surveillance data was integrated with information from the compulsory clinical notification system, which had been administered by the Department of Epidemiology, Statens Serum Institut from 1980. Recent analysis showed that this active and integrated surveillance system was highly efficient, probably identifying more that 95% of all cases of meningococcal disease occurring in the country [7].

METHODS

Laboratory surveillance system

From 1974, a panel of laboratory methods for determination of epidemiological markers was gradually established. When a patient with clinically suspected meningococcal disease is admitted to hospital the attending physician must inform the local Medical Officer of Health (MOH) by telephone and also the Department of Epidemiology at the Statens Serum Institut by mailing a completed Communicable Disease Notification Form. The local Department of Clinical Microbiology refers any meningococcal isolate from the patient to the reference laboratory at Statens Serum Institut as soon as available. Serogrouping is performed without delay and the

MOH as well as the Department of Epidemiology are informed about the receipt of the meningococcal isolate and the result of serogrouping. If the isolate belongs to a serogroup uncommon among invasive strains (W-135, 29E, X, Y, Z) the attending physician is notified that the prevalence of complement deficiency is increased in this selected group of patients. Isolates are stored in liquid nitrogen until further characterization can be carried out, and then lyophilized. The strain collection at the reference laboratory comprises all strains received since 1974; in addition, selected materials from the period 1940–1973 have been kept as lyophilized samples.

The laboratory analyses available fall into three categories: tests performed routinely, tests performed on request and supplementary tests used when discrimination between phenotypically identical strains is needed.

Tests performed routinely

Serological phenotyping

This category comprises serological grouping, typing and subtyping. Serogrouping is performed on receipt of each isolate; the other tests are normally performed every month. The methods used for serological characterization have been described previously [8]. Serotyping using the co-agglutination test was

Table 1. *Serotyping and subtyping of* N. meningitidis *in Denmark 1985–1999*

| | | Panel of monoclonal antibodies used for | |
| | | Typing | Subtyping |
Year(s)	Method		
1985–1986	Co-agglutination	2a, 2b, 15	—
1987	ELISA	2a, 2b, 4, 14, 15	P1.1, P1.2, P1.6, P1.9, P1.15, P1.16
1988–1992	ELISA	1, 2a, 2b, 4, 14, 15, 16	P1.1, P1.2, P1.6, P1.9, P1.15, P1.16
1993–1995	ELISA	1, 2a, 2b, 4, 14, 15, 16	P1.1, P1.2, P1.4, P1.5, P1.6, P1.7, P1.9, P1.10, P1.12, P1.14, P1.15, P1.16
1996–1999	ELISA	1, 2a, 2b, 4, 14, 15, 16, 21, 22	P1.1, P1.2, P1.4, P1.5, P1.6, P1.7, P1.9, P1.10, P1.12, P1.14, P1.15, P1.16

established in 1985; in 1987 the ELISA method was introduced and the panel of monoclonal antibody reagents was expanded to include reagents for serosubtyping (Table 1). The panel of monoclonal antibodies available for typing and subtyping have been updated continuously. Initially, the monoclonal antibodies were provided by the National Institute of Public Health and the Environment, Bilthoven, The Netherlands, and more recently by the National Institute for Biological Standards and Control, Potters Bar, UK.

Antimicrobial susceptibility testing

All strains were tested for susceptibility to penicillin and to sulphonamide (sulphamethoxazole). In addition, all strains were examined for susceptibility to other antibiotics in current use for the prevention of secondary cases of meningococcal disease (rifampicin from 1982 to 1992, ciprofloxacin since 1993). Strain susceptibility to sulphamethoxazole, penicillin, and ciprofloxacin was determined by the agar incorporation method. Resistance to rifampicin was detected by an agar disc diffusion method.

