Chapter 6

Microbiology of non-CF bronchiectasis

J.E. Foweraker* and D. Wat#

Summary

Non-cystic fibrosis (CF) bronchiectasis is a complex disorder characterised by recurrent chest infections and poorly regulated respiratory innate and adaptive immunity. These lead to a “vicious cycle” of impaired mucociliary clearance, chronic infection, bronchial inflammation and progressive lung injury. The most prevalent pathogenic bacteria are Haemophilus influenzae, Pseudomonas aeruginosa, Streptococcus pneumoniae, Staphylococcus aureus and Moraxella catarrhalis although variations in sampling techniques and detection methods have influenced their isolation rates. These organisms can inhibit mucociliary clearance, destroy respiratory epithelium and produce biofilms that promote persistent infection by blocking innate immune defences and increasing antibiotic resistance. While numerous studies have examined the role of different bacteria in CF and chronic obstructive pulmonary disease, little is known about how they contribute to the pathogenesis of non-CF bronchiectasis. There is also a paucity of data regarding the role of respiratory viruses in this condition. This chapter describes the microbiology of non-CF bronchiectasis, defines the bacterial mechanisms that may contribute to persistent infection and airway damage and discusses the potential role for respiratory viruses in this condition. Understanding the pathogenic properties of these microorganisms may allow the development of novel therapies for the management of respiratory exacerbations.

Keywords: Anaerobes, Haemophilus, Moraxella, Pseudomonas, Streptococcus, viruses

Patients with bronchiectasis are commonly colonised with potentially pathogenic microorganisms in the airways [1]. These microorganisms can cause lung infections and may produce a number of inflammatory mediators that can lead to progressive tissue damage and bronchial obstruction. The phenomenon of chronic infection, bronchial inflammation and progressive lung injury is a “vicious cycle” and is also the reason why prompt evaluation of infection is important [2].
Being able to identify the causative bacterium may allow appropriate antibiotic administration to break this vicious cycle.

The most prevalent microorganisms found in non-cystic fibrosis (CF) bronchiectasis are discussed in this chapter and we have included the role of viruses, as well as some recent studies that have investigated microorganisms that are not usually considered to be pathogens in the respiratory tract. Surprisingly, there is little published data on the epidemiology and pathogenesis of infections in non-CF bronchiectasis. However, there are similarities with infections in CF bronchiectasis and chronic obstructive pulmonary disease (COPD). Where the literature for non-CF bronchiectasis is sparse, studies in CF and COPD have been drawn upon as these may aid the understanding of the microbiology; in particular the adaptations that take place to enable microorganisms to establish and maintain chronic infection and the role taken in the development of exacerbations.

Fungal infections, including allergic bronchopulmonary aspergillosis (ABPA), are discussed further by Hilverling et al. [3], while nontuberculous mycobacteria infections are discussed further by Daley [4] in this Monograph.

**Range of bacteria in patients with non-CF bronchiectasis**

Several studies have reviewed the bacteria found in patients with non-CF bronchiectasis (table 1). A similar range of organisms is found in most studies, but the prevalence of each varies. Age, ethnicity, the underlying causes of bronchiectasis and the proportion of patients that were stable or had cultures taken during an exacerbation varies between the different studies and would be expected to affect the microbial flora found. The pattern of antibiotic usage, including long-term prophylaxis, may vary between different centres and could also have an affect on the type of microorganisms cultured. The type of respiratory specimen tested may also determine the rate of positive cultures found. The use of a protected specimen brush to take samples at bronchoscopy yielded the highest positivity rate when compared with sputum specimens in one study [7]. Finally the methodology used for analysis (quantification, culture and identification techniques) will vary between centres and could also affect the result.

*Haemophilus influenzae* and *Pseudomonas aeruginosa* were the most common bacteria found in the majority of the studies and the most likely to cause long-term colonisation [12]. No potentially pathogenic microorganisms were cultured from 18–24% of the patients investigated and an absence of a potentially pathogenic microorganism was associated with the milder disease [9, 13].

**Haemophilus influenzae**

*H. influenzae* has been reported in 14–52% of patients with non-CF bronchiectasis. It is a Gram-negative coccobacillus with specific growth requirements, which can be difficult to isolate in the laboratory if mixed with other flora. Some *H. influenzae* possess a polysaccharide capsule and can be typed using type-specific anticapsule antisera. Those with the type B capsule (Hib) can cause invasive infection with bacteraemia, and are most familiar as a cause of meningitis or epiglottitis. The use of Hib vaccine has greatly reduced the incidence of these life-threatening conditions. *H. influenzae* with capsule types other than type B are relatively rare and are far less pathogenic. The nonencapsulated strains, referred to as nontypeable *H. influenzae* (NTHi), are also less pathogenic than Hib and only rarely cause bacteraemia. They live as commensals in the human upper respiratory tract but can cause otitis media, sinusitis and conjunctivitis, often following a primary viral infection. NTHi are a common cause of lower respiratory infection in patients with underlying respiratory abnormalities including non-CF bronchiectasis [9]. The Hib vaccine does not prevent infection with NTHi as it only contains the *H. influenzae* type B capsule antigen.

NTHi could be an oral contaminant in expectorated sputum; however, studies using a protected specimen brush (PSB) at bronchoscopy found NTHi in significant numbers in non-CF bronchiectasis, confirming its presence in the lower respiratory tract [7]. In contrast
Haemophilus parainfluenzae, a common commensal organism found in the upper respiratory tract, may be cultured from sputum but was not found in PSB samples. In patients with COPD the presence of NTHi in sputum was associated with raised inflammatory cytokines, whereas patients with H. parainfluenzae in sputum had similar levels of cytokines to those who had no microorganisms cultured from their sputum, suggesting that even if present in the lower tract it does not have a direct pathogenic role [14, 15].

There is little published data on the epidemiology of H. influenzae in non-CF bronchiectasis. It may be cultured repeatedly from the same patient over several years, but without typing data it is not known if this is the persistence of a single strain or repeated episodes of infection [9]. In COPD patients, NTHi were found in higher numbers ($>10^6$ colony forming units (CFU)·mL$^{-1}$) during exacerbation compared with when the patient was stable, and exacerbations in COPD may be associated with the appearance of a new strain [16, 17]. A prospective study in COPD using molecular typing of H. influenzae and direct analysis of amplified DNA from sputum showed persistence of the same strain over prolonged periods [18]. This suggests either long-term colonising infection in the lung or persistence in the upper respiratory tract with repeated deposition followed by clearance from the lung. In CF, sequential infection with different strains of H. influenzae was found in some patients and persistence of the same clone in other individuals [19].

NTHi have various properties that can help explain their pathogenicity and ability to persist in the lung. They can adhere to mucus and to various cell types in the human respiratory tract using pili and other adhesion molecules. Virulence factors include the endotoxin lipo-oligosaccharides.
NTHi may be able to evade the immune response by varying its surface antigens. Mechanisms include phase variation of LOS [24], and changes to outer membrane proteins (OMP) either by horizontal gene transfer or point mutations of the immuno-dominant OMP, i.e. P2. Antigenic drift, resulting from change in the P2 gene, has been observed in persistent infections in patients with COPD [25]. NTHi could be protected within host cells as they have been found inside macrophages in the chinchilla otitis media model and in macrophage-like cells in human adenoids. NTHi were able to enter cultured nonciliated respiratory epithelial cells and cross the respiratory epithelium [26]. Using in situ hybridisation, NTHi were identified inside cells in bronchial biopsies taken from patients with COPD [27].

*H. influenzae* (along with other bacteria infecting a bronchiectatic lung) may exist in biofilms in the respiratory tract. These are co-operative populations of bacteria surrounded by an amorphous matrix and could help the organism to survive in a hostile environment by resisting both host defences and antibiotics. The antibiotic resistance observed for bacteria growing in biofilms is in part attributable to its electrolyte content but also by reduced bacterial growth or even dormancy within the biofilm matrix. NTHi from patients with COPD can form biofilms in vitro, and NTHi biofilms were seen in the chinchilla model of otitis media [28, 29]. NTHi cultured from CF patients could form biofilms in vitro and on the surface of cultured airway epithelial cells. Structures consistent with biofilms containing *H. influenzae* were also found in bronchoalveolar lavage (BAL) samples from children with CF [30]. NTHi in biofilms were more resistant to antibiotics in vitro. Sub-inhibitory concentrations of azithromycin were found to reduce the size of both growing and established biofilms [31].

