

REVIEW ARTICLE

# Respiratory infections

*Proceedings of the Eighth Liverpool Tropical School Bayer Symposium of Microbial Disease held on 3 February 2001*

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## Introduction

Acute respiratory tract infections (ARI) are a major cause of morbidity and mortality world-wide. In a global survey of causes of mortality, respiratory tract disease was estimated to be the third commonest cause of death with 4.3 million deaths in 1990 [1]. The two major causes of death were ischaemic heart disease (6.3 million deaths) and cerebrovascular disease (4.4 million) [2]. However, it was also calculated that lower respiratory tract infections were responsible for 112.9 million disability-adjusted life year (DALYS) and as such were the major burden of premature death and disability world-wide, exceeding DALYS due to diarrhoeal disease (99.6 million) and perinatal disorders (92.3 million) [2]. As might be expected, the burden was significantly greater in the developing compared with the developed world [2]. ARI are estimated to be responsible for one third of all childhood deaths in developing countries [3]. It is estimated that the incidence of ARI, at 5–9 episodes/child/year in the first 5 years of life, is the same in developed and developing countries [4]. Although the incidence of ARI does not differ between developed and developing countries, the incidence of acute lower respiratory tract infection (ALRI) is over 12-fold greater in developing countries [5]. Risk factors for progression from ARI to ALRI include young age (0–11 months), gender (male), malnutrition (both macro- and micro-nutrients), lack of breast feeding, HIV infection and environmental factors such as crowding and indoor air pollution [6]. For ARI the major aetiological agents are viruses; in particular, respiratory syncytial virus (RSV) [7], influenza A, B and C virus, parainfluenza viruses and, in unvaccinated communities, measles

virus are the most important. However, in 2001 a ‘new’ virus, human metapneumovirus was described [8]. It is a newly discovered rather than new virus and it appears to have similar epidemiological characteristics to RSV with most subjects having been infected by the age of 5 years. It has been detected in children in Canada [9], Australia [10] and Holland [8], as well as South Africa, Brazil and the UK (Hart *et al.*, unpublished data).

Although RSV and, presumably, human metapneumovirus do cause pneumonia, bacteria such as *Streptococcus pneumoniae* and *Haemophilus influenzae* are also major pathogens [11]. Finally, the airways in cystic fibrosis pose a particular problem of infection with bacteria [12] not usually associated with community-acquired respiratory tract infection. We have attempted to address some of the aspects of the important topic of respiratory infection in the latest Liverpool Symposium on Microbial Disease.

## COMMUNITY-ACQUIRED PNEUMONIA IN CHILDREN

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Community-acquired pneumonia in childhood remains a major cause of morbidity world-wide, and a significant cause of death in developing countries. In the UK, it is generally seen as a much less serious disease, at least in the well child, but is still a not uncommon reason for hospital admission. Rational treatment of pneumonia depends on knowing the most likely pathogens in each community, as the relative frequency varies from one region to another. In most cases, few tests to isolate a causative agent are undertaken, and

Received 25 June 2002; accepted 4 July 2002.

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treatment is empirical. There have been many studies of adult community-acquired pneumonia, and a number of studies of paediatric community-acquired pneumonia in Europe and North America, but there are very few data for children in the UK.

Even where studies have been performed, questions remain concerning the reliability of methods used routinely for identifying pathogens: blood cultures have a low sensitivity, lung taps and transtracheal aspirates are too invasive for routine or perhaps even experimental use, while non culture-based techniques are not yet widely used. Antibiotic use in the community and the difficulty in obtaining sputum samples from young children hinder the isolation of bacteria. Furthermore, because potential pathogens (such as pneumococci) are frequently carried in children's upper respiratory tract, it is difficult to assess the significance of finding such organisms in cultures of nose, throat and nasopharyngeal secretions.

A large study in Finland, conducted between 1981 and 1982 in three neighbouring districts with a population of 8850 children under the age of 16, identified 201 cases of pneumonia – defined as cough fever with radiological changes [13]. Serological testing was used to identify the causative agent, and this was deemed to be bacterial in 102 cases, viral in 51 cases and mixed in 51 cases. *Streptococcus pneumoniae* was responsible for 57 cases, *Mycoplasma pneumoniae* for 51, *Chlamydia* spp. for 29, *Haemophilus influenzae* for 11, RSV for 43 and other viruses for 8. Viruses accounted for 80% of cases seen in children aged <2 years, but only 49% of those aged >2 years. A more recent study in Nottingham conducted between 1996 and 1997 identified 89 cases, although the investigators did not know the size of the childhood population from which these cases were drawn [14]. Children were investigated by blood culture, serology, viral immunofluorescence studies of the naso-pharyngeal secretion aspirates and PCR studies of blood and naso-pharyngeal aspirate secretions. *S. pneumoniae* accounted for 7 cases, *M. pneumoniae* for 14, *Bordetella pertussis* for 6, *Chlamydia* spp. for 1 and a virus for 23 (in 12 cases RSV). PCR testing of blood and naso-pharyngeal secretions proved by far the most sensitive method for detecting a pathogen. The investigators also looked at the pattern of chest X-ray changes and showed that although lobar changes were more common than alveolar or non-alveolar changes, the pattern of X-ray changes did not distinguish between bacterial and viral pneumonia. At a similar time, 136 cases admitted to Newcastle Children's Hospital Units between 1996 and 1998 were studied [15]. Acute blood samples for serology were taken from 122 and convalescent samples from 87, naso-pharyngeal secretions (NPS) or sputum from 98 and nose and throat swabs from 65 patients. Blood was taken for a full blood count, ESR, CRP and cultures. Laboratory studies included immunofluorescence for influenza A and B, RSV,