Sulphamethoxazole. The basic medium used was Danish sensitivity agar [9, pp. 55–56] and the inoculum applied was 10 times less than that used for the other antibiotics tested. The range of concentrations of sulphamethoxazole was from 0·031 to 512 mg/l with four-fold dilution steps. Three control strains were established in 1974 and since then have been included in all experiments: KI [strain SSI 747/74, serogroup C, minimum inhibitory concentration (MIC) 128 mg/l], KII (strain SSI 357/74, serogroup B, MIC 0·5 mg/l) and KIII (strain SSI 2607/74, serogroup A, MIC 8 mg/l). Strains with an MIC $\geqslant 8$ mg/l were designated resistant.

Penicillin and ciprofloxacin. The basic medium was chocolate agar [9, p. 23]. The density of the bacterial suspension used as inoculum was about 10^8 c.f.u./ml. The range of concentrations of penicillin was from 0·01 to 2·4 mg/l, four-fold dilution steps until 1993 and then 0·016–0·5 mg/l, two-fold dilution steps. The traditional use of four-fold dilutions of penicillin incorporated in the plates meant that before 1993 all strains representing the two MIC values generally used as cut-off for grouping strains as susceptible (MIC 0·063 mg/l) or less susceptible ($=$moderately resistant) (MIC 0·125 mg/l) fell into one group, namely those with an MIC at 0·15 mg/l. The range of concentrations of ciprofloxacin was 0·002–0·016 mg/l, two-fold dilution steps. Since an appropriate panel of *N. meningitidis* control strains could not be established, the WHO *N. gonorrhoeae* reference strains B, C and D plus a ciprofloxacin-resistant *N. gonorrhoeae* strain (SSI 141/91) were used.

Rifampicin. The disc content was 30 μg rifampicin at which load the inhibition zones for resistant strains are zero.

Tests performed on request

Counter-immunoelectrophoresis (CIE)

Direct detection of meningococcal capsular polysaccharides A, B and C was performed as previously described [10].

Meningococcal antibody test (MAT)

The MAT is an in-house complement fixation test using heat-killed whole cells from a pool of meningococcal strains as antigen and fresh guinea-pig serum as complement source. The result is expressed as degree of potency (DP). Either seroconversion to
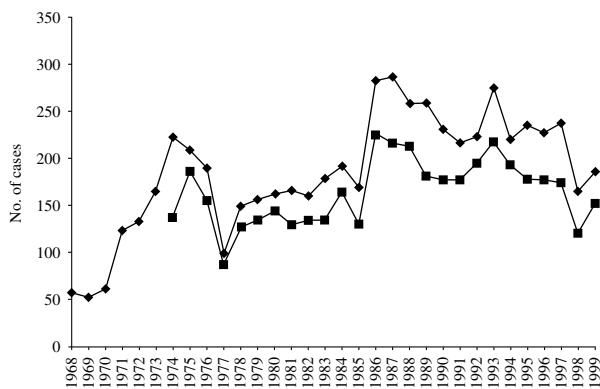
Fig. 2. Meningococcal disease in Denmark 1968–1999: annual numbers of notified (–◆–) and culture-confirmed (–■–) cases.
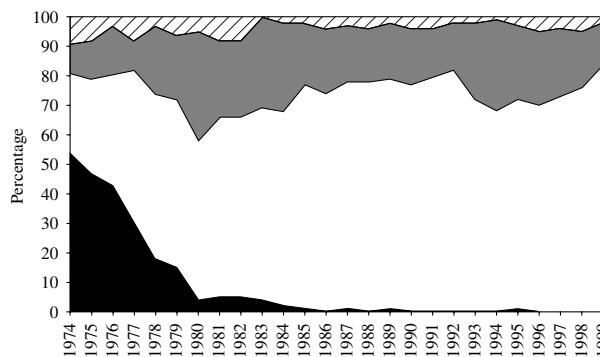

Fig. 3. *N. meningitidis* isolates from patients with meningococcal disease in Denmark 1974–1999: proportions of strains by serogroup. ■, Serogroup A; □, serogroup B; ▨ serogroup C; ▨, other.

⩾3 DP or a change in titre of ⩾3 DP were considered indicative of current meningococcal infection [11].