The prevalence of antibiotic resistant NTHi increases over time in patients with non-CF bronchiectasis [9]. Many are resistant to amidopenicillins (e.g. amoxicillin, ampicillin) either due to production of β-lactamase or alteration of penicillin binding proteins. Quinolone resistance is now recognised and resistance rates to trimethoprim and tetracycline are rising. Antibiotic resistance may occur by horizontal transfer of genetic material from other organisms in the complex polymicrobial environment of the mouth and upper respiratory tract. It may also take place in the lower respiratory tract, which may be polymicrobial in non-CF bronchiectasis. Alternatively, resistance may result from gene mutation. Some NTHi have a higher than usual mutation rate due to a mutation in *mutS*, which is one of the methyl-directed mismatch repair genes (MMR) that corrects errors in DNA. This hypermutability is not usually thought to be advantageous, as many random mutations can reduce bacterial fitness. However, if mutations lead to antibiotic resistance, the hypermutable state may become beneficial to the bacterial population. Hypermutators are generally rare in acute infection but hypermutable *H. influenzae* have been found in patients with CF and these strains have more resistance to antibiotics compared with normo-mutators [19, 32]. Hypermutability is seen in other species causing chronic infection (as is discussed later in this chapter) and may be a general adaptation to long-term survival in the lung. The prevalence and role of hypermutable *H. influenzae* in non-CF bronchiectasis has yet to be assessed.

**Pseudomonas aeruginosa**

*P. aeruginosa* is a versatile nonfermentative Gram-negative bacillus that is found in a range of environments. It is an opportunistic human pathogen that can cause severe, acute and invasive...
infections, such as necrotising ventilator-associated pneumonia and infections in immunocompromised patients often with bacteraemia [33]. It is one of the most common causes of infection in non-CF bronchiectasis and other chronic lung diseases, most notably CF, but may also be important in severe COPD. The epidemiology of P. aeruginosa, the mechanisms of pathogenicity and the genotypic and phenotypic changes in chronic infection have been extensively studied in CF, with fewer publications in non-CF bronchiectasis and COPD. There are many similarities between the infections in these different conditions, suggesting a common route of adaptation to chronic infection in the lung.

**P. aeruginosa in CF**

Early infections in CF are caused by genotypically distinct isolates, suggesting repeated episodes of acquisition. These early *P. aeruginosa* have the typical phenotype of isolates causing acute infections and environmental strains [34]. As chronic infection with *P. aeruginosa* can lead to an accelerated deterioration in lung function, antibiotic treatment regimens were developed to clear early infection and delay the onset of chronic infection [35]. CF patients eventually developed a persistent infection that seldom cleared despite aggressive antibiotic therapy. While there are some mixed infections, most CF patients carry a single genotype of *P. aeruginosa*, often for many decades [36, 37], and exacerbations do not appear to be due to the acquisition of a new strain of *P. aeruginosa* [38]. Early studies in CF showed that individual patients were infected with distinct strains that were thought to have been acquired from the environment. Some siblings shared strains but it was not known whether this was cross-infection or exposure to a common environmental source. More recently there have been reports in several countries of cross-infection between CF patients with what are termed “epidemic” strains. Some, in particular the Liverpool epidemic strain (LES), have been associated with increased morbidity [39, 40]. LES is now the most common epidemic strain in the UK affecting as many as 11% of patients in England and Wales [41].

**P. aeruginosa in COPD**

*P. aeruginosa* has been cultured from 4–15% of patients with COPD and was more prevalent in patients with advanced disease, particularly those requiring mechanical ventilation for severe exacerbations. *P. aeruginosa* infection was associated with steroid use, prior antibiotics and a low forced expiratory volume in 1 second (FEV1) [42]. In a study of 126 patients with moderate-to-severe COPD over an 11-year period, 39 patients grew *P. aeruginosa* from one or more sputum culture. There was a significant association with the culture of a new strain of *P. aeruginosa* and symptoms of an exacerbation. However, of interest, two-thirds of new infections that later cleared from the sputum, did so without the use of specific antibiotic treatment [43]. Only 13 patients had carriage of the same clone for more than 6 months with four patients infected with mucoid strains. Chronic infection is therefore rare in COPD, but when it does occur *P. aeruginosa* has a range of colony forms (morphotypes) and adaptations including increased mutability, reduced motility, reduced protease production and increased antibiotic resistance, similar to those seen in CF [44].

**P. aeruginosa in non-CF bronchiectasis**

*P. aeruginosa* is one of the most common isolates found in 12–43% of non-CF bronchiectasis patients (table 1). Stable patients with *P. aeruginosa* have poorer lung function and more sputum production when compared with patients with other potentially pathogenic microorganisms (PPM) [45] and it has been associated with a poorer quality of life and more frequent hospital admissions [46]. There is debate over whether infection with *P. aeruginosa* leads to a faster decline in lung function as is seen in CF, or whether it is a marker of more damaged lungs [47, 48].

A recent study compared long-term colonisation with *P. aeruginosa* in 21 patients, of which six had CF, 10 had non-CF bronchiectasis and five had COPD. The authors typed 125 sequential
isolates from sputa taken at least 1 month apart. The authors found a similar pattern of colonisation in all three diseases, with a dominant persistent clone, showing that the pattern of infection found in CF could also be shown in other conditions [49].

There have been no studies using genomic typing methods to investigate whether patients with non-CF bronchiectasis share strains of *P. aeruginosa*, but there has been one report of a patient with non-CF bronchiectasis acquiring LES from a relative with CF [50]. Interestingly, early studies using pyocin typing and examining mucinophilic and chemotactic properties of *P. aeruginosa* suggest that specific subpopulations may have a predilection to infect bronchiectatic lungs [51, 52].

**Pathogenicity of *P. aeruginosa***

*P. aeruginosa* possesses a range of virulence factors, although their expression may differ between isolates that cause acute infection and those responsible for chronic infection. Flagella, type IV pili, lipopolysaccharide and exopolysaccharides contribute to the adherence to cells and surfaces. Type I and type II secretion systems export protein toxins, such as alkaline protease, elastase, exotoxin A and phospholipase C, while type III secretion systems inject exoenzymes directly into eukaryotic cells. Other extra-cellular virulence factors include rhamnolipids, pyocyanin and hydrogen cyanide [53, 54]. Another pathogenicity factor is the ability to form alginate-enhanced biofilms [55], which contributes to the persistence of the organism rather than acute tissue damage and, together with other adaptations, promotes chronic infection (refer to later section).

**Antibiotic resistance**

*P. aeruginosa* is intrinsically resistant to many commonly used antibiotics and easily acquires resistance by chromosomal mutation or the acquisition of new genes from other microorganisms by horizontal transfer [56]. In addition, the biofilm mode of growth also protects *P. aeruginosa* from antibiotics by a variety of mechanisms [57].

Little has been published specifically on antibiotic susceptibility of *P. aeruginosa* from patients with non-CF bronchiectasis. In CF the prevalence of resistant *P. aeruginosa* is increasing as a result of repeated antibiotic courses. Resistance rates are significantly higher than for strains originating from patients without CF [58] and pan-resistant bacteria that are resistant to all antibiotics other than the polymyxins have been described.

*P. aeruginosa* can develop resistance by either: 1) producing enzymes that destroy the antibiotic, such as AmpC β-lactamase, carbapenemases or aminoglycoside modifying enzymes; 2) modifying the antibiotic target, such as gyrA for quinolone resistance; or 3) reducing exposure either by a decrease in permeability or increased removal of the antibiotic from the bacterial cell (efflux). Efflux mechanisms often affect more that one class of antibiotics and therefore contribute to multi-drug resistance [56].

Antibiotic resistance and its regulation can be complex in *P. aeruginosa* and various mechanisms that affect resistance to a single antibiotic may be present in the same organism. For example, low level resistance to meropenem may be due to reduced permeability following changes to the membrane porin OprD. More resistance can result from an increase in an efflux pump that can remove the meropenem from the cell. Both mechanisms may be present and additive, leading to high-level resistance. Enzymes that can destroy meropenem (penemases such as VIM) do occur but are currently rare [56].

