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Incidence of invasive pneumococcal disease and distribution of capsular types of pneumococci in Denmark, 1989–94

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SUMMARY

During the period 1989–94, 4620 strains of *Streptococcus pneumoniae* (4063 from blood and 557 from cerebrospinal fluid), from cases of invasive disease in Denmark, were received for capsular typing and penicillin susceptibility testing. During the study period the incidence of bacteraemic pneumococcal disease increased from 10 to 18 cases per 100000 inhabitants per year. The highest rates were seen in the very young, age less than 5 years (23/100000/year, in 1994), and in the elderly, age greater than 60 years (55/100000/year, in 1994). The annual number of cases of meningitis did not vary.

Overall, 92% (93% blood, 87% CSF) of isolates and 94% of all childhood isolates belonged to the 23 vaccine types. The capsular types occurring most commonly among the 4123 pneumococcal strains from adults were types 1, 4, 14, 6A+6B, 7F, 9V, 3, 12F, and 8 (in order of frequency). The ten most frequently occurring types from children (6A+6B, 18C, 14, 1, 7F, 19F, 9V, 4, and 23F) covered 84% of the cases of bacteraemia and meningitis. Reduced susceptibility to penicillin was rare (< 1%).

INTRODUCTION

Streptococcus pneumoniae is divided into 90 types based on differences in antigenicity of the capsular polysaccharides [1–3]. The most serious infections caused by the pneumococcus are meningitis and bacteraemia (septicaemia).

Since 1983, a vaccine containing 23 different pneumococcal capsular polysaccharides has been available and recommended for use in high risk individuals aged more than 2 years. The 23 types cause about 90% of all invasive pneumococcal diseases [4–10].

Strains of *S. pneumoniae* resistant to penicillin have been isolated throughout the world during the last 20 years, mainly in Spain, South Africa and New Guinea [11, 12]. In specific geographic areas resistant pneumococci seem to consists of clusters of pneumococcal strains of the same capsular type (or types), e.g. type 6B in Iceland [13] and type 23F in Spain [14], and molecular typing has shown a clonal relationship [15]. Apart from Iceland, Northern Europe has very low rates of penicillin resistant strains [16, 17], although an increase in resistant strains has been reported in southern Sweden [18]. Prevention of pneumococcal infection by vaccination might reduce the number of resistant strains and prevent introduction of such strains in areas with little resistance.

Capsular polysaccharide vaccines are not immunogenic in children under 2 years of age. Clinical efficacy trials of pneumococcal polysaccharide-protein conjugate vaccines are currently being carried out in the very young [19]. The formulation of such vaccines depends on the distribution of pneumococcal types in the target area.

In this paper we present the distribution of types of *S. pneumoniae* in Denmark during the period 1989–94.

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Table 1. *Number of isolates of pneumococci from blood or CSF*, 1989–94, in Denmark*

Year	Number of isolates (relatively frequency %)			Age†		Incidence per 100000 inhabitants
	Total	Blood	CSF*	0–14	> 14	
1989	520	425	95 (18·3)	51	467	10
1990	642	535	107 (16·7)	73	565	13
1991	749	656	93 (12·4)	73	674	15
1992	821	738	83 (10·1)	91	730	16
1993	962	867	95 (9·9)	101	857	19
1994	926	842	84 (9·1)	93	830	18

* CSF, cerebrospinal fluid.
† Age unknown in 15 cases.

