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Pneumococcal meningitis in the North East Thames Region UK: epidemiology and molecular analysis of isolates

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SUMMARY

One hundred and fourteen cases of pneumococcal meningitis were identified by prospective laboratory based surveillance during 1990–3 in the North East Thames Region. Higher rates of disease were seen in Asians (2·1/100000) than Caucasians (0·8/100000) ($P = 0·002$). The incidence of meningitis was higher in children than adults, while mortality rates were highest in adults over the age of 60 (48%). In 72 cases, both blood and CSF were culture positive. Serotyping of 65 isolates collected identified 22 serotypes (and one non-typable) causing disease, the most common being serotype 6 (13 cases) and serotype 14 (11 cases). Overall, 90% of serotype antigens identified were represented in the 23 valent vaccine. Ribotyping of 62 isolates identified 35 different patterns, of which 26 were single types. Different ribotypes were found among isolates of the same serotypes, with the exception of serotype 14, where 9 of 11 isolates had the same ribotype pattern. Four percent of isolates had reduced susceptibility to penicillin, but no high level penicillin resistance was found.

INTRODUCTION

Streptococcus pneumoniae is an important cause of bacterial meningitis. Invasive pneumococcal disease has a substantial mortality, particularly in the elderly. Until recently pneumococci were the third major cause of bacterial meningitis in the UK [1] and the second in the USA [2]. Since the introduction of universal immunization of infants with conjugate *Haemophilus influenzae* type b (Hib) vaccine the incidence of Hib disease has dramatically fallen while pneumococcal meningitis has not been reduced and has become correspondingly more important [2].

Of the 84 capsular serotypes seen in *S. pneumoniae*, 23 account for between 85 and 94% of all invasive disease [3–6]. In the UK, vaccination with the 23 valent polysaccharide vaccine has been recommended for populations at high risk of pneumococcal disease but not the healthy elderly [7]. In the USA the 23 valent vaccine was introduced in 1983; immunization of high risk groups is recommended and since 1989 has included healthy adults over the age of 65 [8]. However, uptake of this vaccine is low, with only 21% of those at risk in the USA receiving the vaccine [9].

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Penicillin resistance in pneumococci is gaining increasing prevalence in many parts of the world. Molecular analysis of penicillin-resistant pneumococci by a variety of techniques including multilocus enzyme electrophoretic typing, ribotyping and pulsed field gel electrophoresis has identified several clones of penicillin-resistant pneumococci, with some clones showing worldwide spread [10–13].

With the changing epidemiology of bacterial meningitis and the increasing anxiety over pneumococcal resistance, we performed an active surveillance project over 3 years in North East Thames Region (NETR) to assess the picture in an area which includes inner city London, suburban and rural parts of south east England [1].

METHODS

We identified all cases of pneumococcal meningitis occurring over the 3-year period from January 1990 to December 1993 and characterized the isolates obtained by serotype, ribotype and antimicrobial sensitivity.

There are 19 microbiology laboratories in the North East Thames Region. The consultants in microbiology in each laboratory identified all clinical cases of bacterial meningitis where there was documented microbiological evidence of the pathogen responsible for the disease, by culture of blood and/or cerebrospinal fluid (CSF), CSF Gram stain and/or antigen detection.

Where CSF culture was negative or unavailable, cases were included in the study if there was clinical evidence of meningitis.

Cases of septicaemia (and other syndromes of pneumococcal disease e.g. pneumonia), where there were no symptoms or clinical signs of meningitis were not included in this study. Cases of proven bacterial meningitis associated with HIV infection, were excluded. Clinical details were sought by questionnaire from the microbiologist or clinician in charge of the case. If no form was returned within 6 months, details were obtained from the case notes. The microbiologist at each centre forwarded the laboratory results of blood and CSF culture, with the organism, to the coordinating hospital. Isolates were confirmed as *Streptococcus pneumoniae* by the characteristic Gram stain, sensitivity to optochin and bile solubility.

Data were analyzed using EPI INFO 5.01 (CDC, Atlanta, Georgia 30333, USA). Tests of significance were determined using χ^2 and Fishers' exact test. Denominator data from the 1991 census were used.

