***Pseudomonas aeruginosa* Infection in Cystic Fibrosis: Pathophysiological Mechanisms and Therapeutic Approaches**

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**Abstract**

*Pseudomonas aeruginosa* is a remarkably versatile environmental bacterium with an extraordinary capacity to infect the cystic fibrosis (CF) lung. Infection with *P. aeruginosa* occurs early, and although eradication can be achieved following early detection, chronic infection occurs in over 60% of adults with CF. Chronic infection is associated with accelerated disease progression and increased mortality.

Extensive research has revealed complex mechanisms by which *P. aeruginosa* adapts to and persists within the CF airway. Yet knowledge gaps remain, and prevention and treatment strategies are limited by the lack of sensitive detection methods and by a narrow armoury of antibiotics. Further developments in this field are urgently needed in order to improve morbidity and mortality in people with CF.

This review summarises current knowledge of pathophysiological mechanisms underlying *P. aeruginosa* infection in CF. Established treatments are discussed, and an overview is offered of novel detection methods and therapeutic strategies in development.

**Introduction**

*P. aeruginosa* is a highly versatile Gram-negative bacterium, found abundantly in the natural environment and capable of causing opportunistic infections in the human host (Figure 1). Although rarely pathogenic in healthy individuals, *P. aeruginosa* is an important cause of serious infections in patients with malignancy, immunosuppression, burns, and those on mechanical ventilation (1). In people with cystic fibrosis (CF) it is the most commonly isolated and thus, clinically important respiratory pathogen, and is associated with accelerated disease progression.

The ubiquitous ecosystems in which *P. aeruginosa* exists include soil, surface water, rivers and oceans, as well as domestic and clinical wastewater systems (2, 3). Its capacity to thrive in settings of varied nutrient and oxygen availability is imparted by its high metabolic diversity and the potential to adapt to low-nutrient environments (4). At between 5.5 and 7 million base pairs, the *P. aeruginosa* genome is one of the largest of all bacterial genomes; sequencing of the PAO1 laboratory strain (originally from a wound isolate) has revealed a high proportion of regulatory genes, and genes encoding transport systems/ enzymes involved in metabolism and substrate uptake (5). The similar breadth of genes encoding outer membrane proteins involved in motility, adhesion, antibiotic efflux, and virulence factor secretion contribute to the pathogenicity of *P. aeruginosa* and its ability to evade host and antimicrobial attack.

Initial infection of the CF airway by *P. aeruginosa* occurs early. Longitudinal studies in children with CF monitored with annual bronchoscopy have shown a high prevalence of *P. aeruginosa* in bronchoalveolar lavage (BAL) cultures in the first 3 years of life (6-8). In the study by Burns *et al*., in which *P. aeruginosa* antibodies were measured annually until the age of 3 years, the detection of positive antibodies at an earlier mean age than culture detection on BAL, and in many children who were culture-negative during the study period (6), suggests that some early infections may be cleared naturally. If detected early, antibiotic eradication regimes can achieve successful eradication in over 60% of cases (9). However, a pattern of intermittent, and eventually chronic infection, which persists despite antibiotic treatment, is common. Registry data show that by adulthood over 60% of patients with CF are chronically infected with *P. aeruginosa* (10, 11). Such chronicinfection is associated with phenotypic and genotypic adaptation, including transition to a mucoid phenotype, reduction in motility and virulence, biofilm formation and high rates of antibiotic resistance. Pathogen elimination by either host immune responses or current treatments is generally regarded as impossible at this stage. Several studies have shown that chronic *P. aeruginosa* infection has a clear association with faster decline in lung function, greater frequency of pulmonary exacerbations and increased mortality (12-14). Importantly however, extrapulmonary spread or bacteraemia is extremely rare in CF, infection being successfully contained within the airways.

**Why is the CF airway so susceptible to *P. aeruginosa* infection?**

CF is a multisystem disease caused by the autosomal recessive inheritance of mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. Lung disease is the major cause of morbidity and mortality, typified by endobronchial infection, mucus hypersecretion and impaction, and severe neutrophilic inflammation; together these lead to progressive airway obstruction, bronchiectasis, and eventual respiratory failure (15). The basic defect in CF arises from a reduction in functional CFTR protein at the apical membrane of epithelial cells. The exact mechanism by which CFTR dysfunction leads to the manifestations of CF is still incompletely characterised but several principles have been defined. CFTR is an ion channel, reduced activity of which leads to altered chloride, sodium and water transport across airway epithelia, and consequent dehydration of the airway surface (16, 17). Mucociliary clearance (MCC), the primary innate defence mechanism of the airways, is impaired and mucus plaques and plugs build up within which bacteria can thrive (18). Bacteria entering the airways through inhalation or aspiration are ineffectively cleared, and a perpetuating cycle of sputum retention, infection and inflammation ensues.