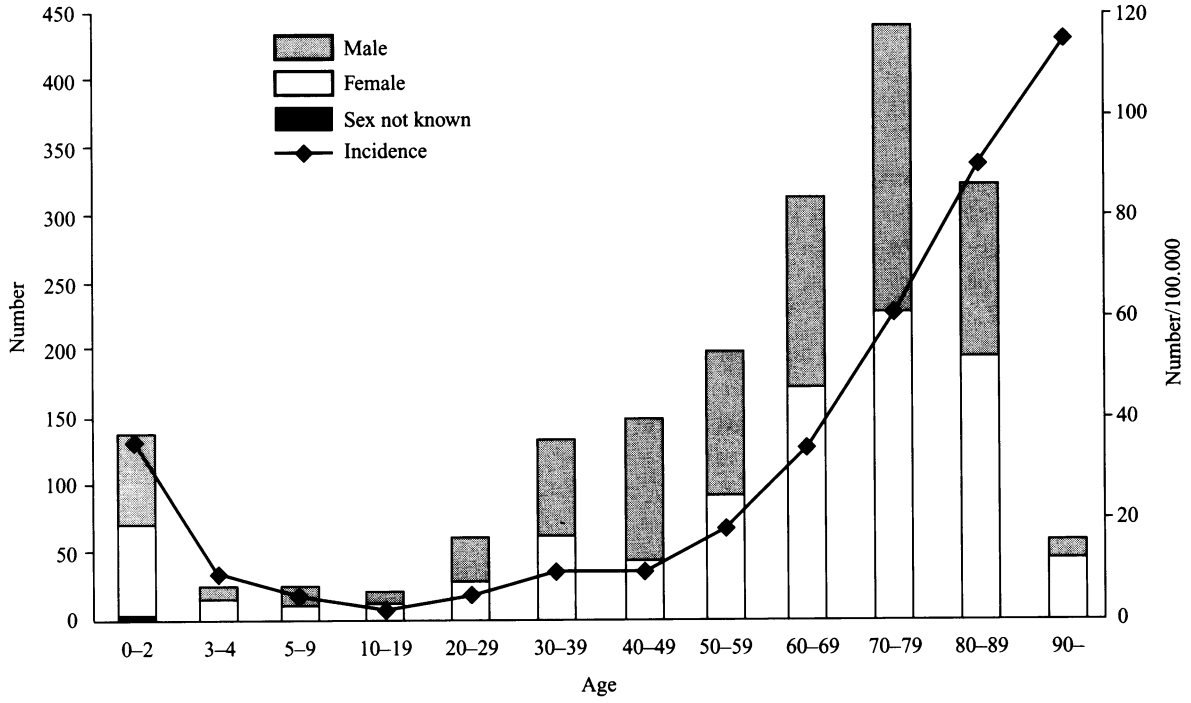

Fig. 1. Incidence, age and sex distribution of invasive pneumococcal disease in Denmark 1993–4.

The incidence of invasive disease, as well as age and sex distribution of the patients in selected years, and susceptibility to penicillin of the pneumococcal strains is also presented.

MATERIALS AND METHODS

Pneumococcal strains isolated from blood and cerebrospinal fluid (CSF) by departments of clinical microbiology in Denmark are routinely sent to the World Health Organization Collaborating Centre for Reference and Research on Pneumococci in Copenhagen, for typing.

This study comprises strains received during 1989–94. Typing of pneumococci was performed by the capsular reaction test (Quellung reaction) using pooled sera, type and group and, when relevant, factor sera for differentiating between types within groups [1]. Multiple isolates of the same type from the same patient were included only once. Strains isolated from both blood and CSF of patients with meningitis were classified as CSF isolates. All isolates were screened for susceptibility to penicillin using an oxacillin disc (1 μg, Neo-sensitabs, Rosco Diagnostica, Denmark) on a 10% blood agar plate (Statens Serum Institut, Denmark) incubated aerobically over-

Table 2. *Pneumococcal types isolated from blood and/or CSF* from patients of all ages during the period 1989–94 in Denmark*

