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Recent advances in the microbiology of respiratory tract infection in cystic fibrosis

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Introduction: Infection is a major cause of morbidity and mortality in patients with cystic fibrosis (CF). Research on CF infection has highlighted differences from other respiratory infections—both in the range and the nature of the organisms—especially in chronic infection. This is a rapidly advancing field of microbiology and is bringing insights into the complexity and adaptations of bacteria causing chronic infection in the respiratory tract.

Areas of agreement and controversy: The epidemiology of some infections in CF has changed, with reduction in spread of *Burkholderia cenocepacia* following patient segregation. Conversely, epidemic strains of *Pseudomonas aeruginosa* have emerged, which spread between patients; previously, most *P. aeruginosa* strains were patient-specific. Studies on hypermutators, quorum sensing, biofilm growth and the development of molecular identification have shed light on pathogenicity, microbial adaptation to the host and complexity of infection in CF. Non-tuberculous mycobacteria are emerging pathogens in CF; however, there is much to learn about pathogenicity and treatment of these infections. Species of aerobic and anaerobic bacteria, more commonly encountered in the upper tract, are found in significant numbers in CF sputum. The significance of this is however under debate. Finally, although the clinical relevance of conventional antibiotic susceptibility testing for chronic CF pathogens has been questioned, there are no clear alternatives.

Emerging areas for developing research: Much has been learnt about pathogenicity, evolution of CF pathogens and development of antibiotic resistance. The need is to focus on clinical relevance of these observations to improve diagnosis, prevention and treatment of CF infection.

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Introduction to infection in cystic fibrosis

Cystic fibrosis (CF) is an inherited disorder due to a range of mutations in the CF gene affecting the cAMP-regulated chloride channel (CF transmembrane regulator or CFTR). In the lungs, this leads to dehydration of the peri-ciliary layer such that the mucociliary escalator cannot perform its function of clearing particles (including micro-organisms) from the airways. Patients develop acute and later chronic respiratory infection leading to a cycle of further damage and less ability to clear infection. The range of micro-organisms causing infection differs from those in patients without CF with the commonest pathogens being *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[1,2] Antibiotics are used to clear early infection, treat acute exacerbations of chronic infection and reduce the frequency of exacerbations (by use of long-term systemic or inhaled drugs). These treatments have had a major impact on the quality and survival of patients with CF. There are still however questions about the best way to diagnose infection and the use of antibiotics in patients who will need repeated courses during their lifetime. Bacteria in chronic infection have different characteristics from those causing acute infection, including atypical phenotypes leading to difficulty in identifying some species in the laboratory. Antibiotic resistance—both from infection with innately resistant bacteria and also the development of multi-resistant strains—is an increasing challenge to CF. Although infection remains the main cause of morbidity and mortality,[3] recent advances in understanding the role of different species and modes of pathogenicity hold much promise for improved treatments.

This review will focus on recent advances in CF microbiology, look at their relevance to the diagnosis and management of infection and highlight areas that need further development. Specific antibiotic treatment strategies or practical guidelines for the diagnosis of infection will not be discussed here but are well reviewed elsewhere.[4,5]

Staphylococcus aureus

Staphylococcus aureus is the most frequent pathogen in young infants with CF, but an uncommon cause of lower respiratory tract infection in patients without CF. It has been found in broncho-alveolar lavage (BAL) fluid in 30% of CF children of average age of 3 months[6] and often persists despite treatment.[7] The use of anti-staphylococcal antibiotics has been clinically effective but the role of long-term prophylaxis for *S. aureus* is uncertain.[8] The small-colony variant (SCV)

phenotype of *S. aureus* is common in CF patients.[7] These bacteria have adaptations including antibiotic resistance and the ability to survive inside host cells, which may contribute to persistence in the CF airways. The colonies are very small, non-pigmented and are slow growing, so may be missed during routine culture, but the use of certain media may make them easier to recognize. The slow growth makes identification and susceptibility testing in automated systems unreliable.

