Flinders University

ANTIMICROBIAL DRUG RESISTANCE IN PNEUMOCOCCUS *STREPTOCOCCUS PNEUMONIAE*

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Dedication

This thesis is dedicated to my wife, Miriam Hansman, who has provided constant, generous and perceptive supportive throughout my medical career.

Summary

The studies described here include the first reports of antibiotic resistance in the pneumococcus *Streptococcus pneumoniae.* The development of resistance was important clinically because of the high prevalence of pneumococcal infections, especially pneumococcal pneumonia, which, in low and middle income countries, was often fatal in both children and adults. When these studies began, in the 1960's, pneumococcal pneumonia was probably the commonest infectious cause of death in these countries
Of other serious infections caused by pneumococci, meningitis was especially important. The pneumococcus was a leading cause of bacterial meningitis in both children and adults, with high mortality rates in all regions. Survivors often suffered from permanent sequelae, including mental impairment and severe deafness.
The first drugs effective and safe in the treatment of pneumococcal infections were the sulphonamides, introduced in the 1930's. These proved useful in a variety of infections, including pneumococcal pneumonia. Although by the early 1940's sulphonamide resistance was becoming more common, penicillin, introduced in 1944, proved even more effective
In the treatment of pneumococcal infections.
When my studies began, in the 1960's, several antibiotics were available for the treatment of pneumococcal and streptococcal infections: tetracyclines, chloramphenicol, erythromycin and lincomycin, as well as penicillin. Resistance to these agents was unknown, so pneumococcal infections, including bacteraemic pneumonia and meningitis, could be treated with these antibiotics, with the confidence that most patients would respond well to therapy.
Parenteral penicillin was the antibiotic usually given in severe pneumococcal infections. Any material decrease in susceptibility was potentially important. This was especially so

in patients with pneumococcal meningitis: because of the blood brain barrier, penetration of penicillin into cerebrospinal fluid [CSF] is poor.

Amongst other antibiotics used to treat pneumococcal infections in the 1960's, the tetracyclines were prominent. In 1963 Evans and I reported the isolation of a tetracycline-resistant pneumococcus, from the CSF of a young child with meningitis. This was soon followed by reports of similar strains from the United Kingdom [UK].

It had long been recognised that pneumococci could cause family and institutional outbreaks of pneumococcal infection. In 1963/64 a hospital outbreak of pneumococcal respiratory disease, including cases of pneumonia, caused by tetracycline-resistant pneumococci of several serotypes occurred in Sydney, which Andrews and I reported. Also in 1963, a similar hospital outbreak caused by a tetracycline-resistant pneumococcus occurred in the UK.

The first report of a pneumococcus with reduced susceptibility to penicillin, from a young woman with hypogammaglobulinaemia, was made in 1967, by Bullen and me.

Subsequently, studies of pneumococci isolated from New Guineans in 1969, and later, showed that pneumococci with reduced susceptibility to penicillin were relatively common in Papua New Guinea [PNG]. These RSP pneumococci, which belonged to several serotypes, were present in coastal, highland and island communities. That such strains could be fully virulent was shown by their isolation from patients with fatal infections.

Some seven years elapsed before reports appeared of RSP strains in other regions. From 1974 pneumococci with reduced susceptibility to penicillin were reported from the United States [US], UK, Canada and South Africa.

In 1978 I reported the isolation of pneumococci showing dual or multiple drug resistance, from patients in Adelaide, Melbourne and Sydney, during the four-year period, 1972 through 1975. Several resistance patterns were encountered, involving chloramphenicol, erythromycin, lincomycin, penicillin and tetracycline.

Once widespread drug resistance had been demonstrated, in the 1970's, clinical laboratories began to test the susceptibility of clinical isolates to penicillin and other antibiotics. This was of particular importance in patients with severe infections, especially bacteraemic pneumonia, meningitis and other invasive infections, so that inappropriate chemotherapy could be avoided.

It could no longer be assumed that pneumococci were invariably susceptible to antibiotics. Fortunately, the introduction of first polysaccharide and then conjugated pneumococcal vaccines provided an effective method of preventing pneumococcal infections. This was especially important in infants and young children in whom the bulk of invasive pneumococcal disease occurs.

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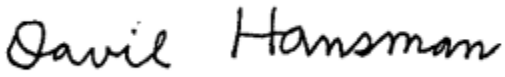
Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university and the research within will not be submitted for any other future degree or diploma without the permission of Flinders University; and to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.



December 20th 2023

List of Abbreviations

COPD	chronic obstructive pulmonary disease
C	chloramphenicol
CSF	cerebrospinal fluid
E	erythromycin
IPD	invasive pneumococcal disease
L	lincomycin
ug	microgram
mg	milligram
ml	millilitre
MBC	minimal bactericidal concentration
MIC	minimal inhibitory concentration
MRP	multiply-resistant pneumococcus
NSW	New South Wales
OM	otitis media
P	penicillin
PNG	Papua New Guinea
RSP	reduced susceptibility to penicillin
SA	South Australia
T	tetracycline
UK	United Kingdom
US	United States

Contextual statement

Pneumococcal infections are among the most common and important bacterial infections, especially in low- and middle-income countries. Despite the availability of antibiotic therapy and pneumococcal immunization, pneumococci continue to be a leading infectious cause of illness and death.

This contextual statement describes the recognition of drug resistance in pneumococcus for the first time and explains my contribution to guidance for appropriate antibiotic therapy in patients whose infections were caused by strains with reduced susceptibility to antimicrobial drugs.

Although resistance to sulphonamides – the first drugs to be useful in pneumococcal infections – had been clearly documented [some five years after their introduction], it had been assumed that pneumococci were uniformly susceptible to antibiotics used in the treatment of pneumococcal infections, notably penicillin and tetracycline.

A consequence was that most clinical laboratories abandoned the susceptibility testing of pneumococci – as well as of other important pathogens – in the early antibiotic era [late 1940's and 1950's], assuming uniform susceptibility of strains. This applied even in the case of patients with serious – and sometimes life-threatening - infections, such as bacteraemic pneumonia and meningitis. This was so not only in Australia but also in other regions, including Europe and North America.

Sulphonamides had become widely available in 1935 and were soon found to be highly. effective in the treatment of pneumococcal pneumonia, then a universally common and often fatal infection. Although less successful in the treatment of pneumococcal meningitis than in pneumonia, there was a highly significant reduction in fatality rates in meningitis, an infection which had been previously almost invariably fatal. It had long been known that small outbreaks of pneumococcal pneumonia occurred in families, hospitals, schools, mines, prisons and other environments where susceptible people were in close contact. In the 1930's knowledge about such outbreaks expanded considerably with the application of pneumococcal capsular serotyping. This enabled the contact tracing of cases and was especially useful in hospitals. Studies in the United States [US] showed how the spread of pneumococci could occur, from patient to patient, patient to staff and staff to patient.

These findings were relevant to my studies, as explained in chapter 2, where I describe how the transmission of pneumococci causing respiratory infections, including pneumonia, occurred in a hospital in Sydney where most of the patients were elderly. Also noteworthy in the US study mentioned above, was the seasonal relationship to spread, with hospital-acquired infection occurring in the cold months, ceasing in summer and then resuming in autumn. Although winters are much milder in Australia, a similar seasonal variation in the number of cases was observed in the hospital outbreak mentioned above. It was caused by pneumococci resistant to tetracycline. This was one of the first published studies of hospital-acquired infection caused by antibiotic-resistant pneumococci. Sulphonamide-resistance had developed in a number of pneumococcal serotypes, which included types 1, 2 and 5 - serotypes capable of causing epidemic infection.

My studies in Australia showed that isolates with reduced susceptibility to penicillin [RSP strains] belonged to types 6, 19 and 23, while isolates from Papua New Guinea [PNG] belonged to a much wider range of types: 2, 4, 6, 11, 14, 15, 16, 19, 23, 24, 34 and 35. These included the epidemic serotypes 2 and 4 and types which commonly cause infections in children: 6, 14, 19 and 23. The types which had developed resistance to tetracycline in Australia: 1, 5, 9, 17, 33 and 36 included epidemic types 1 and 5. The hospital epidemic of pneumococcal respiratory infections referred to above was principally caused by a type 33 strain, which can also be regarded as an epidemic type.

When pneumococci resistant to sulphonamides were met with in the late 1930's and early 1940's, cross resistance between sulphonamides was demonstrated. Thus, pneumococci resistant to sulphapyridine were also resistant to sulphathiazaole, sulphapyrazine and sulphadiazine – all sulphonamides used in clinical practice at the time. Before treatment was begun, for example in pneumococcal pneumonia, pneumococci were almost invariably susceptible to sulphonamides. However, when sulphonamide therapy was prolonged, as in cases of pneumococcal endocarditis or pneumonia complicated by empyema, resistance to a variable degree sometimes developed. By 1943 [shortly before penicillin became widely available] hospital transmission of a pneumococcus, resistant to sulphadiazine [type 8] had been reported.

My findings with pneumococci with reduced susceptibility to penicillin or resistance to tetracycline etc. – and reports from other investigators – showed that resistance did not usually develop during therapy but was detectable before treatment was begun.

When penicillin became widely available in 1944, it gradually superseded sulphonamides for the treatment of pneumococcal infections including pneumonia – although a sulphonamide was often administered with penicillin in the treatment of pneumococcal meningitis. So successful was penicillin therapy that it was widely assumed that pneumococci were invariably susceptible to the drug. Not only were antimicrobial susceptibility tests no longer done, bacteriological studies were discontinued in many centres. It is likely that the early development of resistance to penicillin and other antibiotics used in the treatment of pneumonia and meningitis was missed in some regions.