adenovirus and parainfluenza viruses 1, 2 and 3, together with viral and bacterial culture of secretions. Serological testing was set up for influenza A and B, RSV, adenovirus, *Coxiella burnettii*, *Chlamydia* spp., CMV, *M. pneumoniae*, Epstein–Barr virus and group A streptococcus. Cases were deemed to be definite, probable or possible, depending on the results from these studies. A definite case was one where there was a four-fold rise in antibody titre or a positive immunofluorescence on NPS, or a pure growth of a respiratory virus from a nose and throat swab, or a positive urinary antigen test by counter-current immuno-electrophoresis (CIE). A probable case was one where the convalescent antibody titre to a pathogen was >128, or where bacteria were grown, or detected by CIE from sputum. A possible case was one where pneumolysin PCR was positive, or where bacteria were grown from nose and throat swabs. A virus was detected in 50 cases, a bacterium in 17 and there was a mixed infection in one case. Group A streptococcus was the most common bacterial cause, followed by *M. pneumoniae* and *S. pneumoniae*. RSV was by far the most common viral cause, followed by influenza A, cytomegalovirus and adenovirus.

Paired serology gave the best diagnostic yield at 34%, followed by viral immunofluorescence at 33%. Sputum culture was positive in 5, bacterial culture of nose or throat swabs in 16 and viral culture of NPS in 2. Only one of the 110 blood cultures was positive (group A streptococcus). Viral infection was the most common cause of pneumonia, a virus being isolated from 53 (39%), RSV was the most frequent viral pathogen being identified in 34 (25%) children; 19 babies had clinical features of bronchiolitis but the other 15 cases of RSV infection were in older children with a clinical diagnosis of pneumonia (mean age 33.3 months). Seven of these were diagnosed by serology, 6 by immunofluorescence, one by nose throat swab culture and one by both immunofluorescence and serology. Other viral causes of pneumonia included influenza A (7), cytomegalovirus (4) and adenovirus (2). There was one viral co-infection (adenovirus and CMV) and one mixed infection with both influenza A and group A streptococcus.

Bacterial infection was found in 43 children, but in 14 this was by nose and throat swab culture only, making the significance of the isolate uncertain. Group A streptococcal infection was the most common, being found in 9 (7%) of 136. It was diagnosed by a raised anti-streptolysin O titre in eight cases and in one by blood culture. Three of the children with group A streptococcal infection had pleural effusions. *S. pneumoniae* was a definite or probable pathogen in only 5 (4%) of 136 cases, being isolated from CSF in one baby with pneumococcal meningitis in an acute pneumonia, by a rise in anti-pneumolysin antibody in 3, and from sputum in one patient who also had influenza A virus infection. Paired pneumolysin anti-

body testing was achieved in 51 of these, 6% were positive – all under 2 years of age. There were no differences in the total white count, neutrophil count, ESR and CRP between the different diagnostic groups. Chest X-ray appearances were characterised as being lobar, patchy consolidation, peri-hilar/per-bronchial, pneumonitis or effusion. There was no significant association between type of infection and X-ray appearances.

Recently, a heptavalent pneumococcal conjugate vaccine has become available. Large-scale studies of its use have been conducted, mainly to see if it will prevent or lessen the incidence of otitis media. Nevertheless, the studies have been of such power that it has been possible to look at the incidence of childhood pneumonia. In one study from California, 38 000 children were randomly allocated to receive a conjugate pneumococcal vaccine or a conjugate meningococcal vaccine [16]. Those given the pneumococcal vaccine showed a 35% decrease in pneumonia although, interestingly, in the control group there was only one culture-positive case of pneumococcal pneumonia. As this study looked at children under the age of 2 years, it suggests that at the very most, pneumococcal pneumonia in that population accounted for 35% of the cases of pneumonia. This is in keeping with the UK studies.

Taken overall, viruses are the most common cause of childhood pneumonia in Europe and North America, and in older children RSV often causes lobar pneumonia. Pneumococcal infection may account for up to a third of disease in children <2 years old, but seems to account for much less disease in older children. *M. pneumoniae* occurs in epidemics, and so the incidence will vary from year to year. There is still considerable controversy over the best treatment for childhood pneumonia. In children whose clinical state is not severe enough to warrant admission to hospital, a macrolide may well be sufficient treatment. If a young child is more toxic and has lobar changes, then perhaps penicillin G will suffice, although many prefer to give a third-generation cephalosporin with or without a macrolide. Further studies with modern PCR techniques should help to answer this question.