*AmpC* codes for an inducible cephalosporinase which, when production is increased, can result in resistance to nearly all β-lactam antibiotics except the penems. Treatment with piperacillin or ceftazidime can lead to the selection of bacteria that produce the enzyme constitutively rather than just on induction. These are called derepressed mutants and offer a survival advantage. Imipenem induces the *AmpC* β-lactamase, even though it is not affected by the enzyme, and it also induces genes involved with alginate production [59]. The regulation of *ampC* is exceedingly complex and...
is intimately linked to cell wall recycling [60–62]. Some mutations can reduce biological competitiveness and more work is needed to assess the link between antimicrobial resistance and fitness [61]. AmpR does not just regulate ampC but is a global transcriptional regulator that regulates another β-lactamase PoxB, as well as proteases, quorum sensing and other virulence factors [63]. Antibiotic resistance may therefore be associated with a change in virulence and/or fitness. This could explain why some CF patients respond to treatment for acute exacerbation, even though some of the _P. aeruginosa_ are resistant to the antibiotic used [64].

_P. aeruginosa_ possesses multi-drug efflux pumps that can expel a wide range of antibiotics and are responsible for much of the organism’s intrinsic resistance to antimicrobials. For example, substrates for efflux pump MexAB-OprM include ticarcillin, aztreonam, piperacillin, ceftazidime and tetracycline [65]. MexXY uses the same exit duct OprM and can export aminoglycosides, cepfime and ciprofloxacin. Antibiotic resistance may arise from an increase in the efflux pump activity, e.g. MexXY-OprM over-expression may be due to mutation in the regulatory gene mexZ and/or to mutations in the MexXY translocase genes [66].

Conversely, _P. aeruginosa_ may be hyper-susceptible _in vitro_ to some anti-pseudomonal antibiotics and susceptible to agents such as tetracycline and chloramphenicol to which _P. aeruginosa_ would normally be intrinsically resistant. This has been described in chronic infection in both CF and non-CF bronchiectasis [67, 68], but the clinical relevance of these findings has not been investigated. It was found that 25 out of 46 CF patients had strains hyper-susceptible to ticarcillin due to deficiencies in MexAB-OprM efflux activity, resulting from various gene defects including reduced or abnormal expression of MexB and OprM [66]. It is unclear why this phenomenon exists. Efflux pumps do not just expel antibiotics and therefore reduced efflux may give a selective advantage under certain physiological conditions in the chronically infected lung.

### Adaptations to chronic infection

One of the characteristics of chronic infection with _P. aeruginosa_ is the appearance _in vitro_ of a variety of colony forms (morphotypes) that differ from those seen in environmental strains or those causing acute infection (fig. 1).

Several different morphotypes may be found in the same sputum, even though the isolates are clonally related. These can include colonies lacking the typical pigmentation, mucoid forms, some that look like coliforms, “dwarf” forms and very slow growing “small colony variants”. One of the most easily recognised is the mucoid morphotype. This results from over-production of the polysaccharide alginate, due to mutation in the regulatory genes. Hyper-alginate producers were originally thought unique to CF but they are also found in non-CF bronchiectasis and COPD [45, 49, 69]. They are thought to be an adaptation to chronic infection irrespective of the underlying cause. Alginate may protect against phagocytosis [70] and contribute to the formation of biofilms [71, 72]. Small colony variants (SCVs) have enhanced ability to form biofilms and may also contribute to persistence [73]. SCVs have only been described so far in CF but are easily missed unless cultures are prolonged.

The phenotypic changes found in chronic infection have been studied extensively in CF but not in non-CF bronchiectasis. They include loss of acute virulence factors, such as toxin production (e.g. elastase, phospholipase C, pyoverdin, hydrogen cyanide) and type III secretion [74]. Many virulence factors are regulated by the quorum sensing (QS) system. These are signalling...
molecules that act on the regulators of gene transcription. Some QS molecules depend on population density, and only have their effect when the number of organisms reaches a critical concentration (or quorum). *P. aeruginosa* QS molecules comprise acyl-homoserine lactones and molecules of the PQS system. They can affect a large number of functions including pathogenicity, metabolic adaptation and persistence [75]. *P. aeruginosa* with mutations in QS genes, most frequently *las R*, do not respond to QS molecules and are surprisingly common, they were found in 19 out of 30 CF patients in a study by Smith et al. [76]. *Las R* mutants form characteristic iridescent colonies and have also been cultured from patients with non-CF bronchiectasis (J.E. Foweraker, Papworth Hospital, Cambridge, UK; personal communication). These mutants do not produce the toxins elastase, phenazines or hydrogen cyanide. *Las R* mutants can use a more diverse range of compounds as a source of carbon, nitrogen, phosphorus or sulphur and have a growth advantage over the wild type when grown with phenylalanine, isoleucine or tyrosine. They therefore appear to be less pathogenic but better able to adapt to the local environment. Again, different phenotypes can co-exist so sputum may contain *Las R* mutants and organisms without the mutation.

Longitudinal studies in CF have analysed strains from patients over several years. It is thought that with time the bacteria adapt to a form that is less virulent but better able to persist in the damaged lung [76]. Multiple phenotypic variants of the underlying clonal population of *P. aeruginosa* co-exist and form a complex population in the chronically infected lung. This is described as “adaptive radiation” and is thought to give the bacteria an advantage in that they can rapidly respond to changes in the environment, as individual organisms that have the necessary adaptation may already be present in the population.

**Biofilms**

*P. aeruginosa* is thought to grow in biofilms in chronic infections in both CF and non-CF bronchiectasis. Biofilm formation is thought to be a general adaptation to a hostile environment and may allow persistence of infection by protecting the bacteria from the host response and the effects of antibiotics. Biofilm fragments have been seen in CF sputum [77] and may contain a mixture of *P. aeruginosa* plus other bacteria and even fungi, such as *Candida spp*. The extracellular matrix comprises alginate produced by *P. aeruginosa* plus proteins and DNA from other microorganisms and host cells. The biofilm contains a steep oxygen gradient and is anaerobic just below the surface. Different concentrations of nutrients and waste products will also be found in different areas of the biofilm. Therefore, the biofilm contains a wide range of physiological conditions, by which the bacteria possess a variety of adaptations that enable them to survive within these microniches [78].

Alginate protects *P. aeruginosa* in biofilms from interferon (IFN)-γ activated macrophages [79]. Neutrophils have been observed immobilised in the extra-cellular matrix, unable to penetrate the biofilm [80]. It is thought that neutrophils may actually enhance early biofilm formation, as biofilms formed in vitro in the presence of neutrophils are thicker and contain more bacteria [81]. If *P. aeruginosa* and neutrophils are combined, the bacteria aggregate around necrotic dying neutrophils. If neutrophil apoptosis is induced before the bacteria are added, the neutrophils are intact and the *P. aeruginosa* remain dispersed. Neutrophils can release DNA and F-actin complexed with histones and other cations, and these may form the framework for the biofilm. The combination of DNAase and anionic polymers has a synergistic effect in clearing early neutrophil-associated biofilms in vitro and is being studied as a potential treatment to prevent or disrupt early biofilm formation [81]. Neutrophil lysis is thought to be caused by rhamnolipid, a toxin produced by *P. aeruginosa* under QS control. Rhamnolipid may therefore help to protect the biofilm from disruption by neutrophils, especially in the early stages of formation [82].

Azithromycin is a macrolide antibiotic that does not directly inhibit or kill *P. aeruginosa*, but it can block QS and alginate polymer formation in vitro [83]. It can disrupt early biofilms formed by nonmucoid strains but has less effect on early biofilms formed by hyper-alginate producers.
(mucoid strains) or on established biofilms [84]. Azithromycin also has an anti-inflammatory effect in chronic lung infection and the relative importance of its diverse actions is yet to be established. Other antibiotics may also influence bacterial virulence. For example ciprofloxacin can suppress alginate biosynthesis at concentration well below minimum inhibitory concentration (MIC) [85].

Bacteria cultured in biofilms in vitro are more resistant to most antibiotics than when they are dispersed (planktonic). Several mechanisms have been proposed to explain this resistance [86]. It was thought that the extra-cellular matrix formed a physical barrier but there are channels within the biofilm through which most antibiotics can permeate. Positively charged antibiotics such as colistin may bind to free anionic DNA and therefore not reach the bacteria, and an anionic antibiotic, such as an aminoglycoside (e.g. tobramycin) may bind to the alginate. If the AmpC β-lactamase is over produced by some P. aeruginosa it may form a high local concentration and protect bacteria that can only produce basal levels of the enzyme. Mutability is increased in biofilms, partly because of the presence of hypermutators but also because DNA can be damaged by the increased amounts of reactive oxygen species within the biofilm. The range of metabolic conditions in the biofilm may affect antibiotic susceptibility. P. aeruginosa can survive in the anaerobic environment just below the surface of the biofilm by using nitrogen rather than oxygen as a terminal electron acceptor and aminoglycosides, such as tobramycin, cannot act on organisms that are metabolising anaerobically. Organisms within a biofilm may become dormant and therefore resist quinolones and β-lactam antibiotics [87]. These affects have been shown in an in vitro model of a young biofilm in a flow chamber using live/dead staining. Ciprofloxacin kills organisms on the surface of the biofilm but cannot kill those deeply set within the biofilm, whereas colistin can kill the non-dividing cells in the centre [88]. The two antibiotics appear to be very effective against young biofilms in vitro and may explain why that combination is particularly effective in eliminating early infection with P. aeruginosa in CF.