When Evans and I [1963] reported the first isolation of a tetracycline-resistant pneumococcus, from a young child with meningitis, it was swiftly followed by reports of similar strains, from the US and UK. However, when I and Bullen [1967] described a RSP pneumococcus [type 23] from a young woman with hypogammaglobulinaemia and bronchiectasis [who had received many courses of antibiotics], there was an interval of seven years before similar strains were reported [from the US].

From then on, however, there was an increasing stream of such reports, first from the US and UK and then from other countries, including Canada and South Africa. Pneumococcal resistance to erythromycin, another antibiotic useful in the treatment of pneumococcal infections, was first reported from Canada, soon followed by a report from the US, both in 1967. These isolates were also resistant to lincomycin. I first encountered an erythromycin-resistant strain in 1972; again, the isolate was also resistant to lincomycin. In 1977 I visited Nigeria and studied the antibiotic susceptibility pattern of pneumococcal from nasal carriers. There was a high carrier rate of pneumococci amongst children and some of the strains showed resistance to chloramphenicol [also to tetracycline].

The significance of this was that chloramphenicol was then used extensively in the treatment of pneumococcal pneumonia and meningitis, being inexpensive, readily available and effective by oral administration. The degrees of resistance encountered would have precluded effective therapy.

Reports from South Africa [1978] described the isolation of pneumococci with higher levels of penicillin resistance than had hitherto been described. The isolates were also resistant to chloramphenicol [Durban] or showed multiple resistance, including penicillin, chloramphenicol, erythromycin and tetracycline [Johannesburg].

In 1978 I described strains of pneumococci showing dual or multiple antibiotic resistance from several Australian cities: Adelaide, Melbourne and Sydney. These showed resistance to chloramphenicol, erythromycin, lincomycin, and tetracycline and reduced susceptibility to penicillin [RSP] in various combinations. The report included the description of a pneumococcus resistant to erythromycin and lincomycin, mentioned above.

After reports of significant degrees of drug resistance in pneumococci, involving the key antibiotics used in pneumococcal infections, had appeared in several regions: Australia and PNG, Europe, North America and South Africa, it became apparent that it could no longer be assumed that pneumococci [and other important pathogens] had remained uniformly susceptible to penicillin, tetracycline, chloramphenicol, erythromycin and other antibiotics.

Appropriate bacteriological studies were mandatory, including blood culture in infants and young children, to detect occult pneumococcal bacteraemia and in patients of all ages when pneumonia was suspected. Suitable antimicrobial susceptibility tests were important, capable of detecting reduced susceptibility to penicillin, for example.

In this contextual statement I have documented the development of antibiotic resistance in pneumococci in Australia and PNG, its implications for therapy and the need for appropriate bacteriological studies in patients with pneumonia and other serious pneumococcal infections. Further context is provided in the chapters which follow, including the introductory paragraphs.

Chapter 1

Introduction and early history of chemotherapy and antimicrobial drug resistance in *Streptococcus pneumoniae*

1.1 Pneumococcal infections

Pneumococcal infections are common and potentially serious. Moreover, there is an especially high prevalence of pneumococcal pneumonia in low and middle income countries. When these studies began, in the 1960's, pneumococcal pneumonia was probably the commonest infectious cause of serious illness and death in these countries. Other serious infections caused by pneumococci include meningitis, brain abscess, endocarditis, peritonitis, arthritis and osteomyelitis. Of these, meningitis is especially important, because it is an infection with high mortality rates in all regions. Survivors often suffer from permanent sequelae, notably cerebral impairment and deafness. Localised pneumococcal infections are common in both children and adults.

Otitis media [OM] is encountered frequently in infants and other young children. Acute OM can lead to mastoiditis and other potentially serious complications including epidural abscess, sigmoid sinus thrombosis and meningitis.

In Australia, OM is especially common in aboriginal children; acute followed by chronic OM may result in deafness, with learning difficulties. Acute sinusitis is often caused by pneumococci in older children and adults and pneumococcal conjunctivitis occurs at all ages.

Primary [occult] pneumococcal bacteraemia as a clinical entity was first recognised in the United States, as a consequence of the liberal use of blood culture in febrile children presenting to accident and emergency departments [Torphy and Ray, 1970; Burke, Klein, Gezon et al., 1971]. The serotype most commonly isolated was type 14. In many cases the fever resolved rapidly and uneventfully with antibiotic therapy. However, some children became seriously ill, developing pneumonia, meningitis, otitis media or a rarer complication, such as pneumococcal osteomyelitis [Burke, Klein, Gezon et al., 1971]. It is likely that some

cases are self-limiting and resolve without treatment. Primary pneumococcal bacteraemia also occurs in adults [Musher, 2005].

The studies described here include the first reports of antibiotic resistance in pneumococci. Drug resistance was and is clinically important because pneumococcal infections, as mentioned, are common and, if invasive, are potentially life-threatening, especially in elderly and immune-suppressed individuals.

1.2 Global importance of pneumococcal infections

It is difficult nowadays to realise the importance of bacterial pneumonias, especially pneumococcal pneumonia, in the 19th and early 20th centuries. In his famous textbook, Sir William Osler used Paul Bunyan's moving epithet to describe pneumonia as the 'Captain of the Men of Death' [Osler, 1909]. At the time pneumococcal pneumonia was the leading infectious cause of mortality in the Western World.

Although there are many [~90] serotypes and 46 serogroups of pneumococci, some serotypes cause pneumonia and other pneumococcal infections more frequently than do others. Thus, in the United States [US], types 1, 2 and 3 * caused about one-half [54%] of the cases of pneumococcal pneumonia in the pre-antibiotic era [Heffron, 1939; Davis, Dulbecco, Eisen et al., 1973]. Although most, if not all, types can cause invasive disease, some types – including types 1, 2, 3, 4 and 5 – are more virulent for humans than are others. Contrariwise, some types – including types 11, 13, 16, 17 and 20 – rarely cause infection.

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* I have used the Danish system for the classification of pneumococcal serotypes and sub-types throughout

1.3 Epidemiology of pneumococcal infections

Unlike some other acute infectious diseases [such as measles] the epidemiology of pneumococcal infections, including pneumococcal pneumonia, is complex. Most community-acquired infections appear to arise *de novo* with no apparent source of infection. However, findings indicate that after acquisition of a virulent strain, carriage is followed by infection [Hausdorff, Feikin and Klugman, 2005]. In extensive studies of pneumonia in service recruits, during the three-year-period 1942 through 1945, high rates of colonisation preceded epidemics of pneumococcal pneumonia [Hodges and MacLeod, 1946]. The recruits were housed in poorly ventilated barracks, with inadequate heating during cold winters.

Since the 19th century outbreaks of pneumococcal pneumonia have been recognised in families, schools, hospitals, prisons, barracks and other institutions where people are in close proximity [Finland, 1942].

During the construction of the Panama Canal, in the late 19th and early 20th centuries, many of the workers, who lived under crowded, barrack-like conditions, developed pneumonia, many of whom died [Gorgas, 1907]. When the workers were allowed to build their own huts or live with their families, the problem was alleviated [Austrian, 1985].

After gold was discovered in South Africa, in the vicinity of present-day Johannesburg, there was a gold rush. This was followed by the development of mining on an industrial scale, beginning in 1886 [Austrian, 1985]. Africans were brought in from other parts of South Africa and from neighbouring tropical regions, including present-day Mozambique, to work in the mines. The Africans, if they survived, worked in the mines on a rotating basis, for six to nine months before returning to their villages [Austrian 1985]. At the mine sites, housed in crowded conditions, they often developed pneumococcal pneumonia and many died.

When the lessons learnt in Panama were applied at the Witwatersrand, the barrack-like accommodation was replaced with huts [Gorgas, 1914]. However, this was only a part solution to the problem, and high rates of pneumonia persisted at least until the 1960's [Austrian, 1985].

The application of capsular typing [and other methods of typing] proved useful in tracing the spread of infection in hospitals and elsewhere. In 1937 a community outbreak of pneumonia and OM, caused by a type 1 pneumococcus, occurred in a rural area of western Massachusetts affecting children and adults [Gilman and Anderson, 1938]. As described in chapter 2, a serious outbreak of pneumococcal pneumonia caused by a type 2 pneumococcus had occurred in 1935 in a hospital for veterans in Massachusetts, in which some patients and staff died [Smillie, 1936]. The large epidemics of pneumococcal pneumonia in military recruits, described by Hodges and MacLeod, mentioned above, were caused principally by six serotypes: 2, 1, 5, 7, 12 and 4, of which type 2 predominated. Over a period of 2 ½ years >1600 cases of pneumonia occurred. Remarkably, none of the young men died [Hodges and MacLeod, 1946].

As described in subsequent chapters, in my studies I encountered both hospital and community spread of pneumococci, which involved subjects in Australia and PNG, with transmission of serotypes 33 and 4, respectively.

1.4 Naso-pharyngeal carriage of pneumococci and its consequences

Naso-pharyngeal [nasal] carriage of pneumococci is common in children, especially in infants and younger children. Carriage occurs in 20% to 40% of healthy children and 5 to 10% of healthy adults [Musher, 2005].

In infants and young children, the pneumococci may be acquired from siblings, as shown in a study of families by Hendley and his colleagues at the University of Virginia [Hendley, Sande and Stewart et al., 1975; Gwaltney, Sande and Austrian et al., 1975]. In 1992 four of six children in a 'family child-care home' in Baltimore developed pneumococcal infections. Three of the children [who were aged eight to 26 months of age] developed pneumococcal bacteraemia and a fourth purulent conjunctivitis. A type 12F pneumococcus was isolated from all four children and from the naso-pharynx of the two healthy children [Cherian, Steinhoff, Harrison et al., 1994].