## PNEUMOCOCCI AND THE ALVEOLAR MACROPHAGE

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### Introduction

*Streptococcus pneumoniae* is the most common bacterial cause of pneumonia [17] and meningitis [18] in adults. Normally, pneumococci are commensal bacteria

in the nasopharynx but local infections (otitis media, sinusitis), as well as pulmonary infection and invasive disease (bacteraemia, meningitis and soft tissue infections) occur following a failure of host pulmonary defence [19, 20]. Alveolar macrophages [21] are the most common cells in the lower respiratory tract and have several important roles in pulmonary anti-pneumococcal defence. The interaction of pneumococci with alveolar macrophages is the subject of this report.

### Alveolar macrophages

Alveolar macrophages are resident tissue macrophages found at a density of one per alveolus in the air spaces of the lung parenchyma [21]. They have roles in both the innate and acquired immune response to bacterial infection [22]. Alveolar macrophages function in the innate immune response as the primary lung phagocyte and express multiple surface receptors (e.g., Fc receptor, complement receptors, scavenger receptors) [23]. They are long-lived (20–80 days), active phagocytes that engulf inhaled particles and organisms as well as apoptotic lung cells. Alveolar macrophages are capable of multiple episodes of phagocytosis but can be induced to undergo apoptosis by some bacterial infections [24, 25]. Alveolar macrophages are also important in the acquired immune response as they present antigens to T cells [26, 27] and produce a large number of regulatory cytokines [28] and mediators [29]. It is important to understand, however, that the role of the alveolar macrophage is more often anti-inflammatory than pro-inflammatory. The delicate respiratory interface of the alveoli is continually exposed to allergens and brisk pro-inflammatory responses to this inhaled allergen load would pose a substantial threat to gaseous exchange. The alveolar macrophage has important immuno-regulatory functions to ensure that most stimuli do not produce a damaging inflammatory response [30, 31].

### Alveolar macrophages and pneumococci

The pathogenesis of pneumococcal disease has been reviewed recently [32]. This complex subject has been the focus of extensive research both in animals [33] and man [34]. Briefly, it is understood that pneumococci first colonise the nasopharynx, and are then aspirated into the lower respiratory tract. Bacteria deposited in the bronchi are removed primarily by the scrubbing action of the mucociliary escalator, but bacteria reaching the alveoli escape this defence mechanism. Small numbers of bacteria reaching the alveoli are removed by the action of alveolar macrophages. These macrophages may then remain in the alveoli or be removed in the sputum. Large numbers of bacteria can be cleared successfully from the lung if they are delivered as an aerosol but much smaller inocula can cause disease if introduced as droplets

[35]. This is presumably because droplet infection delivers a large number of bacteria at once to a relatively small number of alveoli. When large numbers of bacteria overwhelm alveolar defences, local inflammation results with exudation of plasma into the air spaces and an intense cellular infiltrate [36]. The characteristic pattern of infiltration causes red (erythrocytes and neutrophils) and then grey hepatisation. The latter is the result of monocyte infiltration – these cells remove the necrotic debris after the initial neutrophil influx.

However, in considering human disease, several caveats must be applied to this basic paradigm. First, there are substantial differences between species in the ability of alveolar macrophages to ingest and kill pneumococci [37, 38]. In particular, rat alveolar macrophages do not ingest or kill pneumococci effectively, but rabbit alveolar macrophages ingest and kill the bacteria very rapidly, whereas guinea-pig macrophages are intermediate between the two. Work on human alveolar macrophages has consistently shown very low rates of pneumococcal ingestion except when non-capsulate organisms were studied [39]. Secondly, there is considerable heterogeneity in the human alveolar macrophage population in any individual sample [40]. This is likely to be the result of different stages of maturation and activation [22, 41], as well as the effect of recent infection [42, 43] and environmental effects [44]. Third, the role of opsonisation by both surfactant and immunoglobulin has been studied with regard to alveolar macrophage function [45, 46]. Recent work in our laboratory has shown that opsonins are required for binding and internalisation of pneumococci by human alveolar macrophages [47]. Pneumococci opsonised with pooled immune human serum bound to human alveolar macrophages three times more efficiently than unopsonised bacteria. Pneumococci opsonised with IgG-depleted serum were bound with half the efficiency of replete serum. When binding differences were equalised by adjusting the inocula of pneumococci in each experiment, bacteria opsonised with serum were internalised and killed equally but non-opsonised bacteria were poorly processed. These data were interpreted as showing that opsonisation is important in alveolar macrophage function against pneumococci, and that while IgG is the optimal opsonin, other opsonins (probably complement) are also functional. Problematically, however, the dilution of alveolar lining material that occurs in broncho-alveolar lavage makes functional assessment of opsonisation by native lavage fluid difficult [48].

### **HIV, pneumococcal infections and alveolar macrophages**

*S. pneumoniae* is a predominant HIV-related pathogen – the epidemiology, clinical presentation and management of HIV-related pneumococcal disease have been re-

viewed recently in proceedings of this meeting [49]. Briefly, the incidence of pneumococcal disease is 20–80 times increased in HIV-infected adults. This excess of infection is found at all stages of HIV infection, albeit to a greater degree in late infection [50]. In particular, a selective increase in invasive pneumococcal disease occurs before classical opportunist infections occur.

