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Gram negative infections in cystic fibrosis: A review of preventative and treatment options

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Abstract

Introduction

The microbial landscape of CF is changing, reflecting advances in microbial detection, CF therapies and an increasingly heterogeneous and ageing population. Gram negative organisms are important to clinical trajectory, however, some have unknown implications on disease course.

Areas covered

This review covers the evolving landscape of the microbial ecosystem of the CF lung and provides an update on current diagnostic and therapeutic options for management of Gram negative bacteria. Evidence for prevention of acquisition of new organisms, and eradication of Gram negative pathogens is reviewed. There is an increasing range of inhaled antibiotic therapies for chronic suppressive antimicrobial therapy, with an urgent need for research into the efficacy of specific combinations. Intra-venous therapy for pulmonary exacerbations requires optimisation, focusing on greater precision, improved clinical outcomes, whilst reducing anti-microbial resistance and long-term side effects. The future role of CFTR modulators, anti-inflammatory agents and novel anti-infectives is also outlined.

Expert opinion

Antimicrobial therapy must evolve to reflect the evolving microbial landscape and the needs of current and future CF populations. With an increasing number of Gram negative organisms, detection methods and therapeutic options, it is critical therapy is targeted appropriately to the organism and the individual.

Key words

Gram negative; *Pseudomonas*; *Burkholderia Cepacia* Complex; Eradication; Antibiotics; Pulmonary Exacerbations

Article Highlights

- * The microbial landscape of cystic fibrosis (CF) is changing, reflecting advances in microbial detection, treatment options and an increasingly heterogeneous, ageing population.
- * Gram negative organisms are important to clinical trajectory, but key pathogens *Pseudomonas aeruginosa* (Pa) and *Burkholderia Cepacia* Complex (Bcc) are reducing in prevalence, with associated increases in other Gram negative organisms, with unknown implications on disease course.
- * It is unlikely acquisition of organisms from the environment can be prevented in totality, but stringent infection control, temporal and geographical segregation are used to reduce

acquisition and transmission of Pa and Bcc, with the possibility similar strategies may be effective to reduce acquisition of other Gram negative organisms in the future.

- * Eradication regimes for Pa demonstrate a positive impact on clinical outcomes and healthcare utilisation, but studies of eradication in other organisms are limited, with further research into effective eradication urgently required.
- * In the context of chronic growth, chronic suppressive antibiotic therapy can reduce clinical decline, and pulmonary exacerbation frequency, but comparative efficacy studies focus on individual inhaled antibiotics, with more research required on the effectiveness of antibiotic combinations.
- * The concept of the poly-microbial ecosystem of the CF lung questions current antibiotic approaches to pulmonary exacerbations, which require greater precision to optimise clinical outcomes, whilst reducing anti-microbial resistance and long-term side effects.
- * Antibiotic strategies in CF must increasingly follow the principles of antimicrobial stewardship, focused on reducing negative antibiotic effects, including resistance.
- * Alternative “anti-infective” approaches including CFTR modulation, anti-inflammatories and biofilm disrupters may play a greater role in the future, targeting the inexorable intertwining of infection and inflammation in the CF lung.

Gram negative infections in cystic fibrosis: A review of preventative and treatment options

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive, life-limiting, multisystem disease characterized by viscid secretions in multiple organ systems [1]. Advances in multi-disciplinary management combined with therapeutic advances, including Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) modulators [2], have significantly improved survival rates, with death prior to adulthood now rare [1]. With increasing numbers of disease causing mutations [2], lifespan [3,4], and population size [4], people with CF (PWCF) are increasingly heterogeneous. As the CF population ages and enlarges, CF care, must evolve to focus on the needs of current and future PWCF [1].

The CFTR ion channel is found on the apical surface membrane of epithelial cells in multiple organ systems. Impaired function leads to disordered regulation of ion and water transport, resulting in the viscid secretions which are the hallmark of CF [1,2]. Data from animal models (Mouse, Ferret and Pig) have furthered understanding of the pathophysiology of organ damage in CF [5]. Respiratory disease remains the major cause of morbidity and mortality in CF. Lung damage is driven by a complex interplay between infection, inflammation and primary host responses. Deficient sodium and bicarbonate secretion reduces the pH of CF mucus, directly impairing host defence [5]. Defective and dysregulated innate and adaptive immune responses are also described [6]. Dehydrated airway surface liquid and viscid secretions impair ciliary function [7]. These combined factors drive acute and chronic infection, host inflammatory response, and associated lung damage through a “vicious cycle” of infection and inflammation [6]. Understanding of microbiological growth within the CF lung is also changing [8], driven by advances in microbial detection, therapeutic advances [9] and a changing CF population [1,10]. Prevention and management of acute and chronic lung infection must also evolve to meet the needs of current and future CF populations.