The commonest serotypes carried by young children include types 6A, 6B, 14, 19F and 23F [Loda et al 1975; Hausdorff, Feikin and Klugman, 2005]. Pneumococci of serogroups 6, 14, 19 and 23 also commonly cause invasive pneumococcal disease [IPD] in this age group [Hausdorff, Feikin and Klugman, 2005]. Acquisition of a virulent pneumococcus may result either in clinical infection or the carrier state. In adults, pharyngeal carriage is more common than nasal carriage [Masters. Brumfitt, Mendez et al., 1958; Hendley, Sande, Stewart et al., 1975].

In Hendley's study of pneumococcal carriage in families, adults were more likely to be carriers if young children were present in the household [Hendley, Sande, Stewart, 1975]. The duration of the carrier state is variable: it may be transient or of several months' duration. During childhood, capsular antibodies develop to some serotypes as a result of either colonisation or infection [Gwalteney, Sande and Austrian et al., 1975].

1.5 Role of child-care [day-care] centres in transmission of pneumococcal infection

Many studies have shown that infants and young children attending child-care centres [CCC] are at an increased risk of acquiring acute respiratory and other infections, including pneumococcal infections. Children in CCC are 2 to 18 times more likely to acquire several infections than children not so exposed; this applies particularly to respiratory infections and OM [Vaughan and Coffin, 2019]. *'CCC attendance has been found to be the most important risk factor for the development of invasive pneumococcal disease'* in infants and young children [Dagan and O'Brien, 2005]. Pneumococcal infections acquired in CCC's include primary bacteraemia, meningitis and OM [Radetsky, Istre, Johansen et al, 1981; Cherian, Steinhoff, Harrison et al., 1994].

1.6 Optochin

The earliest chemotherapeutic agent used in the treatment of pneumococcal pneumonia was ethyldihydrocupreine [Optochin], one of a number of synthetic derivatives of quinine. Quinine itself is obtained from the bark of several species of trees belonging to the genus *Cinchona,* originally growing on the eastern slopes of the Andes. Used regionally in South America to control shivering, it was taken to Europe by Spanish Jesuits, where it was used successfully, in the form of Jesuits' bark [Peruvian bark], to treat malaria, for several hundreds of years. In 1820 the active ingredient, quinine, was extracted from the bark of a *Cinchona* tree. It was synthesised in 1944. Quinine was the first drug to be successful in the treatment of an infectious disease. In the late 19th century with the rise of the chemical industry in Germany and other European countries, sustained efforts were made to develop drugs which were effective in the treatment of bacterial infections.

These continued into the 20th century and in 1911 Morgenroth, working in Berlin, tested the activity of optochin, in the treatment of experimental pneumococcal infections in mice [Morgenroth and Levy, 1911]. Although effective if administered before pneumococcal challenge, optochin failed to prevent death once infection was well established in the mouse. Despite this limitation, optochin was tried in patients with pneumococcal pneumonia in Germany, South Africa and the US [Moore and Chesney, 1917]. Pneumococcal pneumonia was common and often fatal in South African gold miners, as mentioned above [Gorgas, 1914; Austrian, 1985]. However, because of its toxicity: optochin caused temporary tinnitus, partial hearing loss and visual disturbance [and, rarely, blindness] in some recipients, its use was abandoned, except in the treatment of localised infections, such as conjunctivitis. However, optochin was found to be useful in the identification of pneumococci and the optochin test remains in use to-day.

1.7 Sulphonamides

Probably because of improved living conditions, notably less crowding, mortality rates from pneumonia in westernised countries began to decline in the 20th century [Editorial, 1947]. The quest for safe and effective antibacterial agents had continued in Germany. Amongst the drugs studied by Domagk at IG Farbenindustrie was the red dye sulphonamido-chrysoidin [Prontosil]. Prontosil had no activity *in vitro* but protected mice when challenged with a lethal dose of haemolytic streptococci. It was used successfully in patients with streptococcal infections in Germany [1935]. It came as a surprise when Trefouel and his colleagues in Paris [1935] showed that *in vivo* Prontosil was converted to the colourless, simpler compound *p*-amino-benzene sulphonamide [sulphanilamide].

Sulphanilamide was active both *in vitro* and *in vivo* and proved highly effective in the treatment of puerperal fever caused by haemolytic streptococci. Many sulphonamide compounds were now synthesised, including sulphapyridine [M&B 693] in the United Kingdom [UK]. Sulphapyridine was the first drug to be successful in the treatment of pneumococcal pneumonia. A controlled trial using sulphapyridine reduced the mortality rate in pneumococcal pneumonia significantly [Evans and Gaisford, 1938]. Sulphapyridine commonly caused nausea and vomiting but less toxic sulphonamides soon became available, notably sulphadiazine, sulphadimidine and sulphafurazole. Sulphonamides were soon confirmed as highly effective in the treatment of pneumococcal [and streptococcal] infections. Survival rates in patients with pneumococcal pneumonia, including those with severe infections, improved considerably [Anderson and Dowdeswell, 1939; Agranat, Dreosti and Ordman, 1939]. Moreover, for the first time, an effective treatment was available for the treatment of pneumococcal meningitis, especially in older children and adults [Hodes, Smith and Ickes, 1943].

1.8 Development of sulphonamide resistance

About five years after their introduction as therapeutic agents, came the first reports of sulphonamide resistance in pneumococci [Ross, 1939; Lowell, Strauss and Finland, 1940]. On the initiation of sulphonamide therapy, strains of pneumococci were almost invariably fully susceptible to the drugs. Diminished susceptibility to sulphonamides was principally observed in pneumococci isolated from patients with either severe pneumonia, especially when complicated by empyema, or with pneumococcal endocarditis, who had undergone prolonged therapy [Hamburger, Schmidt, Sesler et al., 1943]. Sulphonamide 'cross resistance' was observed, including resistance to sulphadiazine and sulphathiazole.

Resistance developed in many serotypes, including types 2, 3, 5, 7, 8 and 12, all types which can cause severe disease [Lowell et al., 1940; Hamburger, Schmidt, Sesler et al., 1943].

1.9 Penicillin

However, sulphonamide resistance in pneumococcus did not become a serious clinical problem. This was probably because the first antibiotic effective in pneumococcal [and streptococcal] infections – penicillin – became available in substantial amounts for therapeutic use in 1944 [Tillett, Cambier and McCormack, 1944]. Penicillin G proved highly effective in the treatment of both pneumococcal [and streptococcal] infections, including pneumococcal pneumonia. The mortality rate in pneumococcal pneumonia, previously 20% to 50% in in infants and young children and 3% to 5% in older children, was reduced to less than 1%; moreover, with fewer long-term effects [McCracken and Eichenwald, 1969].

Penicillin was more effective than sulphonamides in the treatment of pneumococcal meningitis and for the first time, cases of bacterial endocarditis could be treated successfully. Later, in the 1950's and 1960's, new penicillins were introduced, which provide adequate blood levels after oral administration. Thus, penicillin V, in appropriate dosage, can be used as an effective treatment in pneumococcal pneumonia [Reeves, Garfield and Polasky et al., 1959]. Other new antibiotics had also become available, starting with chlortetracycline and chloramphenicol, both in 1948.

1.10 Tetracycline, chloramphenicol, erythromycin, lincomycin and clindamycin

Both tetracyclines and chloramphenicol proved effective in the treatment of pneumococcal pneumonia. Chloramphenicol also proved useful in the treatment of the major forms of bacterial meningitis, including pneumococcal meningitis, with its excellent penetration into cerebro-spinal fluid [CSF] [Westenfelder and Paterson, 1969] and was used either by itself, for example in patients allergic to penicillin, or in combination with penicillin [Kucers and Bennett, 1987].

Erythromycin, introduced in 1952, was used successfully in the treatment of pneumococcal pneumonia [Waddington, Maple and Kirby, 1954; Romansky, Nasou, Davis et al., 1956].

Lincomycin, introduced in 1964, a drug with a similar spectrum of activity, although chemically not related to erythromycin, provides higher blood levels. In a comparative study in the treatment of pneumococcal pneumonia, lincomycin proved as effective as penicillin [Anderson, Bauman and Austrian, 1967]. Clindamycin [1968] a semi-synthetic derivative of lincomycin is highly active against pneumococci and attains higher blood levels than does the parent drug.

1.11 Bacteriological studies widely abandoned

For three decades pneumococci appeared to remain uniformly susceptible to the antibiotics used in the treatment of pneumococcal infections. This resulted in most clinical laboratories ceasing to test the susceptibility of isolates of pneumococci to penicillin and other antimicrobial drugs, including tetracycline and erythromycin. Even earlier it had been noted that many centres had ceased to perform bacteriological studies in patients with pneumonia except in cases when the response to treatment had been disappointing, as when a complication such as empyema occurred [Editorial, 1947].

1.12 Antibiotic resistance encountered in pneumococcus

This thesis describes the first reports of drug resistance in pneumococcus to tetracycline and to penicillin and the subsequent recording of pneumococci with resistance to other antibiotics including erythromycin, lincomycin and chloramphenicol. It also includes some of the earliest reports of dual and multiple drug resistance. These findings were of clinical importance because of the frequency of severe pneumococcal infection, especially pneumonia and meningitis. This applied particularly to low- and middle-income countries, as mentioned, where pneumococcal infections were common and therapeutic options were more limited [Hansman, 1973].

