EPIDEMIOLOGY OF INFECTION

Pneumococci causing invasive disease in Britain 1982–1990

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A total of 5348 isolates of *Streptococcus pneumoniae* was serotyped and screened for insusceptibility to tetracycline, penicillin, erythromycin and chloramphenicol. Of these, 4238 (79%) were isolated from patients who had pneumonia or meningitis or were bacteraemic. Altogether, 3948 (74%) of the isolates belonged to one or other of the serotypes 1, 3, 4, 6, 8, 9, 14, 19 or 23 with serotypes 6, 14, 18, 19 and 23 being frequent causes of invasive disease in young children. Many isolates of type 1 were isolated from pneumonia and few from meningitis. Some 768 (14%) isolates were insusceptible to one or more antibiotic and 591 of these belonged to serotypes 6, 9, 14, 19 or 23. Representatives of type 14 resistant to erythromycin were prominent from 1986 onwards. There was an increase in the number of multi-resistant pneumococci from 1985. Among these were isolates of type 23 insusceptible to penicillin, chloramphenicol and tetracycline and cultures of type 6 resistant additionally to erythromycin.

Introduction

Nearly all clinical isolates of *Streptococcus pneumoniae* fall into one or other of 46 serogroups or serotypes based on the antigenicity of their capsular polysaccharides. Twenty-one of these, the serogroups, are each composed of a set of antigenically related strains and can be subdivided further giving a total of 90 type designations [1]. An example of the precision of the method is the modulations within serogroup 15 of type 15B to 15C due to loss of an O-acetyl group [2]. For simplicity, serogroups such as group 23 which is composed of types 23F, 23A and 23B – will be referred to here as ‘type 23’ as well as those, such as ‘type 3’ which react with a single factor serum [1].

Serotyping has provided a tool for studies of outbreaks. For instance, in the 1644 cases of pneumococcal pneumonia that occurred in an American Air Force Base over three successive winters, one-third were caused by pneumococcus type 2 [3]. Large outbreaks, comparable to that in the Air Force Base are not known to have occurred in Britain. On the other hand pneumococci comprise 11% of positive blood cultures and 18% of isolates from bacterial meningitis [4].

The distribution of serotypes causing disease differs by time, place and age of patient. Type 2, for instance, was one of the predominant types causing disease in Denmark in the years 1939–1947 [5]. It was not found in that country between 1983 and 1988 [6] but it causes invasive infections in Africa [7]. Type 5 is one of the less common serotypes in Europe and North America. For that reason it was not included in the 14-valent pneumococcal vaccine, but it is prevalent in West Africa [8] and was incorporated in the 23-valent vaccine. The apparently invasive type 46 occurs in South Africa [9] and Papua-New Guinea [10], but is not isolated in Europe [5, 6] or Australia [11]. Among children, serotypes 6, 14, 19 and 23 account for many infections. In Alabama, for instance, over a 15-year period they caused nearly two-thirds of the pneumococcal infections in infants and young children [12].

Insusceptibility to one or more clinically useful antibiotics has been described in representatives of all common types [13]. Worldwide, the problem strains are found most often in serotypes 6, 14, 19 or 23. Disparate levels of susceptibility to penicillin were detected in the very first tests in 1941 [14, 15]. Resistance became a clinical problem with the finding of insusceptibility to tetracycline in 1963 [16], penicillin in 1967 [17], erythromycin and lincomycin in 1967 [18], chloramphenicol in 1970 [19] and multiple resistance – that is resistance to three or

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more classes of antibiotics – in 1977 [20]. In an earlier survey of pneumococci isolated in Britain from invasive disease, resistance to antibiotics was not a major problem [21].

The results presented here are from routine surveillance based upon isolates referred from hospital and public health laboratories in England and Wales. The aims were to follow the distribution of types among isolates causing invasive disease, investigate possible outbreaks of infection and screen isolates for insusceptibility to antibiotics. There was thus a deliberate bias towards the study of isolates of clinical importance. Additional information on pneumococcal infection is available to the Public Health Laboratory Service from laboratory reports to the Communicable Disease Surveillance Centre and also from special surveys [22].

The period beginning in 1982 has been chosen for this report because, apart from special surveys, less information was available from earlier years and techniques were being modified. For example, when screening for insusceptibility to erythromycin was begun in 1979, disks with a content of 0.125 µg of this antibiotic were used. Later, a change was made to disks containing erythromycin 0.5 µg because of the small zone size obtained with susceptible strains with the disks of lower content.

Materials and methods

Isolates

Results from 5348 isolates were used (Table 1). Records for which the referring laboratory had not given both the patient's name and the source of the culture were excluded. If more than one isolate of a single serotype was available from either one episode of infection or from what appeared to be hospital or family spread then an arbitrary choice was made of a single record. For example, an isolate from cerebrospinal fluid (CSF) would be chosen rather than one from blood culture on the same patient. Occasionally, isolates of different serotypes were isolated at different times from invasive disease affecting an individual patient and these were accepted as re-infections.