Longitudinal studies have shown a significantly higher rate of genome alterations in persisting clones of *S. aureus* in CF sputum than those in the nose of healthy individuals, presumably in response to the selective pressures of the host response and repeated use of antibiotics in CF.[9] Potential mechanisms for genome alteration are bacteriophage mobilization and hypermutability. Hypermutator bacteria have mutations in DNA repair or error avoidance genes such that the spontaneous mutation rate is raised. Mutator strains of *S. aureus* have been identified in CF, some due to inactivation of a DNA mismatch repair gene *mutS*.[10] Another mechanism that may help the long-term survival of *S. aureus* in the CF lung is the ability to form biofilms. This may be important for infection of medical devices and prostheses such as artificial heart valves and orthopaedic implants. The role of biofilms in CF is however unclear and needs further investigation.

The increase in methicillin-resistant *S. aureus* (MRSA) in the general population has been a cause of concern in many countries. This is not because these organisms are necessarily more pathogenic, rather than treatment options are limited. The mechanism of resistance to methicillin confers resistance to all β-lactams (penicillins and cephalosporins) and many strains of MRSA have also acquired resistance to unrelated antibiotics, such as quinolones and aminoglycosides. Major attempts are being made to control the spread of resistant *S. aureus*,[11] with some success in the UK. However, the prevalence of MRSA in CF patients is increasing[12] and vancomycin-resistant *S. aureus*, although very rare, are being seen in non-CF patients. Treatment alternatives to the glycopeptides are now available, but experience with them in CF is limited. Several clearance regimens have been proposed, some surprisingly successful (up to 94%).[13]

Haemophilus influenzae

Non-encapsulate and non-type B capsulate *Haemophilus influenzae* are more common in infants and young children with CF than in older patients. There is little information on the epidemiology of long-term infection with *H. influenzae* in CF. A recent study showed that

infection was a dynamic process with most patients sequentially infected with different strains. Repeated isolation of the same clone was only seen in 5 of 27 CF patients.[14] There was an increase in antibiotic resistance over time, with 37% of patients infected with *H. influenzae* resistant to two or more antibiotic classes. Resistance included decreased susceptibility to ciprofloxacin, which is rarely seen outside CF. One-third of patients had hypermutable strains. In another CF study, a hypermutable *H. influenzae* was associated with mutation in *mutS*.[15] Over 11 months, this organism gradually became resistant to the antibiotics used to treat the patient.

Persistence of *H. influenzae* may be linked to ability to form biofilms. This was shown in otitis media in non-CF patients. Structures consistent with biofilms have now been observed in the BAL of asymptomatic CF patients. Clinical concentrations of azithromycin have been shown to inhibit biofilm formation and reduce established biofilms of *H. influenzae in vitro* without affecting bacterial growth.[16]

Streptococcus pneumoniae

Although *Streptococcus pneumoniae* is a common respiratory tract pathogen, it is unusual in CF. Rates of colonization range from 3 to 5% in different series. A recent study showed multi-resistance and possible hypermutators in isolates from CF.[17] *Streptococcus pneumoniae* from CF respiratory samples were better at producing biofilms *in vitro* than blood culture isolates from non-CF patients,[18] which suggests that these organisms have the ability to persist in the CF lung. It is still however unclear whether *S. pneumoniae* acts as a harmless commensal in CF or has a role in acute exacerbation or facilitating infection with other pathogens.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is the most common pathogen cultured from CF sputum. It may be seen early in infancy and is often cultured intermittently during childhood. Eventually, chronic infection develops and this leads to a faster decline in lung function. The age at which infection becomes chronic has increased with better treatment of CF. This is particularly due to the practice of intense treatment with anti-pseudomonal antibiotics when *P. aeruginosa* is first cultured, which can delay the onset of chronic infection and increase life expectancy in CF. The patient may be clinically stable when chronically infected with *P. aeruginosa* but has repeated episodes of acute exacerbation