HIV infects and affects alveolar macrophages [51]. Initially, the viral load in alveolar macrophages was thought to be unusually high and closely related to the clinical status of the patient [52] but it now seems that alveolar macrophages contain a similar viral load to other cells of the macrophage lineage [53]. Nevertheless, HIV infection affects activation [54], receptor expression [55], accessory cell function [56], cytokine production [57], regulatory functions [58, 59] and phagocytosis [60] in alveolar macrophages. Given the pivotal role of the alveolar macrophage in regulating pulmonary defence against pneumococci, we hypothesised that a failure in alveolar macrophage function might contribute to the susceptibility of HIV-infected adults to pneumococcal infection. Surprisingly, however, experiments in which human alveolar macrophages from HIV-infected and uninfected volunteers were challenged *ex vivo* with opsonised pneumococci showed no difference in the binding or internalisation of the bacteria [61]. This may have been due to technical factors (loss of affected macrophages, low HIV load in alveolar macrophages) or due to the critical conditions in the alveolus necessary for optimal alveolar macrophage function, but a similar result has been seen with *Staphylococcus aureus* [62]. Alternatively, alveolar opsonisation may be a critical determinant of susceptibility to pneumococcal disease in HIV infection and this is the subject of ongoing studies.

### **Conclusions**

Animal studies suggest that alveolar macrophages are essential in lung defence. As the predominant cell in alveolar lavage and the only phagocyte in normal lung, it is likely that alveolar macrophages have a key role in human defence against pneumococci. However, available *ex-vivo* data show that human alveolar macrophages ingest pneumococci only at a low rate even in the presence of opsonins. Despite considerable evidence of altered function in alveolar macrophages from HIV-infected patients, no defect in pneumococcal binding and internalisation of opsonised pneumococci has been seen. Further studies on the *in-vivo* regulation of alveolar macrophage function and alveolar opsonisation of inhaled bacteria are required to understand the pathogenesis of this disease.

This work received financial support from the Wellcome Trust of Great Britain (Career Development Fellowship held by S.B.G. – grant number 063675) and forms part of the Malawi-Liverpool-Wellcome Trust Programme of Research in Clinical Tropical Medicine.

## THE ROLE OF ANTIBIOTICS IN COMMUNITY-ACQUIRED PNEUMONIA

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### Relevance of antibiotics in the treatment of community-acquired pneumonia

No randomised controlled trials have ever been conducted to demonstrate that antibiotics are superior to placebo in the management of community-acquired pneumonia. However, Robert Austrian described the effect of the introduction of sulphonamides and penicillins based on historical data derived in New York. Comparison of survival curves over three different decades showed that the case fatality rate dropped from 35% in the era of symptomatic treatment only to 25% during the era of the use of serum therapy, and subsequently to 17–25% during the era of penicillin therapy. However, mortality during the first 5 days in hospital did not appear to be influenced by the introduction of penicillin; rather, 28-day mortality was dramatically improved [63]. Tillet *et al.* reported the early use of penicillin in pneumococcal pneumonia during the 1940s and demonstrated a reduction in the case fatality rate to below 10% with doses of 40 000–100 000 units of penicillin per day, doses which are very low by today's standards [64]. With the modern era, there is some circumstantial evidence to support the efficacy of antibiotics. Woodhead *et al.* [65] have shown that administration of antibiotics by general practitioners before admission reduces subsequent in-hospital mortality. Similarly, delay or inadequate antibiotic therapy after admission to hospital [66] or before admission to the intensive care unit (ICU) [67] is associated with increased mortality in patients admitted to hospital and ICU respectively.

### Therapeutic choices for empiric therapy

Clinicians treating community-acquired pneumonia usually have to do so without knowledge of the precise microbiological cause in any case, and their approach is based on knowledge of the likely microbiological

**Table 1.** Microbiological causes of CAP in adults

Organism	Percent of cases (ranges reported)		
	Out-patient	In-patient	ICU
<i>S. pneumoniae</i>	1–36	7–76	10–36
<i>H. influenzae</i>	0–14	1–11	0–12
<i>Staph. aureus</i>	0–1	0–4	0–22
Legionella	0–3	0–16	0–30
<i>M. pneumoniae</i>	1–26	0–29	0–7
<i>C. pneumoniae</i>	4–16	6–18	?

causes in various settings which are shown in Table 1. The most common cause of pneumonia in most series is *Streptococcus pneumoniae* and the highest mortality rates are associated with pneumonia due to *S. pneumoniae* and also *Legionella pneumophila*. Therefore, empiric therapy should always be capable of covering pneumonia due to *S. pneumoniae* but in severe cases therapy should be tailored to cover the broad range of gram-positive, gram-negative and atypical organisms that can cause disease. Several national societies have published guidelines for empiric therapy of community-acquired pneumonia and the older examples of these are illustrated in Table 2 [68–70]. Most of these have recommended either an aminopenicillin, a macrolide or a tetracycline or a combination of an aminopenicillin and a macrolide in the treatment of patients with mild ambulant disease. In the therapy of severe disease, most guidelines recommend the use of second- or third-generation cephalosporins intravenously with an intravenous macrolide. This approach has never been formally tested, but it is intuitive. Gleason *et al.* [71] validated the American Thoracic Society (ATS) guidelines. The ATS guidelines recommend the use of a macrolide or tetracycline for patients who are <60 years old with no cardiopulmonary morbidities. In a study of 864 outpatients with community-acquired pneumonia including 546 younger patients without co-morbidity, Gleason *et al.* [71] found that 62% of 546 patients with mild, ambulant features were prescribed the recommended ATS therapy, which was mostly erythromycin. They found that there was no difference in outcome compared with those patients who were erroneously prescribed non-ATS therapy, although the costs associated with care were four-fold lower. The ATS guidelines recommend that those