2. The evolution of microbiology in CF

Traditionally, a sequence of chronic bacterial growth in CF airways is described. Acute, and then chronic colonisation with a Gram positive organism, *Staphylococcus aureus* (S.aureus), was followed by emergence of more resistant pathogens, predominantly Gram negative organisms, including *Pseudomonas aeruginosa* (Pa) and *Burkholderia cepacia* complex (Bcc) (Fig 1.) [3]. Colonisation with multi-resistant bacteria resulted in clinical decline, deteriorating lung function and ultimately increased mortality [11,12].

The landscape of CF lung infection is evolving [8,10,12,13]. Pa and *Burkholderia cenocepacia* (part of the Bcc) remain significant pathogens, associated with lung function decline, increased pulmonary exacerbation (PEx) frequency and mortality rates [11,14]. Evidence suggests that some individuals with chronic Pa may be more stable than previously thought, and able to maintain higher levels of lung function, measured by percent predicted Forced Expiratory volume in 1 second (ppFEV₁) [10]. The Bcc comprises 21 genomovars, Bcc strains which are phenotypically indistinguishable, but phylogenetically different, acquired from various environmental sources [15]. Genomovars vary in clinical consequence, virulence and pathogenicity [15]. *Burkholderia cenocepacia* and *B. multivorans* account for the majority of Bcc infections in CF, with chronic growth associated with both stability and

clinical decline, dependent on the individual [12,15]. With 19 other genomovars now described, and a paucity of evidence on their individual impact, the clinical consequences of Bcc infection are much less predictable [12,15].

Stringent infection control precautions limit the transmission of Pa, Bcc and other transmissible bacteria between PWCF [10]. Rates of Pa are declining secondary to infection control precautions, improved stability across the CF population and focused eradication regimes [10,13,16]. With reduced Pa prevalence, an increase in other Gram negative bacteria has been observed (Fig 1.) [8,16]. Alongside a growing understanding of the polymicrobial environment of the CF lung, the concept of a sequence of bacterial growth has been replaced by that of a dynamic evolving ecosystem [17,18].

Haemophilus influenza (H.influenzae) is a common respiratory pathogen associated with less severe disease, greater lung function preservation and lifespan in PWCF [12,19]. A range of other Gram negative bacilli, including *Achromobacter* species (spp.), *Stenotrophomonas maltophilia* (S.maltophilia), and *Ralstonia* spp., are emerging as pathogenic organisms, rising in prevalence and clinical importance (Fig 1.) [8,12,16,20]. Other less common Gram negatives, with unknown clinical effects include *Inquilinus limosus*, and various species of *Bordetella*, *Cupriavidus*, *Acinetobacter*, and *Xanthomonas* (12). As the majority of these bacteria are not included in registry reports, it is difficult to estimate their prevalence, and influence on clinical course [3]. The impact of these emerging pathogens on disease trajectory and anti-microbial strategies requires further investigation to optimise therapy targeted to these pathogens [8,16].

Resistant Gram positive organisms, in particular, Methicillin Resistant *Staphylococcus aureus* (MRSA) are also more common, especially in the United States (US) [12]. Increasing rates of Non-Tuberculous Mycobacteria (NTM), in particular *Mycobacterium abscessus* (M.abs), have impacted sampling methodology and infection control measures [21]. Joint guidance on the management of NTM in CF was recently published by the European CF Society (ECFS) and US CF Foundation (CFF) [21].

Figure 1. Chronic Microbial Colonisation in UK CF population in 2018. Intermittent growth of S.aureus and Pa, becomes increasingly chronic with age. Prevalence of MRSA, Bcc and Aspergillus fumigatus increases with age, whilst the prevalence of H. influenzae reduces. From UK CF Trust Registry Annual Report 2017 [3]

2.1 Microbial detection methods

Non-culture based microbial detection has expanded our knowledge of the microbial landscape of the CF lung. These techniques have identified a significantly larger number of organisms present within the CF lung, including anaerobes [17,18,22], than previously identified by standard microbial culture [23,24].