The cultures that form the basis of Table 1 can be divided into those from sites that are normally sterile and those from the vagina, urinary tract, wounds (including burns), eyes, ears, sputum or nasal swabs where pathogenicity cannot be assumed. The isolates classed as coming from patients with pneumonia were from blood cultures of patients with this clinical diagnosis or were isolated from pleural aspirates, lung tissue or pericardial fluid. Those from meningitis were from CSF, blood or both. Bacteraemia isolates were from patients with positive blood cultures for whom clinical diagnoses were either not supplied or in whom

Table 1. Sources of 5348 isolates of pneumococci sent to the Streptococcus Reference Unit, 1982–1990

Source	Year									Total
	1982	1983	1984	1985	1986	1987	1988	1989	1990	
Pneumonia	270*	146	163	130	187	186	265	266	283	1896
	23	7	10	8	13	15	26	18	22	142
Bacteraemia	75	117	139	126	139	128	154	228	223	1329
	4	8	5	9	14	5	21	25	35	126
Meningitis	72	75	109	97	121	115	143	134	147	1013
	2	0	3	6	7	11	7	8	19	63
Arthritis or osteomyelitis	5	4	9	4	6	5	9	3	9	54
	0	0	0	0	1	0	2	1	1	5
Endocarditis	2	2	5	1	3	3	3	2	3	24
	0	0	0	0	0	0	1	0	1	2
Cellulitis	5	4	4	2	4	6	4	7	13	49
	1	2	1	0	0	0	0	1	3	8
Abdominal infection	5	6	8	1	4	2	4	9	9	48
	1	0	0	0	0	0	0	1	0	2
Neonatal sepsis	0	3	4	2	6	5	8	11	9	48
	0	0	0	0	2	0	2	0	1	5
Vagina	0	4	2	3	8	8	5	5	4	39
	0	1	0	0	1	0	0	1	2	5
Urinary tract	3	0	1	2	4	2	3	2	0	17
	0	0	0	0	0	0	0	0	0	0
Wounds	1	0	0	6	1	0	10	4	2	24
	1	0	0	1	0	0	0	1	0	3
Eye	3	7	12	16	14	10	18	37	25	142
	0	1	0	2	1	4	8	11	18	45
Ear	6	8	8	4	8	8	11	11	20	84
	0	1	0	1	2	2	3	4	13	26
Sputum or nasal swabs	71	29	11	20	45	74	92	81	158	581
	12	7	9	16	28	53	46	53	112	336
Total	518	405	475	414	550	552	729	800	905	5348
	44	27	28	43	69	90	116	124	227	768

*Total from this condition with number insusceptible to one or more antimicrobial agent given below.

there was no evidence of localisation. For arthritis or osteomyelitis, pneumococci were isolated from blood culture, joint fluid or bone tissue. Endocarditis strains were solely from blood cultures. Isolates from cellulitis – including orbital cellulitis and epiglottitis – and peritonitis were isolated from either blood culture or tissue fluid or both. The category neonatal sepsis was restricted to organisms isolated from the blood or CSF or neonates for whom a strain of the same serotype was isolated from the genital tract of the mother. Nearly all the isolates from eyes were from conjunctival or corneal swabs. The exceptions were two from endophthalmitis. Two cultures only were from middle ear fluid, all other in this category were from ear swabs.

Identification

On reception isolates were cultured on horse blood agar incubated in air supplemented with CO₂ (5% v/v). If cultures were thought to be mixed further subcultures were made. Nearly all pneumococci isolated from invasive disease are susceptible to optochin (ethylhydrocuprein), are lysed by bile or other detergents (activation of N-acetylmuramic acid, L-alanine amidase) and are agglutinated by one or other of the currently available typing sera. Exceptions occur: pneumococci can develop resistance to optochin and non-capsulate pneumococci may not be bile-soluble [23]. Conversely, occasional isolates that clearly are not pneumococci are bile soluble. An example is isolate R985, *Streptococcus suis*, serological group R [24]. Finally, cross-reactions between various oral streptococci and pneumococcal serotypes have been documented [25].

Optochin susceptibility and serotyping were used routinely. Disks containing optochin 5 µg (Mast Laboratories, Bootle, Merseyside) were applied to blood agar and zone sizes > 12 mm were expected. Isolates that could not be serotyped were tested for bile solubility.

Typing

Sera for typing were bought from the State Serum Institute, Copenhagen. Routinely, the pooled sera A–I and the individual sera 1–48, excepting the numbers 26 and 30, were employed [26]. Isolates were subcultured in serum glucose infusion broth. The bacterial growth was deposited by centrifugation, resuspended in a few drops of the supernate and tested for agglutination in, first, the pooled sera, then the components of the pool agglutinating the isolate. Typing with factor sera, as used by Smart [27], was not done. Isolates that did not react with any of the pools were tested for agglutination with polyvalent 'OMNI-serum'. If agglutinated by that reagent the procedure with individual pools and sera was repeated. If not, bile solubility was determined.