**Table 2.** Older guidelines for empirical therapy of CAP

Society	Mild ambulant CAP	Mild out-patient CAP with risk factors shown	In-patient CAP
American Thoracic Society (1993) [68]	Macrolide <b>or</b> tetracycline	>60 years/cardiopulmonary disease: 2nd-generation cephalosporin, TMP-SMX <b>or</b> co-amoxiclav orally	Intravenous 2nd/3rd-generation cephalosporin <b>or</b> co-amoxiclav
British Thoracic Society (1993) [69]	Aminopenicillin ( <b>plus</b> erythromycin in patients with atypical features) <b>plus</b> flucloxacillin if <i>Staph. aureus</i> suspected	Not specified	Intravenous aminopenicillin; erythromycin if atypical features; flucloxacillin if <i>Staph. aureus</i> suspected
European Respiratory Society (1998) [70]	Aminopenicillin, <b>or</b> new tetracycline, <b>or</b> newer FQ, <b>or</b> oral streptogramin <b>or</b> macrolide	In patients with COPD: co-amoxiclav	Intravenous 2nd/3rd-generation cephalosporin <b>or</b> co-amoxiclav <b>or</b> aminopenicillin <b>or</b> macrolide

PFQ, fluoroquinolone; TMP-SMX, co-trimoxazole; COPD, chronic obstructive pulmonary disease.

patients older than 60 years or who do have cardiopulmonary morbidity should receive a more sophisticated antibiotic, i.e., a cephalosporin. However, Gleason *et al.* [71] found that in patients aged  $\geq 60$  years who were first prescribed the sophisticated antibiotic there was no difference in morbidity or mortality, but the treatment was associated with 10-fold higher costs.

Guidelines published much more recently by the Infectious Disease Society of America [72] and the Canadian Infectious Disease Society [73] are shown in Table 3. These differ markedly from the older guidelines in that they include a new fluoroquinolone with activity against *S. pneumoniae* in the choice of treatments for patients with mild out-patient disease. Newer fluoroquinolones are also recommended for the treatment of in-patients and also as part of the therapeutic choice in patients with severe community-acquired pneumonia. Older fluoroquinolones, such as ciprofloxacin, were unsuitable for the treatment of community-acquired pneumonia because the activity of these drugs against the pneumococcus was relatively poor. Newer fluoroquinolones, e.g., moxifloxacin, gemifloxacin and gatifloxacin, are much more potent against the pneumococcus and are currently being deployed world-wide. Their inclusion in therapeutic guidelines is a consequence of the emergence of penicillin- and macrolide-resistant strains of *S. pneumoniae*.

### The emergence of penicillin-resistant pneumococci

Clinically significant penicillin-resistant pneumococci (PRP) emerged during the 1970s and 1980s. Resistance is due to stepwise mutation of the penicillin-binding proteins. Resistant strains emerging within a given clone are capable of rapid spread within a community and between communities. Within a community, the incidence of PRP is related to antibiotic consumption [74] and is encouraged by lengthy therapies of low dose penicillins [75]. The past 20 years has seen a rapid emergence internationally of PRP and also macrolide-resistant pneumococci [76].

Data from the Centers for Disease Prevention and Control (CDC) have demonstrated the rapid emergence

of PRP in the USA during the mid-1990s. Data derived from 12 045 isolates of *S. pneumoniae* isolated from invasive sites in patients across the USA demonstrated a relentless increase in resistant isolates (*S. pneumoniae* resistant to penicillin, cefotaxime, erythromycin and tetracycline) between 1995 and 1998. When penicillin resistance was defined as an MIC  $>0.125$  mg/L, the incidence of PRP in the USA during 1998 was found to be 27% [77]. Factors associated with PRP in the USA were shown to be childhood, race (white people yielded PRP isolates more commonly than black people) and geography (PRP rates were highest in Tennessee and Georgia). There was little difference in the rates of PRP isolation from hospital in-patients and community out-patients. Pneumococci are highly transformable, and penicillin resistance is often accompanied by erythromycin, chloramphenicol or tetracycline resistance. In the same study from the CDC, rises in cefotaxime, erythromycin and tetracycline resistance were observed exclusively in those isolates that were penicillin-resistant. Erythromycin resistance was  $>50\%$  among PRP. Fortunately, in the same study, 88% of PRP from children  $<5$  years and 76% from people over the age of 5 years were of serotypes included in the new 7-valent conjugate vaccine [77].