ELISAs

The assays used for detection of serogroup B-specific (IgM) and serogroup C-specific (IgM and IgG) antibodies have been described previously [12].

Supplementary tests

Multilocus enzyme electrophoresis (MEE) and DNA fingerprinting (DFP) were performed as previously described in detail [13], pulsed-field gel electrophoresis (PFGE) was performed as described by Bygraves and Maiden [14] and ribotyping was performed using an automated system (Ribo Printo, Qualicon, USA) as described by Jensen et al. [15].

RESULTS

Overall 4257 (79 %) of 5356 cases of meningococcal disease notified from 1974 to 1999 were confirmed by culture of *N. meningitidis*. Except in 1974, the year in which the laboratory surveillance system was initiated, the proportion of known culture-confirmed cases ranged from 70 % in 1989 to 89 % in 1980. The curves in Figure 2 illustrate that the annual number of notified cases of meningococcal disease and the annual number of culture-confirmed cases concordantly reflect changes in the prevalence of disease. During the whole period the referral rate was close to 100 % and very few strains were lost before or during transport to the reference laboratory (Lind, I. & Berthelsen, L., unpublished data). In total we received 5157 isolates from 4283 patients. Thus, 26 patients with culture-confirmed meningococcal disease (0·6 %) had not

been notified. Roughly the diagnosis was confirmed by culture of meningococci from cerebrospinal fluid (CSF) in half of the patients, from blood in one third and from both these sites in the remaining sixth part. In a few cases isolates from petechiae ($n = 18$), joint fluid ($n = 2$), eye secretions ($n = 3$), etc. were received, most often together with an isolate from blood or spinal fluid. *N. meningitidis* strains isolated from nasopharyngeal swabs from patients hospitalized with meningococcal disease were also accepted for serogrouping.

Determination of epidemiological markers

In general, all isolates received were examined for all epidemiological markers applied at that time and where epidemiologically relevant.

Serological phenotyping

In 1974 serogroup A was predominant in Denmark, but it disappeared rapidly and during the past 15 years only a few sporadic cases have occurred (Fig. 3). Serogroup A strains were replaced by serogroup B, and partly by serogroup C strains; these two serogroups accounted for more than 95 % of all cases of meningococcal disease. The remaining cases were caused by serogroups W-135, 29E, X, Y, Z and by non-groupable strains. A high proportion of these cases occurred in complement-deficient persons [16] and in recent years, also in HIV-seropositive patients. From 770 patients, two or more isolates were subjected to serogrouping. Simultaneous isolates from CSF and blood were available from 491 patients; in only two patients (0·4 %) were discrepant results found and in both cases the isolates from the spinal fluid

Table 2. *Prevalence of non-typable invasive N. meningitidis serogroup B strains 1985–1999*

| Year | Number | Percentage | | |
		NT	NST	NT/NST
1985	99	46	—	—
1986	166	42	—	—
1987	167	25	13	2
1988	165	24	8	2
1989	146	13	9	< 1
1990	137	22	9	3
1991	142	15	20	6
1992	160	24	16	3
1993	158	11	7	3
1994	130	16	6	4
1995	128	15	4	0
1996	123	11	9	3
1997	127	13	8	2
1998	91	11	2	1
1999	128	16	7	3

NT, Non-typable; NST, non-subtypable.

Table 3. *Prevalence of non-typable invasive N. meningitidis serogroup C strains 1985–1999*

| Year | Number | Percentage | | |
		NT	NST	NT/NST
1985	27	52	—	—
1986	49	73	—	—
1987	41	12	27	5
1988	39	26	36	13
1989	35	31	37	14
1990	33	33	39	15
1991	29	24	28	10
1992	31	23	39	16
1993	55	5	15	2
1994	57	7	9	2
1995	44	7	11	2
1996	45	9	16	2
1997	40	13	8	3
1998	23	0	4	0
1999	21	10	24	5

NT, Non-typable; NST, non-subtypable.

were groupable and the isolates from blood non-groupable. From 186 patients we received a pharyngeal isolate together with an isolate either from CSF or blood; in one patient (0·5%) the pharyngeal isolate belonged to another serogroup (Y) than that found in the CSF (B).