1.13 Renewed importance of pneumococcal serotyping

With the continuing importance of pneumococcal infections in all countries, despite the availability of chemotherapy, initiatives to develop effective pneumococcal vaccines to prevent infection, gathered momentum, driven by Austrian in the US. The development of drug resistance to the antibiotics used in the treatment of pneumococcal infection added impetus to this goal. It therefore became essential to resume the serotyping of pneumococci, in order to determine type prevalence in major regions and including low, middle- and high-income countries. To ascertain which types predominated was especially important in the case of infants and young children [children under five years of age] in whom most of the cases of IPD occurred. Studies in Oxford in the 1990's showed that types 14, 19, 6A, 6B, 18, 1, 9 and 23, in that order, were the commonest serotypes causing IPD in children less than five years of age in the Oxford region [Sleeman, Knox, George et al., 2001].

For all ages the predominant types were 14, 9, 6, 4, 23, 3, 8 and 12. Findings were similar in other regions. From a global perspective, serogroups 1, 6, 14, 19 and 23 were amongst the most prominent [Hausdorff, Feikin and Klugman, 2005].

Polysaccharide pneumococcal vaccines proved effective in preventing bacteraemic infections caused by the constituent capsular types in adults. To prevent serious infections In the under five -year-old age group – in whom IPD predominates – conjugated vaccines were successfully developed. This was fortunate as drug resistance in pneumococcus, as explained in later chapters, was becoming an increasing and universal problem.

Chapter 2

On the Development of *Streptococcus pneumoniae* resistance to Tetracyclines

2.1 First reports of tetracycline resistance in pneumococci

This chapter presents three articles which reported tetracycline resistance, published in the 1960's and 1970's, including the first report of infection caused by a tetracycline-resistant pneumococcus. These findings were significant because tetracyclines were widely used in the treatment of respiratory infections, including pneumonia.

The tetracycline antibiotics had been introduced in 1948, with chlortetracycline first in the series, followed by oxytetracycline in 1950 and then tetracycline in 1953. Other congeners were synthesised later; those introduced into clinical practice included doxycycline and minocycline. Because they could be administered orally, the tetracyclines became a popular alternative to penicillin in the treatment of pneumococcal [and streptococcal] infections. As with penicillin, it was assumed that pneumococci were uniformly susceptible to tetracyclines. An article from the UK on the treatment of chronic bronchitis [now considered a form of chronic obstructive pulmonary disease [COPD] stated:

'*The pneumococcus was accepted as being invariably sensitive to the drugs used in the trial* [*penicillin and tetracycline*]' [Francis, May and Spicer, 1961].

In 1962 an infant with meningitis in Sydney yielded a pneumococcus resistant to tetracycline from a sample of cerebrospinal fluid. I confirmed and quantified the degree of resistance to tetracycline. The infant was treated with parenteral penicillin and sulphadimidine by mouth, which was a standard regimen for the treatment of bacterial meningitis at the time. The infant recovered. This was the first report of a pneumococcus resistant to tetracyclines [Evans and Hansman, 1963].

Tetracyclines were widely used in the treatment of pneumonia, including pneumococcal pneumonia, so the finding had clinical importance.

Evidence soon mounted that pneumococci resistant to tetracycline could be fully virulent. And it became evident that such strains were not restricted to Australia.

2.2 Tetracycline-resistant pneumococci from other regions

Soon afterwards the isolation of tetracycline-resistant pneumococci was reported from the UK; these strains were from four older patients with either recurrent or chronic bronchitis. Three of the isolates, from patients in London, were type 9 and the fourth, from a patient in Southend, was type 11 [Richards and Rycroft, 1963]. Next year cases of pneumonia caused by tetracycline-resistant pneumococci of types 6 and 14 were recorded from the US [Schaedler, Choppin and Zabriskie, 1964]. In 1966 a fatal case of pneumonia caused by a tetracycline-resistant type 14 pneumococcus was reported, also from the US [Schaffner, Schreiber and Koenig, 1966].

2.3 Hospital-acquired pneumococcal infections

Over a hundred years ago, perceptive observers had realised that pneumococci could cause outbreaks of pneumonia. In France, Netter recognised family outbreaks of respiratory disease, including pneumonia and the role of the pneumococcus [Netter, 1888, cited by Finland, 1942].

In later editions of his textbook, Osler classified pneumococcal pneumonia amongst the infectious diseases [Osler, 1909]. By the 1930's there had been many reports of hospital-acquired pneumococcal infection [Finland, 1942]. These included the serious outbreak in a hospital for veterans in Bedford, Massachusetts in 1935, mentioned in chapter 1, where 17 cases of pneumonia caused by a type 2 pneumococcus occurred [Smillie, 1936].

The first phase of the epidemic, beginning in February and ending in April, affected 10 patients and staff, of whom six died. There were no cases during summer but the epidemic recurred in September, with a further seven cases during autumn and winter, of whom two died, before the epidemic ended in December. Carrier studies showed that each case was linked to a naso-pharyngeal pneumococcal carrier; case to case transmission could not be demonstrated.

In 1941 a patient with lobar pneumonia in Battle Creek, Michigan, caused by a sulphonamide sensitive type 8 pneumococcus, initially responded to therapy with sulphadiazine but relapsed during therapy and a sulphadiazine-resistant type 8 pneumococcus was isolated on blood culture. A patient nearby later developed pneumonia due to the same strain; both patients responded to combined treatment with type specific horse serum and sulphafurazole [Frisch, Price and Myers, 1943].

2.4 Hospital outbreak of respiratory infection in Sydney caused by tetracycline-resistant pneumococci

In 1963 Andrews and I encountered a hospital outbreak caused by tetracycline-resistant pneumococci amongst mostly elderly patients in a hospital in Sydney where conditions were crowded. The crowding probably facilitated the spread of respiratory pathogens and notably pneumococci. Many of the affected patients suffered from an exacerbation of COPD but 13 of the patients developed pneumonia, which in two cases was fatal.
The resistant pneumococci isolated belonged to five serotypes [5, 9, 17, 33 and 36] but predominantly [~80%] to type 33, which was isolated from 30 patients.

This finding was of interest since type 33 was not one of the then recognised 'epidemic' serotypes – types 1, 2, 4, 5, 7 and 12 - which were known to cause outbreaks of pneumococcal disease. However, since then the spectrum of known epidemic types has increased [Hausdorff, Feikin and Klugman, 2005].

During October and November 1963, four patients in the same ward yielded a type 33 - tetracycline-resistant pneumococcus, preceded by a single case in August and followed by a single case in December. Over the next three months no cases occurred, followed by two cases in the same ward in April, 1964. There was a significant increase in the number of cases over the winter and spring with a total of 21 cases in the six months period June through November. The minimal inhibitory concentration [MIC] of the resistant strains ranged from 24 to 100 ug tetracycline per ml, levels of resistance which would preclude successful therapy with tetracycline [Hansman and Andrews, 1967]. The seasonal nature of the outbreak was of interest, being reminiscent of the type 2 outbreak of pneumococcal pneumonia which had occurred in Bedford, Massachusetts in 1935; this outbreak had begun in winter, ceased in summer and then resumed in autumn.

2.5 Hospital outbreaks of respiratory infections cause by tetracycline-resistant pneumococci in other regions

The outbreak we had witnessed in Sydney occurred in 1962/1963. A similar but more deadly outbreak of respiratory infection, also caused by a tetracycline-resistant pneumococcus, occurred in a hospital in Liverpool, UK, in 1963. The resistant pneumococcus was type 7. Of ten patients, five died. The potential danger of large open wards enabling transmission of respiratory infections was noted [Turner, 1963].

A subsequent report, also from Liverpool, UK, described two small outbreaks of hospital-acquired respiratory infections caused by strains of tetracycline-resistant pneumococci belonging to types 3 and 9, respectively [Percival, Armstrong and Turner, 1969]. Hospital outbreaks caused by pneumococci [type 19A] resistant to several antibiotics, including penicillin and tetracycline were reported subsequently, from South Africa [Jacobs, Koornhof and Robins-Browne et al., 1978].

2.6 A case of bacteraemic pneumonia caused by a type 1 tetracycline-resistant pneumococcus.

Ten years after the first reports of pneumococci resistant to tetracycline, Bullen and I reported a case of bacteraemia pneumonia caused by a type 1 pneumococcus. This occurred in a man aged 57 years, whose pneumonia had been preceded by an influenza-like illness for two weeks. The pneumococcus, isolated on blood culture, was highly resistant, MIC 50ug tetracycline per ml. [Bullen and Hansman, 1975]. Resistance was also shown to doxycycline and minocycline. The significance of this finding is that type 1 pneumococci often cause severe disease, including segmental pneumonia [Heffron, 1939; Davis, Dulbecco and Eisen et al., 1973].

Subsequently, pneumococci resistant to tetracycline were reported from many regions. These included South Africa [as mentioned] and several European countries, notably Poland and Spain [Jacobs, Koornhof and Robins-Brown et al., 1978; Cybulkska, Jeljaszewicz and Lund et al., 1970; Garau, Linares and Dominguez, 1981]. Some of the chloramphenicol-resistant pneumococci I encountered in Nigeria in 1977 [chapter 4] were also resistant to tetracycline [Hansman, 1978].