In addition to impaired MCC, there is evidence from studies in CF pigs (19) and ferrets (20) of a direct bacterial killing defect. *In vivo* and *ex vivo* studies have demonstrated impaired bacterial elimination in the airways of newborn CF pigs compared to wild-type, and that the airway surface liquid (ASL) in CF pigs is more acidic than in wild type animals (19, 21). Activity of ASL antimicrobial factors including defensins and collectins is impaired at lower pH, and restoration of ASL pH to wild-type values restored bacterial killing (21, 22). Data confirming that this is a relevant mechanism in humans is currently scant and somewhat confusing (23, 24) but the question is being actively investigated by our group and others.

A characteristic pattern of infection occurs from early in life, with *Staphylococcus aureus* and *Haemophilus influenzae* being the most frequently isolated pathogens in the first 3 years, and *P. aeruginosa* becoming increasingly common thereafter (10, 11). Several mechanisms have been suggested to explain the specific vulnerability of the CF airway to infection with *P. aeruginosa*, although many uncertainties remain. The close association could relate to increase risk of initial acquisition, or a favourable environment for chronic persistence, or both.

With regard to initial acquisition, it has been proposed that the CFTR protein acts as a receptor for *P. aeruginosa*, which binds to and internalises the bacterium with subsequent sloughing of the epithelium, a hypothesis supported by reduced *P. aeruginosa* uptake in cultured CF epithelial cells compared to wild-type cells (25, 26). This is unlikely to be a major mechanism in our opinion; unlike the epithelium of the bladder for example, sloughing off of respiratory epithelium has not been demonstrated as a host response to other infections; indeed, denuded areas of mucosa appear to be more prone to infection than intact regions. An alternative hypothesis, which attracted some attention in the 1980-90’s was that *P. aeruginosa* binding to epithelial cells was increased due to an abundance of asialoganglioside M1 (asialo-GM1) caused by the intracellular deficiency of CFTR function. Both pili and flagella are known to function as adhesins on *P. aeruginosa*, and pili attachment to asialylated glycolipids on the surface of epithelial cells was shown *in vitro,* resulted in inflammation (27, 28) and could be reduced by *CFTR* gene therapy (29). Whether this is of any relevance to the *in vivo* situation is unclear. There is evidence from autopsies of patients with end-stage CF lung disease that *P. aeruginosa* colonies exist within luminal mucus rather than attached to epithelium (30), although the host-pathogen interaction in early infection may differ substantially.

Isolation of *P. aeruginosa* from the healthy upper airway has been reported, although as sampling is rarely undertaken, incidence and prevalence are unknown. It may be that the ‘risk’ in CF relates more to the organism’s ability to survive and establish chronic disease. Plugs of CF mucus have been shown to be hypoxic (31), an environment encouraging both mucoidy and biofilm formation, discussed further below. The high viscosity and solid-concentrations within the CF mucus have been shown to impair the activity of soluble host defence factors such as lactoferrin and encourage high local bacterial densities (18).

**Sources of *P. aeruginosa* acquisition**

The predominant source of early *P. aeruginosa* acquisition is thought to be the natural environment, as genetic analysis has shown most early acquired strains to be distinct, and to reflect the genotype diversity found in environmental strains (6, 32, 33). Analysis of clinical isolates from two large European centres revealed that the five most common *P. aeruginosa* clones identified in CF patients belonged to the 20 most common environmental clones (34). Studies comparing particular environmental exposures with the risk of *P. aeruginosa* acquisition have yielded variable results and may be location-specific. For example, there was evidence from a Belgian study that living closer to natural open water was associated with greater risk of chronic infection (35), but this appeared not to be the case in a large registry study of North American patients (36). In the latter study, risk was increased by geographical and meteorological factors including altitude, humidity and temperature (36).

Since the 1980’s, evidence that cross infection is an important route of acquisition has emerged from the identification of highly prevalent transmissible strains among patients attending the same CF centres (37-40), as well as between geographically distinct centres (41, 42). Airborne transmission has been proposed at the main mechanism of cross-infection, supported by isolation of epidemic strains from air and surface samples from clinical areas (40, 43, 44). This is also made plausible by observations that viable *P. aeruginosa* is viable in cough aerosols from CF patients, which can travel for 4 meters and persist for up to 45 minutes (45). The identification of epidemic strains in CF centres on different continents remains incompletely understood, but may relate to mixing of patients in summer camps prior to these strains being discovered. These findings have since driven widespread introduction of patient segregation measures in CF clinical environments.