Concurrently with the introduction of serosubtyping in 1987, the typing method was changed from co-agglutination to ELISA. All strains from 1987 were serotyped by both methods; the change of method resulted in a significant decrease in the proportion of non-typable (NT) strains (Tables 2 and 3), especially for serogroup C strains, among which 64% and 12% were NT by co-agglutination and ELISA respectively. Most of this decrease was attributable to a better performance of the type 2a monoclonal antibody by the latter method. The percentage of NT strains among serogroup B strains as well as among serogroup C strains decreased from 1992 to 1993 without any associated change in method or in the panel of typing reagents employed. However, a new kit of type-specific monoclonal antibodies was used from January 1993. The introduction of type 21 and type 22 reagents in 1996 brought no changes. The number of serosubtyping reagents was raised in 1993. Subsequently, the percentage of non-subtypable (NST) strains within both serogroups decreased. NT/NST strains became as rare among serogroup C

strains as they had already been among serogroup B strains since 1987 (Tables 2 and 3).

In 1985 a retrospective collaborative study on meningococcal serotype and serogroup B disease in North-West Europe [17] included approximately 20 Danish serogroup B strains from each year from 1974 to 1983. This study showed that the phenotype B:15 was introduced in Denmark in 1976 followed by a rise in prevalence of meningococcal disease (Fig. 2). Within few years B:15 (B:15:P1.7,16) strains accounted for 50–60% of all serogroup B strains including the periods before and after 1985–1986, where a 60% increase in the incidence of meningococcal disease occurred (Fig. 2). In 1999, 56% (72/128) of all serogroup B strains were still B:15:P1.7,16. The remaining 56 strains represented 33 different serologically defined phenotypes, among which type 4 strains accounted for 7%. During the whole period 1987–1999 the annual percentage of type 4 strains among serogroup B strains ranged from 5% to 16%.

Among serogroup C strains type 2a strains predominated (69%) in 1987. As shown in Figure 4, type 2a was replaced by type 2b in the early 1990s and from 1995 and onwards also by type 15.

The phenotypes associated with major outbreaks in Denmark were B:15:P1.7,16 in 1986–1987 [13] and 1987–1989 [13, 18] and C:2a:P1.2,5 in 1983–1984

Table 4. *N. meningitidis strains from patients with meningoccal disease: distribution according to MIC of sulphamethoxazole at different points between 1940 and 1995*

	Number of strains at each MIC level					
MIC (mg/l)	1940–42	1963–64	1971–72	1975	1985	1995
⩽0·031	0	0	1	1	6	2
0·125	14	3	2	22	12	14
0·5	21	23	8	52	30	46
2	14	9	12	12	9	8
8	7	0	1	53	7	22
32	1	0	2	37	54	76
128	1	0	3	7	11	9
512	0	0	0	1	0	1
>512	0	0	0	0	0	0
Total	58	35	29	185	129	178

MIC, Minimum inhibitory concentration.

Fig. 5. *N. meningitidis* strains from patients with meningococcal disease 1975: distribution according to serogroups and MICs of sulphamethoxazole. ■, Serogroup A ($n = 84$); □, serogroup B ($n = 67$); ■, serogroup C ($n = 27$); ▨ other ($n = 7$).

Fig. 4. Prevalence (%) of serotypes 2a (–◆–), 2b (–■–) and 15 (–▲–) among *N. meningitidis* serogroup C strains 1987–1999.

Fig. 6. *N. meningitidis* strains from patients with meningococcal disease 1995: distribution according to serogroups and MICs of sulphamethoxazole. ■, Serogroup A ($n = 1$); □, serogroup B ($n = 128$); ■, serogroup C ($n = 44$); ▨ other ($n = 5$).

[13, 19], 1986 [13] and 1989 (Zoffman, H. & Lind, I., unpublished data).