Order of frequency	Type†	Number of isolates: CSF* (% total)	Number of isolates: Blood and CSF* (% total/ % accumulated)
1	*1*‡	17 (3·1)	575 (12·4/12·4)
2	*4*§	39 (7·0)	397 (8·6/21·0)
3	*14*	37 (6·6)	387 (8·4/29·4)
4	*7F*	51 (9·2)	340 (7·4/36·8)
5	*9V*	26 (4·7)	292 (6·3/43·1)
6	*3*§	32 (5·7)	250 (5·4/48·5)
7	*12F*§	28 (5·0)	218 (4·7/53·2)
8	*6B*††	45 (8·1)	212 (4·7/57.8)
9	6A††	31 (5·6)	173 (3·7/61·6)
10	*8*§	17 (3·1)	172 (3·7/65·3)
11	*18C*††	31 (5·6)	168 (3·7/68·9)
12	*23F*	22 (3·9)	167 (3·6/72·5)
13	*19F*	25 (4·5)	145 (3·1/75·7)
14	*5*‡	8 (1·4)	131 (2·8/78·5)
15	*9N*§	10 (1·8)	110 (2·4/80.9)
16	*19A*‡	4 (0·7)	102 (2·2/83·1)
17	*22F*†	13 (2·3)	98 (2·1/85·2)
18	*20*§	15 (2·7)	95 (2·1/87·3)
19	24F	26 (4·7)	86 (1·9/89·1)
20	38††	17 (3·1)	71 (1·5/90·7)
21	*11A*	6 (1·1)	62 (1·4/92·0)
22	16F	7 (1·3)	55 (1·2/93·2)
23	*10A*	8 (1·4)	51 (1·1/94·3)
24	*33F*	9 (1·6)	49 (1·1/95·4)
25	*17F*	8 (1·4)	26 (0·6/95.9)
26	35F	3 (0·5)	24 (0·5/96·5)
27	23A	4 (0·7)	22 (0·5/96·9)
28	*15B*	1 (0·2)	19 (0·4/97·3)
29	15A	5 (0·9)	18 (0·4/97·7)
30	31	0	17 (0·4/98·1)
31	12A	0	10 (0·2/98·3)
32	15C	3 (0·5)	9 (0·2/98·5)
33	34	0	9 (0·2/98·7)
34	*2*	1 (0·2)	8 (0·2/98·9)
35	13	1 (0·2)	7 (0·2/99·0)
36	29	1 (0·2)	5 (0·1/99·1)
37	21	2 (0·4)	4 (0·1/99·2)
38	11B	0	4 (0·1/99·3)
39	42	1 (0·2)	4 (0·1/99·4)
40	18A	0	3 (0·1/99·5)
Subtotal		554	4595
Other types**		4§§	25
Total		558	4620

* CSF, cerebrospinal fluid.

† Types present in the currently available pneumococcal vaccine are italicized.

‡ Types isolated significantly more often from blood than from CSF. $P < 0·05$, χ^2 test.

§ Types isolated significantly more often from patients above 14 years of age compared with patients aged 0–14 years. $P < 0·05$, χ^2 test.

†† Types isolated significantly more often from CSF than from blood. $P < 0·05$, χ^2 test.

** 2 each: 7C, 10F, 17A, 18F, 23B, 35C, 36; 1 each: 7A, 7B, 18B, 22A, 27, 28A, 33A, 33B, 35B, 37, 45.

§§ 1 each: 7B, 10F, 23B, 28A.

night at 37 °C. Susceptibility was registered as being reduced if the diameter of the inhibition zone was less than 20 mm.

The incidence of invasive disease was determined by the number of cases per 100000 persons in each age group in Denmark. Data from 'Statistics Denmark' on age distribution of the Danish population were used [20].

Differences in the frequency of types were compared by the χ^2 test. $P < 0·05$ was considered significant.

RESULTS

During the period 1989–94 we received 4620 strains of *S. pneumoniae* for typing isolated from patients with invasive disease in Denmark; 4063 were from blood and 557 from CSF. A total of 482 (10%) of the patients were 0–14 years of age and 4123 (89%) were older; the age of 15 patients was unknown (Table 1). The incidence of invasive pneumococcal disease increased during the study period (Table 1); in the elderly (> 60 years), from 27/100000 (55% of all isolates) in 1989 to 55/100000 (62% of all isolates) in 1994, and in children (< 5 years), from 13/100000 in 1989 to 23/100000 in 1994.