### Consequences of infection with penicillin-resistant pneumococci

Although the advent of penicillin resistance amongst pneumococci is alarming, data have suggested that in hospitalised patients at least, infection with PRP or cephalosporin-resistant pneumococci does not result in increased mortality, even when patients with such strains are treated with penicillins or cephalosporins, respectively [78, 79]. Metlay *et al.* [80] measured the impact of penicillin susceptibility on medical outcomes for adult patients with bacteraemic pneumococcal pneumonia in a retrospective cohort study of 192 patients with invasive pneumococcal disease, of whom 23% were infected with PRP. Compared with patients infected with penicillin-susceptible pneumococcal strains, patients whose isolates were not susceptible did not suffer any increase in mortality, respiratory failure or rates of admission to ICU. However, they were significantly more likely to have suppurative complications as a result of their illness (relative risk 4.8% [1.2–18.8]). In another study by the CDC the

**Table 3.** More recent guidelines for empiric therapy of CAP

Society	Mild ambulant CAP	Mild out-patient CAP with risk factors shown	In-patient CAP
Infectious Disease Society of America (2000) [72]	Doxycycline <b>or</b> a newer FQ <b>or</b> a macrolide	Suspected drug-resistant <i>S. pneumoniae</i> , <b>or</b> older patients, <b>or</b> patients with an underlying disease: a newer FQ	A macrolide <b>or</b> extended-spectrum cephalosporin <b>or</b> co-amoxiclav (alone)
Canadian Infectious Disease Society/Canadian Thoracic Society (2000) [73]	Macrolide	Recent antibiotics or orally administered steroids within the last few months: newer FQ; suspected macroaspiration: amoxicillin $\pm$ a macrolide	Newer FQ

FQ, fluoroquinolone.

epidemiological factors affecting mortality from pneumococcal pneumonia during 1995–1997 were investigated. Patients with invasive pneumococcal disease were stratified according to the MIC of penicillin for the pneumococcal isolate (Table 4) [81]. For the overwhelming majority of isolates there was no increased risk of death in patients with PRP, but when risk of death in in-patients who died >4 days after admission to hospital was measured, there was a significantly higher relative risk of death in patients with PRP but only when the PRP had an MIC of penicillin >4 mg/L. Patients infected with PRP with MICs less than this did not suffer significantly different outcomes [81].

### A new dawn for fluoroquinolones?

Widespread concern about the advent of PRP has resulted in a flourish of fluoroquinolone use internationally. It is to be hoped that therapeutic use of these valuable agents will be judicious. Unfortunately, data from Canada suggest that even conservative use of fluoroquinolones (in this case ciprofloxacin) can lead to a rise in fluoroquinolone resistance. Chen *et al.* [82] demonstrated an increase in fluoroquinolone resistance from 1.5% to 2.9% during the 1990s in Canada. Resistance correlated with age (fluoroquinolone resistance was more common in the elderly), the source of the isolates (respiratory tract isolates were more likely to be resistant than blood isolates) and the regional consumption of fluoroquinolones. It is to be hoped that physicians exercise caution in the years ahead.

### Conclusions

Antibiotics are an important component of the modern management of community-acquired pneumonia. In most cases, therapeutic choice is based on empirical grounds as the microbiological cause is usually not available at the time the patient presents to the doctor. Numerous therapeutic guidelines have been published by national societies, which essentially recommend aminopenicillins, macrolides, tetracyclines or the newer fluoroquinolones for patients with relatively mild disease. The dominant factor affecting therapeutic choice over the coming years will be the emergence of penicillin- and macrolide-resistant strains throughout the world. Data suggest that the incidence of such strains is rising rapidly, although there is no evidence that penicillin-resistant pneumococci are associated

with increased mortality at least for strains with a penicillin MIC <4 mg/L. New fluoroquinolones have been developed and are being recommended for patients with community-acquired pneumonia. It is to be hoped that these new drugs are used prudently to avoid the emergence of fluoroquinolone resistance.

### CYSTIC FIBROSIS: PSEUDOMONAS AERUGINOSA AND BURKHOLDERIA CEPACIA

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### Introduction

Cystic fibrosis (CF) is the commonest autosomal recessive disorder of Caucasians. In the UK the incidence of CF is 1 in 2500 births with a carrier rate of *c.* 1 in 20 [83]. The disease results from defects in a gene on the long arm of chromosome 7. The gene encodes the cystic fibrosis transmembrane conductance regulator (CFTR) which regulates and facilitates transport of electrolytes across cell membranes [84, 85]. The gene is 250 kb long with 27 exons and the CFTR protein has 1480 amino acids. Over 1000 naturally occurring mutations in CFTR have so far been described. These can be by frameshift or deletion or point mutations leading to amino acid loss or substitution. However, *c.* 60% of CF patients have  $\Delta F508$ : a three base (codon) deletion at phenylalanine 508. These mutations can result in no synthesis of CFTR, a block in processing so the protein does not get to the plasma membrane (e.g., in  $\Delta F508$ ), a block in regulation or altered conductance or reduced synthesis of CFTR. It is less clear how this absence or decreased expression of CFTR results in the clinical features of CF. This is in part because CFTR interacts with a number of other transmembrane transporters of water and electrolytes to up- or down-regulate their activity.