Currently, culture based methods remain the mainstay of microbial detection [25]. CF guidelines suggest regular surveillance of sputum for common organisms [21,26]. Culture identifies the majority of organisms present in the CF lung [27], with the addition of mass spectrometry (Matrix Assisted Laser Desorption/Ionization-Time of Flight) (MALDI-TOF) increasing bacterial identification and assisting strain typing [25]. This has increased the detection of other Gram negatives, including *Ralstonia* spp., which may commonly be mis-identified as Bcc by culture alone [28]. Anaerobic culture can further increase the detection of organisms, but is not performed routinely in all clinical laboratories [22,27].

A major advantage of culture based detection is the ability to assess antibiotic susceptibility. Standard susceptibility testing may be enhanced by resistome analysis, synergy and biofilm susceptibility testing [19,25]. However, clinical response to antibiotic therapy does not always correlate with antibiotic sensitivities, and further evidence of the clinical benefits of enhanced susceptibility testing is needed [8,25].

Routine culture may significantly underestimate the number of organisms present in the CF lung [23], but changes to culture based methods can increase microbial detection rates [29]. Extended culture methods are capable of identifying a much wider range of organisms, including most identified by non-culture based methods [27,30]. However, extended culture is costly, time laborious and may identify bacteria with unknown clinical consequences, requiring clinical laboratories to focus on common established pathogens [27,30]. Regular review and updating of laboratory methodology is necessary to ensure culture based practice keeps pace with the changing landscape of CF microbiology.

2.2 Non-culture based microbiological detection

2.2.1 The microbiota

Culture independent techniques detect bacterial 16s rRNA genes through high throughput sequencing. Capable of identifying a significantly larger number of organisms, including bacteria not previously described in CF, the complexity of the microbiological communities present in the CF lung is now evident [18]. This generated the concept of the CF lung as a complex poly-microbial ecosystem - the lung microbiota [17,23].

This greater breadth of detection has changed understanding of the environmental niches and fluctuant microbial ecosystem of the CF lung [18]. A chronic inflammatory state, fluctuating between stability and instability, combined with CF mucus acidity, and structural variation, including anaerobic niches, create a unique setting for microbial evolution [18]. Airway microbial diversity increases during the first decade of life, peaks during late adolescence, then declines [31]. Reductions in diversity are associated with dominance of resistant organisms, including Pa and Bcc, which can drive prolonged inflammatory responses. Antibiotic pressure increases with progression of lung disease, leading to reductions in microbial diversity, but not density [18,32]. Microbial communities vary significantly within, and between individuals, over time and between differing lung regions [32-34].

However, the clinical relevance of many organisms identified by microbiota analysis remains unknown [18,22]. The role previously unidentified organisms, including anaerobes [22], and bacteria previously not considered of importance in CF, for example Streptococci spp., play in disease progression requires further investigation [33]. Individual organisms may play a less significant role, than the interplay between organisms, and dynamics of the microbial community as a whole [18,33].

There are considerable challenges when considering the application of microbiota analysis to clinical care. Repeated sampling at multiple time points is necessary to establish an overall picture of an individual's airway microbiota [32,33]. Expertise in sample processing and bioinformatics analysis, combined with cost, limit the feasibility of this being achieved in all clinical laboratories, but may be achievable in regional or reference laboratories [8,35].

2.2.2 Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) testing is capable of rapid detection of viruses and bacteria. There is interest in expanding the role of PCR and other rapid non-culture based methods of bacterial detection within clinical care [8,36]. Quantitative PCR can approximate the bacterial burden, grossly correlating with quantitative culture results [35,36]. Quantification of bacterial burden may be a useful addition to assessing community structure when investigating changes in the lung microbiota [8,31]. At times of clinical deterioration, rapid detection of bacterial and viral pathogens may be useful to determine management [35,36]. PCR based approaches may also be effective at detecting less common Gram negative species, such as *Ralstonia*, commonly misidentified or overgrown by other species in culture [28,30]. Further research is needed to optimise the role of PCR and other rapid detection methods in clinical scenarios [35,36].