**Theearly stages of infection**

The evidence suggests that *P. aeruginosa* infects the CF airway intermittently prior to the onset of chronic infection, although the limitations of sampling methods need to be considered. Longitudinal studies have shown that initial infection typically occurs with unique isolates, which are non-mucoid and antibiotic-susceptible (6, 33, 46). In a large study of *P. aeruginosa* infection in pre-school children, initial acquisition occurred at a mean age of 2.4 years, and despite successful eradication in over 90% of cases, re-infection occurred in 44% of children during a mean follow-up period of 2.8 years (33). In those who underwent BAL sampling, the majority of re-infecting isolates were of different genotypes from the original strains (33). The continued pattern of re-infection eventually leads to persistence of either a single dominant or multiple co-infecting clones (34).

The importance of the sinonasal cavity as a reservoir for *P. aeruginosa* is increasingly recognised. Data showing genotype concordance between isolates obtained from the nasal cavity or paranasal sinuses and contemporaneous sputum isolates support the hypothesis that bacteria migrate from the sinuses to the lower airways (47-49). There is additional evidence that *P. aeruginosa* harboured in the oral cavity may act as a source from which pulmonary infection can be seeded (50, 51). Persistence of sinonasal or oral *P. aeruginosa* may be an important source of re-infection following eradication from the lower airways, and has been implicated in causing descending infection following lung transplantation (52).

***P. aeruginosa* behaviours facilitating survival in the CF airway**

*Surface structures*

*P. aeruginosa* possesses a broad range of virulence factors (Table 1), which enable it to adapt to and persist within the CF airway. These include surface structures such as lipopolysaccharide (LPS), flagella and type IV pili, which contribute to pathogenicity and trigger host immune activation. LPS is an immunogenic virulence factor with a high degree of structural heterogeneity in its lipid A component and O-antigen side chains (53). Isolates of *P. aeruginosa* from chronically infected CF patients have been shown to differ from environmental isolates in their loss of LPS O-antigen side chains and selection of specific penta- and hexa-acylated lipid A structures (53, 54), illustrating bacterial adaptation within the CF lung. Flagella and type IV pili are important for swimming and twitching motility respectively, as well as attachment to host epithelium and biofilm formation (55, 56). Flagellin, the primary component of bacterial flagella, binds to Toll-like receptor (TLR)-5 expressed on host epithelium, which activates nuclear factor NF-kappaB to stimulate production of TNF-α and other pro-inflammatory cytokines (57). Thus, flagella are both important to the early pathogenicity of *P. aeruginosa* and also trigger host immune responses; the latter may explain the downregulation of flagella synthesis seen in *P. aeruginosa* isolates in the setting of late stage infection (58).

*Secretion systems*

An extensive array of secreted proteins constitute a major component of *P. aeruginosa* virulence. The various secretion systems used by *P. aeruginosa* differ in complexity, and in the mode by which effector proteins are delivered into the extracellular environment or injected directly into the host cell cytosol (59). Secreted proteases (alkaline protease and elastase), toxins and haemolysins degrade host tissue and disrupt the host inflammatory response. Elastase is able to cleave host immunoglobulins, complement activation products, and surfactant proteins A and D, further compromising host immunity (60, 61). Exotoxin A, one of several secreted exotoxins, is a potent ADP-ribosylating agent that inhibits protein synthesis in eukaryotic cells, causing cell death (1). In a rodent model of lung infection, exotoxin A has been shown to be important in systemic pathogenicity (62). A number of secreted secondary metabolites exert detrimental effects on host epithelium. The phenazine derivative pyocyanin (responsible for the blue-green pigmentation of *P. aeruginosa* cultures) is a potent redox-active compound capable of generating reactive oxygen species, thereby exposing host cells to oxidative stress (63). Pyocyanin and hydrogen cyanide secreted by *P. aeruginosa*, have both been shown to decrease ciliary beat frequency, which may further impair innate airway defence mechanisms (64, 65).

One of the more complex secretion systems utilised by *P. aeruginosa* is the type III secretion system (T3SS), in which toxic effector proteins are injected directly into host cells by a macromolecular syringe. Over 36 genes have been identified in association with the T3SS, coding proteins integral to needle assembly, pore formation in the eukaryotic cell, and effector functions (66). Four effector proteins have been described in *P. aeruginosa*; ExoS, ExoT, ExoU, and ExoY, of which exoU has the most potent cytotoxic activity. The genes encoding these effectors vary between strains, with ExoS being the most prevalent in clinical isolates from CF patients, and rarely co-existing with ExoU (67-69). The strain phenotype during infection is dependent on effector protein, with ExoU-secreting strains causing rapid lysis of host cells, and ExoS-secreting strains initiating a sequence of cell death mimicking apoptosis (70). The highly-complex type VI secretion system (T6SS) has most recently been described; T6SS and biofilm growth have been shown to be co-regulated and T6SS component proteins have been identified in the sputum of CF patients with chronic *P. aeruginosa* infection (71). One of the characteristics of the T6SS is the killing of other Gram-negative bacteria, which may contribute to the ability of *P. aeruginosa* to outcompete other species in complex microbial infections (this theme is discussed further later). It has been postulated that certain components of these secretion systems could be future therapeutic targets, limiting the virulence of infecting bacteria (59).