Patients with CF present in a number of different ways and at varying times after birth but with the discovery of the CFTR gene more and more are being detected by genetic screening. Many clinical manifestations are related to defects in epithelial transport of  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$  and thus of fluid. Thus, destruction of the exocrine pancreas is thought to result from excessively viscid secretions plugging the pancreatic ducts. This can lead to meconium ileus and malabsorption because of an insufficiency of pancreatic digestive enzymes. Later in life, diabetes mellitus is associated with destruction of the endocrine pancreas secondary to the destruction by exocrine duct plugging. However, it is in the lung that the most dramatic and life-threatening manifestations occur [12, 85, 86]. Most of the deaths are related to lung infection. Nevertheless, with improved recognition and case management,

**Table 4.** Relative risk in penicillin-resistant pneumococcal infection [81]

Penicillin MIC (mg/L)	Risk of death	Risk of death >4th hospital day
>4	2.3	7.1
2	1.3	0.7
0.12–1	1.4	1.0

survival in CF has increased dramatically. For patients born in the 1950s the mean survival was 9 months; for those born between 1968 and 1976, it was 17 years; in 1997 the UK mean survival was 31 years and the predicted mean survival of a child born in 2000 with CF is currently >40 years [87].

In general, the broncho-alveolar tree should be sterile below the vocal cords. This state is maintained by powerful innate defence systems that include the mucociliary escalator, antimicrobial peptides (defensins, cathelicidins), lactoferrin, neutrophils and the alveolar macrophage. In the CF airway, the mucin is highly viscid, sulphated and readily forms aggregates [88]. This means that the ciliary escalator cannot propel the mucus and clearance of any particles reaching the lower airways is greatly impaired. This allows pathogens to persist for much longer. Following an observation that the airways surface liquid (ASL) in the CF bronchial tree is less efficient at killing bacteria [89], it has been suggested that the ionic composition of the ASL inhibits the activity of defensins and cathelicidins [90, 91]. Despite the above, the airways in the CF lung exhibit an extremely active inflammatory response with large quantities of neutrophils, macrophages and inflammatory mediators including tumour necrosis factor (TNF)- $\alpha$ , interleukin (IL)-1 and IL-8 (a neutrophil chemoattractant). This inflammatory response is certainly greatly enhanced by the presence of bacteria but does occur early in life and perhaps occurs as an intrinsic feature of CF epithelial cells [92, 93].

It is thought that infections with *Haemophilus influenzae* and *Staphylococcus aureus* occur early in the evolution of CF lung disease and that *Pseudomonas aeruginosa* and *Burkholderia cepacia* occur later. However, the risk of *S. aureus* in CF lung disease is not entirely clear; viral infections (including rhinovirus) [94] are important in clinical exacerbation and *P. aeruginosa* infection can occur in the first year of life [95]. Although a plethora of bacteria [96] such as *Stenotrophomonas maltophilia* and other pseudomonads [12, 97, 98], *Alcaligenes xylosoxidans*, non-tuberculous mycobacteria and fungi such as *Aspergillus* spp. do cause infections, the most important pathogens are *P. aeruginosa* and *B. cepacia*.

### ***Pseudomonas aeruginosa***

Most studies indicate that 70–80% of CF patients are infected with *P. aeruginosa* by their teens. In an American study that employed broncho-alveolar lavage, it was found that almost all (97.5%) of the children in three CF centres were infected by 3 years of age [95]. What affects the prevalence and age of onset of infection with *P. aeruginosa* is not known but there is evidence that continuous prophylactic administration of anti-staphylococcal antibiotics is associated with a

higher rate of acquisition [99]. This contrasts with the finding that inhaled gentamicin prophylaxis delays acquisition of *P. aeruginosa* [100]. However, it is clear that infection with *P. aeruginosa* has a deleterious effect on lung function, causes more frequent hospitalisations and induces increased and more rapid death [101]. The earlier the infection occurs the greater the effect [102]. Previously it has been assumed that the bacteria are acquired sporadically, perhaps from the inanimate environment, with a minor contribution of person-to-person spread – for example between siblings [103]. It is now clear that patients can be infected by two, three or more different genotypes concurrently or sequentially. Furthermore, there are some lineages that infect a large proportion of patients attending a particular CF clinic. Such lineages have been described in CF units in Denmark [104], Liverpool [105], Manchester [106] and Melbourne, Australia [107]. The strains from Liverpool, Manchester and Melbourne are genetically distinct. The Liverpool strain has been shown not only to cross-infect but also superinfect, infecting a patient already infected with *P. aeruginosa* and displacing the original strain [108]. Furthermore, the Liverpool strain has also caused pneumonia in the parents of one of the infected CF patients [109]. *P. aeruginosa* persists for many years in the CF airways and can show tremendous phenotypic variation with rough, smooth and mucoid colonial variants of varying colonial size. The mucoid phenotype is particularly associated with infection in cystic fibrosis. This phenotype results from production of a mucoid exopolysaccharide or alginate [86]. It is a polyanionic heteropolymer of mannuronic and glucuronic acids. The phenotype is unstable but is expressed in the CF lung, where it aids in the production of alginate biofilms of *P. aeruginosa* and other micro-organisms. The production of biofilms and antibiotic resistance are closely related in *P. aeruginosa* [110]. Formation of a biofilm is also associated with a phenomenon termed quorum sensing [111]. At high bacterial densities, *P. aeruginosa* secretes high concentrations of diffusible auto-inducers, N-butyryl homoserine lactone (HSL) and N-oxododecanoyl HSL. These signal to the whole bacterial population to co-ordinate expression of virulence factors, alginate production and biofilm formation. Approximately 4% of the 6000 genes in *P. aeruginosa* can be controlled by quorum sensing signals. Within the biofilm the bacteria are well protected from the external environment including from antimicrobial peptides, neutrophils and antibiotics.