with increased breathlessness, sputum production and feeling unwell. These exacerbations are also marked by a reduction in lung function. Exacerbations are generally treated with a combination of systemic antibiotics.[5] Inhaled antibiotics, most commonly colistin or tobramycin, can be used to help clear early infection. They can be effective as long-term prophylaxis, reducing frequency of acute exacerbation once infection is chronic. More recently, long-term azithromycin prophylaxis has been used in CF. This followed experience with treating diffuse pan-bronchiolitis, a condition described in Japan that also leads to chronic infection with *P. aeruginosa*. The benefit of long-term oral azithromycin has been shown in CF,[19] with fewer exacerbations, better lung function and a reduction in inflammatory markers. Macrolide antibiotics may act because of their anti-inflammatory activity or by modifying the pathogenicity of *P. aeruginosa* (see below).

Early epidemiological studies showed that cross-infection with *P. aeruginosa* was rare and that CF patients were infected with their own unique strains presumably acquired from the environment. Some siblings did have the same strain but it was unclear if this was due to cross-infection or acquisition from a common source. In contrast, more recent studies revealed the spread of the so-called epidemic strains, some of which were multi-resistant. This would not necessarily be a particular concern except that some, for example, the Liverpool Epidemic Strain (LES), may be more pathogenic and can superinfect patients already colonized with other strains of *P. aeruginosa*. Infected patients had more exacerbations, time in hospital and courses of antibiotics.[20] Furthermore, when patients were first infected with multi-resistant epidemic *P. aeruginosa* early aggressive treatment often failed.[21] This led to a policy in the UK of segregating patients with epidemic strains. The LES genome was recently sequenced and work is progressing to determine the basis of transmissibility and enhanced pathogenicity. Most strains of LES are hyper-producers of pyocyanin. This is a putative pathogenicity factor with effects including reduced beating of respiratory epithelium cilia and induction neutrophil apoptosis, and is regulated by quorum sensing (QS) (*lasA*).[22]

QS systems have been extensively studied in *P. aeruginosa*. Molecular signals allow communication between bacteria.[23] They are secreted and at a certain concentration can trigger expression of genes. In *P. aeruginosa*, these include those that code for pathogenicity factors, biofilm formation and motility. It is thought that 5% of genes in *P. aeruginosa* are regulated by QS and this mechanism allows precise regulation of genes in response to environmental stimuli and cell density. Some plants naturally form antagonists to QS molecules (acylated

homoserine lactones) as a defence mechanism. QS is therefore a potential target for new antimicrobial therapies.

In the laboratory, bacteria isolated from chronically infected patients differ from the usual *P. aeruginosa* cultured from acute infection. Clonal bacteria can form colonies with different appearance referred to as colonial morphotypes. The classical CF isolate is mucoid, due to hyperproduction of alginate. Other morphotypes include bacteria that look like coliforms, non-pigmented forms and slow-growing SCVs.[24] These latter are hydrophobic, have reduced swimming motility and form good biofilms *in vitro*. They may be missed in the routine laboratory because they can take more than 48 h to appear. Phenotypic variation is not restricted to colonial morphology but includes type III secretion, auxotrophy, lipopolysaccharide (LPS) modification (smooth to rough) and both resistance and hypersusceptibility to antibiotics. Variability in antibiotic susceptibility in a population of bacteria from a CF sputum includes differences between bacteria of the same clone and morphotype. As a result, susceptibility testing is poorly reproducible as the result may depend on which bacteria are picked for testing.[25]