The age and sex distribution of invasive pneumococcal disease and the age-specific incidence in 1993 and 1994 are shown in Figure 1. Of a total of 1875 (99·6%) patients, whose sex was reported, 918 (49%) were male. In those aged over 59 years, the number of cases was higher in females than in males, whereas in those aged 20–59 years it was the opposite. This was most apparent in those aged 40–49 years, where the difference was significant ($P < 0·05$, χ^2 test). Because women on the average live longer than men, the annual incidence of invasive pneumococcal infections, when age- and sex-adjusted to the Danish population over 60 years of age, was 56 cases per 100000 men and 53 cases per 100000 women.

Pneumococci with reduced susceptibility to penicillin were rare, less than 1%, in all of the 6 years studied.

Capsular types of pneumococci isolated from blood

Table 3. *Pneumococcal types isolated from blood or CSF* from patients aged 0–14 years during the period 1989–94 in Denmark*

Order of frequency	Type†	Number of isolates (% total % accumulated)
1	*18C*‡	67 (13·9/13·9)
2	*14*‡	60 (12·4/26·3)
3	*6B*‡	58 (12·0/38·4)
4	*1*	48 (10·0/48·3)
5	*7F*	44 (9·1/57·5)
6	*6A*‡	34 (7·1/64·5)
7	*19F*‡	34 (7·1/71·6)
8	*9V*	29 (6·0/77·6)
9	*4*	16 (3·3/80·9)
10	*23F*	13 (2·7/83·6)
11	*5*	9 (1·9/85·5)
12	*19A*	9 (1·9/87·3)
13	24F	8 (1·7/89·0)
14	38	6 (1·2/90·2)
15	*12F*	5 (1·0/91·3)
16	*3*	4 (0·8/92·1)
17	*22F*	4 (0·8/92·9)
18	*10A*	4 (0·8/93·8)
19	*8*	3 (0·6/94·4)
20	*33F*	3 (0·6/95·0)
21	21	3 (0·6/95·6)
Subtotal		461
Other types§		21
Total		482

* CSF, cerebrospinal fluid.

† Types present in the currently available pneumococcal vaccine are italicized.

‡ Types isolated significantly more often from patients aged 0–14 years than from patients over 14 years of age. $P < 0{\cdot}05$, χ^2 test.

§ 2 each: *9N*, 15A, *15B*, 15C, *17F*, 23A; 1 each: 10F, *11A*, 16F, 18A, 23B, 27, 34, 35F, 42.

and CSF from patients of all ages and from patients under 15 years of age are given in Tables 2 and 3, respectively. Among the 20 most frequently occurring types isolated from blood or CSF from patients of all ages, types 6A, 6B, 18C, 24F, and 38 were isolated significantly more often from CSF than from blood, whereas types 1, 5, and 19A were isolated significantly more often from blood than from CSF. Types 6A, 6B, 14, 18C, and 19F were isolated from blood significantly more often from patients aged 0–14 years than from patients over 14 years of age, whereas the opposite was true for types 3, 4, 8, 9N, 12F, 20, and 22F.

Comparing the period 1983–8 [16] with the study period 1989–94, we found that types 5, 9V, and 38 had been isolated significantly ($P < 0{\cdot}05$, χ^2 test) more frequently during the latter period and type 3 had decreased from 7·8 to 5·4 per cent of all invasive isolates (data not shown). In our study some year-to-year variation was seen in the numbers of different pneumococcal capsular types as a percentage of all isolates: types 1 (10·7–16·4%), 4 (6·9–10·2%), 7F (5·3–10·0%), 12F (2·5–9·2%) and 14 (7·3–10·1%), but overall there was no significant difference between the frequency of these predominant types during 1983–8 and 1989–94 ($P > 0{\cdot}05$, χ^2 test). Type 2, a vaccine type not recovered at all in Denmark during 1983–8, was isolated eight times during 1989–94. Type 24F, a non-vaccine type, isolated more frequently in Denmark compared with other countries, was 19th in the overall order of frequency but was the 9th most common cause of pneumococcal meningitis (4·7%).