Antimicrobial susceptibility testing

Sulphonamide. Preliminary experiments, which provided the basis for the methodology applied and the establishment of the three reference strain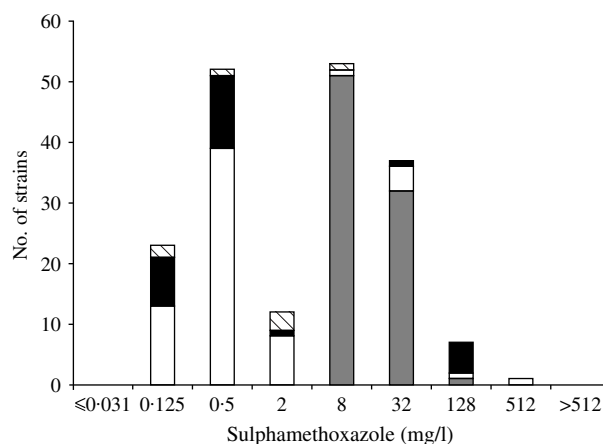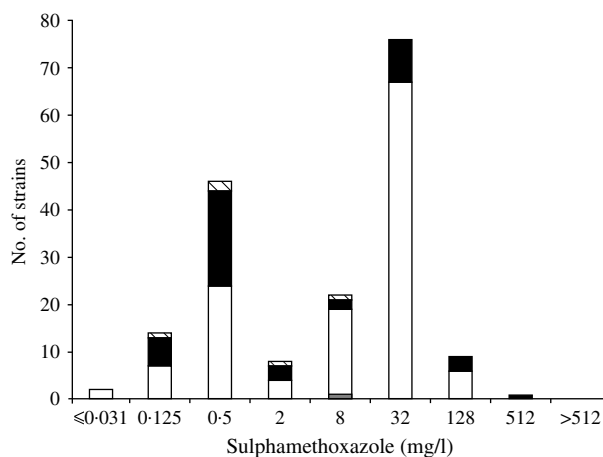s, comprised duplicate testing of 199 invasive meningococcal strains with two different inocula on 22 experimental days (Lind, I., unpublished data, 1974–1975). The reference strains have been included in each experiment ever since and they have proved to be stable. Table 4 illustrates that sulphonamide-resistant meningococcal strains occurred in Denmark in the early 1940s, disappeared in the 1960s and then emerged again in 1972 [20]. The old strains were tested in 1982; they originated from patients with meningococcal disease and were available at the strain collection kept at the reference laboratory. All serogroup A strains isolated in 1974 were sulphonamide resistant, but as the serogroup A strain disappeared,

Table 5. *N. meningitidis strains from 178 patients with meningococcal disease 1995: distribution according to MIC of sulphamethoxazole for B:15:P1.7,16 compared to other phenotypes*

MIC (mg/l)	Number (%) of strains among			
	B:15:P1.7,16	Other sero-group B	All sero-group C	Other sero-groups
⩽0·031	0	2 (3·3)	0	0
0·125	0	6 (9·8)	6 (13·6)	2
0·5	0	26 (42·6)	20 (45·5)	0
2	0	4 (6·6)	3 (6·8)	1
8	11 (16·4)	7 (11·5)	2 (4·5)	2
32	51 (76·1)	15 (24·6)	9 (20·5)	1
128	5 (7·5)	1 (1·6)	3 (6·8)	0
512	0	0	1 (2·3)	0
>512	0	0	0	0
Total	67 (100)	61 (100)	44 (100)	6

MIC, Minimum inhibitory concentration.

sulphonamide-resistant serogroup B and C strains emerged and the proportion of sulphonamide-resistant strains was constantly between 50% and 60% as illustrated by data from 1975, 1985 and 1995 (Table 4). The associations between serogroups and susceptibility to sulphonamide in 1975 *vs.* 1995 are illustrated in Figures 5 and 6. The only phenotype consistently associated with sulphonamide resistance was B:15:P1.7,16 (Table 5).