3. Prevention and Eradication

3.1 Prevention

Preventative strategies to reduce the chronic growth of pathogenic Gram negative bacteria, have focused on patient isolation to reduce spread, and attempted eradication treatment upon first organism detection. The possibility of therapeutic approaches based on microbiota analysis, targeted at maintaining bacterial communities to “prevent” emergence of future Gram negative bacteria is unknown.

Outbreaks of Bcc, with confirmed spread between PWCF, precipitated strict patient separation and infection control measures which reduced bacterial organism transmission between PWCF, now viewed as a standard of care [37,38]. Confirmation of *Pa* spread between children with CF at CF summer camps, in clinic and on hospital wards, confirmed person-to-person transmission was also applicable to other CF organisms [39,40]. With new-born screening leading to earlier diagnosis, PWCF are now routinely geographically and temporally segregated, even prior to the development of any chronic bacterial growth. Clear guidelines on infection control are available [21,41], and combined with greater clinical stability provided by new therapies, have led to a reduction in the prevalence of chronic *Pa* and Bcc [10,13].

Infection control guidance re-affirms the need for segregation, but emphasises opportunities to further reduce the risk of bacterial transmission between PWCF. This includes wider use of contact precautions, monitoring of adherence to infection control policy, and enhanced ventilation/decontamination of healthcare environments. Bacteria can survive on hard surfaces, in clinical areas for hours, and in some cases days [41]. Improving the air filtration of clinical rooms, leaving appropriate intervals between patients, and optimised cleaning of clinical areas are now recommended practice [41]. With aerosolised bacterial cough droplets capable of travelling 4 metres, the wearing of masks outside specific isolation rooms, and in other areas of the hospital, could further reduce transmission of organisms [41,42]. This is recommended in the latest CFF guidance, but has yet to translate into routine clinical practice [41,42].

3.2 Environmental acquisition

Whilst stringent infection control reduces transmission between PWCF, environmental acquisition of organisms continues. Genotype and CFTR function, may play a greater role in age of first acquisition of Pa, than any modifiable environmental risk factor [43]. Most Gram negative organisms, including Pa and Bcc are prevalent in the environment [12,44]. The most appropriate strategies to reduce environmental acquisition have yet to be identified. PWCF are informed of environments where bacterial acquisition may be higher, including hot tubs, hydrotherapy pools, water sprinklers and mud kitchens, so they can make an informed choice on use [41,45]. Seasonality and warmer ambient temperatures increase Pa acquisition, with acquisition highest in summer and autumn, lowest in spring [46,47]. Higher rainfall, and living in a tropical climate are both associated with increased Bcc acquisition [44]. Whilst, *S.maltophilia* is also environmentally acquired, no specific environmental risk factors have been identified. Factors associated with first acquisition include younger age (<18 years), dysglycaemia, intra-venous or oral quinolone antibiotics, lower oral antibiotic exposure, and lack of chronic growth of Pa/Bcc [48-50]. It is hypothesised that acquisition of other Gram negatives from the environment, may be associated with more severe disease and increased antibiotic pressure, driving the microbiota towards these more resistant organisms after exposure [51].

Concern over acquisition of new organisms, can lead to restricted activities, particularly in children with CF, impacting on quality of life and raising parental stress levels [52]. Currently evidence is insufficient to formally restrict activities, and physician advice on preventative measures to reduce environmental acquisition vary [45]. Any advice given must be balanced against the actual, and perceived impact on quality of life [45].

The concept of poly-microbial environment of the CF lung, and interactions between organisms means existing organisms may impact acquisition of new pathogens and the future microbiota. *Aspergillus fumigatus* growth increases the risk of acquisition of Pa, Bcc and *S.maltophilia*. Chronic growth of Pa reduces the likelihood of future growth of Bcc, *S.maltophilia* and *Achromobacter xylosoxidans*. *S.aureus* and MRSA reduce the risk of future Pa [53]. These interactions may impact treatment for existing organisms. For example, antibiotic prophylaxis for *S.aureus*, in children with CF, may lead to earlier Pa acquisition [54]. A UK registry based, open label trial, CF-START, investigating the safety and efficacy of Flucloxacillin prophylaxis is enrolling, with the primary outcome age at first growth of Pa (CT 2016-002578-11).