**Hypermutators**

One of the drivers of variability and adaptation seen in persistent infection in bronchiectasis is thought to be the presence of hypermutator (HM) bacteria [89]. These are P. aeruginosa with a higher than usual spontaneous mutation rate and are thought to accelerate bacterial evolution. P. aeruginosa usually mutates at a frequency of one in $10^8$–$10^9$, while mutation rates in HM bacteria can be as high as one in 100. HM P. aeruginosa were found in 37% of chronically infected CF patients. This was the highest prevalence that had been described for a naturally occurring population [90]. In comparison a HM prevalence of 1% in Escherichia coli and Salmonella spp. had previously been considered high [91]. In a longitudinal study of CF patients in Denmark, none of the bacteria from early infections were found to be HMs but after 20 years of colonisation 65% of patients were infected with HM P. aeruginosa [92]. HM P. aeruginosa were described in 57% of chronically infected patients with COPD or non-CF bronchiectasis, suggesting that hypermutability is a general adaptation to long-term survival in the lung [93]. KENNA et al. [94] suggests that hypermutability is an extremely rare finding in environmental P. aeruginosa and in isolates from newly infected CF patients.

Most of the information on HM P. aeruginosa comes from work on isolates from CF [95]. Hypermutability usually results from a primary mutation in genes of the MMR system, most commonly mutS and mutL, or defects in the GO system (mut M, Y and T). The function of these systems is to detect and repair DNA replication errors and repair oxidative damage. MMR also inhibits recombination between moderately diverged sequences and therefore reduces the acquisition of exogenous DNA through horizontal gene transfer [96].

HMs are uncommon in most bacterial populations because many of the mutations are deleterious. HM P. aeruginosa had reduced virulence and fitness both in vitro and in an animal model [97, 98]. However, in changing environments or stressful conditions HM bacteria may be selected because they have adaptive mutations, such as antimicrobial resistance (referred to as “hitchhiking”).
The sequential acquisition of resistance to multiple antibiotics is seen in infection with *P. aeruginosa* in CF, and several studies in CF, non-CF bronchiectasis and COPD have shown that HM are more likely to be antibiotic resistant than isolates with normal mutation rates [93, 99]. In a study of 29 CF patients over a 5-year period, mutations accumulated at an average mutations rate of three per year in HM *P. aeruginosa* compared with 0.25 per year in non-mutators. HM had more mutations leading to antibiotic resistance but also more mutations in other genes such as lasR [89]. Therefore, other adaptations may provide a selective advantage for HM isolates, not just antibiotic resistance.

Two recent studies have shown that CF patients with HM had poorer lung function (FEV1 predicted), but longitudinal studies are needed to determine if this was due to infection with a HM or just an association, both being the result of prolonged infection [99, 100]. Work is needed on the role of HM in non-CF bronchiectasis.

### Chronic *P. aeruginosa* infection and the clinical microbiology laboratory

One practical implication of the range of phenotypic diversity of *P. aeruginosa* from non-CF bronchiectasis is that some isolates may be difficult to identify. Colonies of *P. aeruginosa* from chronic infection may lack pigmentation, grow very slowly and may mimic other species.

Commercial identification schemes that use biochemical reactions and assimilation tests are not reliable in identifying atypical *P. aeruginosa* and some of the other nonfermenting Gram-negative bacilli found in chronic infection, and therefore identification methods, such as species-specific PCR or sequencing of the 16S ribosomal RNA gene may be required [101].

Another consequence of phenotypic diversity is that a range of antimicrobial susceptibility patterns can be found in a population of *P. aeruginosa* in a single sputum sample (fig. 2). Bacteria with the same morphotype may have different susceptibility and therefore resistant sub-populations may be missed, depending on which colony is picked for testing [68].

In CF, once a chronic infection is established the range in the antibiotic susceptibility of *P. aeruginosa* in a single sputum is so diverse that susceptibility testing methods are unreliable [102, 103]. It is currently unclear whether these findings can equally be applied to chronic infection in non-CF bronchiectasis.

Finally it has been questioned whether current methods used for testing antimicrobial susceptibility are relevant for bacteria that may be present in biofilms in the chronically infected lungs. A variety of methods are being developed for testing biofilm susceptibility; however, their clinical relevance still needs to be determined.

### Streptococcus pneumoniae

*S. pneumoniae* is a Gram-positive coccus appearing in pairs and in short chains. It may be a harmless commensal in the oro-pharynx but can cause severe and invasive disease (pneumonia or meningitis). It can also cause otitis media or sinusitis, or lower airway infections in patients with damaged lungs such as non-CF bronchiectasis or COPD, but it is rare in CF. Although *S. pneumoniae* can be found in up to 37% of patients with non-CF
bronchiectasis, very little has been published on its role in this condition. In COPD, *S. pneumoniae* has been cultured from both stable patients and those with exacerbation [20, 104]. The patient with non-CF bronchiectasis due to an underlying antibody deficiency may be particularly susceptible to recurrent infections with *S. pneumoniae* [105]. Bronchiectasis in primary and secondary immunodeficiency patients is discussed further in the chapter by Brown et al. [106].

*S. pneumoniae* has a polysaccharide capsule that helps evade opsonisation, and isolates lacking the capsule are avirulent. There are over 90 capsule types and the capsule type may be one of several factors that determine the pathogenicity of an individual strain [107]. A polyvalent vaccine containing the most common serotypes is available and recommended for use in patients with chronic lung disease.

*S. pneumoniae* can use a wide variety of molecules to adhere to host cells and produces an IgA protease and a toxin, pneumolysin that can promote invasion, inflammation and tissue damage [108]. Pneumolysin is proinflammatory and has many actions including cytolysis, inhibition of ciliary beating, and direct activation of the classical complement cascade. Although it is not a common pathogen in CF, isolates of *S. pneumoniae* from CF sputum have characteristics that may be associated with adaptation to persistence in the lung, *i.e.* hypermutability and the ability to form biofilms [109, 110]. Further work is needed to clarify the role of the different virulence factors in order to understand why *S. pneumoniae* may be a harmless commensal or cause non-invasive respiratory tract infection (in COPD or bronchiectasis) or produce severe invasive disease with bacteraemia.

The prevalence of antibiotic resistant *S. pneumoniae* has increased and in some countries very high rates of resistance to penicillin, macrolides and tetracyclines limit the treatment options. Penicillin resistance is due to modifications to penicillin binding proteins not by the production of a β-lactamase and, therefore, amoxicillin–clavulanate is ineffective.

**Moraxella catarrhalis**

*M. catarrhalis* is a Gram-negative diplococcus that was previously named *Branhamella* or *Neisseria catarrhalis*. Like NTHi it is a common commensal organism in the upper respiratory tract and can cause otitis media or sinusitis. It was not reported in studies of non-CF bronchiectasis in the 1960s as it was considered an oral contaminant rather than a PPM. However, it can be cultured in significant numbers from sputum or PSB in up to 27% of patients with non-CF bronchiectasis [7]. It is also considered a significant pathogen in COPD but is only rarely isolated in CF.

A longitudinal study of *M. catarrhalis* in 29 patients with non-CF bronchiectasis found that patients were colonised with a variety of strains with average colonisation duration of 2.3 months for each strain. No association between strain acquisition and exacerbation was found and as *M. catarrhalis* was often in mixed culture with other PPMs (*H. influenzae* or *S. pneumoniae*), it was difficult to determine whether it had an independent pathogenic role [111]. In a study of 50 patients with COPD, the average time from acquisition to clearance of a new strain of *M. catarrhalis* was 1 month and re-infection with the same strain was rare, suggesting that there was an effective immune response. Of the new acquisitions, 47% were associated with an exacerbation [112]. Acquisition of *M. catarrhalis* led to an increase in airway inflammation, characterised by a rise in sputum neutrophil elastase, interleukin (IL)-8, tumour necrosis factor (TNF)-α and a reduction in secretory leukocyte protease inhibitor (SLPI) [113].