Until 1981 isolates were serotyped by two methods, namely agglutination and the capsular swelling reaction. The same result was obtained by either method and the simpler agglutination procedure was adopted for routine use. Both methods are used during production of typing sera [28].

Susceptibility to antibiotics

A cotton wool swab was used to transfer growth from the initial culture to a second horse blood agar plate (Blood Agar Base supplemented with Yeast Extract 0.3% w/v; Oxoid) and disks (Oxoid) were applied. The agents used are listed, followed first by the drug content of each disk, second the zone size obtained with most susceptible strains and third the critical zone size used to define insusceptible strains. They were optochin: 5 µg, > 12 mm, ≤ 9 mm; oxacillin: 1 µg, > 20 mm, ≤ 15 mm; benzylpenicillin: 0.6 µg, > 25 mm, ≤ 25 mm; tetracycline: 5 µg, > 20 mm, ≤ 18 mm; erythromycin: 0.5 µg, > 14 mm, ≤ 14 mm; chloramphenicol: 10 µg, > 20 mm, ≤ 15 mm. All came within the description 'low content' as used by Snell and colleagues [29]. A reduction in zone size about the oxacillin disk was used to indicate insusceptibility to penicillin [30]. The methods used would not distinguish between moderate- and high-level resistance and the term 'insusceptible' will be used for both.

Results and Discussion

Type distribution – general

The serotype distribution of the cultures is given in Table 2; 3693 (69%) fell into one or other of nine serotypes, each of which represented at least 5% of the strains studied. They were serotypes 1, 3, 4, 6, 8, 9, 14, 19 and 23. Another nine serotypes – 7, 10, 11, 12, 15, 17, 18, 22 and 33 – each accounted for 1–5% of the total. This distribution of most frequent types did not differ greatly from those recorded in Denmark [6] or Australia [11], although types 7 and 12 were relatively more common in the former study and 7 and 18 in the latter. There were few isolates of 22 serotypes, including types 2 and 5 (Table 2). No isolates of serotypes 40, 43, 44, 45, 46 or 47 were received. There were no obvious trends in the distribution of serotypes from laboratories that referred isolates over several years.

Age

Of the total of 5348 isolates, 4238 (79%) were from patients who had pneumonia, meningitis or were bacteraemic (Table 1). Pneumococcal meningitis or bacteraemia tended to occur more often in younger persons than pneumococcal pneumonia. The ages of 2936 patients were available for the years 1986–1990. This was 83% of the total of 3536 patients for those years. Of these, 2257 belonged to one or other of

Table 2. Serotype, source of isolates and number insusceptible

Serotype	Source						Total
	Pneumonia, empyema	Meningitis, brain abscess	Bacteraemia	Other invasive states*	Infections: possible pathogens†	Sputum, nasal swabs	
1	208‡	13	83	30	7	7	348
	18	0	3	0	1	0	22
3	264	61	120	21	41	45	552
	5	3	6	0	2	9	25
4	124	43	81	11	7	6	272
	1	1	3	0	0	1	6
6	98	127	112	15	49	111	512
	5	10	11	3	21	84	134
7	96	56	48	4	5	16	225
	1	0	0	2	1	5	9
8	143	44	50	7	8	3	255
	4	2	0	1	1	0	8
9	138	63	112	6	17	30	366
	7	1	5	1	8	16	38
10	19	25	10	2	2	11	69
	2	0	1	1	0	3	7
11	43	12	25	5	7	18	110
	1	0	0	1	0	6	8
12	28	25	27	6	1	2	89
	1	0	0	1	0	0	2
14	288	137	205	26	29	45	730
	60	32	57	5	14	30	198
15	21	26	19	4	4	13	87
	3	2	2	0	1	4	12
17	14	15	13	2	3	6	53
	1	1	1	0	0	0	3
18	45	77	80	10	6	13	231
	3	0	3	0	0	3	9
19	128	91	114	25	39	54	451
	10	5	9	1	5	27	57
22	33	21	33	10	4	3	104
	1	0	0	0	1	0	2
23	83	86	86	21	37	149	462
	9	3	11	3	18	130	174
33	27	20	19	3	8	8	85
	1	1	1	0	0	3	6
Others§	96	71	92	15	32	41	347
	9	2	13	3	6	15	48
Total	1896	1013	1329	223	306	581	5348
	142	63	126	22	79	336	768

* Isolates from arthritis, endocarditis, osteomyelitis, cellulitis, neonatal septicaemia and peritonitis.

† Organisms isolated, usually not in pure culture, from wounds, eye or ear swabs, the vagina or urinary tract.

‡ Total of this serotype with number insusceptible to one or more antimicrobial agent below.