Serotyping

Cultures were grown in 4·5 ml of Difco Todd–Hewitt broth, supplemented with 0·5 ml of 20% (w/v) glucose. The cultures were centrifuged at 3000 rpm for 20 min and the supernatants discarded. The cell deposit (antigen) was vortexed and slide agglutination was performed against the capsular typing sera (Statens Serum Institut, Denmark) [14].

Ribotyping

DNA was extracted by the method of Pitcher and colleagues [15] exactly as described elsewhere [16]. DNA was digested with *Eco*R V and separated by electrophoresis in 0·9% agarose with TBE buffer (0·9 M Tris-borate, 0·004 M EDTA) at 120 V by standard procedures [17]. The DNA was denatured and transferred to a Hybond-N membrane (Amersham) using positive pressure (Posiblot, Stratagene). Southern blots were probed with digoxigenin cDNA to *E. coli* ribosomal RNA [16] and developed with the DIG-non radioactive detection system (Boehringer Mannheim Biochemicals) to generate ribotypes. Indistinguishable ribotype patterns were grouped by number designations.

Susceptibility testing

Minimum inhibitory concentrations were determined by the agar dilution method using Isosensitest agar (Oxoid CM 471) supplemented with 5% lysed horse blood. The antimicrobial agents were supplied as pure powders and solutions were prepared on the day of use. Four hour broths of the organisms were diluted to produce an inoculum of 10^4 cfu/ml. The Oxford *Staphylococcus aureus* was used as a control with each batch of isolates tested. Organisms were delivered by a multipoint inoculator (Denley) and incubated overnight a 37 °C in 5% CO_2. The results were read the next day.

RESULTS

Epidemiology and description of cases

A total of 114 cases of *S. pneumoniae* meningitis were identified over the 3-year period. In the 108 cases where gender was recorded, there were 59 (55%) cases in males and 49 (45%) in females. Ethnicity data were available for 96 cases over the 3-year period (3 cases were of mixed race and not included in the analysis). There were significantly higher rates of pneumococcal

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Table 1. *Incidence of pneumococcal meningitis (n = 93)* by ethnic group North East Thames Region 1991–3 inclusive (rates and 95% confidence intervals calculated from 3-year totals)*

	S. pneumoniae meningitis cases/100000/year		
	Caucasian	Black	Asian
Rates	0·8	0·6	2·1
95% confidence intervals	0·62–0·98	0·01–1·28	0·94–3·18
3-year totals	76	4	13
Denominator†	3179997	206829	210521

* Ethnicity unknown in 18 cases, 3 cases were of mixed race and not included in analysis.
† Denominator data taken from 1991 census.

Table 2. *Age specific case fatality ratios for pneumococcal meningitis North East Thames Region, 1991–3**

Age group (years)	Case fatality rate % (deaths/cases)
< 1	3·8 (1/26)
1–4	12·5 (2/16)
5–19	10 (1/10)
20–39	28 (4/14)
40–59	18·8 (3/16)
60+	47·8 (11/23)
Total	21·9 (22/105)

* Mortality data unavailable in seven cases of pneumococcal meningitis.
Age unknown in one patient with pneumococcal meningitis who died.

meningitis overall in the Asian population (2·1/100000) when compared with Caucasians (0·8/100000) and Blacks (0·6/100000, $P = 0·002$) (Table 1). There was no difference in the rates of disease in the Black population when compared with Caucasians.

Details of age were collected for 105 cases. There were 42 (40%) children under the age of 5 years and of these 26 (43%) were under the age of 1 year. Fifty-three (50%) cases occurred in adults over the age of 19 years and 23 (43%) of these were in people over the age of 60 years. Although the incidence of meningitis was greatest in the paediatric population, case fatality rates were higher in those over 60 (48%) compared with children (Table 2).