Putative virulence factors of *M. catarrhalis* include several outer membrane proteins plus LOS and these affect cell adhesion, epithelial cell invasion, serum resistance and biofilm formation [114]. More work is needed to understand the pathogenesis of infection in both COPD and non-CF bronchiectasis.

More than 90% of *M. catarrhalis* produce a β-lactamase (BRO-1 or BRO-2) and are resistant to ampicillin. Acquired resistance to other antibiotics is rare with most remaining susceptible to macrolides, tetracyclines, amoxicillin-clavulanic acid and quinolones [115].
**Staphylococcus aureus**

*Staphylococcus aureus* is a Gram-positive coccus found in clusters that may be part of the normal flora in the anterior nares, throat and on moist skin sites such as groin and axilla. Infection is characterised by abscess formation, particularly in skin and soft tissues. It is a rare cause of respiratory tract infection, but can cause severe pneumonia after influenza. It is a common cause of early infection in CF but is less common in non-CF bronchiectasis where its presence may indicate undiagnosed CF [10]. There is also an association of *S. aureus* with ABPA in non-CF bronchiectasis [116].

*S. aureus* produces a range of exotoxins that can cause tissue damage. It is also thought to form biofilms on prosthetic devices and thereby evade the host response and resist antimicrobial therapy [117]. Biofilm-like aggregates of *S. aureus* surrounded with the polysaccharide poly-N-acetyl-glucosamine have been observed in anaerobic conditions in CF mucus and can resist nonoxidative killing [118]. Persistence of *S. aureus* in CF and prosthetic infections has also been related to the presence of small colony variants. These tiny colonies are difficult to identify in vitro. They are associated with treatment with trimethoprim/sulphamethoxazole or aminoglycosides, are more antibiotic resistant than the typical forms in the same sputum and may survive within host cells [119].

The ability of *S. aureus* to rapidly adapt and persist in the lung may be a result of genomic instability due to mobilisation of bacteriophages. Isolates from the anterior nares of CF patients had a higher frequency of genomic alterations than those from healthy controls [120]. A higher proportion of hypermutable strains of *S. aureus* were found in CF patients when compared with isolates from bacteraemia or other respiratory infections. As with other species with high mutation rates, many of these had defects in *mutS* [121].

Meticillin resistant *S. aureus* (MRSA) are resistant to all penicillins, cephalosporins and penems and are often also resistant to other classes of antibiotics (macrolides, fluoroquinolones and aminoglycosides). They can be difficult to treat, partly because oral options are limited but also because the active parenteral options (glycopeptides) may be less effective compared with the use of a β-lactam antibiotic to treat a susceptible isolate. It may be difficult to clear MRSA carriage from patients with bronchiectasis, but there is data from CF that shows that a combination of systemic treatment with skin antisepsis and inhaled antibiotics may be effective [122].

**Burkholderia spp. and other non-fermenters**

*Burkholderia spp* are plant pathogens and are a major cause of morbidity and mortality in CF but are rarely encountered in other conditions. In spite of the frequent presence of these bacteria in the environment, and their propensity for spread between CF patients, there are only two case reports of infection in non-CF bronchiectasis, one with *Burkholderia cepacia* complex (not speciated) and another with *Burkholderia gladioli* [123, 124].

A wide variety of other nonfermentative Gram-negative bacilli can occasionally act as opportunistic pathogens in the human lung. Species of the genera *Achromobacter*, *Stenotrophomonas*, *Ralstonia*, *Pandoraea* and *Inquilinus* can cause infection in the CF lung, and *S. maltophilia* and *Achromobacter* (previously *Alkaligenes*) *xylosoxidans* have been reported in non-CF bronchiectasis (table 1). Many are both intrinsically resistant to some antibiotics and easily acquire resistance. They can be difficult to identify in the laboratory and molecular methods are recommended to ensure accurate identification [101]. In particular it is important to differentiate these organisms from the *Burkholderia spp*. because of the need to prevent cross infection. There is too little experience with these microorganisms to comment on their propensity for colonisation, infection, or role in exacerbation of non-CF bronchiectasis.
Anaerobes and other bacteria considered normal upper respiratory tract flora

Sputum may contain microorganisms other than the PPM. These have been considered either contaminants from the upper respiratory tract or harmless commensals colonising the sputum. This assumption has been challenged following studies using both conventional culture methods and culture-independent techniques.

Sputum is not routinely cultured for anaerobes partly because they are present in large numbers in saliva and can easily contaminate expectorated sputum, but also because some are very difficult to culture. Following the observation of a rapid drop in oxygen partial pressure just below the surface of a CF sputum plug, investigators began to look for anaerobes in the sputum from CF and non-CF bronchiectasis [125]. Obligate anaerobes in particular *Prevotella spp.* were found in significant numbers, far more than would be expected from oral contamination [126–128]. Their significance in disease has been questioned as high numbers of bacteria were found in a stable CF patient in one study, and the numbers of anaerobes remained constant during successful treatment of a clinical exacerbation [129].

It has been proposed that members of the *Streptococcus milleri* group may have a role in chronic lung infection. One study followed the changes in the microbial flora during and between pulmonary exacerbations of CF using both culture and culture-independent methods. The group identified members of the *S. milleri* group as of potential importance in exacerbations both in CF and in two patients with non-CF bronchiectasis [130].

Following an observation that a range of upper respiratory tract flora were seen in large numbers in sputum from CF patients, a *Staphylococcus sp.* (not *S. aureus*) and a viridans-type *Streptococcus sp.* were further studied. While not intrinsically pathogenic, they were able to enhance the virulence of *P. aeruginosa* in an animal model and increase the expression of certain virulence genes of *P. aeruginosa in vitro*. This could be reproduced using an inter-species QS molecule, Auto Inducer-2 (AI-2) [131]. Of interest, the oral anaerobe *Prevotella* also produces AI-2 [127]. The complex pattern of interaction between microorganisms in ecosystems other than the lung has been described and it is known that microorganisms can enhance or inhibit growth of other cohabitants [132]. The studies in CF show that interactions may also enhance pathogenicity [133].

The CF lung, therefore, may contain a mixture of microorganisms that includes those that are directly pathogenic, those that behave as commensals and those that are not directly pathogenic but may increase the virulence of other organisms. Although this has not been studied in non-CF bronchiectasis, microorganisms other than PPMs are regularly observed in sputum cultures in combination with PPMs and further work on these potential interactions is needed.

Culture-independent studies of microbial flora in the lung

There have been attempts to describe the composition and diversity of the microbes in the lung irrespective of the ability to culture individual microorganisms. One approach is to analyse the gene encoding 16S rRNA. This is present in all true bacteria and the sequence variation is sufficient to identify most genera and many species. Genetic material can be extracted from a clinical sample, and the 16S rRNA gene amplified by PCR. The product may be analysed looking at terminal restriction fragment length polymorphisms (TRFLP). This compares the size of the terminal fragment of rRNA after cutting with a restriction enzyme; the length of the fragment being characteristic of certain species. Alternately the PCR product can be cloned, sequenced and compared with databases containing sequence data from a wide range of microorganisms. These methods and other variations have been applied to patients with CF and non-CF bronchiectasis to describe the diversity of microorganisms, and have revealed species not previously found in respiratory samples using traditional culture methods [12, 134–136]. While the presence of nucleic acid does not necessarily indicate the presence of viable organisms, a comparison of RT-TRFLP
with TRFLP showed that a high proportion of the bacterial species detected in CF sputum were metabolically active [137].

There have been major technical advances facilitated by the development of next generation sequencers plus developments in bioinformatics. These have allowed direct analysis of amplified DNA without the cloning step, and greater depth of sequencing of 16S rRNA DNA [138, 139]. An alternative approach is to attempt whole genome sequencing directly from the clinical sample [140]. This could include analysis of nucleic acid from eukaryotes and viruses in sputum as well as bacteria [141]. Such techniques can provide an enormous amount of information that is a great challenge to process. However, they offer the potential for a far more sophisticated analysis of the genetic variability found in single species and the variety of microorganisms in chronic lung infection.

**Respiratory viruses**

The role of viruses in non-CF bronchiectasis is not known and remains an important area for future research. Some data exists that viral infections in childhood may predispose to the development of bronchiectasis in later life [142], whether it is through the development of bronchiolitis, disruption of small airway associated innate/adaptive immunity, damage of airway epithelia or compromise of mucociliary clearance, it is unclear.