2.7 Clinical significance of tetracycline resistance in pneumococcus

The degree of resistance to tetracycline has varied, with MIC's ranging from 10 to > 100 ug per ml. Because peak plasma values with normal oral dosage are in the range of 2 to 4 ug tetracycline per ml [Garrod and O'Grady, 1971], infections caused by strains with even relatively low MIC values, such as 10 ug/ml, would be unlikely to respond to tetracycline therapy. This was verified by reports of patients with pneumonia caused by tetracycline-resistant pneumococci treated with tetracycline who failed to respond to treatment [Schaedler, Choppin and Zabriskie, 1964; Schaffner, Schreiber and Koenig, 1966].

I have been unable to find comparable reports of cases of meningitis caused by tetracycline-resistant strains of pneumococci, where tetracycline therapy had also failed. However, at least by the 1970's, tetracycline was probably rarely used for this purpose. It is of historical interest, however, that, earlier, tetracyclines had been used successfully to treat patients with bacterial meningitis [Koch and Hansen, 1957]. It was noted at the time that parenteral administration produced 3 to 6 times higher levels of tetracycline in blood and CSF than did comparable oral dosage. In csf, levels of 0.75 to 3 ug tetracycline/ml were attained.

Many serotypes of pneumococci- types 3 through 11, 14, 17 through 19, 23, 24, 31 and 33 - had developed resistance to tetracycline by 1973, ten years after our first report, as shown:

Table 2.1: tetracycline resistant serotypes by region

Region	Serotypes showing resistance					Reference
London, Southend, UK	9	11				Richards, Rycroft, 1963
Liverpool, UK	7					Turner, 1963
New York, US	6	14				Schaedler et al., 1964
New York, US	14					Schaffner et al., 1966
Sydney, NSW	5	9	17	33	36	Hansman, Andrews, 1967
Liverpool, UK	3	4	7	8	9	
New Haven, US	17	19	23	31	33	Percival et al., 1969
Poland	7	10	18	23	24	Bizzozero, Andriole, 1969
	6A, 6B, 14		19	23		Cybulska et al.,1970

These resistant pneumococci included the epidemic types 4, 5, 7 and putatively, type 33.

In the UK Holt and his colleagues studied pneumococci isolated from children from 1961 to 1968. Tetracycline-resistant pneumococci, first encountered in 1962, were consistently found thereafter in small numbers [Holt, Evans and Newman, 1969].

By the 1970's, tetracycline resistance in pneumococcus had been recognised in many regions and was of an order which precluded successful therapy with this useful antibiotic. Resistant pneumococci had been shown to be fully virulent and capable of causing pneumonia and meningitis, including fatal infections. It was now prudent not to treat cases of pneumonia or other forms of severe pneumococcal infection with this antibiotic.

Chapter 3

Development of *Streptococcus pneumoniae* with decreased susceptibility to Penicillins

3.1 Penicillin therapy in pneumococcal infections

This chapter summarises a series of 11 articles, including the first reports of pneumococci with decreased susceptibility to penicillins. These findings were important because systemic pneumococcal infections, especially pneumonia and meningitis, were [and are] common, often severe and sometimes fatal. Moreover, in both westernised and 'developing' countries penicillin was the agent of choice in treatment.

3.2 Penicillin in pneumococcal pneumonia

When Penicillin G [penicillin] became available for clinical use in 1944, it was soon shown to be highly effective in the treatment of pneumococcal infections, including pneumococcal pneumonia [Tillett, Cambier and McCormack, 1944]. Pneumococci were highly susceptible to penicillin, with MIC values ≤ 0.02 ug/ml. Cases of pneumococcal pneumonia could be treated successfully with standard doses as low as 6 to 15 mg [10,000 to 25,000 units] *penicillin, 3 hourly [Witt and Hamburger, 1963]. At this time penicillin was in relatively short supply but as the antibiotic became more freely available, dosage was increased. Before penicillin became available, the drugs used in the treatment of pneumococcal infections were the sulphonamides. It was fortunate that penicillin became available at this time because sulphonamide resistance was becoming more common in pathogenic bacteria, including pneumococci [Hamburger, Schmidt and Sesler, 1943].

3.3 Limitations of penicillin therapy in bacterial meningitis

Penicillin penetrates relatively poorly into cerebrospinal fluid [CSF] even when the meninges are inflamed, as occurs in meningitis [Radetsky, Istre and Johansen et al., 1981].

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* Penicillin G 1000 units = 0.6 mg 1 million units = 600 mg 1 mg = 1666 units

In the absence of meningeal inflammation, CSF levels of penicillin are in the order of 1% to 2% of serum levels [Hieber and Nelson, 1977]. In meningitis, penicillin levels attained in CSF are from 5% to 20% of serum levels [Choi, 2001]. Peak levels of about 1 ug/ml of penicillin are attained in CSF. For this reason, a sulphonamide – and when it became available, chloramphenicol – was often used in conjunction with penicillin in the treatment of pneumococcal meningitis, because of the superior ability of these drugs to pass the blood/brain barrier and so ensure effective levels of antibacterial activity in CSF.

3.4 First reports of pneumococci with reduced susceptibility to penicillin

In 1967 a pneumococcus with reduced susceptibility to penicillin [RSP] was isolated from the sputum of a 24-year-old woman in Sydney with hypogammaglobulinemia and bronchiectasis who had received much antibiotic therapy, including penicillin and tetracycline [Hansman and Bullen, 1967]. The isolate [type 23] was relatively non-susceptible to penicillin and tetracycline [Table 3.1].

Table 3.1 Results of Quantitative Antimicrobial tests with Penicillin and Tetracycline

	ug/ml	controls *	resistance ratio #
Penicillin	0.6	0.03	20
Tetracycline	5	0.5	10

* Drug-susceptible pneumococci # MIC (ug/ml) isolate / MIC control strains

This was the first report of reduced susceptibility of pneumococci to penicillin. However shortly afterwards I encountered a penicillin non-susceptible pneumococcus [type 6], MIC 1 ug/ml, which had been isolated by Rountree [Rountree, 1969] from an Aboriginal girl in Ernabella [now Pukatja], eastern Musgrave Ranges, a remote region of northern South Australia [Hansman, Devitt and Miles et al., 1974]. The isolate was from a nasal swab. Aboriginal children often showed a high carriage rate of pneumococci. High carriage rates may be linked to frequent episodes of pneumococcal OM, which, as mentioned above, are common in aboriginal children.

3.5 Definitions of grades of reduced susceptibility to penicillin

From my studies it soon became apparent there was a continuous gradient in reduced susceptibility to penicillin from slight to moderate. The following degrees of resistance became recognised:

MIC penicillin G ug/m	degree of reduced susceptibility
0.1	slight
0.2 to 1.0	intermediate
> 1	resistant

How should pneumococci with reduced susceptibility to penicillin be recorded?

My early articles referred to such strains as ‘pneumococci with increased resistance to penicillin’ [Hansman et al. 1971]. Other investigators called them ‘pneumococci with decreased susceptibility to penicillin’ [Naraqi, Kirkpatrick and Kabins, 1974] or ‘relatively penicillin-resistant’ [Howes and Mitchell, 1976] while others again used my earlier term ‘Increased resistance to penicillin’ [Paredes et al., 1976]. Later the abbreviation PRP, for penicillin-resistant pneumococci, was suggested but was not widely used [Pallett and Strangeways, 1988]. In this thesis I will adopt for this purpose RSP, reduced susceptibility to penicillin.

3.6 Studies in Papua New Guinea

In 1969 I visited Papua New Guinea [PNG] to help conduct bacteriological studies in patients with pneumonia. As was once the case in Australia, Europe and North America, pneumococcal pneumonia was common in PNG and, unlike in westernised countries, often affected young men, sometimes with fatal results [Douglas and Riley, 1970, 1971]. At the time of my visit a clinical trial was in progress, to ascertain whether a monthly dose of procaine penicillin would reduce the incidence of pneumococcal pneumonia, which was common in the region [Douglas and Sturt, 1975]. The subjects were young men in two villages; the inhabitants of one village served as controls. The study was conducted at Anguganak, Sepik District, a remote area of PNG. I showed subsequently that RSP pneumococci were commonly carried by men in both villages [Hansman, Glasgow and Sturt et al., 1971a]. Although many serotypes were encountered amongst pneumococcal carriers in PNG, all RSP strains were identified as serotype 4. Pneumococci of this 'epidemic' serotype commonly cause severe infections, including pneumonia [Hodges and MacLeod, 1946]. Relative resistance to methicillin and to the cephalosporins, cephaloridine and cephalothin, was also demonstrated [Hansman et al, 1971b]. Subsequent studies with RSP pneumococci showed that this applied also to cephazolin [Hansman, 1978].

The type 4 pneumococci with reduced susceptibility to penicillin from the Anguganak villagers showed MIC's of 0.5 ug/ml which represented a 25 fold increase in MIC compared with penicillin- susceptible pneumococci tested as controls.

In 1970 I visited the Kiriwina, Trobriand Islands, in the Solomon Sea, north of mainland PNG, during a separate study of respiratory disease in that region. Smooth pneumococci were isolated from 39 of 64 [61%] of children. Sixteen serotypes were encountered, including the epidemic types 1 and 2, types responsible for severe infection and not commonly found in

carriers [Hausdorff, Feikin and Klugman, 2005]. RSP strains of types 19 and 23 were encountered [Hansman, 1972]. These types frequently cause infections, including serious infections, in children. The level of reduced susceptibility was low level, MIC 0.1 ug/ml, resistance ratio 5, whereas the Anguganak type 4 strain, which was 25 less susceptible than control strains, with an MIC 0.5ug/l, could be regarded as showing a moderate level of reduced susceptibility. Several years earlier Rountree and her colleagues had found a high carriage rate of pneumococci amongst children [65%] on Kiriwina, with a lower rate in adults [16%] [Rountree, Beard and Arter et al., 1967].