Details of underlying and predisposing factors where available in 105 cases. A prior history of

Table 3. *Underlying or predisposing factors for pneumococcal meningitis, North East Thames Region, 1991–3**

Disease/condition	Number of cases with underlying disease or predisposing factor (%)
Immune deficiency†	7 (6)
Sickle cell disease	1 (1)
Diabetes mellitus	2 (2)
Rheumatoid arthritis	1 (1)
Polycystic kidneys	1 (1)
Pregnancy	2 (2)
Splenectomy	1 (1)
Cardiac surgery‡	2 (2)
Trauma/neurosurgery¶	4 (4)
ENT surgery	4 (4)

* Details of underlying illness unavailable in nine cases. Two patients with diabetes mellitus underwent ENT surgery and are counted in both categories.
† Oral corticosteroid therapy 2, neutropaenia 1, congenital nephrotic syndrome 1, Hodgkins lymphoma 1, unspecified 1.
‡ Both cases of congenital heart disease (Fallot's tetralogy, atrial septal defect).
¶ Road traffic accident 1, fractured skull 1, stitch abscess post meningioma 1, transphenoidal pituitary resection 1.

conditions which may have predisposed to pneumococcal infection was given in 23 patients (22%) (Table 3). Two patients had both diabetes mellitus and underwent ENT surgery. Concurrent clinical syndromes were seen in 33 cases: pneumonia in 18 and otitis media in 15. There was no prior history of disease or concurrent clinical syndrome in 49 patients.

Microbiological analysis

Seven-two of the 114 cases identified (63%) had both positive blood and CSF cultures (Table 4). CSF culture alone was positive in 16 cases and there were 21 (18%) cases where CSF was culture negative. In these cases identification of the pathogen was by positive blood culture in 12, and by antigen detection and/or gram stain of the CSF in 9. In 5 cases, CSF culture was not performed and blood culture was positive.

CSF antigen detection was performed in 79 cases (Table 4, ii). There were 16 cases where CSF was culture positive, antigen detection negative. In 14 cases, CSF was culture negative and antigen detection was positive. The laboratories used a variety of different commercially available antigen detection test kits: Wellcome: 11 (Wellcome Diagnostics, Dartford,

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Table 4(i). *Results of CSF culture and blood culture in cases of pneumococcal meningitis, North East Thames Region, 1991–3*

Blood culture	CSF culture		
	Positive	Negative	Not done
Positive	72	12	5
Negative	11	7*	0
Not done	5	2*	0

* In these 9 cases the CSF antigen detection and Gram stain were both positive in 5, CSF antigen detection alone positive in 3 cases, Gram stain alone positive in 1 case, where CSF antigen detection was not performed.

Table 4(ii). *CSF antigen detection and CSF culture results in cases of pneumococcal meningitis, North East Thames Region, 1991–3**

CSF antigen	CSF culture		
	Positive	Negative	Total
Not done	28	2†	30
Positive	44	14	58
Negative	16	5	21
Total	88	21	109

* In five cases, CSF samples were not taken.
† In these 2 cases, the diagnosis was made on CSF Gram stain (1), and positive blood culture (1).

UK, DA1 5AH), Murex: 3 (Murex Diagnostics Inc, 3075 Northwoods Circle, Norcross GA 30071 USA), Biomerieux: 2 (Marcy-L'Etoile, 69752 Charbonnieres-les-Bains, Cedex, France), Phadebact CSF test: 2 (Boule Diagnostics AB, Huddinge, Sweden). One laboratory did not perform antigen detection tests.

Serotyping identified 22 serotypes of 18 different serogroups, plus 1 non-typable in the 65 isolates tested. The most commonly isolated serotypes were serotype 6 (13 cases) and serotype 14 (11 cases). Both serotypes caused more disease in the paediatric population (Fig. 1). The number of different serotypes causing disease in children under 5 was relatively restricted; 11 serotypes of 7 serogroups were identified among the 28 isolates collected from children (1, 6, 9, 14, 15, 18, 19). Twenty serotypes caused disease in those of 5 years and older (Fig. 1). Overall, capsular antigens of 80% of isolates identified are present in the 23 valent vaccine, with a further 10% covered by cross protective antigens, so that a total of 90% of meningitis isolates from this study would be covered by the current vaccine. In children under 5, 88% of the serotypes identified are found in the vaccine and the remaining 12% are covered by cross protective antigens.