Penicillin. Tables 6 and 7 show the distribution according to MICs of penicillin during the periods 1975–1992 and 1993–1999 respectively. The introduction of a more precise MIC determination in 1993 (use of two-fold *vs.* four-fold dilutions of penicillin in the media) did not reveal any changes in an upwards direction. From 1975 to 1992 the MIC_{50} was found to be 0·15 mg/l and the MIC_{90} was 0·15 mg/l except for the years 1979, 1980 and 1984 showing a four-fold higher value (0·6 mg/l). During the subsequent years (1993–1999) using two-fold dilution steps the MIC_{50} was 0·063 mg/l and the MIC_{90} was 0·25 mg/l.

Rifampicin. During the 10-year period (1983–1992) in which rifampicin treatment was recommended for prevention of secondary cases among household and similar close contacts, a total of three (0·12%) of 2359 invasive strains were found to be rifampicin resistant and only one of these strains was from a secondary case in a rifampicin-treated sibling of a girl in whom onset of disease occurred 1 week before and

Table 6. *N. meningitidis strains from patients with meningococcal disease 1975–1992: annual distribution according to penicillin MIC*

Year	No. of strains tested	MIC (mg/l)				
		⩽0·01	0·04	0·15	0·60	2·4
1975	179	0	3	179	15	1
1976	155	0	5	135	15	0
1977	86	0	2	81	3	0
1978	128	0	5	112	11	0
1979	134	0	8	110	16	0
1980	144	0	2	118	24	1
1981	130	0	10	112	8	0
1982	133	0	3	117	13	0
1983	134	0	1	121	12	0
1984	171	0	1	139	29	2
1985	130	0	5	115	10	0
1986	227	1	3	202	21	0
1987	216	0	6	198	12	0
1988	213	0	7	189	16	1
1989	183	0	5	169	9	0
1990	177	0	7	162	8	0
1991	173	0	9	151	12	1
1992	193	1	14	167	11	0

MIC, Minimum inhibitory concentration.

Table 7. *N. meningitidis strains from patients with meningococcal disease 1993–1999: annual distribution according to penicillin MIC*

Year	No. of strains tested	MIC (mg/l)					
		⩽0·016	0·032	0·063	0·125	0·250	0·500
1993	218	2	23	125	56	11	1
1994	193	1	16	102	62	12	0
1995	178	0	11	117	45	4	1
1996	177	3	0	85	74	15	0
1997	174	2	5	103	57	6	0
1998	120	3	20	81	12	3	1
1999	152	3	10	84	44	11	0

MIC, Minimum inhibitory concentration.

from whom a phenotypically identical, rifampicin-susceptible strain was encountered [21].

Ciprofloxacin. All 1212 strains isolated from patients with meningococcal disease in the period 1993–1999 were fully susceptible to ciprofloxacin (MIC ⩽0·004 mg/l). Further, 390 invasive strains isolated from November 1990 to the end of 1991, and 1992, i.e. before ciprofloxacin became first-choice drug for prophylaxis, were fully susceptible.

MEE and DNA-based analyses (*fingerprinting, PFGE, riboprinting*)

The usefulness of these methods for the study of the epidemiology of meningococcal disease in our setting has been discussed previously [13, 22, 23]. Our conclusions were that invasive strains from epidemiologically related cases (outbreaks, clusters) were reliably identified by phenotyping whereas determination of the relationship between carrier strains and invasive strains or with epidemiologically unrelated strains of identical phenotypes required application of one or more supplementary tests.

Ascertainment of suspected cases

Serology

MAT (*meningococcal antibody test*). In a study on the usefulness of the MAT in the diagnosis of meningococcal disease [11] 90% of patients with culture-confirmed meningococcal disease were found to be seropositive 10–15 days after onset of disease; the majority of patients were MAT-negative at onset of disease, i.e. on the day of hospitalization. Since 1992 MOHs and the Department of Epidemiology have been informed if seroconversion or a significant increase in antibody titres was demonstrated in a patient who had not been identified through the laboratory surveillance system. Since then an increasing proportion of cases notified without culture-confirmed diagnosis have been registered as serologically confirmed [11]. In 1999 22 out of 35 cases (63%) not confirmed by culture of *N. meningitidis* were recorded as serologically confirmed [24].