§ Serotypes with 1–48 representatives brought together here. They are, with the number susceptible to one or more antibiotic in parentheses: 2, 1(0); 5, 39(17); 13, 14(2); 16, 23(0); 20, 48(1); 21, 10(2); 24, 23(1); 25, 2(2); 27, 7(0); 28, 3(0); 29, 14(1); 31, 18(1); 32, 4(1); 34, 26(0); 35, 16(5); 36 2(0); 37, 6(0); 38, 14(2); 39, 14(0); 41, 3(0); 42, 5(2); 48, 2(0); 29 + 35, 1(0); 35 + 42, 4(0); non-typable, 48(11). No isolates of serotypes 40, 43, 44, 45, 46 or 47 were handled.

the three main disease categories. More than one-third with meningitis (38%) or bacteraemia (35%) were aged ≤ 5 years. For a diagnosis of pneumonia only 6% were aged ≤ 5 years and 66% were > 60 years.

A comparison was made of the serotype distribution between, on the one hand, those aged ≤ 5 years and, on the other, older children or adults (Table 3). Among isolates from invasive disease, representatives of serotypes 6, 14, 18, 19 and 23 were more common in young children. The same five together with type 4 caused most cases of systemic infection in children in Australia [11]. Some serotypes, in particular types 11 and 15, have been isolated more often in carriage studies on children than from invasive disease [21,

31]. In contrast to the findings in young children, isolates of serotypes 3, 8, 11, 12, 17 and 22 were relatively more common in those aged ≥ 6 years (Table 3).

For the strains of types 1, 3, 8, 14, 18 and 23 that are included in Table 3 the distribution by age of patient, in 10-year bands, is shown in Fig. 1. The general increase, with age, of types 3 and 8, and the predominance of type 18 in children is evident. Something like a bimodal distribution occurred with types 14 and 23. Type 1 is illustrated because percentages were relatively evenly distributed by age. Of the total of 309 isolates of these six serotypes from children aged < 10 years of age, only 22 were isolated from those aged 6–9 years.

Table 3. Isolates received from 2936 patients of known age, 1986–1990

Type	≥ 6 years of age		≤ 5 years of age		Total
	Invasive	Non-invasive	Invasive	Non-invasive	
1	171 (9.2)*	8	32 (5.6)	4	215
3	207 (11.1)	22	17 (3.0†)	14	260
4	126 (6.8)	5	22 (3.8)	1	154
6	106 (5.7)	51	99 (17.2‡)	50	306
7	88 (4.7)	2	17 (3.0)	3	110
8	111 (6.0)	3	2 (0.3)	1	117
9	173 (9.3)	19	29 (5.0)	11	232
10	23 (1.2)	3	4 (0.7)	1	31
11	39 (2.1)	5	1 (0.2)	5	50
12	43 (2.3)	2	3 (0.5)	1	49
14	270 (14.5)	22	147 (25.5)	25	464
15	27 (1.5)	7	7 (1.2)	4	45
17	25 (1.3)	2	3 (0.5)	0	30
18	49 (2.6)	5	52 (9.0)	0	106
19	118 (6.3)	25	74 (12.8)	33	250
22	47 (2.5)	0	2 (0.3)	3	52
23	103 (5.5)	82	37 (6.4)	46	268
33	34 (1.8)	6	8 (1.4)	1	49
Others§	102 (5.5)	24	20 (3.5)	2	148
Total	1862	293	576	205	2936

*Total of this serotype and for invasive isolates percentages in that age group in parentheses.

†Italics indicate percentages one-half, or less, of the percentage from patients aged ≥ 6 years.

‡Bold type used for a percentage more than double the percentage from patients aged ≥ 6 years.

§All types with from 1 to 23 representatives brought together here. It is composed of serotypes, 5, 13, 16, 20, 21, 24, 27, 29, 31, 32, 34, 35, 37, 38, 41, 35 + 42 and non-typable isolates.

Scott [32] has brought together the results of studies performed in different countries. He calculated the relative risk of invasive disease caused by type 1 and found that it declined throughout life. Most of his data on type 1 were from studies in Brazil. In one of these sets [33] only two isolates of type 1 were from patients aged ≥ 50 years. In contrast, 86 (42%) of the 203 isolates of type 1

included in Fig. 1 were from patients aged ≥ 50 years.

Pneumonia

Altogether, 1896 (35%) of the isolates were isolated from patients with pneumonia (Table 1). The distribution of serotypes may be different in patients

Fig. 1. Serotype of pneumococcus and age distribution, in 10-year bands, among 1198 patients with invasive infection. Number of cases: type 1, 203 (60% males); 3, 224 (62% males); 8, 113 (62% males); 14, 417 (52% males); 18, 101 (57% males); 23, 140 (60% males).

with pneumococcal pneumonia but negative blood cultures. Of the isolates causing pneumonia types 1, 3 and 14 each contributed at least 10% of the total (Table 2). Type 1 was unique because it had the largest proportion (208 of 348 isolates; 60%) from pneumonia and the smallest (only 13; 4%) from meningitis. This association of type 1 with pneumonia and infrequency in meningitis has been noted by others [6, 11]. An outbreak of bacteraemic pneumonia caused by type 1 pneumococci occurred among Australian aborigines [34]. As in other outbreaks, carriage of type 1 strains was uncommon unless the subject had invasive disease or was in contact with cases of acute infection.