Ribotyping was performed on 62 of the 65 isolates serotyped using *Eco*R V digests of genomic DNA. *Eco*R V does not appear to cut within the ribosomal operon, generating four large fragments in most cases. Patterns generated were diverse; ribotyping of 62 isolates identified 35 distinct types, of which 26 were represented by single isolates. Seven isolates of 4 different serotypes had ribotype 1 (see examples in Fig. 2). Nine isolates, all serotype 14, had ribotype 2 (Fig. 2). Ribotype 3 was represented by 6 isolates of which 5 were serotype 6B and one serotype 9V. Ribotype 19 had 4 isolates of 4 different serotypes. Five further ribotypes were represented by 2 or 3 isolates.

Analysing the results by serotype, 9 of 11 serotype 14 isolates were ribotype 2, 5 of 9 serotype 6B isolates were ribotype 3 and 2 were ribotype 5, 2 of 4 serotype 3 isolates were ribotype 13, 2 of 2 serotype 15C isolates were ribotype 1, and 2 of 4 serotype 18C isolates were ribotype 15. All other sets of isolates of the same serotype had different ribotypes.

Two (4%) of the 55 isolates tested had intermediate susceptibility by benzylpenicillin (MIC $\geqslant$ 0·12 mg/l) (Table 5) No isolates with high level resistance to penicillin were detected (MIC $\geqslant$ 1 mg/l). Reduced susceptibility to cefotaxime (as defined by the NCCLS guidelines of susceptibility to cefotaxime in CNS infections of MIC $\geqslant$ 0·5 mg/l) was not detected. However two (4%) isolates had cefotaxime MICs of 0·25 mg/l which is several dilutions higher than the modal MIC of 0·015 mg/l and close to the definition of reduced susceptibility. One of these isolates also had reduced susceptibility to benzylpenicillin. None of the isolates was resistant to chloramphenicol (MIC $\geqslant$ 16 mg/l). There were 6 (11%) isolates resistant to erythromycin (MIC $\geqslant$ 1 mg/l). All of the erythromycin resistant isolates were serotype 14 and ribotype 2. Two (4%) isolates were resistant to tetracycline (MIC $\geqslant$ 4 mg/l).

DISCUSSION

The work described forms part of a 3-year study on all causes of bacterial meningitis in North East Thames region from 1990–3. We identified 114 cases of pneumococcal meningitis in this period, representing an overall incidence of 0·8/100000/year. The in-

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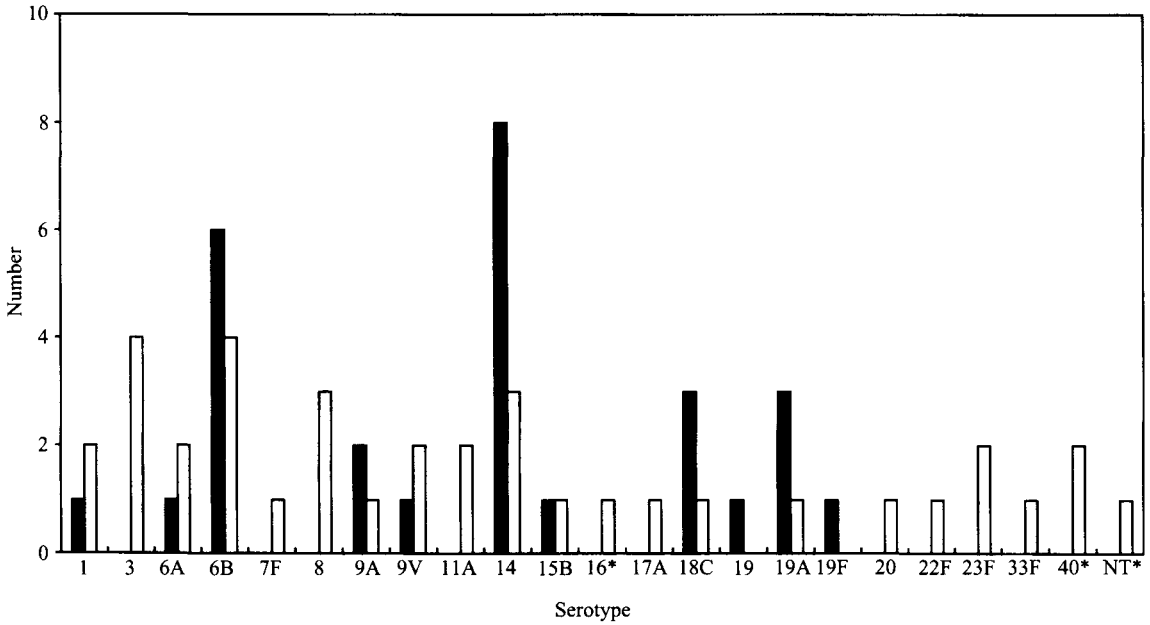