The role of antimicrobial susceptibility testing for helping the clinician who chose the antibiotics to treat acute exacerbations in CF is controversial. There are anecdotal reports of poor correlation between antimicrobial susceptibility and treatment of acute exacerbation. Methods to improve the detection of resistant sub-populations have been described.[25,26] However, the only big study to correlate antibiotic resistance to clinical response showed that lung function (forced expiratory volume in one second) improved in patients in spite of treatment with antibiotics to which their *P. aeruginosa* were resistant *in vitro*.[27] Antibiotics seldom if ever eradicate chronic infection with *P. aeruginosa* from the CF lung. Even the resolution of symptoms and signs of an exacerbation may not be matched with a significant reduction in bacterial load. It may be therefore that antibiotics are effective without actually inhibiting or killing the bacteria—the so-called sub-minimum inhibitory concentration (MIC) effect. This may also explain the successful treatment of infection with antibiotics, even though the bacteria are resistant. For example, at sub-MIC concentration, ceftazidime can inhibit attachment to cells and ciprofloxacin can reduce alginate production.[28] Although *P. aeruginosa* is intrinsically resistant, azithromycin may reduce pathogenicity by inhibition of QS and biofilm formation.

Pseudomonas aeruginosa is innately resistant to many antibiotics and acquired resistance is increasing.[29] Synergy testing has been advocated as a way to find combinations of antibiotics effective against multiresistant *P. aeruginosa*. Time-kill curves, chequerboard methods and the multiple combination bactericidal test (MCBT) are the most

commonly used methods and synergy can be shown *in vitro*. Different results can however be obtained with different methods and there is no accepted reference standard.[30] There are few studies that attempt to correlate the results of synergy testing with the clinical outcome. One randomized prospective study compared the MCBT with traditional single antibiotic susceptibility testing for determining treatment of acute exacerbation with a range of multi-resistant species in CF including *P. aeruginosa*, *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans* and *Burkholderia cepacia* complex (Bcc). This showed no better outcome when antibiotics were chosen on the basis of the result of MCBT.[31] The role of current methods of synergy testing is controversial, but there is a definite need for clinically validated *in vitro* tests to guide treatment of multi- and pan-resistant bacteria at acute exacerbation and when patients receive lung transplants.

The rapid development of antibiotic-resistant *P. aeruginosa* may be partly due to the presence of hypermutator strains. These bacteria have defects in their ability to correct mistakes in DNA replication because of a range of mutations, including in the mismatch repair genes such as *mutS*. Strong mutators may have a mutation rate as high as 1×10^{-5} to 10^{-4}, compared with the normal rates of 1×10^{-8} to 10^{-7}. One study showed hypermutators in 37% of CF patients with chronic infection with *P. aeruginosa*.[32] This was the highest prevalence described in a natural system. Since then hypermutators have been shown in other chronic lung diseases.[33] Hypermutator strains were found amongst isolates of *P. aeruginosa* early in infection suggesting that they may have an advantage in initiating infection as well as in chronic infection, however they were infrequent.[34] It was questioned whether mutator strains would be at a disadvantage because of the high rate of damaging spontaneous mutations. However, their ability to rapidly adapt to a changing environment becomes an advantage in an ecological niche repeatedly exposed to antibiotics. In the large population of bacteria in the CF lung, an organism with a mutation for antibiotic resistance may be present even before the antibiotic is given. It has been practice in CF to give two anti-pseudomonal antibiotics to reduce the emergence of resistant strains. The recent observation of hypermutators in CF shows the potential importance of that recommendation.

In common with other bacteria seen in CF, some *P. aeruginosa* form biofilms *in vitro* and can be observed in biofilm-like aggregates in expectorated sputum. Biofilms are communities of bacteria within an acellular matrix, usually attached to a surface—in CF, the respiratory epithelium. In the patient, biofilms may also contain other species of bacteria and host-derived products. Bacteria in biofilms are difficult to eradicate as they can resist the host immune system and are more antibiotic resistant by a variety of mechanisms. Antibiotics may bind to the

extracellular matrix or have reduced activity in areas of poor oxygenation or low pH. Also the bacteria in a biofilm live in a gradient of oxygen, nutrients and metabolites. They are therefore diverse, having adapted to the different micro-environments. The growth phase (actively dividing or dormant) may affect their susceptibility to some antibiotics.[35] The variability in antibiotic susceptibility seen in the population of bacteria cultured from a single sputum under the same *in vitro* conditions may be because bacteria are present in biofilm fragments in the sputum. These organisms may have adapted to a range of different active concentrations of antibiotic encountered in different levels of the biofilm. Concern that laboratory methods developed to measure the antibiotic susceptibility of planktonic bacteria causing acute infections may not be relevant to CF has led to development of methods to test antibiotic susceptibility in a biofilm. Evaluation of one method to determine the optimum antibiotics to clear chronic infection is in progress.[36]