The susceptibility of AIDS patients to pneumococcal infection is indicated by the frequency of pneumococcal pneumonia in them, the many such infections that are bacteraemic and the number of recurrences of infection [35]. The first cultures handled, four in all, from patients recorded as being infected with HIV were referred in 1986. Since then, cultures from HIV-infected patients comprised one from an infant with fatal septicaemia and six isolates from five individuals with chest infections. All five cultures handled in 1986 and 1987 were susceptible to antibiotics. The isolate received in 1989 was resistant to tetracycline, but four of the five isolates received in 1990 were insusceptible to penicillin, tetracycline and either chloramphenicol or erythromycin.

Bacteraemia

The clinical states seen in pneumococcal bacteraemia range from a passing febrile illness in which recovery occurs without treatment [36] to an acute septicaemic illness. Of the 1329 cultures from bacteraemias there were 205 of type 14 (15% of the total), followed by types 3, 6, 9 and 19 – each about 9% of the total (Table 2). Among individual serotypes, 80 (35%) of 231 of the cultures of type 18 were from bacteraemias; a smaller total but a larger proportion.

In adults, pneumococcal bacteraemia is often associated with a pre-disposing condition [37], one of which is splenectomy [38], and another is splenic dysfunction [39]. Twenty patients were known to have had previous splenectomy and of these nine presented with meningitis, eight with septicaemia, two with pneumonia and one with a post-surgical abscess. Strains of type 23 were isolated from five patients; types 9, 18, 19, 24 and 33 were each isolated from two subjects; and single cultures of types 11, 20, 22, 38 and 42 were handled. Two patients, one infected with type 9, the other with type 19, had received the pneumococcal vaccine.

During the years 1982–1992 there were reports to the Public Health Laboratory Service, Communicable

Disease Surveillance Centre, London (CDSC) of 11 299 isolations of *S. pneumoniae* from blood cultures. The overall rate of pneumococcal bacteraemia in males exceeded that in females by 26%. During this period the proportion of infections at the extremes of age – those aged ≤ 1 year and those > 75 years – rose three-fold [4].

Meningitis

From 1982 to 1992, 3500 cases of pneumococcal meningitis were reported to CDSC and 1023 (29%) were in infants aged ≤ 1 year. For both meningitis and bacteraemia the first week of life is the time of greatest risk for babies [4].

An unknown proportion of the 1013 cultures handled by us from meningitis, 19% of all cultures, would have been included in the CDSC figures. The largest number of isolates from meningitis (137; 14%) were, again, of type 14. Serotypes with the largest proportion of isolates from meningitis were type 10 (25 of 69 isolates; 36%), type 18 (77 of 231 isolates; 33%) and type 15 (26 of 87 isolates; 30%). Only 13 of 348 cultures of type 1 (4%) were from meningitis. This comparative rarity of type 1 has been noted in an earlier survey in this country [40]. However, type 1 was the most common cause of meningitis in West Africa [8], while in Denmark from 1947 to 1962 types 1, 3 and 7 were common in adults with meningitis and types 4, 6 and 18 in children [41]. In South Africa, for the years 1979–1986, types 1, 6, 14 and 19 were the most numerous isolates from pneumococcal meningitis [7].

There were 24 patients with recurrent meningitis and among these, a head injury had been recorded for 14 patients. The set of 24 cultures was composed of five representatives of type 6, three of type 23, two each of types 10, 14, 15, 19 and 33 together with single isolates of types 8, 9, 20, 34, 39 and a non-typable isolate.

Vaccine

The current pneumococcal vaccine contains 23 capsular polysaccharides. A total of 5001 isolates, forming the body of Table 2, belonged to 18 of the serotypes or serogroups numbered between 1 and 33. They represented 93.5% of the isolates studied and all are incorporated in this vaccine. Because of the lack of cross-protection within each of the serogroups 9 and 19, capsular polysaccharides from serotypes 9N, 9V, 19F and 19A are used in the vaccine. The remaining three components of the vaccine, serotypes 2, 5 and 20, are at present uncommon in this country. They are listed in a footnote to Table 2 and comprised 1.6% of the isolates studied.

In a randomised trial among young African gold-

miners, immunisation with pneumococcal polysaccharide gave *c.* 80% protection against bacteraemic infection caused by a serotype present in the vaccine [42]. By studying the distribution of serotypes causing infection among immunised and non-immunised populations, indirect cohort analysis, similar levels of protection appear likely in elderly subjects who are immunocompetent [43]. In contrast, little or no protection could be detected in, for example, patients with sickle cell disease, leukaemia or multiple myeloma [43, 44]. Among children with sickle cell disease, penicillin prophylaxis has protected against pneumococcal septicaemia [39, 44].