*Mucoidy*

The most frequently observed phenotypic change in chronic *P. aeruginosa* infection is conversion to the mucoid phenotype, which occurs relatively late during infection and is associated with accelerated clinical decline (46, 72). The mucoid switch is caused by overproduction of the exopolysaccharide alginate, associated with inactivating mutations in the anti-sigma factor *mucA* gene, which normally regulates activity of the alternate sigma factor AlgU gene and its dependant alginate biosynthetic pathways (73). There is evidence that the mucoid conversion in *P. aeruginosa* is driven by the host inflammatory response, specifically reactive oxygen species produced by activated polymorphonuclear leucocytes, which have been shown to induce mutations in *mucA* along with excess alginate production (74). Alginate overproduction is considered to confer protection from oxygen radicals as well as to impede opsonisation, phagocytosis and complement-mediated bacterial killing. It is also associated with enhanced microcolony formation and biofilm maturation (75).

*Biofilm growth and Quorum sensing*

In the early stages of infection, *P. aeruginosa* exists in the CF airway in planktonic (free floating) form, but there is evidence that as infection progresses, bacterial colonies adapt to a biofilm mode of growth (76, 77). Biofilms are microcolonies of sessile bacteria encased in a self-produced polymeric matrix, which exhibit high resistance to host immune clearance and antibiotics (Figure 2) (78, 79). Transition between motile and sessile growth involves complex regulatory signalling networks, some of which act via the second messenger cyclic-di-GMP, to influence bacterial gene expression, virulence and mode of growth (80). Features of biofilms include reduced oxygen tension and nutrient availability at the biofilm centre, where reduced metabolic activity and replication have been demonstrated (81). Under anaerobic conditions, encountered in CF mucus, *P. aeruginosa* alginate production and biofilm growth are further enhanced (31, 82).

Control of biofilm and many other genotypic and phenotypic adaptations in *P. aeruginosa* is influenced by a system of intercellular molecular signalling known as quorum sensing (QS). This is mediated by highly-diffusible acylated homoserine lactone (AHL) signalling molecules, which regulate target gene expression once a critical bacterial population density is reached. The core components are the *las* and *rhl* systems, which are tightly interrelated, with the *las* having a regulatory role over the *rhl* system (83-87). Evidence that QS signalling systems are important in CF airway infections has stemmed from identification of AHLs in CF sputum, correlated to transcript accumulations of QS target genes (88, 89). The *las* and *rhl* systems have been shown to modify expression of multiple virulence genes in the *P. aeruginosa* genome, regulating biofilm maturation, swarming motility and expression of antibiotic efflux pumps (90-93). A third system, the *Pseudomonas* quinolone signal (PQS) also contributes to *P. aeruginosa* QS in regulation with both *las* and *rhl*, and has been shown to further modulate virulence factor expression (94).

The importance of QS in chronic *P. aeruginosa* infections is debatable. Loss-of-function mutations in the QS regulator *lasR* have been shown to be common in isolates from chronically infected patients (95, 96), along with reduced production of AHLs (97), in keeping with a reduction in virulence. The *mucA* mutation seen in mucoid *P. aeruginosa* isolates also leads to down-regulation of AHLs and PQS-dependant signalling systems, with evidence of associated complex changes in virulence factor secretion, such that cyanide and pyocyanin production is reduced as bacteria enter stationary phase, but continued during prolonged stationary phase (98). Together the evidence supports a down-regulation of QS signalling and modification of virulence as chronic infection becomes established.

*Antibiotic resistance*

As with other Gram-negative bacteria, *P. aeruginosa* resists antimicrobial killing through a number of intrinsic and acquired resistance mechanisms. Intrinsic factors include low antibiotic permeability of the bacterial outer membrane, drug elimination through surface-expressed efflux pumps, and enzymatic drug inactivation by chromosomally-expressed β-lactamases. Acquisition of plasmid and integron-coded extended-spectrum β-lactamases and carbapenamases is well described in *P. aeruginosa*, enabling the bacteria to hydrolyse a wide range of β-lactam antibiotics (99, 100). Acquired resistance to carbapenems has been shown, associated with loss of the outer membrane protein OprD, (either through inactivating mutations or downregulated gene expression) as well as over-expression of efflux pumps (101). In clinical practice, it is not uncommon for adults with CF who have received multiple courses of systemic antibiotics to have pan-resistant strains of *P. aeruginosa.*