A recent study using whole genome analysis showed an accumulation of mutations in 68 genes of *P. aeruginosa* over 8 years in a patient with CF.[37] This evolutionary divergence was thought to favour adaptation. Early and late isolates of *P. aeruginosa* from 29 patients were compared to look at these genes. One of the commonest mutations was in *las R*, the principle QS regulator in *P. aeruginosa*. This interferes with many pathogenicity factors including biofilm formation. This is difficult to reconcile with the proposed importance of biofilms in persistence of infection. Other genes commonly mutated after chronic colonization are also involved in pathogenicity e.g. *exo A*, the regulator of type III secretion and antibiotic resistance (genes coding for components of multi-drug efflux pumps). The picture that may be emerging is therefore of an organism with a great ability to adapt to its environment that becomes less pathogenic with time, presumably in itself a device for long-term survival in its host. A recent study has shown that antibiotic-resistant genes are not over-represented in hypermutators and that hypermutability may play an important more general role in genome evolution and adaptation in the CF lung.[38]

Pseudomonas aeruginosa and *Burkholderia* spp. (see below) in CF have a range of strategies to enable them to persist in the CF lung, including constantly adapting to a changing environment.[3] Not only are these bacteria difficult to eradicate, but also the phenotypic diversity observed in the laboratory makes the design and validation of new tests of antibiotic susceptibility very difficult. Future research is needed to understand the mechanisms of pathogenicity and persistence, treatment to prevent or clear infection and the development of appropriate laboratory test of susceptibility relevant to clinical outcome.

Burkholderia cepacia complex, *Burkholderia pseudomallei*

Isolates of the species *B. cepacia* (previously *Pseudomonas cepacia*) have been shown to consist of phenotypically similar but genotypically distinct genomovars.[39] Subsequently, the number of genomovars has increased and all have achieved species status. Presently, the so-called Bcc comprises at least 15 species.[40] They are natural soil bacteria, found in the rhizosphere and can be plant symbionts or pathogens. While they can be opportunist pathogens affecting patients who are severely immuno-suppressed or following contamination of fluids or medical devices, they only cause significant primary infection in patients with CF or chronic granulomatous disease (CGD). While patients with CGD have a specific immune defect in the oxidative burst pathway, the reason for the association with CF is still unknown. Patients with non-CF bronchiectasis do not get infected with Bcc. These are complex organisms with a range of pathogenicity factors, many regulated by QS systems, similar to *P. aeruginosa*.[41] *Burkholderia* spp. are resistant to colistin and often multi- or even pan-resistant. Some combinations of antibiotics are effective *in vitro* but there is little evidence to correlate the results with the clinical response to treatment.[31]

The importance of Bcc in CF was first recognized following outbreaks in the 1980s.[42] By the 1990s, the ET12 strain of *Burkholderia cenocepacia* was the most common in the UK and Canada. This was highly transmissible and pathogenic and led to many deaths due to a rapidly progressing pneumonia sometimes with bacteraemia, referred to as the 'cepacia syndrome'. Bcc survives well in natural and health-care environments and some strains most notably the ET12 lineage of *B. cenocepacia* easily spread between patients. Controls to reduce cross-infection including segregating patients have been the main reason that infection and mortality has declined significantly. In the UK, the most common species seen is *Burkholderia multivorans* and most new infections are caused by unrelated clones, suggesting an environmental source rather than person-to-person spread. Infection with Bcc is more common in older CF patients but can occur at any time in the patient's life. Cepacia syndrome can occur when the organism is first acquired or unexpectedly many years after initial infection. It is most frequently due to *B. cenocepacia*; however, both *B. multivorans* and *Burkholderia dolosa* can also cause the 'cepacia syndrome'.[43]