What is also unclear is the role that viral infection plays in triggering infective exacerbations and progressive lung damage in patients with non-CF bronchiectasis where no studies have to date been carried out. Therefore, only cautiously can parallels be drawn from studies examining the role of viruses in asthma, COPD and CF.

**Viral infections in asthma, COPD and CF**

Viral exacerbation of asthma has been well published. In a study by Johnston et al [143] using PCR and viral culture, viruses were detected in 80% of episodes of wheeze or reduced peak expiratory flow in children aged 9–11 years with asthma. Rhinovirus accounted for 61% of the viruses detected, coronavirus 16%, influenza 9%, parainfluenza 9% and respiratory syncytial virus (RSV) 5%. Similarly, Nicholson et al. [144] found that respiratory viruses accounted for 44% of asthma exacerbations in adults. Respiratory viruses were also present in most patients hospitalised for life-threatening asthma and acute not life-threatening asthma [145].

The application of molecular diagnostic methods has improved the understanding of viral epidemiology. Respiratory viruses may induce asthma exacerbations via direct effects on the airway epithelium as well as through a systemic immune reaction.

Rhinovirus is the most common respiratory virus and represents two-thirds of all upper respiratory tract infections. It also accounts for 50% of asthma exacerbations in children [146]. Traditionally, rhinovirus is thought to infect the upper respiratory epithelium. However, rhinovirus is also capable of replicating in the lower airway cells during experimental infection [147]. Papadopoulos et al. [148] showed that both rhinovirus genomic material and replicative strand RNA were detectable in bronchial biopsies using in situ hybridisation in 50% of adult volunteers subjected to an experimental rhinovirus upper respiratory infection.

The mechanism by which viruses cause bronchoconstriction is not fully understood, but it is likely to involve cytokine production in response to viral replication in the lower airways, which includes upregulating the expression of a range of proinflammatory mediators. The proinflammatory cytokine IL-1β is detectable in experimental infected individuals. IL-8, a key mediator in neutrophil-mediated acute inflammation, is also detected in naturally occurring infections correlating with neutrophilia in blood and nasal samples in children with virally precipitated asthma or experimental infection [149]. Other mediators induced by rhinovirus infections include neutrophil-activating peptide (which induces neutrophil migration), eotaxin and RANTES.
RSV was detected in only 5% of asthma episodes in the study by JOHNSTON [143]. However, it is known to be a potent cause of wheezing, particularly in infancy. It has been shown that G-glycoprotein of RSV appears to stimulate T-helper cell (Th) type 2 immune response in the upper airway, whether or not if the infant is atopic [150]. Th2 cytokine patterns are known to be associated with viral immunopathology and allergic-type responses, in contrast to Th1 cytokine patterns, which are classically associated with viral elimination. Interestingly, the nasal cytokine responses to other viruses are of the predominant Th1 type (except RSV). This could explain the tendency for RSV to cause wheezing, but not the association between other respiratory viruses and wheezing.

Influenza A infection induces large amounts of intrapulmonary IFN-\(\gamma\) and enhances both later allergen specific asthma and dual Th1/Th2 responses [151]. TERAN et al. [152] also demonstrated that the eosinophil product, major basic protein (MBP), and RANTES increased with viral infections, and there was a correlation in the concentration of RANTES with clinical symptoms. In addition, epithelial cells infected with influenza in vitro were associated with an increase in eotaxin [153]. Eotaxin can in turn lead to an exaggerated inflammatory response by binding to eosinophils, T-cells and basophils. These are all key factors in asthma exacerbation.

Patients with asthma are no more susceptible to upper respiratory tract rhinovirus infections than healthy people, but suffer from more severe consequences of the lower respiratory tract infection. Recent epidemiological studies suggest that viruses provoke asthma attacks by additive or synergistic interactions with allergen exposure or with air pollution. An impaired antiviral immunity to a rhinovirus may lead to impaired viral clearance and hence prolonged symptoms. Indirect prevention strategies focus on the reduction of overall airway inflammation to reduce the severity of the host response to respiratory viral infections. There is a lack of specific antiviral strategies in the prevention or reduction of viral-triggered asthma exacerbations. Recent advances in the understanding of the epidemiology and immunopathogenesis of respiratory viral infections in asthma may provide opportunities or the identification of specific targets for antiviral agents and strategies for management and prevention.

COPD is the fourth leading cause of mortality worldwide and is an important cause of global burden of disease [154]. The disease is associated with intermittent exacerbations characterised by acute deterioration in symptoms, lung function, and quality of life [155, 156]. Exacerbations have major effects on health status and are associated with considerable morbidity and mortality that can lead to hospital admission with high treatment costs [157].

Infectious agents are recognised as a major pathogenic factor in exacerbations. Bacteria have a role in the pathogenesis [158, 159] and the exacerbations of COPD. However, bacteria are absent in about 50% of exacerbations and the frequency of isolation does not increase during exacerbation [160].

Early studies looking at respiratory viruses and COPD have stated a 20% detection rate in COPD exacerbations [161, 162]. However, these studies were limited by using less sensitive methods in viral detection. SEEMUNGAL et al. [163] detected respiratory viruses from nasal samples and blood of patients with COPD using a combination of culture, serology and PCR. They showed that 64% of COPD exacerbations were associated with a cold occurring up to 18 days before exacerbation. In total, there were 168 episodes of COPD exacerbation in 53 patients and 77 viruses (39 were rhinoviruses) were detected. Viral exacerbations were associated with frequent exacerbations, increased symptoms, a longer median symptom recovery period (up to 13 days) and a tendency towards higher plasma fibrinogen and serum IL-6 levels. RSV has also been shown to be an important virus in COPD exacerbations and was detectable in 11.4% of patients admitted into hospital [164]. Patients with stable COPD may carry respiratory viruses. Non-RSV respiratory viruses were detected in 11 (16%), and RSV in 16 (23.5%) out of 68 stable COPD patients, with RSV detection being associated with higher inflammatory marker levels [161, 164].
Early studies looking at respiratory viruses in CF relied on repeated serological testing, either alone [165], or in combination with viral cultures for viral detection [166–170]. These methods are relatively insensitive and more recent studies have utilised molecular based methodologies [171–175]. All these studies produced different results in terms of prevalence of respiratory viruses in CF, these differences could be due to the different methodologies utilised. It is also likely that there are differences in the populations studied, as the prognosis for CF has improved with each successive birth cohort.

It has now been 25 years since Wang et al. [169] described the relationship between respiratory viral infections and the deterioration in clinical status in CF patients. Viruses were identified through repeated serology and nasal lavages for viral isolation in 49 patients with CF (mean age 13.7 years) over a 2-year period. Although the CF patients had more respiratory illnesses than the sibling controls (3.7 per year versus 1.7 per year), there were no differences in virus identification rates (1.7 per year). The rate of proven virus infection was significantly correlated with the decline in forced vital capacity (FVC) and FEV1, Shwachman score, and frequency and duration of hospitalisation.

More recent studies suggest no difference in the frequency of either upper respiratory tract illness episodes [166] or proven respiratory viral infections [168] between children with CF and healthy controls; however, children with CF have significantly more episodes of lower airway symptoms than controls [166, 168]. Ramsey et al. [168] prospectively compared the incidence and effect of viral infections on pulmonary function and clinical scores in 15 school children with CF aged 5–21 years and their unaffected siblings. Over a 2-year period, samples were taken at regular 2-month intervals and during acute respiratory illnesses for pharyngeal culture and serology for respiratory viruses. There were a total of 68 acute respiratory illness (ARI) episodes that occurred in the patients with CF, in 19 of these episodes an associated virus identified. A total of 49 infective agents were identified either during ARIs or at routine testing in the patients with CF; 14 were identified on viral isolation (rhinovirus on 11 occasions), whilst 35 were isolated on seroconversion (parainfluenza virus on 12, RSV on nine and Mycoplasma pneumoniae on six occasions). There was no significant difference in the rate of viral infections between the patients with CF and their sibling controls, as measured either by culture or serology. The rate of viral infections was higher in younger children (both CF and controls), and the rate of decline in pulmonary function was greater in the younger children with CF with more viral infections. At the time of an ARI, the virus isolation and seroconversion (four-fold increase in titres) rates were 8.8% and 19.1%, respectively, in children with CF compared with 15% and 15%, respectively, for the siblings not affected. In contrast the rates for virus isolation and seroconversion at routine 2 monthly visits were 5.6% and 16.2%, respectively, for children with CF and 7.7% and 20.2%, respectively, for the siblings not affected.