**The response of the CF host**

Persistence of *P. aeruginosa* in the CF airway is enabled through a combination of defective innate host defences and the ineffective host inflammatory response. Bacterial presence drives an inflammatory response typified by epithelial secretion of interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor (TNF)-α, as well as other pro-inflammatory mediators, which collectively stimulate an excessive neutrophil influx (102, 103). There is some evidence that phagocytosis, a key mode of bacterial killing by neutrophils, is impaired in the CF airway, related to abnormal production of the hypochlorous acid in the phagolysosomal compartment (104). Bacterial killing by neutrophil elastase extracellular traps (NETs), whereby pathogens are bound by chromatin and nuclear proteins released from dying neutrophils, has been shown to be equivalent to non-CF neutrophils in culture conditions, but diminished against *P. aeruginosa* isolates obtained from chronically infected CF patients (105). The mechanism by which *P. aeruginosa* becomes less vulnerable to NET-mediated killing during chronic infection has not yet been defined. A secondary defect in neutrophil function has also been demonstrated in the CF airway, with unopposed protease activity leading to cleavage of the chemokine receptor CXCR1, thereby disabling neutrophil bacterial killing capacity (106). With recurrence and persistence of infection in the CF airway, the ineffectual but enhanced inflammatory response leads to progressive structural lung damage (107). Release of neutrophil elastase and other proteases leads to digestion of structural lung components (collagen and elastin) and degradation of immunoglobulins and other opsonins, causing further tissue destruction and limiting the host immune response. Release of high-molecular weight DNA from degraded neutrophils further increases the viscosity of airway secretions and perpetuates the cycle of airway obstruction and inflammation.

**The interaction between *P. aeruginosa* and other pathogens**

Recent intriguing work has demonstrated that *P. aeruginosa* possesses strategies to manipulate the host immune response to its advantage, expanding its available niche by killing other bacterial pathogens in the locality. As already mentioned, the characteristic pattern of sequential infection in CF children is early *S. aureus* and somewhat later, *P. aeruginosa*. Whilst some older patients are co-infected with these organisms, it is striking how many appear to have ‘grown out’ of the former once *P. aeruginosa* has established itself. Pernet *et al.* have recently demonstrated that infection with *P. aeruginosa* induces the host airway epithelia to express type-IIA-secreted phospholipase A2 (sPLA2-IIA), an enzyme to which *S. aureus* is sensitive and which leads to bacterial death, whereas *P. aeruginosa* itself is resistant (108).

In addition to the impact of pseudomonas-induced host proteins, there may be a competitive advantage conferred by antagonistic interactions between *P. aeruginosa* and other organisms: the pseudomonal endopeptidase, LasA, selectively disrupts *S. aureus* peptidoglycan, leading to lysis and death (109). Hydrogen cyanide has been mentioned earlier for its potentially damaging effects on host epithelia. It may also endanger other pathogens, which do not possess the de-toxification systems present in *P. aeruginosa*, and which allow the latter to survive in high concentrations of the toxin. *P. aeruginosa* has also been reported to exert anti-fungal effects, although the exact mechanism is unknown (110). In other experiments, co-culture with *Aspergillus fumigatus*, a fungus frequently isolated from the CF airway, enhanced the virulence of *P. aeruginosa* via increased elastase production (111). Conversely, *Candida albicans* (frequently cultured from CF secretions and mostly ignored, perhaps mistakenly) has been shown to reduce the virulence of *P. aeruginosa* by suppression of toxins including pyoverdin (112). Finally, an area of increasing research is the role of viral infections in CF airway disease: clinical convention is to use broad-spectrum antibiotics during periods of (likely viral) upper respiratory tract symptoms to protect against secondary bacterial infection of a damaged mucosa. The common respiratory syncytial virus (RSV) has been reported to increase *P. aeruginosa* adherence to epithelial cells *in vitro* and rhinovirus to liberate planktonic organisms from biofilms of *P. aeruginosa* (113), potentially thereby facilitating seeding of infection to other areas of the respiratory tract.

These observations are likely the tip of the iceberg, as increasingly we are becoming aware of the huge array of organisms within CF airways; clearly much further work is still to be done before these relationships are fully understood.

**Diagnosis *of* *P. aeruginosa* infection in clinical practice**

Standards of care in CF include frequent surveillance for airway infection. As *P. aeruginosa* is the organism with the strongest evidence base supporting early eradication, timely detection is key. As mentioned above, acquisition in early life is common, yet babies and young children produce little sputum and are usually unable to expectorate. BAL is considered the gold standard for sampling the lower airway, but is invasive, requires a general anaesthetic in children and can therefore not be undertaken frequently; there are also limitations related to geographical variability as sampling all airways is time consuming and may be more likely to lead to systemic side effects such as fever and hypoxaemia. Moreover, the routine use of BAL to direct anti-pseudomonal therapy in preschool children has not been shown to be superior to oropharyngeal cultures (114).