3.3 Eradication

It is unlikely the acquisition of organisms from the environment can be prevented in totality. Therefore effective strategies to screen for, rapidly detect and eradicate known pathogens after acquisition are necessary. Currently, guidelines recommend respiratory samples are taken at all clinic attendances, and screened for common pathogens using bacterial culture [21,26]. In older adults who can spontaneously expectorate sputum, sufficient screening samples can be obtained. This can be more challenging in children, and those with less severe disease. Alternative sampling methods include cough swabs and broncho-alveolar lavage (BAL) samples, which have limitations in regard to sensitivity and procedural risks respectively. Induced sputum may be a suitable alternative, having demonstrated equivalent sensitivity and bacterial detection to BAL [55].

PCR has the advantage over culture, of rapid detection and greater detective sensitivity [36]. However, the place of PCR within CF care has yet to be established, and further evidence is needed to prove that more rapid detection of organisms leads to improved clinical outcomes [35].

Once suitably detected, an effective eradication regime may be used to prevent chronic growth. Evidence for the benefits of eradication in Pa are used as proof of principle that eradication of other pathogenic organisms should be attempted [56]. Whilst definitions of successful eradication vary, three negative samples, over 6 months from cessation of eradication therapy is widely accepted as sufficient evidence of clearance [56,57]. Successful Pa eradication has positive effects on clinical outcomes, but also reduces healthcare utilisation, positively affecting healthcare resources and costs [56]. Eradication for Pa may be successful in up to 96% cases, dependent on the sensitivity of the strain at first isolation [56]. Eradication can be repeated if not successful, or if a sufficient time interval has elapsed between isolations.

Eradication regimes for Pa vary, with oral, intravenous and nebulised antibiotics all used, alone or in combination [56,57]. Debate continues as to the optimal eradication regime for Pa. Open label studies of 28 days nebulised Tobramycin and Aztreonam lysine, both demonstrated successful Pa eradication rates between 65%-75% [58,59]. A randomised controlled trial (RCT) comparing initial intravenous (Ceftazidime/Tobramycin) to oral antibiotics (Ciprofloxacin), both followed by 3 months nebulised Colistimethate sodium has recently completed, with full published results awaited (CT 2009-012575-10-Torpedo). Presented results suggest no significant difference in eradication rates between regimes, with a trend towards higher percentage eradication using oral Ciprofloxacin [60].

Bcc, particularly *B.cenocepacia* is intrinsically resistant to the majority of antibiotics, including polymyxins, aminoglycosides and most beta-lactams, and capable of acquiring further resistance *in vivo* [61]. This limits antibiotic options for attempted eradication. The majority of UK centres routinely attempt eradication for Bcc [62], using combinations of IV, oral and nebulised antibiotics, but only one randomised trial of eradication therapy for Bcc has been performed [63]. This randomised cross-over study compared nebulised Taurolidine to 0.9% saline, demonstrating no evidence of successful eradication or significant change in lung function in participants [63]. A second small (n=6) US single centre study demonstrated 100% Bcc eradication at one year, after a 21 day induction regime of IV Ceftazidime/Tobramycin, with oral Co-trimoxazole/Azithromycin and inhaled Tobramycin, followed by a 2 month consolidation regime of oral and nebulised therapy [64].

The impact of *Achromobacter* spp. on clinical trajectory is variable. Two studies demonstrated no evidence of increased risk of PEx, or significant effect on lung function [65,66]. One study detected an increased risk of death or need for transplantation [65]. Over a longer study duration (13 years), compared to CF controls, those chronically infected with *Achromobacter* spp, showed a more rapid rate of lung function decline, and significantly higher frequency of PEx [51]. This difference may be driven by differing *Achromobacter* spp., or different clones within a species [67]. In light of potential deterioration after first isolation, one study assessed the benefits of inhaled antibiotics (Ceftazidime, Colistimethate sodium or Tobramycin) in eradicating *Achromobacter* spp. showing effective eradication at three years in 55% participants [67].

There is limited, or no evidence on eradication of other Gram negative bacteria in CF [12,28]. This is likely due to the proportionally small numbers growing these bacteria, and the limited evidence on pathogenicity in some cases. Taking into account the interactions between bacterial species in the CF

lung, other bacteria may contribute to the success or failure of eradication regimes, and the impact of eradication of one organism on the risk of future organisms must also be considered [53].

4. Development of chronic growth

If attempts at eradication fail, chronic growth of the organism is likely and a long-term management approach is required. Chronic infection is commonly defined as positive culture of an organism in >50% samples over a 12 month period [68]. To develop persistence in the airways bacteria undergo a series of morphological changes, which impact on virulence, antibiotic resistance and clinical outcomes. Most notable are the development of mucoid phenotypes and biofilm growth, environmental adaptations associated with airway chronicity, and crucial to bacterial defence against antibiotic therapy [14].