ELISAs (*antibodies specific to meningococcal polysaccharides B and C*). The performances of the ELISAs have previously been described in detail [12]. Among patients with culture-confirmed meningococcal disease, antibodies specific for the capsular polysaccharides can be demonstrated in 79–92%. ELISAs are less sensitive than the MAT for the diagnosis of meningococcal disease [25], but allow serological discrimination between serogroup B and C disease. Since 1992 sera from culture-negative, serologically confirmed, i.e. MAT-positive, cases were examined by ELISA and the results suggested that the proportions of serogroup B and C disease were the same as among culture-confirmed cases of meningococcal disease.

Counter-immunoelectrophoresis (*CIE*). The test was made available for use on serum or CSF from patients in whom early antibiotic treatment had ruled out the possibility of recovering *N. meningitidis* by culture. In addition to a previous study on CSF samples [10] we carried out investigations on acute serum samples (Berthelsen, L., unpublished data) – 134 samples from patients with culture-confirmed meningococcal disease and 75 samples from patients with serological evidence of meningococcal disease (seroconversion in MAT), but in whom the diagnosis had not been confirmed by culture. All sera were collected during the period 1986–1990. For culture-confirmed cases the overall sensitivity was 44%; for sera drawn on days 0, 1 or 2 after onset of disease the sensitivity was 44, 61 and 18% respectively. For serologically confirmed cases it was 25%; acute samples from 44 patients with meningitis caused by various other microorganisms were negative.

In 1992 we decided further to assess whether CIE performed routinely on all acute serum samples received for MAT would contribute to an early diagnosis in suspected cases. The material comprised sera from 140 patients: 36 culture-confirmed cases, 15 with serological evidence of meningococcal disease and 89 without meningococcal disease. Doubtful results, i.e. those with weak indistinct precipitate with the serogroup B antiserum turned out to be a problem. Regardless of whether the doubtful results were considered positive or negative, the sensitivity of CIE was lower than that found previously, namely 22% and 24% respectively. The predictive values of the positive test results under the same conditions were 61% and 44%. CIE is, therefore, only offered as a supplementary test on request. MAT-positive sera have never been found positive by CIE.

DISCUSSION

When laboratory surveillance of culture-confirmed meningococcal disease in Denmark was established in 1974, the primary objective was to ensure optimal and prompt use of the newly developed serogroup A and C capsular polysaccharide vaccines [4, 5] and to monitor the susceptibility of invasive meningococcal strains to antibiotics used for treatment and for the prevention of secondary cases. Due to the poor immunogenicity of the serogroup B capsular polysaccharide, attempts to develop a vaccine protecting against serogroup B disease have involved many bacterial components, including the proteins

determining type and subtype specificity. The considerable antigenic diversity among serogroup B meningococcal strains has resulted in a variety of serotype/subtype combinations. Antibodies to any single protein are unlikely to provide broad protection against serogroup B meningococcal disease [26]. Approaches to vaccine development have been based on proteins from epidemic strains as B:15:P1.7,16 or on serosubtype-determining proteins from the most predominant endemic, invasive strains. To address the potential usefulness of such vaccines it was considered important to monitor the prevalence of serogroup B phenotypes as defined by their serotype/serosubtype patterns. Monoclonal reagents for typing and subtyping became available in the mid-1980s and have since been used in the majority of European reference laboratories. To assess the performance of these reagents an international comparative study [27] was carried out. The study concluded that serological typing could be standardized, and thus, be a useful tool in vaccine development projects. It is a prerequisite that the panel of antibodies is broad and currently updated. However, the inclusion of serotypes 21 and 22 reagents in 1996 did not decrease the percentage of NT strains in spite of the fact that type 22 made up more than 60 % of serogroup B strains in a neighbouring country (Poland) at that time [28]. Since the typing system was found useful in the assessment of clusters and outbreaks it has been maintained, although potent serogroup B vaccines seem still to be unattainable in the near future.