Oropharyngeal (OP), throat or cough cultures are typically used in such settings where sputum cannot be obtained, although their positive predictive value (PPV) for identifying lower airway infection is variable. Studies comparing OP cultures against BAL report PPV values of 42-69% and negative PV values of 82-97% (6, 33). Emerging evidence indicating sinonasal and oral reservoirs as important sources of *P. aeruginosa* suggests that upper airway sampling (for instance by nasal lavage) could require greater attention than previously given, particularly as BAL or OP cultures and have been shown to be poor predictors of organisms present in the sinuses (115).

Measurement of IgG antibodies against *P. aeruginosa* surface components is an attractive detection method, in use in some CF centres, although the diagnostic value in detecting early infection has proven disappointing (116). The utility of *P. aeruginosa* antibodies for predicting response to eradication therapy is potentially more promising (117).

The limitations of currently available sampling techniques highlight the need for alternative, preferably non-invasive, detection methods for *P. aeruginosa*. Based on the production by *P. aeruginosa* of volatile organic compounds, breath analysis would seem an attractive option. Hydrogen cyanide and 2- aminoacetophenone have been reported by some, but in a recent study, our group found significant overlap in a number of markers detected by mass spectrometry between CF patients with and without *P. aeruginosa* infection (118). Confounders may be contamination from the mouth (119) and the contribution of a host-derived signal. Separate efforts to detect *P. aeruginosa* in exhaled breath condensate by polymerase chain reaction have been unsuccessful (120). Rapid evaporative ionization mass spectrometry (REIMS) is capable of rapidly identifying different bacterial pathogens (121) and with some optimisation may be applicable for non-culture based assessment of patient samples. This is an area which in our opinion requires significant further work, but may hold promise. Benefits would not only include rational therapeutic decision making for individual patients, but there could be significant impacts on cross-infection and segregation practices (122).

Whereas, based on culture alone, we may regard CF patients as infected with a relatively narrow range of pathogens, advances in microbiological techniques with culture-independent methods of bacterial identification are rapidly changing this view. 16s rRNA PCR and other molecular methods are increasingly revealing a massive array of bacterial and fungal species in the lower and upper airway. Indeed, the healthy human airway is far from sterile, as has been previously thought. In CF patients, diversity of the microbiota appears to decrease with advancing disease stage, which may related to antibiotic pressures (123, 124). This is a very active area of research, which seeks to address important questions of clinical relevance and implications for management.

**Current treatments for *P. aeruginosa* lung infection**

*Eradication of initial or early infection*

Much emphasis has been placed in recent years on detection and eradication of *P. aeruginosa* at the early stages. High success rates have been reported with a number of approaches, the optimal of which is unknown (9). In Europe, convention is to use an inhaled antibiotic, most commonly colomycin, with systemic (either oral or intravenous) drugs. Whether one of these options is superior to the other is currently being investigated in the UK Torpedo trial (http://www.torpedo-cf.org.uk). In many other parts of the world including the US, single agent tobramycin via inhalation is the most common approach (125), with the ELITE trial demonstrating no significant advantage of 56 days therapy over 28 days (126). While the use of prophylactic antipseudomonal antibiotics to prevent *P. aeruginosa* infection might seem attractive, in a single RCT this approach was found not to be of benefit (127), and is therefore not recommended (125).

*Treatment of pulmonary exacerbations*

Pulmonary exacerbations (PEx) are poorly understood ‘flare-ups’ during which previously stable patients experience increased symptoms (cough, sputum production, breathlessness, sometimes fever) and decreased lung function. The detrimental effects of PEx are well described; patients with frequent PEx experience more rapid decline in lung function (12, 13, 128), and are at greater risk of death or transplantation (129). Importantly, it is recognised that between ¼ and ⅓ of patients do not regain their baseline lung function following a PEx (130, 131) In childhood, the role of viruses is increasingly recognised, although in later life PEx occur in the absence of new infection and indeed the burden of existing bacterial infection does not alter much. It is possible that PEx are rather caused by changes in bacterial behaviours or in host response. This lack of understanding means that the only treatment available is antibiotics. For milder PEx, these will often be oral, but more severe episodes require a combination of intravenous agents. Interestingly, *in vitro* antibiotic sensitivity testing (Figure 3) correlates poorly with clinical response, a well-recognised but poorly understood phenomenon. Laboratory synergy testing has also been shown not to translate into clinical benefit (132).