An additional problem is the poor response of young children to some polysaccharides. To provide protection for them, conjugate immunogens of the serotypes 6B, 14, 19F and 23F have been suggested with the possible later addition of types 4, 9 and 18 [45]. These seven serotypes comprised 78% of isolates from invasive disease in young children (Table 3).

Arthritis or osteomyelitis

Isolates were received from 44 cases of septic arthritis. The ages of 27 of these patients were available and eight were aged ≤ 2 years: the next four were aged 10, 32, 45 and 51 years. Four of the 44 were infected at the same time at more than one site, two with polyarthritis. Previous meningitis was recorded for one patient. Before antibiotics were available infections involving more than one site were apparently common [46]. The 44 isolates from arthritis were distributed across 14 serotypes including: type 14 (eight isolates), types 4 and 22 (five isolates each) and types 1, 6 and 19 (four isolates each).

In one series of cases of pneumococcal osteomyelitis [46] strains of serotype 19 were unusually common, comprising four of the 11 isolates. The same serotype, five of 10 referrals, was predominant among our isolates from this infection.

Abdominal infection

Pneumococcal peritonitis can follow conditions such as appendicitis [47], liver failure or bacteraemia. Primary pneumococcal peritonitis occurs, albeit infrequently, in young girls [48]. The infection has occurred in women fitted with intra-uterine contraceptive devices [49] and the infection has been produced experimentally by the introduction of the organism into the vagina of monkeys [50].

Some 48 isolates were received from patients with abdominal infections and they belonged to one or other of 13 serotypes with 16 isolates in type 1, seven in type 3 and five in type 14. The referring laboratories described 10 of the isolates as coming from patients with primary peritonitis, all but one

of whom were female. In contrast, the six isolates from appendicitis were distributed equally between types 6, 14 and 19 and only one patient was female.

Endocarditis

The recognised suppurative complications of bacteraemic pneumococcal pneumonia include meningitis, empyema, endocarditis and less often arthritis or peritonitis [51], 24 isolates of 16 serotypes were received from patients with endocarditis, an unknown proportion of whom had had pneumonia. Types 14 (four isolates) and types 6 and 23 (three isolates each) were the most numerous.

Cellulitis

There were 49 isolates in the collection from patients with cellulitis and for 13 of these periorbital cellulitis was specified. Other conditions included epiglottitis, necrotising fasciitis, blistering cellulitis and cellulitis surrounding a burn. Altogether, 21 different serotypes were represented, including type 23 (eight isolates), type 14 (seven isolates) types 3 and 19 (five isolates each) and type 18 (four isolates). At least nine of the periorbital infections occurred in children and among the pneumococci recovered from this condition there were three isolates each of types 14 and 18, and two each of types 19 and 23.

Neonatal septicaemia

Pneumococci have been reported as causing 2–10% of cases of neonatal sepsis [52]. Isolates were received from 48 neonatal infections in which cultures of the same serotype were isolated from vaginal swabs of the mother and blood cultures or CSF of the baby. The isolates were distributed between 18 serotypes, with seven representatives in each of types 1 and 19, six of type 23, five of type 3, three each of types 6, 8, 18 and 27, two of type 4 and one each of types 5, 9, 11, 12, 14, 15, 20, 22 and 24.

The organism is isolated infrequently from vaginal swabs [53]: only 39 cultures in this study were exclusively from the site. Some of these cultures were presumably saprophytic, but most were associated with vaginitis, infection of Bartholin's glands or endometritis. The finding of neonatal septicaemia caused by an organism that is not often found in the vagina has been attributed to invasiveness.

Wound infections

Pneumococci are one of the less common causes of wound infection [11]. Of the 24 isolates from wound pus, nine were from infected burns. The set was composed of 13 serotypes but 13 isolates belonged to one or other of the serotypes 3, 14, 19 or 23.

Conjunctivitis

There can be problems in the identification of some pneumococci, or pneumococcus-like streptococci, associated with conjunctivitis [54]. In a study covering selected years from 1935 to 1974, Finland and Barnes [55] found that one-quarter of the strains from eyes were untypable: their largest source of non-typable strains. Similarly in a study in this country [56] all strains from invasive disease could be serotyped, but 2% of strains from sputum or the nasopharynx and 12% of strains from eyes could not be serotyped.

Of the 142 isolates from eyes (Table 1) 14 were untypable, usually because growth in broth was granular. In 1985 cultures were received from 24 adolescents affected in a school outbreak of conjunctivitis. Seventeen of these cultures could not be serotyped, five were of type 33 and two of type 34. One record from each category was used here.

Ear Swabs

Unlike isolates from the eyes, all 84 cultures from the ears could be serotyped. Of the 84 cultures, 16 were of type 3, 13 of type 6, 11 of type 14, 10 of type 19, nine of type 7 and five of type 23. The same six serotypes were dominant also in Orange and Gray's set of 228 strains from otitis media [12]. There were two important differences. First, the latter strains were all isolated from fluid collected after tympanocentesis, ours with two exceptions were from ear swabs, and second, type 3 was fifth in their rank order.