3.7 Further studies in Australia and PNG

Over the three-year period, 1968 through 1970, 62 of 518 [11.9%] of pneumococci tested from New Guineans showed reduced susceptibility to penicillin. These strains were from six regions of PNG: Port Moresby; Madang; Anguganak, Sepik District; Lufa, Eastern Highlands; Tari, Southern Highlands and Kiriwina, Trobriand Islands, comprising coastal, highland and island communities. Ten serotypes: 4, 6, 11, 14, 15, 16, 19, 23, 34 and 35 were represented. MIC values ranged from 0.1 to 1.0 ug penicillin/ml. This finding suggested that a stepwise reduction in susceptibility to penicillin had occurred [Hansman, Devitt and Miles et al., 1974]. In Australia, three of 1098 [0.27%] of strains, over a period of four years, 1967 through 1970, were RSP isolates.

3.8 Infections caused by pneumococci with reduced susceptibility to penicillin

Before long came evidence that RSP pneumococci could cause serious infections in children and adults. In 1977 my colleagues and I reported three cases of such infections, two of which were fatal. These cases had occurred in 1970 and 1971. A 19-year-old New Guinean in Madang, whose diagnosis of multi-segmental pneumonia had been delayed, died despite

the initiation of penicillin therapy. A fatal infection also occurred in a 10 -week-old female infant in Geelong whose purulent meningitis had been undetected during life. A two-year-old Aboriginal girl from Maryvale, Northern Territory with extensive bronchiectasis, treated first at the Alice Springs Hospital and later transferred to the Adelaide Children's Hospital, suddenly developed fever shortly after bronchoscopy. Blood culture yielded a pneumococcus. She was treated successfully with penicillin. These isolates belonged to types 6, 16 and 19, with MIC's of 0.1, 0.2 and 1.0 ug penicillin/ml., respectively [Devitt, Riley and Hansman, 1977].

3.9 Mouse virulence of RSP pneumococci

Early studies had shown that pneumococci isolated from humans were usually virulent for rabbits and mice, less so for guinea pigs. For several decades, especially in the US, the inoculation of mice with clinical specimens was used as a diagnostic test [Hendley, Sande, Stewart et al., 1975]. Pneumococci could be recovered in pure culture in this way. Detailed studies by Lund in Denmark showed that mouse virulence varied considerably, depending on serotype [Lund, 1943]. Thus, pneumococci of types 1 through 6 were highly virulent, whereas type 14 and 16, showed low degrees of virulence. Less commonly, some serotypes, including type 35, showed an intermediate degree of virulence.

In tests of representative RSP strains, mouse virulence varied from low [types 11, 14, 16, 19, 23, and 34] to high [types 4, 6 and 15] [Hansman, Devitt and Miles et al., 1974]. It was concluded that mouse virulence and reduced susceptibility to penicillin were probably independent characteristics.

3.10 Results of quantitative susceptibility tests; effect of inoculum size; bactericidal tests

Further studies of antimicrobial susceptibility, with a set of isolates belonging to ten serotypes, confirmed that RSP pneumococci also showed relative resistance to penicillin V, methicillin and cephalosporins [Hansman, 1975]. However, such strains were either fully susceptible to ampicillin or showed slightly reduced susceptibility [Hansman, Devitt and Riley, 1973; Hansman 1975]. In quantitative tests, the size of the inoculum had little effect on the MIC value of either fully sensitive or RSP strains. In tests of bactericidal activity, the minimal bactericidal concentration [MBC] of penicillin varied with the strain tested. Thus, for one RSP strain [type 23], the MBC equalled the MIC at 0.1 ug/ml. However, for another RSP strain [type 15] the MBC was much higher than the MIC [MIC 0.2 ug/ml and MBC > 5ug/ml, respectively, for 99.9% killing] [Hansman, 1975]. These findings had obvious implications for the treatment of patients with meningitis, where peak penicillin concentrations in CSF are of the order of 1ug penicillin/ml [Hieber and Nelson, 1977].

The serotypes of RSP pneumococci included types 4, 6, 14, 19 and 23, which often cause human infections [Hansman, 1976]. Later, in 1978, I reported RSP pneumococci of serotypes 2 and 24, from Tari and Madang, both in mainland PNG. Type 2 strains may cause epidemic infection. The least susceptible strains in this study [which belonged to serotypes 6 and 24, from Tari and Madang respectively], showed an MIC of 2.0 ug/ml. [Hansman, 1978]. These strains, 100 fold less susceptible than fully sensitive strains, could be regarded as resistant to penicillin.

3.11 Reports of RSP pneumococci from other regions

There was considerable delay before RSP pneumococci were reported from other regions. One of the earliest such reports was from New Mexico in 1974, seven years after our first article on the topic. Of 131 isolates of pneumococci from Navajo Indians, 19 [14%] showed MIC values from 0.1 to 0.5 ug penicillin/ml. These pneumococci belonged to 11 serotypes, including the important types 3, 7 and 8 [Tempest, Carney and Eberle, 1974]. Some of the isolates were from 'body fluids' suggesting that these strains were virulent.

In the same year came further evidence of virulence in RSP strains. The case of a young boy with sickle cell anaemia who developed relapsing pneumococcal meningitis was reported from Chicago. Despite treatment with penicillin in standard doses, the child suffered a clinical and bacteriological relapse; a pneumococcus [type 23] with increased resistance to penicillin was re-isolated after ten days of therapy [Naraqi, Kirkpatrick and Kabins, 1974]. Therapeutic success was attained eventually by administering larger doses of penicillin. In 1974 a RSP pneumococcus type 9 was encountered in Canada [Dixon, 1974].

As mentioned above, RSP pneumococci were often almost fully susceptible to ampicillin. An infant with pneumococcal meningitis in Oxford, who had failed to respond to penicillin therapy, despite an increase in dosage, was treated successfully with ampicillin. The meningitis was caused by a type 14 pneumococcus which showed reduced susceptibility to penicillin, but was almost fully susceptible to ampicillin [Howes and Mitchell, 1976].

A similar case, caused by a type 6B pneumococcus relatively resistant to penicillin but less so to ampicillin, occurred in Montreal in 1978. The affected infant's meningitis responded well to ampicillin, given in combination with gentamicin [Lapointe and Joncas, 1983]. After the time gap mentioned, reports of RSP pneumococci increased in number in the mid 1970's, principally from Europe and North America. Also in 1978, an eight-month-old male infant in Pittsburgh with meningitis caused by a type 23 RSP pneumococcus initially responded to treatment with penicillin 300,000 units [180 mg]/kg/ day in divided doses but relapsed on the third day of therapy, with positive blood and csf cultures. He responded to treatment when chloramphenicol was added to the regimen. The pneumococcus showed an MIC 0.5 ug/ml [Gartner and Michaels, 1979]. Extensive studies in Canada, of pneumococci from patients in Alberta and the North-West Territories, during 1974 through 1976, revealed that 101 [2.3%] strains of 4319 isolates examined showed reduced susceptibility to penicillin. The RSP isolates, which showed penicillin MIC values of 0.16 to 0.32 ug/ml, belonged to serotypes 6, 9, 10 and 19 [Dixon, Lipinski and Graham, 1977].

In 1977 pneumococci with high levels of resistance to penicillin had been encountered in South Africa, first in Durban and then in Johannesburg, where they caused serious and sometimes fatal infections in children, most of which had been hospital-acquired. Many of these strains showed multiple drug resistance, including resistance to chloramphenicol, erythromycin and clindamycin [Appelbaum, Bhamjee, and Scragg et al., 1977; Jacobs, Koornhof and Robins-Browne et al., 1978].

In Minnesota a three-year-old girl with an immune deficiency, who earlier had suffered several serious bacterial infections, developed a severe bacteraemic pneumonia caused by a type 14 pneumococcus, resistant to penicillin, with MIC values to penicillin and ampicillin of 4 ug/ml; her near fatal infection was treated successfully with a combination of chloramphenicol and rifampicin [Cates, Gerrard and Giebink GS et al., 1978].

3.12 Global spread of RSP pneumococci

In summary, within a decade of the first report of a RSP pneumococcus, such strains had been recognised in Africa, Europe and North America and isolates had belonged to many serotypes, including epidemic types. Moreover, the degree of resistance to penicillin had increased considerably. It is probable, of course, that RSP pneumococci had occurred earlier in some regions, notably in South Africa but also in Europe and North America, without being recognised. Gradually, and progressively, RSP pneumococci were recognised in other regions, including Asia and South America. For example, the first report of pneumococcal meningitis caused by a RSP pneumococcus -in a 10-month-old boy – in Brazil was published in 1988 [de Sousa Marques, Yamamoto and Sakane et al., 1988].

Chapter 4

Streptococcus pneumoniae resistant to chloramphenicol, erythromycin, lincomycin and other antibiotics; dual and multiple antibiotic resistance

4.1 Pneumococci resistant to chloramphenicol, erythromycin and other antibiotics

This chapter summarises my studies of strains of *S. pneumoniae* resistant to two or more of the antimicrobial drugs which were commonly used in the treatment of pneumococcal infections. These drugs included chloramphenicol [C], erythromycin [E], lincomycin [L], penicillin [P] and tetracycline [T]. The 'original' isolate of a RSP pneumococcus [type 23], also showed diminished susceptibility to tetracycline [Hansman and Bullen, 1967]. Several further patterns of resistance were identified subsequently In Australian isolates. During the four-year period, 1972 through 1975, the resistance combinations encountered were CT, CPT, EL, ELT and PT.