Multiple mechanisms of resistance are described across the spectra of bacteria seen in CF contributing to persistent bacterial growth. Antibiotic exposure is a major driver for later resistance, which may increase with greater antibiotic pressure over a longer lifespan [19,30,69]. Resistance mechanisms range from production of enzymes to inactivate or alter antibiotic targets, to reduced cell permeability, or efflux pump expression, with individual resistance mechanisms capable of conferring resistance to multiple antibiotics [19]. Anaerobic niches, the acidity of CF mucus and bacterial species interaction via quorum sensing also contribute to resistance development [14,19]. With the potential for horizontal transfer of resistance genes and interactions between organisms, the resistance patterns of both key pathogens, and the rest of the microbiota may have to be considered when designing future chronic suppressive antibiotic strategies [19,53].

4.1 Chronic suppressive therapy

In the case of *Pa*, chronic suppressive therapy with inhaled antibiotics (IA), and long-term oral azithromycin therapy is beneficial in reducing lung function decline, reducing PEx frequency, and improving clinical outcomes (Table 2) [70,71]. Long-term Azithromycin therapy has risks of reversible oto-toxicity, cardiac arrhythmia and may generate macrolide resistance, so proof of ongoing benefit must be evident to justify prolonged therapy [71].

The efficacy of chronic suppressive therapy for Bcc may vary by genomovar. With 21 genomovars, and limited studies examining suppressive therapy for Bcc it is not possible to stratify response [12]. One randomised study in PWCF ≥ 6 years, with chronic growth of Bcc, compared 24 weeks of nebulised Aztreonam lysine to placebo, followed by 24 weeks open label treatment. Whilst, there was no evidence of a significant increase in ppFEV₁ compared to placebo, or during the open label treatment period, there was a trend towards a reduction in PEx and hospital admission [72]. Other genomovars, including *B. Multivorans* may show sensitivity to oral agents, particularly tetracycline's, but the efficacy of long term oral suppressive therapy is unknown [15].

The evidence for effectiveness of chronic suppressive therapy for other organisms is also limited. Acquisition of chronic, but not intermittent *S. maltophilia* is an independent risk factor for PEx, and need for hospitalisation [20,49]. Whether chronic *S. maltophilia* is a marker of more severe disease or itself generates greater lung function decline is less clear [20,49]. Benefit from chronic suppressive therapy could be postulated, but no evidence exists on specific antimicrobial regimes. *S. maltophilia* has multiple mechanisms of resistance, but may be sensitive to Tetracycline's, Co-Trimoxazole,

Levofloxacin, Ticarcillin-clavulanic acid and Colistimethate sodium [73], offering potential options for oral or nebulised suppressive therapy [49]. The benefits of chronic suppressive therapy for other Gram negatives including *Achromobacter* spp., *Ralstonia* spp. and *Pandorea* have not been studied [12,28].

4.1 Inhaled antibiotic regimes

Individual IA have demonstrated efficacy in clinical trials, but with a substantial increase in the number of IA options, and combination therapy in common use, further study of IA combinations is needed to optimise clinical efficacy (Table 2 and Fig 3). An alternate month approach to IA therapy is used, with either no antibiotic or an alternate antibiotic used at 28 day intervals. In the UK, an escalating approach to therapy is primarily driven by licensing restrictions and cost [74]. Across countries, differences in licensing restrictions alter treatment combinations. For example, the UK first line antibiotic Colomisthetate Sodium (Colomycin™) is unlicensed in the US, so initial therapy is primarily with Tobramycin. Tobramycin is available in two licensed preparations (Bramitob™/Tobi™), with two further biosimilar preparations now available [74]. This may lead to greater competition, with potential impact on long-term costs of care. Treatment with a nebulised liposomal formulation of Aztreonam, Aztreonam Lysine (AZLI™/Cayston™) leads to a reduction in PEx, and increase in lung function, in common with other nebulised antibiotics [75]. Prescribed thrice daily, it also demonstrates clinical efficacy when taken only twice daily, which may be of importance in individuals with variable adherence [76]. A nebulised liposomal levofloxacin formulation, Aeroquin™/Quinsair™ is the latest IA to be licensed in the EU and Canada [74]. It demonstrates non-inferiority to other licensed IA in regard to lung function, PEx frequency and the need for hospitalisation [74].