*Suppression of chronic Pa infection*

Once infection is chronic, most commonly defined as >50% of airway samples over the previous 12 months (133), the emphasis switches from aggressive attempts at eradication, to long-term suppressive strategies, which it is hoped, will reduce the damaging host inflammatory response. These most commonly involve either single or rotating (on a monthly basis) inhaled antibiotics such as colomycin, tobramycin and aztreonam. The formulation of previously nebulised solutions into dry powder devices means shorter delivery times and is a significant recent advance. Other conventional antibiotics are being developed using novel technologies, for example the formulation of amikacin into a liposome which leads to sustained release.

As disease advances and patients require increasing amounts of antibiotics, antimicrobial resistance increases, as too does the potential for adverse events including drug allergies and renal/ ototoxicity. Alternatives to conventional antibiotics are actively being researched.

**Novel therapeutic approaches for of *P. aeruginosa* lung infection**

**Biofilm and QS inhibitors**

Targeting quorum-sensing (QS) molecules involved in the formation of biofilms is an attractive strategy. This is considered to be a possible mode of action for the macrolide, azithromycin, which is effective in patients with CF and in widespread clinical use (134). Garlic has been shown to possess QS inhibitory activity *in vitro* and in animal models; a small pilot trial did not show any microbiological or clinical effects (135) but further work is being undertaken. The ability of nitric oxide to disperse biofilms has been demonstrated, related to stimulation of phosphodiesterase and consequent reductions in intracellular cyclic-di-GMP (136). These observations have led to the development of NO-donor drug strategies, including those activated by bacterial enzymes and coupled to antibiotics (137). The seaweed-derived alginate oligomer, OligoG, has been shown *in vitro* to reduce *P. aeruginosa* biofilms and also possesses favourable mucolytic properties. It is currently in multicentre phase 2 clinical trials for CF patients both with *P. aeruginosa* and *Burkholderia cepacia* complex infections (ClinicalTrials.gov identifier NCT02157922 and NCT02453789 available at <https://www.clinicaltrials.gov> )

**Bacteriophages**

Bacteriophages are naturally-derived viruses that can specifically bind to pathogenic bacteria, multiply within them, and lyse the bacterium, thereby amplifying at a site of need (Figure 4). Having been somewhat ignored by western medicine since the advent of the earliest antibiotics, increasing concern over antimicrobial resistance has reawakened interest. There has been one small study reporting positive outcomes using bacteriophage to treat resistant otitis externa infections (138). We, and others have used murine models to confirm the efficacy of bacteriophage against *P. aeruginosa* airway infections (139, 140), but clinical data in patients with CF are currently lacking. The use of phage mixes or ‘cocktails’ is likely to reduce the development of resistance, but this issue will need to be carefully addressed in clinical trials.

**Immunotherapies**

Both passive and active approaches to immunisation have been explored. With regard to the former, immunoglobulin Y derived from the yolks of *P. aeruginosa*-exposed hens’ eggs is being administered as a gargle in a multicentre European trial (NCT01455675). Kalobios are testing a monoclonal antibody against PcrV, a component of the type III secretion system (MabKB001-A) and Panobacumab, a human monoclonal anti-LPS antibody, is being used in a clinical trial in non-CF patients following encouraging data of efficacy in mice (141). Active immunisation with a preventative vaccine has been explored for some time, but to date there is insufficient evidence of efficacy (142).

**Conclusion**

A significant proportion of the world’s CF population is affected by *P. aeruginosa* infection. The past decade has witnessed a significant expansion in our understanding of the mechanisms of infection, both from the pathogen and host perspective, some of which are translating into the development of new treatments. Further advances in this field, and in that of detection systems, are urgently needed if we are to create the step change needed in efficacy, in the context of the global concerns over antibiotic resistance.

**Expert Commentary**

In the CF airway, a ubiquitous environmental organism, usually regarded as relatively non-virulent, is able to cause major problems. We understand aspects of both the host defence defects and the pathogen features which lead to this, but in our opinion, do not as yet fully appreciate their relative contributions, nor to what extent other organisms within the respiratory tract play a role. It is also likely that genetically determined host factors, so-called modifier genes, influence both early and persistent infection; these remain particularly poorly understood.

Although eradication of early infection is relatively successful, two major challenges exist: first, timely detection of the organism is likely to increase the success rate of eradication strategies, and yet young children and babies are the least likely to yield useful samples. Although likely some way off, non-invasive detection tools, for example based on breath samples, such as those used routinely for *Helicobacter pylori* infection, would be a significant contribution to management. Secondly, even after an apparently successful eradication, *P. aeruginosa* frequently recurs in a relatively short space of time. In some cases, this may reflect a failed eradication but in others, detailed genetic analysis confirms that infection is with a distinct organism. Thus, eradication does not in any way protect the CF airway from subsequent re-infection. Given the poor outcomes associated with the infection, attempts at prevention must surely be better than partially-effective treatments, and yet we are a long way from any prophylactic regime.