The isolates showing these resistance patterns belonged to serotypes 9, 11, 14, 19 and 23 [Hansman,1978]. As mentioned earlier, pneumococci of types 14, 19 and 23 commonly cause infections in children, including pneumonia, meningitis and other severe infections. All of these resistant strains had been isolated in Australia. Resistance to chloramphenicol, tetracycline and other oral antibiotics used in the treatment of pneumococcal infections had not been encountered in PNG during the study period.

4.2 Multiply-resistant pneumococci in Australia

Pneumococcal resistance to erythromycin had been first reported from Canada and then from the United States; the pneumococci were also resistant to lincomycin [Dixon, 1967; Kislak, 1967]. This was also the case with the Australian isolates, some of which were also resistant to tetracycline [Hansman, 1978]. Dual and multiply resistant strains were isolated from patients in Adelaide, Melbourne and Sydney, showing a wide geographical spread of resistant strains in Australia.

The degree of resistance to erythromycin and tetracycline was marked and to chloramphenicol and lincomycin was moderate, as shown in Table 1 of the article [Hansman, 1978]. Resistance to chloramphenicol alone was not observed. Strains showing dual resistance [CT, EL, PT] belonged to types 9, 11 and 19 while multi-resistant strains [CPT and ELT] were of types 9 and 19.

4.3 Multiply-resistant pneumococci in Poland and South Africa

Pneumococci resistant to chloramphenicol had been first reported from Poland, and showed high degrees of resistance, with MIC values of 50 ug /ml; the resistant strains belonged to types 6A, 6B, 19 and 23 [Cybulska, Jelaszewicz, Lund et al., 1970]. Some of the Polish strains showed either dual [CT] or multiple [CET] drug resistance, in serotypes 6A, 6B and 19; and 23, respectively.

Later, In South Africa, chloramphenicol-resistant strains, encountered in Durban and Johannesburg, showed either dual [CP] or multiple drug resistance [MDR]. The latter included a CEPT resistant type 19 A strain, which was also resistant to clindamycin and co-trimoxazole [Appelbaum, Bhamjee, Scragg et al., 1977; Jacobs, Koornhof, Robins-Brown et al., 1978].

4.4 First report of chloramphenicol-resistant pneumococci in West Africa

In 1977 I visited Nigeria, West Africa and studied the antimicrobial susceptibility of pneumococci in children and adults who were nasal carriers [Hansman, 1978]. There was a high carriage rate of pneumococci amongst sick children attending the outpatients department [44% of 99 children] of the University College Hospital, Ibadan.

Of 50 strains of pneumococci isolated in all, seven [14%] were resistant to both chloramphenicol and tetracycline. The MIC's for the chloramphenicol-resistant strains ranged from 10 to 20 ug/ml.

The importance of this observation lies in the fact that pneumococcal meningitis was common in West Africa and even moderate decreases in susceptibility to chloramphenicol have clinical significance [Appelbaum, Bhamjee, Scragg et al., 1977]. In developing countries chloramphenicol was a drug much used in the treatment of serious infections, including bacterial meningitis, because it was usually highly effective, freely available and could be given orally. It was therefore important to test isolates of pneumococci from patients with serious infections for susceptibility to chloramphenicol [Radetsky, Istre, Johansen, 1981].

4.5 Meningitis caused by a chloramphenicol-resistant pneumococcus in Australia

In 1979 colleagues in Melbourne and I reported a case of pneumococcal meningitis in a boy aged 10 months whose family had arrived from Yugoslavia recently [Hansman, Hewstone and Ritchie, 1978]. Despite treatment with penicillin and chloramphenicol, the child's condition deteriorated. A type 23 pneumococcus, which had been isolated from CSF, showed reduced susceptibility to penicillin and to chloramphenicol. As the pneumococcus was susceptible to lincomycin, treatment with this drug was begun. However this regimen [penicillin had been continued] failed to sterilise the CSF, which again yielded a pneumococcus on several occasions, including a month after treatment had begun.

Eventually, rifampicin was administered, which resulted in rapid improvement.

This case illustrates several points: clinical and microbiological failure in the face of resistance to chloramphenicol and reduced susceptibility to penicillin; and failure of lincomycin therapy despite *in vitro* susceptibility, presumably due to failure of lincomycin to reach bactericidal concentrations in CSF.

4.6 Pneumococci showing multiple drug resistance in Spain, with spread to UK

In 1978 the isolation of a multiply-resistant pneumococcus from a woman who had returned recently from a holiday in Spain, was reported from the UK. The strain, type 23, was resistant to chloramphenicol and tetracycline [MIC values 20 ug/ml] and also showed reduced susceptibility to penicillin [MIC 0.25 ug/m], [Meers and Matthews, 1978]. As the authors pointed out, a patient with meningitis caused by such a strain would be unlikely to respond to treatment with either chloramphenicol or penicillin.

This finding in the UK, together with encountering a patient whose pneumococcal meningitis had failed to respond to treatment with chloramphenicol, led workers in Barcelona to make a systematic study of pneumococci isolated from patients in their hospital [Garau, Linares and Domingurez, 1981]. Of 140 isolates tested from September, 1978 to January, 1981, 20 [14%] showed MIC values of 8 to 16 ug/ml chloramphenicol and 67 [47%] MIC's of >/= 32 ug/ml. Most chloramphenicol resistant strains were also resistant to tetracycline. The chloramphenicol-resistant strains belonged to 11 serotypes: types 1, 3 through 6, 8, 9, 11, 15, 19 and 23. These included epidemic types 1, 4 and 5 and the 'paediatric' types 6, 19 and 23.

These concerning findings – the highest incidence of chloramphenicol-resistant pneumococci reported to date – and affecting a number of important types - could probably be related to the *'uncontrolled and widespread usage of chloramphenicol over many years in Spain'* [Garau, Linares and Domingurez, 1981].

4.7 Spread of multiply-resistant pneumococcus from Poland to the UK

In 1981 the isolation of a multiply-resistant pneumococcus type 23 pneumococcus, from the nose and throat of a one-year-old girl, was reported from Birmingham [George, Mulligan and Rycroft et al., 1981]. The infant had developed pneumonia while in hospital in Poland, where she had been admitted with gastro-enteritis at the age of six months. The pneumococcus was unusually resistant to penicillin [MIC 4 ug/ml] and ampicillin [MIC 8 ug/ml] and was resistant to chloramphenicol [MIC 16 ug/ml], tetracycline and erythromycin. It could be assumed that the infant had become a carrier of this multi-resistant strain while in hospital in Poland.

By 1987 multiply drug resistant [MDR] pneumococci had become more common in the UK, where all the MDR strains reported belonged to types 6, 19 or 23 [George, Cooper and Erdman, 1987]. As mentioned above, dual or MDR had developed earlier in Australia, in types 19 and 23 [Hansman, 1978].

4.8 Further international spread of multiply-resistant pneumococci

These studies illustrate a progressive increase of drug resistance in pneumococcus: from diminished susceptibility to penicillin and resistance to tetracycline, evolving to chloramphenicol, erythromycin and lincomycin resistance. Some strains had now developed resistance to two or more of these antibiotics.

As mentioned in chapter 3, the degree of resistance to penicillin had increased: strains of pneumococci with higher levels of resistance to penicillin were being encountered, with MIC's exceeding 2 ug/ml. Moreover, there had been transnational spread of multiply-resistant pneumococci, from Spain and Poland to the UK [Meers and Matthews, 1978; George, Mulligan and Rycroft, 1981].

In 1992 the isolation of pneumococci resistant to penicillin, MIC 2 ug/ml, was reported from Reykjavic – from 1988 to 1991 the proportion of pneumococci with reduced susceptibility to penicillin increased from < 1% to 8% and comprised types 3, 6, 14, 18, 19, 23 and 36. Many of these isolates were resistant to other antibiotics, notably a multiply-resistant [PCET] type 6 strain. Like other Nordic countries, Iceland had a restrictive antibiotic policy and it was postulated that the strains had been introduced from abroad. Later it was shown that an identical MDR type 6A strain was common in Spain [Kristinsson, Hjalmarsdottir and Steingrimsson, 1992; Soares, Kristinsson and Musser et al, 1993].

4.9 Recognition of value of antimicrobial drug susceptibility testing

It was increasingly accepted that *'antibiotic susceptibility testing should be routinely performed in all CSF, blood and other significant isolates'* [Garau, Linares and Dominguez, 1981]. An editorial in 'The Lancet' in 1988 suggested that susceptibility testing should be extended further, to include all isolates of pneumococci [Editorial, 1988]. This was reinforced by the results of studies in Hungary which showed a concerningly high incidence of pneumococci resistant to penicillin with 23% and 35% relatively resistant and resistant, respectively. These included isolates from blood and CSF; some strains showed MIC values =/> 5.0 ug/ml.

Multiple drug resistance, also, was exceptionally high in Hungary: about *'70% of the penicillin-resistant isolates were also resistant to tetracycline, erythromycin and co-trimoxazole and ~30% to chloramphenicol'.* As in Spain, control of antibiotic prescribing was lax in Hungary [Marton, Gulyas, Munoz et al., 1991].

So, by the 1990's, strains of pneumococci had developed resistance in some regions to all of the drugs principally used in the treatment of severe pneumococcal infections.