A recent discovery has been that the ability to evolve rapidly is a survival trait possessed by *P. aeruginosa* that allows them to persist in the CF lung [112]. Thus 36% of *P. aeruginosa* strains isolated from 30 CF patients were hypermutable compared with none of 75 isolates from non-CF patients.

*P. aeruginosa* has an array of potential pathogenicity

determinants [12, 85, 86]. These include various hydrolytic enzymes including proteases, elastase, lipase, phospholipase, alkaline phosphatase and mucin sulphatase. The phospholipase C can catalyse the breakdown of surfactant and elastase, elastin and immunoglobulins. The CF mucin polysaccharide moieties are frequently capped by sulphate which normally prevents bacterial glycosidase enzymes from degrading such mucin. Both *P. aeruginosa* and *B. cepacia* have mucin sulphatase activity [113] and this might enable persistence by exposing new receptors for the bacteria. *P. aeruginosa* can also produce other factors such as pyocyanin, haemolysins, cytotoxins and siderophores to aid pathogenesis. It has been shown recently that *P. aeruginosa* possesses at least two pathogenicity islands. One, PAGI-1 is found in most virulent strains of *P. aeruginosa* [114]. The other encodes a type III secretion system which injects, for example, exoenzymes (ExoS and ExoT) directly into host cells. ExoS induces the transcriptional activation of a number of pro-inflammatory cytokines and chemokines [115].

### ***Burkholderia cepacia***

There are several potential CF pathogens in rRNA group II, including *Ralstonia taiwanensis*, *R. pickettii* and *Pandoraea* spp. [97, 98]. However, *Burkholderia cepacia* is perhaps the most important. *B. cepacia* is named after Burkholder who, in 1950, described it as the cause of onion rot (cepia is Latin for onion). Up to the 1980s, *B. cepacia* was thought of as a rare opportunist causing infections in immunocompromised hosts. Then it was found to infect and cause the death of patients with cystic fibrosis. Originally *B. cepacia* isolates were subdivided into a number of genomovars but now many of the genomovars have been assigned to species (Table 5). The most recently described are *B. anthina* and *B. pyrrocinia* [116], but there will undoubtedly be more to come. Of the *B. cepacia* complex, some are highly transmissible and some can cause lethal infection in CF patients [12, 85, 86]. One highly transmissible lineage called ET-12 (Edinburgh Toronto [12]) has spread in CF clinics world-wide and is responsible for the fatal cepacia syndrome of necrotising pneumonia and bacteraemia in some of the infected CF patients [117]. This is now known to be a member of *B. cepacia* genomovar IIIa. It

possesses two genomic markers called BCESM (*B. cepacia* epidemic strain marker) and *cbIA* gene (that encodes the production of cable pili). Cable pili appear specific to *B. cepacia* genomovar III strains but BCESM is present in some epidemic and sporadic strains including *B. cepacia* genomovar I and III as well as *B. multivorans* and *B. stabilis* [118]. However, *B. cepacia* ET-12 is arguably the most important pathogen in causing cepacia syndrome, spreading person-to-person with ease, superinfecting and displacing other *Burkholderia* spp. [119] and even infecting a parent of a chronically infected CF patient [120]. It has also caused chronic mastitis in sheep [121]. The reservoir of *B. cepacia* appears to be the inanimate environment including soil [122, 123].

*B. cepacia* adheres to the CF airway via one or more of at least five morphologically distinct pili [124]. Of these the cable pili have been most studied. These attach *B. cepacia* genomovar III to the epithelial cells via a cytokeratin 13 receptor which is enriched on the CF airways epithelium [125]. Binding appears to enhance uptake of *B. cepacia* into epithelial cells and rapidly promotes release of IL-8 [126]. In the inflammatory milieu of the CF airway, *B. cepacia* is particularly well adapted for survival. It is naturally resistant to the human antimicrobial peptides [127], can resist oxidant killing [128, 129] and can even produce a haemolysin that induces neutrophil degranulation and apoptosis [130].

Like *P. aeruginosa*, *B. cepacia* elaborates an impressive array of potential virulence determinants including protease, lipase, haemolysins, mucin sulphates and cytotoxins [85, 86, 113, 130]. Some strains can produce a mucoid exopolysaccharide [131] and others can invade lung epithelial cells [132]. Finally, there is evidence that strains of the *B. cepacia* complex (other than genomovar I) have pathogenicity island genes encoding a putative type III secretion system [133] which may also be related to virulence. The full genome of a *B. cepacia* genomovar IIIa strain is currently being sequenced. This will further enhance the understanding of *B. cepacia* pathogenicity.

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**Table 5.** Species and genomovars of *Burkholderia*

Genomovar	Species
I	<i>B. cepacia</i>
II	<i>B. multivorans</i>
IIIa	<i>B. cepacia</i> (ET12)
IIIb	<i>B. cepacia</i>
IV	<i>B. stabilis</i>
V	<i>B. vietnamiensis</i>
VI	<i>B. cepacia</i>
VII	<i>B. ambifaria</i>
VIII	<i>B. anthina</i>
IX	<i>B. pyrrocinia</i>

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