Fig. 1. Serotypes of *S. pneumoniae* isolated from patients aged under 5 years (■) and 5 years and over (□). NT is non-typable. * Not in current 23 valent vaccine.

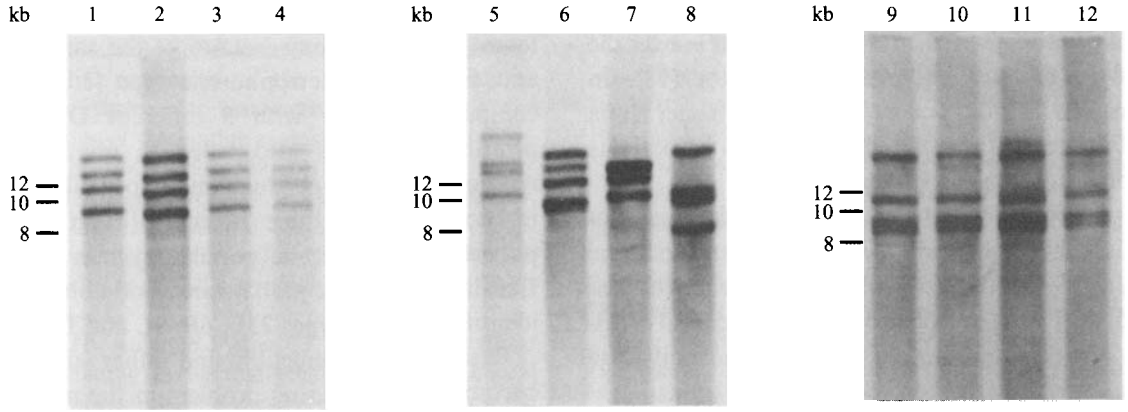

Fig. 2. Examples of ribotype patterns from different isolates: lane 1, ribotype 1, serotype 18; lanes 2–3, ribotype 1 serotype 15; lane 4, ribotype 1, serotype 19; lane 5, ribotype 20 serotype 23F; lane 6, ribotype 22 serotype 19F; lane 7, ribotype 25, serotype 11A; lane 8, ribotype 4, serotype 9A, lanes 9–12, ribotype 2, serotype 14. Positions of 8, 10 and 12 kilobase molecular size markers are indicated

Table 5. *Antimicrobial susceptibility testing for* S. pneumoniae (*n* = *55*), *North East Thames Region, 1991–1993**

	% of isolates with MIC (mg/l)										
Antimicrobial	⩽ 0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	⩾ 8
Benzylpenicillin	11	49	4	32	2	0	2	0	0	0	0
Cefotaxime	22	67	2	5	0	4	0	0	0	0	0
Chloramphenicol	0	0	0	0	0	0	2	6	50	42	0
Erythromycin	0	0	0	52	37	0	0	0	0	2	9
Tetracycline	0	0	0	0	7	56	29	4	0	0	4

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cidence of pneumococcal meningitis is approximately seven times higher in children under the age of 5 (incidence 5·5/100000) than in the general population, but individuals of all ages were affected [1]. In the Asian population the incidence is nearly three times that for Caucasians. Interestingly the incidence of *H. influenzae* meningitis, but not meningococcal meningitis, was also higher in the Asian population [1]. The number of cases in each ethnic group is small. However, it may be that this difference in incidence may be due to a number of interrelating variables including poverty, overcrowding and reduced access to health care. The number of cases in Blacks in this survey is too small for statistical analysis. Other studies have shown higher incidence of pneumococcal disease in blacks than non-Hispanic whites [2].