Similarly Hiatt et al. [166] assessed respiratory viral infections over three winters in 22 infants less than 2 years of age with CF (30 patient seasons) and 27 age matched controls (28 patient seasons). The average number of acute respiratory illness per winter was the same in the control and the CF groups (5.0 versus 5.0). However, only four of the 28 control infants had lower respiratory tract symptoms in association with the respiratory tract illness, compared with 13 out of the 30 infants with CF (OR -4.6, 95% CI 1.3–16.5; p-value <0.05). Seven of the infants with CF cultured RSV, of whom three required hospitalisation. In contrast, none of the controls required hospitalisation. Pulmonary function measured by rapid chest compression technique was significantly reduced in the infants with CF after the winter months and was associated with two interactions; RSV infection with lower respiratory tract infection and male sex with lower respiratory tract infection.

From previous reports, two viral agents appear to have the greatest effect on respiratory status in CF, namely RSV and influenza, possibly because the uses of viral culture and serology have underestimated the effects of rhinovirus (due to the vast amount of serotypes). In younger children, RSV is a major pathogen resulting in an increased rate of subsequent hospitalisation. Abman et al. [176] prospectively followed up 48 children with CF diagnosed through newborn
screening and documented the effect of RSV infection. 18 of the infants were admitted into hospital a total of 30 times over a mean follow-up period of 28 months (range 5–59 years). In seven of these infants RSV was isolated, and their clinical course was severe with three requiring mechanical ventilation and five necessitating chronic oxygen therapy. Over the next 2 years these infants had significantly more frequent respiratory symptoms and lower chest radiograph scores than non-RSV identified infants. In another prospective study of repeated BAL in 80 infants identified through CF newborn screening over a 5-year period, 31 infants were hospitalised for a respiratory exacerbation, 16 (52%) of which had a respiratory virus identified with the most common being RSV (n=7).

In older children and adults with CF, influenza seems to have the greatest effect. Pribble et al. [167] assessed acute pulmonary exacerbation isolates from 54 patients with CF. Over the year of the study 80 exacerbations were identified, of which 21 episodes were associated with an identified viral agent (influenza A: five episodes; influenza B: four episodes; RSV: three episodes) with most agents identified by serology. Compared with other agents, infection with influenza was associated with a more significant drop in pulmonary function (FEV1 decreased by 26% compared with 6%, respectively). A retrospective study in older patients with chronic P. aeruginosa infection reported an acute deterioration in clinical status in association with influenza A virus infection [177].

Collinson et al. [171] followed 48 children with CF over a 15-month period using a combination of viral culture and PCR for picornaviruses alone [178]. 38 children completed the study and there were 147 symptomatic upper respiratory tract infections (2.7 episodes per child per year), with samples available for 119 episodes. Picornaviruses were identified in 51 (43%) of these episodes, of which 21 (18%) were rhinoviruses. In those children old enough to perform spirometry there were significant drops in both FVC and FEV1 in association with upper respiratory tract infection, with little difference in the severity of drop whether a picornavirus was identified or not. Maximal mean drop in FEV1 was 16.5%, at 1–4 days after onset of symptoms, but a deficit of 10.3% persisted at 21–24 days. Those with more upper respiratory tract infections appeared to have a greater change in total Shwachman and Crispin–Norman scores over the study. Six children isolated a P. aeruginosa for the first time during the study, five at the time of a upper respiratory tract infection and only one was asymptomatic at the time of first isolation. The data from this study has to be handled with care as the term “upper respiratory tract illness (URTI)” did not necessarily imply a positive viral isolation.

Punch et al. [173] used a multiplex RT-PCR assay combined with an enzyme-linked amplicon hybridisation assay (ELAHA) for the identification of seven common respiratory viruses in the sputum of 38 CF patients. 53 sputum samples were collected over two seasons and 12 (23%) samples from 12 patients were positive for a respiratory virus (influenza B n=4, parainfluenza 1 n=3, influenza A n=3, RSV n=2). There were no statistical associations between virus status and demographics, clinical variables or isolation rates for P. aeruginosa, S. aureus or Aspergillus fumigatus.

Olesen et al. [174] obtained sputum/laryngeal aspirated from children with CF over a 12-month period in outpatient clinics. They achieved a viral detection rate of 16%, with rhinovirus being the most prevalent virus. However, this virus did not seem to have any devastating impact on lung function. However, the other viruses detected were associated with significant reduction in lung function. The authors failed to show a positive correlation between respiratory viruses and bacterial infections in their studied population, as the type or frequency of bacterial infection during or after viral infections were not altered. They also demonstrated that clinical viral symptoms had a very poor predictive value (0.39) for a positive viral test.

Wat et al. [179] utilised “real-time” nucleic acid sequence-based amplification (NASBA) to examine the role of respiratory viruses in CF. They achieved a rate of 46% for respiratory viruses in their paediatric CF cohort during reported episodes of respiratory illness. The results compare favourably with previous studies, this may be due to earlier studies relying heavily on repeated serological testing either alone [165] or in combination with viral isolation [166–170]. These traditional
methods are relatively insensitive and once again may have underestimated the prevalence of viruses in CF.

Detection of respiratory viruses

The principal laboratory methods utilised for the diagnosis of respiratory viruses, rely upon the detection of the virus in respiratory secretions and therefore an important factor in respiratory viral diagnosis is the necessity for the submission of an appropriate sample for testing. Inadequate or improper specimen collection and transport account for the largest source of error in the accuracy of viral detection results [180]. Nasal swabs, nasopharyngeal aspirates, nasal wash and sputum specimens are generally considered as the specimens of choice for the detection of respiratory viruses [173, 180–183]. A prospective study by Heikkinen et al. [184] showed that the sensitivity of nasal swabs was comparable to nasopharyngeal aspirates for the detection of all major respiratory viruses by tissue culture, with the exception of RSV.

Molecular techniques have superseded many “conventional” methods utilised for respiratory viral detection, such as viral culture and serology analysis, due to the rapid turn-around time for the results. Molecular assays have particular advantages where the starting material available is acellular (swab) or where surveillance samples have a low copy number of the viral target. The rapid turn-around time of results allows diagnostic virology to have an impact on patient management, thereby avoiding prescribing the inappropriate use of antibiotics and allowing the correct prescription for anti-virals. It may also play an important role in infection control in the hospital setting.

Interaction between bacteria and viruses

There is very little known about the interaction between respiratory viruses and bacteria in non-CF bronchiectasis but a number of publications suggest that respiratory viruses may precipitate secondary bacterial infection in CF. In a 25-year retrospective review from the Danish CF clinic, the most likely first isolation of *P. aeruginosa* was found to be occur between October and March [185], coinciding with the peak of the RSV season. This observation implies a causal relationship between respiratory viral and bacterial infection.

The first bacterial isolation of a given organism in CF has also been shown to often follow a viral infection. In the 17-month prospective study reported by Collinson et al. [171], five of the six first isolations of *P. aeruginosa* were made during the symptomatic phase of an upper respiratory tract infection or 3 weeks thereafter. In contrast only one of the six initial infections with *P. aeruginosa* was identified during the asymptomatic period. Similarly, *H. influenzae* was recovered for the first time from three children within 3 weeks of an upper respiratory tract infection and the one new *S. aureus* infection was identified immediately following a viral infection.

Armstrong et al. [170] have reported that 50% of CF respiratory exacerbations requiring hospitalisation are associated with the isolation of a respiratory virus. In their prospective study of repeated BAL in infants over a 5-year period, a respiratory virus was identified in 52% of the infants hospitalised for a respiratory exacerbation, most commonly RSV. 11 of the 31 hospitalised infants (35%) acquired *P. aeruginosa* in the subsequent 12–60-month follow-up period, compared with three out of 49 (6%) non-hospitalised infants (relative risk 5.8).