Included in this category were five isolates from infections of the mastoid. They comprised two strains of type 14 and one each of types 3, 9 and 22. When otitis media or mastoiditis led to meningitis the cultures were placed in that category.

In one study middle ear infections accounted for 1.5% of all visits to general practitioners [57]. *S. pneumoniae* and *Haemophilus influenzae* are the predominant primary pathogens in acute otitis media and aspects of such infection caused by pneumococci have been reviewed by others [45].

Sputum or nasal swabs

Isolates from sputum or nasal swabs were the fourth largest category (Table 1). That 336 of 581 such cultures (58%) were insusceptible to one or more antibiotics provides a clear indication of the reason for referral of these isolates. Many were representatives of types 6, 14 or 23 (Table 2).

The most prevalent pneumococcal serotypes in the upper respiratory tract are not the same as those isolated from invasive disease [58]. The upper respiratory tract is a reservoir of antibiotic-resistant pneumococci [59].

Insusceptibility to antibiotics

There were 768 isolates insusceptible to one or other, or any combination, of the antibiotics penicillin, tetracycline, erythromycin or chloramphenicol (Table 1) with a general increase in prevalence through the study period. Altogether 591 (77%) of the 768 isolates belonged to one or other of five serotypes, namely 6, 9, 14, 19 or 23 (Table 2). This clustering was most noticeable in the years 1989 and 1990, when representatives of these serotypes made up 86% and 87%, respectively, of the insusceptible strains.

Optochin. Optochin has not been used clinically for many years, but the occurrence of resistance to this compound among pneumococci could lead to misidentification of clinical isolates. Mixed populations, susceptible and resistant to optochin, have been observed in fresh clinical isolates of types 3 and 6 [60].

Resistance to optochin was detected in 23 isolates distributed among 15 serotypes. All exhibited the colony forms expected of pneumococci. If the organism was resistant to optochin alone it is not included among the resistant isolates in the tables. Sixteen isolates were obtained in pure culture from invasive disease; there were four representatives of type 6 and three of type 3. In 1985 two cultures of type 3 were collected from throat swabs taken in one incident – one was insusceptible to optochin and tetracycline, the other to tetracycline alone. Again, in 1990, of two isolates of type 9, this time from sputum samples from a single patient, one was insusceptible to penicillin and the other to optochin and penicillin.

Tetracycline, chloramphenicol. Insusceptibility to tetracycline was found alone or in combination in 444 (8% of all isolates) (Table 4). Of these, 116 were insusceptible to tetracycline alone and they were distributed among 23 serotypes. Some 69 of these 116 (59%) were other than the serotypes 6, 9, 14, 19 or 23 and included the only resistant isolates of the serotypes 24, 25, 31 or 42. The serotypes 1 and 3 accounted for 16 and 11 isolates, respectively.

Insusceptibility to chloramphenicol alone was detected in six isolates and combined with tetracycline in a further 25 isolates. Although isolates of type 5 were uncommon, this serotype contained the highest proportion of isolates insusceptible to antibiotics: 17 of 39 isolates (Table 2). Of these 17, all of which were isolated from invasive disease, eight were insusceptible to tetracycline alone, three to chloramphenicol and six to both chloramphenicol and tetracycline.

Spread of tetracycline-resistant pneumococci in a British hospital was seen first in Liverpool in 1963 [61] and by 1968 almost one-quarter of the pneumococci isolated in the Liverpool Royal Infirmary were

Table 4. Distribution of resistance in serotypes 6, 9, 14, 19, 23 and other types (N)

Antibiotic resistance	Serotype						Total
	6	9	14	19	23	N	
Pen	10	30	15	16	12	26	109
Tet	12	3	17	13	2	69	116
Eryth	10	3	145	4	1	16	179
Chlor	2	0	2	0	1	6	11
Pen/Tet	5	2	7	4	3	9	30
Pen/Eryth	0	0	3	3	1	1	8
Pen/Chlor	1	0	1	1	8	2	13
Eryth/Tet	8	0	2	2	3	3	18
Eryth/Chlor	0	0	1	0	0	2	3
Chlor/Tet	3	0	1	4	1	25	34
Pen/Chlor/Tet	16	0	1	4	135	6	162
Pen/Eryth/Tet	12	0	1	2	2	2	19
Pen/Chlor/Eryth	0	0	0	1	0	0	1
Eryth/Chlor/Tet	4	0	0	2	0	10	16
Pen/Chlor/Tet/Eryth	42	0	2	0	5	0	49
Total	125	38	198	56	174	177	768

Pen, Penicillin; Tet, tetracycline; Eryth, erythromycin; Chlor, chloramphenicol. Bold type indicates the most common pattern in this serotype.

resistant to the drug [62]. In 1975 the proportion of tetracycline-resistant pneumococci ranged from 3% to 37% depending on the hospital [63], but then fell [64].