A total of 58 of the 90 known types was encountered (Table 2). The 23 vaccine types represented 92% of all isolates, 94% of isolates from patients less than 15 years of age (Table 3), and 87% of the CSF isolates (Table 2). In this calculation type 6A is considered together with type 6B because they have been shown to offer cross-protection [21].

DISCUSSION

The annual incidence of diagnosed pneumococcal bacteraemia in Denmark continued to increase from 1989 to 1993–4 as it also did during the period 1983–8 [16]. Notably, the annual number of cases of meningitis ascertained (defined as number of CSF isolates) did not change during the period 1989–94.

Patient admissions to hospitals have increased in Denmark and the number of blood cultures performed per 1000 admissions to Danish hospitals has also increased during the last 30 years (unpublished observation, Niels Frimodt-Møller, manuscript submitted). This might have contributed to the increase in the number of cases of pneumococcal bacteraemia. However, there has been a more than tenfold increase in verified cases of systemic pneumococcal disease in Denmark over the last 20 years [22] and only a twofold increase in both the number of blood cultures performed and the number of hospital admissions. The waxing and waning in the incidence of invasive infections caused by capsulated bacteria seen over decades is probably attributable to changes in immunity in the population.

The highest rates of invasive pneumococcal disease were found in the very young and in the elderly as

described previously by others [23]. The incidence of bacteraemic pneumococcal infections in children less than 5 years of age nearly doubled during the study period (from 13 cases per 100000 in 1989 to 23 cases per 100000 in 1994). Some workers have recently reported higher incidences of systemic pneumococcal disease among young children [24, 25], whereas our results agree well with those of others [26–28].

More cases of invasive disease have been reported to occur in males than in females [27]. During the 1993–4 period of our study, 49% of the pneumococci were recovered from males (Fig. 1). Our study shows that the difference in sex ratio in different studies probably can best be explained by the age of the study population.

The overall frequency of types included in the 23-valent vaccine causing invasive disease in Denmark during 1989–94 corresponds with that found in other studies [5–10]. During the study period 92% of the pneumococcal strains isolated from blood or CSF belonged to types included in the vaccine, whereas vaccine types represented 87% of isolates from CSF only. This compares with 91% and 87%, respectively, during the period 1983–7 [16].

The distribution of pneumococcal types varies with the age of the patient, geographical area, period of time, and source of infection [23]. The variation in order frequency of the predominant types (1, 4, 7F, 12F, and 14) during the study was not statistically significant, whereas that of types 5, 9V, 38, and 3 was. Type 24F, a non-vaccine type, as in the previous study period, 1983–8, was isolated more frequently in Denmark than in other countries.

Group 6 and pneumococcal types 7F, 14, 18C, 19F, and 23F are reported to be the major childhood types world-wide. In our study, types 6A, 6B, 14, 18C and 19F were isolated significantly more often from patients aged 0–14 years than from patients aged over 14 years. Confirming results from others, Butler and colleagues found that seven pneumococcal capsular types (14, 6B, 19F, 18C, 23F, 4, and 9V) could potentially prevent 86% of all bacteraemia and 83% of all meningitis cases in children less than 6 years of age in the United States [29, 30]. A vaccine containing these types would cover only 65% of all isolates from cases of invasive disease in children less than 14 years of age in Denmark, whereas types 6B, 18C, 14, 1, 7F, 19F and 9V would cover 78% (6A included). The formulation of a conjugate vaccine with a limited number of types for use in children, will in part depend upon the diseases targeted for prevention, and also upon the country or countries in which the vaccine is meant to be used. Continuous surveillance of the capsular types of disease-causing pneumococci in various locations is necessary.

The prevalence of pneumococci with reduced susceptibility to penicillin in our study was less than 1%, presumably because of strict control of antibiotic use in Denmark.

The data presented here argue for an active pursuit of the international recommendation for vaccination of the population aged 60 years or more in Denmark.

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