Of the potential pathogenicity factors (LPS, specific cytotoxins and the ability to form biofilms) many are controlled by QS. In addition, Bcc are able to survive in cultured human epithelial cells and macrophages. Severity of disease may be strain rather than species-specific;

however, it may also be dependent on patient factors and interactions with other pathogens. Of note, neither the presence of cable pili nor the presence of *B. cepacia* epidemic strain marker is now considered as a reliable indicator of virulence or transmissibility.[42]

Chronic infection may develop with acute exacerbations and a gradual decline in lung function. While transmissible species can replace other *Burkholderia* spp. causing chronic infection, exacerbations are not associated with acquisition of a new species or strain.[44]

Factors that lead to more severe disease with certain species or strains are still unclear. The relevance of other members of the complex is unclear. Prompt and accurate laboratory identification of Bcc is very important because of the implications for the patient's care and the need to prevent cross-infection. Commercial identification systems are not reliable but the use of selective culture media and molecular identification methods have greatly improved detection.

The epidemiology of *B. pseudomallei* is totally different from Bcc. This is a well-recognized human pathogen that can cause melioidosis with severe pneumonia in individuals who are immuno-competent and do not have the CF gene defect. There are reports of infection in CF patients living in or travelling to endemic areas.[45] Not all infections lead to significant disease. The bacteria may however persist in the CF lung with the risk of later reactivation.

Other Gram-negative bacilli (e.g. *Achromobacter xylosoxidans, Stenotrophomonas maltophilia, Pandoraea apista*)

Some Gram-negative bacilli are either more prevalent in CF now (*S. maltophilia*) or have been recognized more recently due to improvements in laboratory identification (*A. xylosoxidans*, *Pandoraea apista*). These bacteria may have been selected by prolonged use of anti-pseudomonal antibiotics with the increasing life expectancy of CF patients.

Recent studies of *S. maltophilia* support earlier observations that this organism may be of limited clinical significance in CF.[46] Despite increasing culture from CF patients with more severe disease, it is not associated with pulmonary deterioration. It is intrinsically resistant to meropenem and can acquire resistance to many other antibiotics. While co-trimoxazole is the treatment of choice in symptomatic infection, the best management of co-trimoxazole-resistant infections is uncertain and *in vitro* susceptibility testing of other agents with current methods is not reliable enough to be used to determine treatment.

Like *Stenotrophomonas*, *Achromobacter* (previously *Alcaligenes*) *xylosoxidans* may chronically colonize the CF lung but has not been shown to lead to significant deterioration in lung function, but more data are needed.[47]

Pandoraea apista has only recently been identified in CF sputum. As it is intrinsically resistant to colistin, it may also be mis-identified as a *Burkholderia* sp. More information on the clinical relevance of this organism is needed, but cross-infection with clinical deterioration has been described in six patients in a Danish CF centre.[48]

A wide range of other Gram-negative bacteria can be isolated from CF sputum including species of the genera *Ralstonia*, *Chryseobacterium*, *Comamonas*, *Moraxella*, *Acinetobacter*, *Bordetella*, *Inquilinus* and members of the family *Enterobacteriaceae*.[49] There are insufficient data to comment on the clinical relevance of these bacteria and they may be harmless colonizers. It is important however that they are correctly identified. This is so that any association with significant disease may be recognized but also because some may be mistaken for known pathogens. UK reference laboratory reports suggest that around 10% bacteria referred as members of the Bcc were initially mis-identified. A similar mis-identification rate occurs between *A. xylosoxidans* and atypical multi-resistant forms of *P. aeruginosa*. A recent paper highlights the difficulty identifying CF bacteria with atypical phenotypes using traditional methods (colony morphology and biochemical identification) and the authors advocate the use of nucleic acid-based identification methods such as species-specific PCR or ribosomal RNA gene sequencing.[50]