Respiratory viruses can disrupt the airway epithelium and precipitate bacterial adherence. For example influenza A infection results in epithelial shedding to basement membrane with submucosal oedema and neutrophil infiltrate [186], while both influenza and adenovirus have a cytopathic effect on cultured nasal epithelium leading to the destruction of the cell monolayer [187]. This epithelial damage results in an increase in the permeability of the mucosal layer [188, 189], possibly facilitating the bacterial adherence. Bacteria can also utilise viral glycoproteins and other virus-induced receptors on host cell membranes as bacterial receptors, in order to adhere to virus infected cells [190, 191].
The lower respiratory tract is protected by local mucociliary mechanisms that involve the integration of the ciliated epithelium, periciliary fluid and mucus. Mucus acts as a physical and chemical barrier onto which particles and organisms adhere. Cilia lining the respiratory tract propel the overlying mucus to the oropharynx where it is either swallowed or expectorated. Influenza viral infection has been shown to lead to the loss of cilial beat, and shedding of the columnar epithelial cells generally within 48 hours of infection [192]. Pittet et al. [193] showed that a prior influenza infection of tracheal cells in vivo does not increase the initial number of pneumococci found during the first hour of infection, but it does significantly reduce mucociliary velocity, and thereby reduces pneumococcal clearance during the first 2 hours after pneumococcal infection at both 3 and 6 days after an influenza infection. The defects in pneumococcal clearance were greatest 6 days after an influenza infection. Changes to the tracheal epithelium induced by influenza virus may increase susceptibility to a secondary *S. pneumoniae* infection by increasing pneumococcal adherence to the tracheal epithelium and/or decreasing the clearance of *S. pneumoniae via* the mucociliary escalator of the trachea, and thus increasing the risk of secondary bacterial infection.

De Vrankrijker et al. [194] showed that mice that were co-infected with RSV and *P. aeruginosa* had a 2,000 times higher CFU count of *P. aeruginosa* in the lung homogenates compared with mice that were infected with *P. aeruginosa* alone. Co-infected mice also had more severe lung function changes. These results suggest that RSV can facilitate the initiation of acute *P. aeruginosa* infection.

RSV has also been shown to increase adherence of NTHi and *S. pneumoniae* to human respiratory epithelial cells in vitro [195]. This increase adherence could be explained by an upregulation of cell surface receptors for bacteria, such as intercellular adhesion molecule-1 (ICAM-1), carcinoembryonic adhesion molecule 1 (CEACAM1) and platelet activating factor receptor (PAF). Another study also showed that NTHi and *S. pneumoniae* bind to both free RSV virions and epithelial cells transfected with cell membrane-bound G protein, but not to secreted G protein. Pre-incubation with specific anti-G antibody significantly reduce bacterial adhesion to G protein-transfected cells [196].

Stark et al. [197] showed that mice that were exposed to RSV had significantly decreased *S. pneumoniae*, *S. aureus* or *P. aeruginosa* clearance between 1 to 7 days after RSV exposure. Mice that were exposed to both RSV and bacteria had a higher production of neutrophils induced peroxide, but less production of myeloperoxidase compared with mice that were exposed to *S. pneumoniae* alone. This suggests that functional changes in the recruited neutrophils may contribute to the decreased bacterial clearance.

More recently, Chatteraj et al. [198] demonstrated that acute infection of primary CF airway epithelial cells with rhinovirus liberates planktonic bacteria from biofilm. Planktonic bacteria, which are more proinflammatory than their biofilm counterparts, stimulate increased chemokine responses in CF airway epithelial cells which, in turn, may contribute to the pathogenesis of CF exacerbations.

Collectively, these findings suggest that respiratory viruses may lead to epithelial disruption, destruction of mucociliary escalator, increased cytokine production, neutrophil influx and increased neutrophil induced peroxide release, indirectly facilitating bacterial infection of the airway. Whether these are the mechanisms for infective exacerbations in the context of non-CF bronchiectasis remains to be seen.

### Prevention and treatment of infection with respiratory viruses

Influenza associated death is between 13,000 to 20,000 incidents per year throughout the winter months in the UK [199], though some of the deaths may be attributed to RSV. Influenza vaccines are the only commercially available vaccines against respiratory viruses. Recent vaccines contain antigens of two influenza A subtypes, strains of the currently circulating H3N2 and H1N1 (Swine flu) subtypes, and one influenza B virus. The waning of vaccine-induced immunity over time requires annual re-immunisation even if the vaccine antigens are unchanged. Influenza vaccination...
is recommended to those with chronic respiratory diseases including non-CF bronchiectasis. Despite this recommendation, there is neither evidence for, nor against, routine annual influenza vaccination for children and adults with non-CF bronchiectasis from a recent Cochrane review [200].

Although there is no licensed RSV vaccine to date, prophylaxis using a humanised mouse monoclonal antibody, Palizivumab, which has been shown to reduce the rate of RSV associated hospitalisation in premature infants [201].

Amantadine has been the conventional anti-viral against of influenza. Its mode of action involves interfering with viral protein M2, thereby inhibiting the replication of influenza viruses by interfering with the uncoating of the virus inside the cell. However, it is strain specific as it is only effective against influenza A and has common side-effects such as insomnia, poor concentration and irritability. Amantadine has now been almost completely replaced by neuraminidase inhibitors (NI), except for some NI-resistant influenza.

**Neuraminidase inhibitors**

NIs such as Zanamivir and Oseltamivir are licensed for the treatment of influenza A and B, avian flu (H5N1) and Swine flu (H1N1). They work by inhibiting the function of the viral neuraminidase protein, thus preventing the release of the progeny influenza virus from infected host cells, a process that prevents infection of new host cells and thereby halts the spread of the infection in the respiratory. Early initiation of these therapies within 48 hours from the onset of symptoms can reduce the duration of common cold symptoms by 1–2 days [202, 203]. Zanamivir has a poor oral bioavailability and intranasal application has been shown to be effective in treating experimental influenza infection, by the reduction in symptoms caused, virus shedding and the development of otitis media [204]. A phase III study is currently underway that looks at the efficacy of intravenous Zanamivir preparation. However, compassionate use of i.v. Zanamivir could be considered to treat critically ill adults and children having a life-threatening condition, due to suspected or confirmed pandemic Influenza A (H1N1) infection or infection due to seasonal Influenza A or B virus, who are not responding to oral or inhaled neuraminidase inhibitors. A recent systematic review meta-analysis showed that neuraminidase inhibitors only have modest effectiveness (Oseltamivir and Zanamivir 61 and 62%, respectively) against flu-like symptoms in previously healthy subjects [205].

**Ribavarin**

Ribavarin, a synthetic guanosine nucleoside that has a broad spectrum of antiviral activity, is approved treatment for lower respiratory tract disease caused by RSV [206]. It can be incorporated into RNA as a base analog of either adenine or guanine, it pairs equally well with either uracil or cytosine, inducing mutations in RNA-dependent replication in RNA viruses. Controlled studies also show that the use of ribavarin is effective in reducing the clinical severity score, duration of mechanical ventilation, supplemental oxygen use and days of hospitalisation [207].

**Macrolides**

Although rhinovirus is the major cause of colds, its vast amount of serotypes has made development of anti-virals against it problematic. 90% of rhinovirus serotypes gain entry into epithelial cells using ICAM-1 cellular receptors and blockade of these receptors in experimental studies have shown reduced infection severity [208]. Macrolide antibiotics, bifilomycin A1 and erythromycin, have been shown to inhibit ICAM-1 epithelial expression and hypothesis about their potential as anti-virals have yet to be proven, more clinical proof is required [209].

**Other anti-virals**

Recently there has been a report regarding the use of an anti-rhinoviral agent known as Plecoranil. This anti-viral binds to a hydrophobic pocket of the VP1, the major shell protein for the
rhinoviruses, thereby preventing the virus from exposing its RNA and also prevents the virus from attaching itself to the host cell [210]. The rhinovirus 3C protease inhibitors, Rupintrivir [211] and soluble recombinant ICAM-1 Tremacamra [212], have shown promising results but they are currently not widely available.

Conclusions

The role of bacteria and viruses in non-CF bronchiectasis is not presently fully understood. Through necessity, evidence from studies in CF and COPD is used and applied to bronchiectasis. More research using both conventional microbiological techniques as well as newer molecular diagnostic approaches, is urgently required to address a number of important questions in non-CF bronchiectasis. 1) What is the cause of infective exacerbations? 2) What is the role of anaerobic bacteria and how do normal commensal bacteria interact with pathogenic bacteria? 3) How can we clear chronic infection? 4) What proportion of exacerbations is triggered by viral infection? 5) How do viruses influence bacterial behaviour in chronically infected airways?

A greater understanding of bacterial communal behaviour and their interaction with epithelial cells and viruses will be critical in further developments in the management of non-CF bronchiectasis.

Statement of interest

J.E. Foweraker received a consultancy fee from Novartis Pharma AG for advice on a submission to the European Medicines Agency for licensing of Tobramycin inhaled powder and a consultancy fee from Gilead Sciences International Ltd for advice on an application to European Medicines Agency for licensing of Aztreonam lysine.

References