Although underlying illness is often implicated in pneumococcal disease, only 23 cases (22%) had an identifiable underlying predisposing factor. This small percentage may be the result of concentrating on pneumococcal meningitis rather than pneumococcal disease as a whole where higher rates of disease were seen in children [1]. Steven and Wright reported that 64% of patients with pneumococcal infection over the age of 65 had no underlying risk factor (18). In contrast, in a study of pneumococcal bacteraemia from Finland during 1979–89, only 11% of patients were previously healthy [19].

We identified an overall mortality rate for pneumococcal meningitis of 22%, with the highest rate in the elderly; 48% in those over 60 died. In children less than 5 years, the incidence of pneumococcal meningitis is higher than that of adults, the mortality rate was much lower (7%).

In this study 90% of cases were caused by serotypes that are either contained in the 23 valent vaccine or covered by cross protective antigens. Both serotyping and ribotyping revealed that a diversity of strains caused pneumococcal meningitis. The two serotypes most frequently isolated were serotype 6B and serotype 14, and these were particularly frequent in children. Recent surveys of *S. pneumoniae* around the world suggest that there are distinct geographical variations in the frequency of isolates of different serotypes, although types of specimens analyzed vary between studies. However, either or both serotypes 6B and 14 were among the most common in many countries [20–23].

Grouping of isolates by ribotype corresponded with serotype in some but not all cases. Of particular note, the 9 isolates of ribotype 2 were all of serotype 14, only 2 serotype 14 isolates having different ribotypes. All 6 of the isolates in this cluster tested for susceptibility to erythromycin were resistant. There was also an association of ribotype 3 with serotype 6B. On the other hand the 7 isolates of ribotype 1 were of 4 different serotypes; the extent of genetic similarity between these isolates requires further investigation.

The available epidemiological data on cases from which the 9 serotype 14, ribotype 2 isolates were obtained reveal no epidemiological links. The patients, both children and adults were admitted to 6 different hospitals at different times in the study period. Similarly there appeared no epidemiological links in the 5 cases with isolates of serotype 6B ribotype 3.

Previous studies on DNA-based typing of *S. pneumoniae* have also given mixed results on the association of DNA type with serotype. Viering and Fine [24] demonstrated using densitometric scanning of DNA restriction endonuclease digests that there were substantial differences in patterns among different serotypes and significantly greater similarity among isolates of a given serotype. Pulsed field gel electrophoresis used in another study revealed different patterns among isolates of the same serotype and multilocus electrophoretic type [25]. A recent comparative study with 5 different DNA fingerprinting techniques also failed to reveal any correlation with serotype [26]. Among penicillin resistant isolates however, there is evident that spread of pneumococci within a population may be clonal. Localized and more global spread of clones has been identified for serotypes 23F, 6B, 9L and 19A [10–13].

Resistance to penicillin and other antibiotics is becoming an increasing problem in the management of pneumococcal infection. Data from isolates sent to the PHLS identified a four times increase in the number of pneumococci with reduced susceptibility to penicillin over a 5-year period [27]. In some parts of Europe very high rates of penicillin resistance have been reported: 58% in Hungary [28], 40% in Spain [29]. Reduced susceptibility to penicillin was reported in 5% of isolates collected in the USA during 1979–87, with only one isolate with an MIC of 4 μg/ml [29]. In a collection of isolates from 1991–2 in the USA, penicillin resistance was reported in 6·6% overall, with high level resistance reported in 1·3% of isolates [31]. Recent identification of cefotaxime resistance and reports of treatment failures with this antibiotic are of concern [32, 33]. The rates of 4% overall resistance found in this study will only serve as a conservative estimate, a comparatively small number

of organisms were collected from a limited geographical area.

Continued active surveillance is an important mechanism to identify trends in resistance patterns in *Streptococcus pneumoniae*, monitor the prevalence of different serotypes and determine who is at risk of the disease in order to target the existing and any future vaccines most appropriately.

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