Non-tuberculous mycobacteria

Non-tuberculous mycobacteria (NTM) are being increasingly recognized as a cause of infection in CF. This may be partly because more clinicians are sending samples for NTM culture and laboratories are getting better at isolating NTM. However, there is also thought to be a genuine increase in the prevalence of lung infection with NTM as patients with CF survive longer. Automated liquid systems allow more rapid culture of NTM and may be more sensitive than the traditional use of Lowenstein Jensen agar slopes. The high number of *P. aeruginosa* in CF sputum can however cause problems with decontamination and make NTM culture difficult.[51]

NTM are ubiquitous in the environment and can be found in dust, food and drinking water. CF patients are therefore repeatedly exposed to NTM and it is not surprising that their sputum can be infected. Infection in CF is restricted to the lungs without the disseminated NTM disease found in patients with AIDS.

In a study of 21 USA CF centres, 128 (13%) of 986 CF patients over 10 years old had NTM cultured from at least one of three sputa over 12 months.[52] Of these 25 (2.5%) met the ATS criteria for NTM disease, although the relevance of these criteria for patients with CF has not been assessed. Infection with NTM was more common in older patients and was associated with better lung function and co-infection with *S. aureus* rather than *P. aeruginosa*. There appears to be a geographical influence on the overall prevalence; this ranged from 24% in New Orleans to 7% in Boston. It is uncertain however whether these differences relate to different climate or vegetation or reflect the antibiotic and steroid usage in those CF clinics. Members of the *Mycobacterium avium* complex were most frequently isolated (72% of patients), with *M. avium*, the predominant species. Almost all patients had unique strains with no evidence of cross-infection and 10% had mixed species. *Mycobacterium abscessus* only accounted for 16% of NTM cultured in the US study but has been increasingly reported in CF patients in Europe.[53] This seems to be associated with a more severe infection, and research on pathogenicity is needed. *M. abscessus* colonies can either be rough or smooth depending on the expression of glycopeptidolipid (GPL). It has been proposed that the smooth form (with GPL) forms biofilms *in vitro* and is less pathogenic—favouring colonization whereas the rough form produces little GPL, infects human monocytes and causes persistent invasive infection in a mouse model.[54,55] A similar relationship between colonial morphology and virulence has been proposed for *M. avium* and *M. kansasii*. There have however been no studies to test the clinical relevance of these observations. In common with other bacteria that may persist in biofilms, *M. abscessus* appears less susceptible to antibiotics when grown in biofilms than in planktonic cultures.[56]

The clinical relevance of NTM can be difficult to assess in CF as these patients usually have other pathogens in their sputum. In addition, NTM of different species may be cultured on different occasions such that it can be difficult to distinguish repeated acquisition and loss from the environment from established infection. All isolates therefore need to be identified rather than assuming that repeat positive cultures of *Mycobacterium* spp. indicate persistent infection. Difficulties with identification and changes in taxonomy mean that previous infection attributed to *M. fortuitum* complex or with *M. chelonae* may have been due to *M. abscessus*.

The role of *in vitro* susceptibility testing of *M. abscessus* is in question as correlation with clinical response is poor. Only susceptibility testing to the macrolides (clarithromycin, azithromycin) is recommended for *M. avium* complex.[57]

Areas that need more work include reliable culture and identification of NTM, clinically relevant tests to measure antibiotic susceptibility and more understanding of pathogenicity mechanisms, in particular, for *M. abscessus*. Building on the ATS guidelines, more specific definitions for CF to distinguish transient carriage, colonization and clinical infection are needed for trials of treatment.