There is an increasing armamentarium of anti-pseudomonal therapies, some of which, for example dry powder inhalers, are improving quality of life for patients, but all of which to date are conventional antibiotics. The global concerns over antimicrobial resistance, together with significant cumulative toxicity, mean we really must consider alternatives. Current interest in anti-biofilm strategies, passive immunisation and bacteriophages provide some encouragement in this regard.

**Five year view**

The last few decades have seen major advances in our understanding of the complex relationship between bacterium and host, particularly perhaps with regard to chronic infection and persistence, but there remain major areas of uncertainty: do the innate defence defects in the CF airway predispose selectively to *P. aeruginosa*, or are they broader, the high incidence of *P. aeruginosa* infection simply reflecting high levels of the bacteria in the human environment? What are the relative contributions of host and pathogen factors in establishing and maintaining infection? How do we seek a deeper understanding of bacterial, viral and fungal behaviours within the complex ‘zoo’ of the CF airway?

Until recently, we have been severely hindered in attempts to address these outstanding questions by the lack of a good animal model. Observations arising from clinical samples led to hypotheses that could really only be tested *in vitro*, under conditions bearing little or no resemblance to the human lung. The many transgenic mouse models developed during the 1990’s were largely disappointing with regard to airway disease. However the recent development of the CF pig and ferret provide new opportunities; already much has been learned and we would anticipate that these, and other evolving CF models, will contribute to further expansion of knowledge with regards airway infection over the next few years.

Over the last five years, huge efforts have been focussed on describing the ‘microbiome’, detected by culture-independent, genetic analysis tools, and there is now widespread acceptance that pathogens conventionally regarded as dominant in the CF airway, may not in fact be so, merely a small part of a complex milieu. We hope and anticipate that over the next five years, questions around the clinical relevance of these observations are addressed. We understand that diversity decreases as disease progresses, but does this necessarily mean that restoring diversity will improve outcomes? When organisms are detected with PCR-based methods, should they be treated and if so, how? What does success look like in this regard? Shifts in infecting populations need to be accompanied by changes in other, clinically meaningful outcomes, before we can assume benefit.

The activity in areas of non-antibiotic therapies is encouraging; one or more of these strategies is likely to come of age over the next few years. It seems unlikely we will reach the stage where antibiotics are not needed, but the use of adjuncts, for example anti-biofilm molecules or bacteriophages, could reduce the amount needed or the duration of use, thereby reducing the adverse consequences of these drugs.

Finally, although outside the scope of this article, significant advances have been made recently in therapies targeting the basic defect in CF. The CFTR potentiating drug, ivacaftor, leads to rapid and sustained improvements in lung function in the minority of patients with suitable gene mutations. Of more relevance for this review, a reduction in *P. aeruginosa* infection has been reported in patients taking the drug over several years (143); these findings arose from observational and registry data and are therefore potentially subject to bias, but are theoretically plausible. If CFTR function is restored in the airway (and, as the drug is administered systemically, likely phagocytic cells also), host defence may be improved and bacterial clearance achieved.

Thus, we would like to predict the future of *P. aeruginosa* in CF, perhaps not achievable in the next five years, but possible in the longer-term: incidence of *P. aeruginosa* infection will be significantly reduced by the widespread availability of highly-effective therapies targeting the basic defect; non-invasive surveillance tools will have advanced to the stage where patients can self-monitor in the community, allowing rapid detection of new infection; and for those patients who do acquire *P. aeruginosa*, a combined approach, aggressive to the organism whilst gentle on the host, with conventional antibiotics and novel non-antibiotic antimicrobials working synergistically, may improve long term outcomes.

Reference for figure 2 (144)

**Key issues**

* *Pseudomonas aeruginosa* is a ubiquitous Gram-negative bacterium able to survive in a range of natural and man-made environments.
* In people with Cystic Fibrosis (CF), *P. aeruginosa* is the most commonly isolated respiratory pathogen, and chronic infection is associated with an adverse prognosis.
* The characteristic pattern of intermittent followed by chronic infection arises due to a complex interaction of pathogen-specific adaptive factors and an ineffectual host inflammatory response.
* As culture-independent molecular tools enhance our understanding of the bacterial community dynamics within the CF airways, the complexity of interactions between *P. aeruginosa* and other pathogens is increasingly apparent.
* Early detection of *P. aeruginosa* is key to enable targeted eradication therapy. The evidence that early eradication can be achieved with antibiotics is good although the optimal approach is unknown.
* Detection of *P. aeruginosa* infection is particularly challenging in young children and those who do not produce sputum. Development of novel detection methods, which are sensitive and non-invasive, is a research priority.
* Alternative treatments to antibiotics are urgently needed to avoid the increasing challenges of toxicity and antibiotic resistance. Strategies being explored include inhibitors of biofilms and quorum sensing, passive immunisation and bacteriophages.

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