Chapter 5

Significance of studies for the successful treatment of pneumococcal infections and an afterword on further developments in antibiotic drug resistance in *Streptococcus pneumoniae*

5.1 Significance of findings

Earlier chapters of my thesis describe the first isolations of pneumococci with diminished susceptibility to two key antibiotics used in the treatment of pneumococcal infections: penicillin and tetracycline. At the time, penicillin was the drug principally used in serious pneumococcal infections, including pneumonia and meningitis. A tetracycline was a convenient alternative for patients with pneumonia who were allergic to penicillin, and which had the advantage of oral administration. As mentioned in chapter 2, soon after Evans and I reported the isolation of a tetracycline- resistant pneumococcus from an infant with meningitis [Evans and Hansman, 1963], came reports of similar isolates from the UK and US. This suggested that such strains had occurred in regions other than Australia but had been overlooked.

In 1967 Bullen and I had reported the first isolation of a pneumococcus with reduced susceptibility to penicillin [RSP] [Hansman and Bullen, 1967]. Seven years elapsed before such strains were recognised elsewhere. However, I identified a similar strain in an aboriginal girl in northern South Australia, followed by the finding that such strains were relatively common among New Guineans [Hansman, Sturt, Glasgow et al., 1971 a, b].

Our report of RSP pneumococci in PNG, published in 'The New England Journal of Medicine' [Hansman, Glasgow, Sturt et al., 1971a] was accompanied by an editorial [Editorial, 1971], which probably helped to draw attention to the fact that one could no longer assume that pneumococci were invariably susceptible to penicillin. The incidence of RSP pneumococci was low in Australia [<1% of strains], but it was much higher in PNG [12% strains], [Hansman, Devitt, Miles et al., 1974]. In the 1970's reports appeared from other regions of RSP pneumococci causing serious infections in children [Naraqi, Kirkpatrick and Kabins, 1974; Howes and Mitchell, 1976], in the US and UK, respectively.

5.2 Implications of drug resistance for patients with pneumococcal pneumonia

What were the therapeutic implications for patients whose systemic infections were caused by RSP pneumococci? Penicillin G could still be used in patients with pneumococcal pneumonia provided the antibiotic was employed in sufficiently large doses such as 600mg 6 hourly by intra-muscular [IM] injection, because this dosage yields peak plasma concentrations of ~12 ug/ml. [Kucers and Bennett, 1987]. However, in low income countries, such as PNG, pneumonia was usually treated with procaine penicillin in doses which yielded peak plasma concentrations in the order of 2 ug/ml. Such levels approximated the MIC of the least susceptible strains of pneumococci at the time [Hansman, 1976]. A similar potential problem existed using oral treatment with penicillin V , which also results in relatively low plasma concentrations, with peak levels of ~ 2ug/ml [Kucers and Bennett, 1987].

5.3 Implications for treatment of patients with pneumococcal meningitis

In patients with meningitis, the problem was more serious, as CSF levels of penicillin are only 5% to 20% of plasma levels [Choi, 2002]. Despite meningeal inflammation, CSF levels of penicillin are low, ranging from 0.1 to 1.0 ug/ml [Hieber and Nelson, 1977]. Furthermore, during treatment penicillin levels in CSF probably fall, despite constant dosage, as inflammation lessens. This may explain the clinical and microbiological relapses reported in children with meningitis caused by RSP pneumococci [as mentioned in chapter 3]. After an initial improvement, clinical deterioration occurred during penicillin therapy and pneumococci were re-isolated from CSF [Naraqi, Kirkpatrick and Kabins, 1974; Howes and Mitchell, 1976; Paredes, Taber and Yow, 1976; Gartner and Michaels, 1979].

Therapeutic success was achieved when ampicillin was substituted for penicillin [Howes and Mitchell, 1976], the dosage of penicillin was increased [Naraqi, Kirkpatrick and Kabins, 1974] or chloramphenicol was administered [Paredes, Taber, Yow et al., 1976; Gartner and Michaels, 1979; Marques, Yamamoto, Sakane et al., 1988].

5.4 Implications for children with acute otitis media, mastoiditis and other complications of OM

With the introduction of antibiotics effective in OM, the incidence of serious complications – mastoiditis, epidural abscess, sigmoid sinus thrombosis, chronic purulent OM etc – fell markedly [Poole, 1995]. However, when drug resistance developed, treatment failures became more common, resulting in cases of unresolved acute OM [[Zielnik-JUrkiewicz and Bielicka, 2015]. Studies of such cases in Poland and Spain showed a high prevalence of pneumococci resistant to penicillins, including amoxycillin [the drug usually used in the treatment of acute OM] [Pumarola, Mares and Losada et al., 2013; Zielnik-Juriewicz and Bielicka, 2015]. Resistance has also developed to other drugs commonly used in OM including cefuroxime, erythromycin, clindamycin and co-trimoxazole [Zielnik-Juriewicz and Bielicka , 2015]. Meanwhile *S. pneumoniae* continues to be the commonest cause of acute OM.

5.5 Third generation cephalosporins

When third generation cephalosporins, including cefotaxime and ceftriaxone, became available, these drugs proved highly effective in the treatment of bacterial meningitis, including pneumococcal meningitis [Begue, Floret and Mallet et al., 1984]. Most strains of *S. pneumoniae* were highly susceptible to cefotaxime and ceftriaxone, with low MIC and MBC values [Kucers and Bennett, 1987; Jacobs, Wells and Steele et al., 1985].

With cefotaxime, for example, sterilisation of the CSF was achieved by the second day of treatment [Begue, Floret and Mallet et al., 1984]. Importantly, RSP strains showed only slightly reduced susceptibility to cefuroxime, cefotaxime and ceftriaxone [Marton,Gulyas and Munoz et al., 1991].

5.6 Infections caused by pneumococci resistant to cefotaxime and ceftriaxone

However, in 1992, the case of an infant with pneumococcal meningitis, treated with cefotaxime, was reported from Memphis, when, after an initial clinical improvement, relapse occurred on the fifth hospital day. A penicillin- and cefotaxime-resistant type 23F pneumococcus with MIC and MBC values of 0.25 and 32 ug/ml respectively, was isolated. Despite the addition of vancomycin to therapy, the child developed cerebral infarcts, surviving with severe brain damage. Two other children with meningitis, caused by cefotaxime-resistant pneumococci, also type 23F, were encountered, who also failed to respond to cefotaxime but were treated successfully with a combination of vancomycin and chloramphenicol [Sloas, Barrett, Chesney et al., 1992].

A similar case occurred in Madrid, in an adult with meningitis caused by a RSP pneumococcus, MIC values for penicillin and cefotaxime 1.0 ug/ml, who was treated successfully with intravenous and intrathecal vancomycin [Catalan, Fernandez and Vasquez, 1994]. Systemic infections caused by multiply-resistant pneumococci [MRP] were especially prominent in Spain and Hungary [Linares Palleres and Alonso et al., 1992; Marton, Gulyas, Munoz et al., 1991].

In South Africa the problem of multiple drug resistance had continued. Such infections pose a therapeutic challenge. Vancomycin does not penetrate into CSF when meninges are not inflamed; in meningitis levels of the order of 3ug/ml are attained [Gump DW, 1981]. Pneumococci show vancomycin MIC values of 0.25 to 1.0 ug/ml [Kucers and Bennett, 1987]. Although patients with meningitis caused by MRP, including instances where resistance includes third generation cephalosporins, have been treated successfully with vancomycin, treatment failure may occur [Viladrich, Gudiol and Linares et al, 1991].

5.7 Therapeutic options for the treatment of severe pneumococcal infections

Now, three decades on, the therapeutic options for the treatment of severe pneumococcal infections remain much the same:

For systemic infections, including meningitis, caused by antibiotic-susceptible pneumococci, either penicillin G or a third-generation cephalosporin, such as cefotaxime.

For infections with RSP pneumococci either a third-generation cephalosporin or vancomycin.

For infections caused by MRP [resistant to penicillins, cephalosporins and chloramphenicol] either vancomycin alone or in combination with a third generation cephalosporin [because of the relatively poor penetration of vancomycin into CSF]. Broadly, these were the regimens recommended in 1997 [Quagliarello and Scheld, 1997] and remain essentially unchanged to the present.

In the 1990's drug-resistant pneumococci, especiallly serotypes 6A, 6B, 9V, 14, 19F and 23F, rapidly spread globally. Fortunately, serogroups 6, 9, 14, 19 and 23 etc. had been included in pneumococcal vaccines, firstly in polysaccharide vaccines and later in conjugated vaccines. These vaccines have proved efficacious, notably in children, which has led to a striking reduction in the incidence of severe pneumococcal infections caused by serotypes included in the vaccine [Kaplan SL, Mason EO and Wald ER et al., 2004].

5.8 Global importance of pneumococcal infections

Although pneumococcal conjugated vaccines have proved effective in reducing the burden of invasive pneumococcal disease, *S. pneumoniae* remains one of the leading pathogens causing illness and death globally, as shown in the Global Burden of Disease Study [GBD Antimicrobial Resistance Collaborators, 2022]. This study, conducted in 2019, showed that *S. pneumoniae* was the leading cause of years of life lost [~40 million years] from infectious diseases and *'was associated with the most deaths among young children post-neonatal to age 4 years'.* In the under-five age group the pneumococcus was the pathogen responsible for most deaths. Antibiotic drug resistance contributes to this problem. More needs to be done to expand the use of conjugated pneumococcal vaccines, especially in infants and young children. The need is greatest in low-income countries, notably Africa.

The studies surveyed here, demonstrating for the first time that pneumococci had begun to develop resistance to the antibiotics used in the treatment of pneumococcal infections, had played a role in encouraging the development of pneumococcal vaccines.

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