Fungal infections

Various species of *Aspergillus*, most commonly *Aspergillus fumigatus*, can be cultured from CF sputum. The fungal spores are common in the air and water, and are usually derived from rotting vegetation. *Aspergillus* may contaminate sputum containers or even laboratory cultures. In CF, it can cause allergic broncho-pulmonary aspergillosis (ABPA). Diagnostic criteria for ABPA in CF have been published[58] and patients are treated with steroids and antifungals. Aspergillomas have also been seen in CF but are rare. A recent paper has described a bronchitis in six patients with infection with *Aspergillus* without the features of ABPA who responded to antifungal therapy.[59]

Scedosporium apiospermum has also been associated with the ABPA-like picture in CF.[60]

Candida spp. are commonly cultured from CF sputum. This is not unexpected given that much antibiotic treatment in CF is broad spectrum and can lead to an overgrowth of *Candida* spp. on mucosal surfaces. Some cultures are therefore due to oral contamination of sputum but colonization of sputum is also possible. There is no current evidence of a significant role for *Candida* spp. in lower airway infection.

The possible role of normal oral flora in cystic fibrosis sputum

Bacteria usually labelled as normal flora in the mouth and upper airways are often grown from sputum and even from samples taken at bronchoscopy. These were thought to be due to contamination with saliva, however these bacteria may be present in such large numbers that it is more likely that sputum was seeded during small-volume aspiration and multiplication takes place in the sputum.[61] Two main lines of investigation have focused on these bacteria. Careful culture techniques have shown the large number and diversity of anaerobes that can be cultured from CF sputum.[62] The use of 16s rRNA gene profiling by terminal restriction fragment length polymorphisms

directly from CF sputum has shown a great diversity of anaerobic and aerobic species.[63] What is the significance of these observations? It may be that these flora are just bystanders (commensals). Alternately, some may actually be pathogenic and have been missed because they are present in a mixture with an assumed pathogen such as *P. aeruginosa*. This could even explain why CF patients may improve when treated with antibiotics to which the conventional pathogen is resistant *in vitro*.[27] Another explanation is that members of the 'normal' flora may interact with the conventional pathogen. In one study, a viridan-type *Streptococcus* was shown to enhance the pathogenicity of *P. aeruginosa* in an agar bead lung infection model in rats.[61] This viridans-type *Streptococcus* and a coagulase-negative *Staphylococcus*, both isolated from CF sputum, also upregulated *P. aeruginosa* genes. These included those coding for pathogenicity factors such as elastase (*lasB*).

Respiratory viruses

Respiratory viral infections are common in children, but there have been relatively few well-controlled studies on the specific role of respiratory viruses in CF.[64] This is partly because the techniques for identifying viruses by culture or serology have been too insensitive. Respiratory viruses are thought to be a trigger or co-factor in acute infective exacerbations of COPD[65] and it has been proposed that they may play a role in developing chronic infection with bacterial pathogens. Early Danish data showed that CF patients were more likely to acquire their first *P. aeruginosa* or develop chronic pseudomonal infection between October and March—the peak season for respiratory viruses. This finding was consistent over 25 years in spite of major changes in treatment of bacterial infection. The rapid technical advances, for examples, with the use of micro-arrays, should allow more in-depth studies of the role of viral infections in CF.[66]

Influenza A can have a significant impact in patients with CF. Anti-viral drugs are recommended in the UK for patients with chronic lung disease including CF, both for treatment and prevention after exposure. Annual vaccination is also recommended for patients with CF.

Conclusions

The microbiology of CF is complex. A pattern of acute, intermittent and eventually chronic infection is seen. Both familiar pathogens and bacteria not normally seen in the respiratory tract are encountered. The

relevance of new insights into the pathogenicity and epidemiology of old pathogens in CF is being assessed and the significance of other micro-organisms, previously not recognized in CF, is being investigated. Although there have been exciting advances in basic science, we still need to understand what triggers exacerbations, how to test bacteria from CF in the laboratory for the most accurate and clinically relevant results, and what are the best options for prevention and treatment of infection. Lesson learned from the complex interactions between bacteria and adaptations to chronic infection seen in the CF lung hold promise for better management of infection in CF. These may also help develop strategies to improve the outcome of other chronic infections, both in the lung and elsewhere.

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