Dry Powder Inhaler (DPI) devices offer greater flexibility, ease of use and reduced cleaning times aimed at improving adherence. Dry powder alternatives to nebulised Colomisthetate sodium and Tobramycin are licensed, with evidence of non-inferiority to other nebulised antibiotics in clinical trials and health technology appraisals [74,77]. A Ciprofloxacin DPI phase 2 trial demonstrated no effect on ppFEV₁, and failure to sustain a reduction in Pa density, so despite a trend towards a reduction in PEx, this is not progressing to a phase 3 trial in CF [78,79].

Table 2. Table to show the delivery, dosing, costs, anti-microbial cover and adverse effects of inhaled antibiotics for CF. Currently licensed inhaled antibiotics are shown above, with those in the pipeline (phase 2/3 trials) shown below. With the exception of liposomal amikacin for NTM, current licenses for inhaled antibiotics are specifically for the treatment of Pseudomonas aeruginosa infection based on the available clinical trial data. As many have broader antimicrobial cover, inhaled antibiotics may be used “off license” to manage other CF lung infections. (Abbreviations: Pseudomonas Aeruginosa (Pa); Burkholderia Cepacia complex (Bcc); Stenotrophomonas Maltophilia (STM); Achromobacter species (Ach spp); Other Gram negative bacteria (GNB))

Other IA in the therapeutic pipeline include nebulised liposomal amikacin (Arikace™) and Vancomycin inhalation powder (Aerovanc™). Arikace™ has a US license, for management of *Mycobacterium avium*. A phase three trial demonstrated a higher rate of sputum culture conversion compared to placebo, when Arikace™ was added to a standard treatment regime [80]. A phase 2 trial of Arikace™ to treat Pa in PWCF, demonstrated a significant increase in ppFEV₁, and reduction in PA density compared to placebo after 28 days of treatment [81]. A phase three study of Arikace™ versus Tobramycin Inhalation solution has completed enrolment, with full results awaited (NCT01315678).

AerovancTM has been designed to treat MRSA lung infection, showing a significant reduction in MRSA sputum density in a phase two trial. A phase three trial is currently underway (NCT03181932).

With six licensed IA options, two bio-similars and more options in the pipeline potential combination regimes are now numerous. Whilst cost, and licensing restrictions provide a basic framework for escalation (Fig 3.), tolerability, adverse effects and antibiotic resistance also determine individual regimes. Clinical trial data for individual antibiotics demonstrates safety, efficacy and non-inferiority to alternatives [74-77]. Comparative efficacy analyses compare treatment and cost effectiveness between individual antibiotics [74,77], but neither data type provide the much needed evidence on the efficacy of combination therapy (Fig 3.).

Figure 3. Summary of inhaled antibiotic options for chronic suppression of Pa in CF. Fig 3a. represents the standard UK layered approach of initiating therapy with a Colistimethate sodium preparation (nebulised or DPI), then stepping up to an inhaled tobramycin preparation (nebulised or DPI). Tobramycin is used initially as either a replacement for Colistimethate sodium or on an alternate month basis with Colistimethate sodium. In the event of intolerance or ongoing clinical decline current UK licensing, driven by cost and efficacy analysis, then allows the addition of Aztreonam Lysine, to Colomycin or Tobramycin or a swap to Aztreonam Lysine alone. If clinical deterioration continues, or other inhaled antibiotics are not tolerated then Levofloxacin inhalation solution can be considered as a fourth line. In countries with differing licensing restrictions, and in the UK, it is common for people not to follow this stepwise pattern, but to cycle between, or try various inhaled antibiotic combinations driven by tolerability, resistance patterns and clinical course. Based on an alternating month regime, this leads to 19 potential inhaled antibiotic combinations, illustrated in Fig 3b.

5. Management of PEx

PEx have a significant impact on health related-quality of life, morbidity and mortality [82]. Clinical factors associated with an increased risk of PEx include female sex, lower ppFEV₁, age, poor adherence [83], and chronic growth of Pa or S.maltophilia [84,85]. Around 25% patients do not recover their ppFEV₁ after such episodes [86]. Failure to recover lung function is associated with chronic growth of Bcc or MRSA, a greater drop in ppFEV₁ at presentation, female sex, and worse nutritional status [86].