Determination of susceptibility to sulphonamides was an integrated part of the surveillance system from its establishment in 1974. The reference strains were selected and evaluated in that year and have been used in all tests since. The MIC results were highly reproducible. The reappearance of sulphonamide-resistant strains in 1972 coincided with both an increased prevalence of serogroup A strains and the introduction of cotrimoxazole on the Danish market. Sulphonamide resistance is nearly always present in epidemic strains [13, 18–20, 22] and is a useful epidemiological marker.

Penicillinase-producing *Neisseria gonorrhoeae* (PPNG) emerged in Denmark in March 1976. Since 1 April 1976, the susceptibility to penicillin of all invasive meningococcal strains has been determined; strains from 1975 and the first three months of 1976 were tested retrospectively. Penicillin-resistant meningococcal strains were not detected. Worldwide production of penicillinase in *N. meningitidis* isolates from patients with invasive meningococcal disease has been reported in only four cases occurring in South Africa [29] and Spain [30]. There was no trend over time in the distribution of penicillin MICs among invasive *N. meningitidis* (Tables 6 and 7). The proportion of strains from 1993 to 1999 that might be designated less susceptible or moderately susceptible (MIC 0·125–1 mg/l) [31] was constant. This is in contrast to the changes recorded in Spain [32] and Australia [33].

During a 10-year period from 1982 to 1992 rifampicin was recommended for the prevention of secondary cases. Within this period only three resistant strains (0·12 %, 3/2456) were recovered; one of the three was found in a patient with meningoccal disease who had received prophylactic treatment with rifampicin 1 week before onset of illness [21]. Over the 10-year period about 10 000 close contacts of sporadic cases of meningococcal disease had received rifampicin prophylaxis. Thus, the standard regimen used for prevention of secondary cases did not seem to confer any significant risk of increasing the prevalence of resistance; the same conclusion has been drawn from surveillance studies in other countries [31, 32]. Rifampicin treatment prevents secondary cases [18, 34, 35] and when a more liberal definition of the target group is used, it may also modify the curves of epidemics [18, 35].

Since 1993 a 500-mg single dose of ciprofloxacin has been recommended for prophylactic use in Denmark. All strains tested during the preceding 2-year period as well as all strains isolated during 1993–1999 were fully susceptible. Only a single report of decreased susceptibility to ciprofloxacin in one *N. meningitidis* isolate (MIC 0·25 mg/l) has been published [36].

The introduction of supplementary tests (MEE- and DNA-based analyses) in the assessment of clusters and outbreaks was based on the studies of Weis & Lind [13, 22]. These methods also permitted a more precise identification of distinctive clone complexes [15] associated with an increased case-fatality rate among patients with phenotype B:15:P1.7,16 and C:2a:P1.2,5 meningococcal disease [37]. Similarly, the supplementary tests facilitated identification of virulent strains among carriers of potentially invasive phenotypes [19, 22, 23]. The overall conclusion is that in the epidemic situation phenotyping is sufficient for decision making [22, 38] whereas tracing of potentially virulent strains among carriers requires DNA-based characterization.

Around 1990 the linkage between the clinical notification system and the laboratory surveillance system had been developed to its present status [38]. An improvement had been obtained in the clinical notification system, which received 20–40% of all notifications after a reminder based on data from the reference laboratory. Furthermore, since 1992 both the MOH and the Department of Epidemiology have been informed about all culture-negative patients, in whom serological evidence of meningococcal disease was demonstrated. With these methods, the completeness of the notification system was estimated to be 96% (95% CI 93–98) [7].

It is unlikely that the introduction of new, non-culture DNA-based diagnostic methods will add significantly either to the proportion of confirmed cases or to the number of recognized cases of meningococcal disease in our setting. In order to allow timely and reliable epidemiological management and prevention strategy we advocate moving from passive to active surveillance when outbreaks or clusters of meningococcal disease are suspected.

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