Erythromycin. Erythromycin is often used by family doctors for the empirical treatment of community-acquired pneumonia because it is active against mycoplasmas as well as pneumococci. Cross-resistance to lincomycin was found in the first isolates of erythromycin-resistant pneumococci [18].

From 1986, there was a striking increase in the number of referred pneumococci that were resistant to erythromycin. Of the total of 179 such isolates, 145 were of serotype 14 (Table 4). In the nine years 1982–1990, the sequence of erythromycin-resistant isolates of type 14 was: 1, 0, 1, 0, 8, 16, 31, 37 and 51. One erythromycin-resistant isolate of type 1 was isolated from pleural fluid 10 days after a susceptible isolates, also of type 1, had been found in blood cultures of that patient.

Elsewhere different patterns prevailed. In Belgium, for example, erythromycin-resistant isolates of type 6 were prevalent and most of these were resistant also to tetracycline [65]. There is some evidence of a linkage of genes for resistance to erythromycin and tetracycline, because co-transfer occurs in transformation tests [66]. Among our isolates of type 6, 10 were resistant to erythromycin alone and eight to both drugs (Table 4).

Penicillin. The 109 isolates that were insensitive to penicillin alone were distributed among 19 serotypes, but 83 (76%) belonged to one or other of the five serotypes 6, 9, 14, 19 or 23. The screening tests used would not have been distinguished pneumococci that were insensitive (MIC 0.1–1.0 mg/L) or resistant

(MIC $\geq$ 2 mg/L) to penicillin. More detailed studies of isolates were carried out by the Antibiotic Reference Unit [67].

Usually when a zone size of < 15 mm was found around an oxacillin disk (1 μ g) then the zone size about the benzylpenicillin disk (0.6 μ g) was < 25 mm. However, there were exceptions. In 1982, for example, an isolate of type 6 from sputum exhibited zones of inhibition of 9 mm to oxacillin and 29 mm to penicillin. In retrospect these cultures may have been comparable to the recently described intermediately oxacillin-resistant pneumococci [68].

Multi-resistance. Multi-resistance, to penicillin, chloramphenicol and tetracycline, or penicillin, chloramphenicol, tetracycline and erythromycin, was prominent from 1985 onwards. Most isolates with the former pattern belonged to type 23 and selected isolates referred to the State Serum Institute, Copenhagen (Dr J. Henriksen) were all found to be type 23F. The numbers of such isolates of type 23 for the years 1982–1990 were 1, 1, 2, 8, 18, 28, 22, 22 and 33. In 1986, for the first time, cultures resistant to all four antibiotics were handled. In that year there were two of these cultures, one of type 6, the other type 23. In later years there were 2, 4, 10 and 25 isolates of type 6 and a total of four isolates of type 23.

In 1990 there were 85 multi-resistant isolates, 9% of the total of 905 isolates for that year. This figure is higher than the nine (1.7%) multi-resistant isolates found in 1990 among 530 cultures from 52 British laboratories [22]. This sample was composed of all pneumococci isolated over a 2-week period.

The base sequence of the gene coding for the penicillin-binding protein 2B was available for three

of the type 23 isolates resistant to penicillin, chloramphenicol and tetracycline [69]. The sequences were the same as two type 23 strains from Spain. All could be distinguished from strains of other serotypes isolated, for instance in Papua-New Guinea or Africa, or a type 19 strain isolated in the UK.

Sources. The proportion of isolates that were insusceptible to antibiotics differed by source as well as serotype. Of the 581 cultures from sputum or nasal swabs (Table 1), 336 (58%) were insusceptible to one or more agent. In contrast, of the 4238 isolates from patients with pneumonia, meningitis or bacteraemia 331 (8%) were insusceptible. Among the isolates of type 23, 104 of the 135 cultures insusceptible to penicillin, chloramphenicol and tetracycline were isolated from sputum or nasal swabs, 15 from pneumonia, meningitis or bacteraemia, 10 from sporadic cases of conjunctivitis in babies and six from other sources. Such strains have spread within hospitals [70]. Of the total of 255 isolates of type 23 from cases of pneumonia, meningitis or bacteraemia, 23 (9%) were insusceptible to one or more antibiotic. A greater proportion of insusceptible isolates of type 14 were from the same three conditions: 149 (29%) of 630 cultures. Most were insusceptible to erythromycin alone.

During the period of this study there was an increase in the number of antibiotic-resistant pneumococci and this worrying trend continues [22]. The association of types 9 and 19 with insusceptibility to penicillin, type 14 with erythromycin resistance and types 6 and 23 with multi-resistance suggests the possibility of nationwide spread of a few resistant strains. It may be significant that representatives of these serotypes often caused invasive disease in children. Apart from the need to continue surveillance by reference laboratories there is a need for more general research. Possible areas include continuation of studies on microbial pathogenicity [71]; development of new vaccines, including conjugated preparations; the definition of groups that would, or would not, benefit from immunisation and the development of additional methods for the diagnostic laboratory, such as an enzyme test to replace the bile solubility test.

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