5.1 Microbiology of PEx

Relatively few PEx are due to acquisition of new organisms [87]. Clonal expansion of existing Pa strains may contribute in a few cases [87]. Viruses may trigger up to 65% of PEx [88], with Rhinoviruses, Influenzae and Respiratory Syncytial Virus the predominant pathogens [88]. Changes in detection methods from serological assays, to PCR have increased detection of viral triggers for PEx [8,36,89]. Microbiota analysis has changed perception of the alterations in microbial communities around PEx [27,33]. No increase in bacterial density is demonstrable pre-PEx [90], with variable changes in microbial diversity [27,33]. Exposure to common respiratory pathogens, or subtle changes in the microbiota, may trigger shifts in stable microbial communities, resulting in increased inflammation, clinical deterioration and PEx [18].

5.2 Antibiotic strategies for PEx

Evidence to support current clinical practice for the treatment of PEx is limited [82]. Broad spectrum IV antibiotics over 10-14 days, in combination with increased physiotherapy and nutritional support are standard practice [26,82,91]. Traditional antibiotic approaches targeted reduced bacterial growth, of either new invading organisms, or presumed increases in chronic bacteria, now thought to be lesser players in the aetiology of PEx [90].

Current therapeutic approaches require optimisation and greater personalisation, with further investigation required to guide effective therapy [82]. The optimal length of IV treatment for PEx remains unknown, and may vary between individuals [82,92,93]. During treatment, the relative efficacy of IV antibiotics versus increasing airway clearance, nutritional, and psycho-social support in assisting recovery has never been determined. Future PEx management needs to reflect individual responses, the heterogeneity of the CF population and changes in our understanding of the aetiology of PEx [82].

In response to antibiotic therapy for PEx, within 72 hours, there is a temporary decrease in Pa density, bacterial richness and diversity, which returns to baseline within 7 days [27,33,94,95]. The impact of antibiotics may be greater on less abundant organisms, including anaerobes, than dominant pathogens such as Pa [42,94,95]. With reducing rates of Pa and increasing prevalence of other organisms [10,13] antibiotic strategies are changing [8,16], but are limited by a relative paucity of data on bacterial communities dominated by other organisms [33,94]. Whilst, the benefit of antibiotic strategies designed to target the microbiota has been postulated, the first trial to examine this approach demonstrated no clinical benefit over current antibiotic strategies [96]. This study, CF MATTERS, compared microbiota directed antimicrobial therapy to “standard of care” for PEx. Whilst no difference in clinical outcomes was evident, the feasibility of microbiota analysis to direct antibiotic therapy was shown. Samples were successfully processed, analysed, and a microbiota directed antibiotic strategy provided within 42 days [96].

This makes it difficult to determine an optimal approach to antibiotic therapy for PEx (Fig 4). Antibiotic choice is driven by chronic bacterial growth, previous antibiotic susceptibilities, local hospital policies and previous clinical response, including antibiotic allergies and intolerances. Bacterial susceptibility testing, augmented by biofilm or synergy testing could assist in targeting therapy, but evidence of clinical benefit is limited [8]. As well as varying regimes, antibiotic doses and dosing intervals vary between CF care centres [93,97]. The adverse effects and risks of end-organ damage generated by multiple IV antibiotic courses over a longer lifespan must be considered. With new IV antibiotics, with action against Pa and other Gram negatives now in use, it is increasingly important therapy is targeted appropriately, and the risk: benefit profile of regimes considered [69,98]. Increased antibiotic exposure increases the risk of fixed drug reactions, and hypersensitivity [69]. All broad spectrum IV antibiotics have gastro-intestinal side effects, and may impact the gut microbiota [69]. Aminoglycosides, including Tobramycin, are oto- and nephrotoxic, with cumulative dosing effects [69]. Beta-lactams, can cause bone marrow suppression [69].

Anti-microbial stewardship is an increasing part of CF care, with establishment of an ECFS/CCF strategic working group, and all centres encouraged to review their antimicrobial prescribing practice [69]. Whilst dual IV anti-pseudomonal antibiotics were considered “standard of care” in a PWCF with chronic Pa [26,91], in this changing microbial landscape, future regimes are unlikely to be this simplistic. A randomised open-label cross-over study comparing IV Colistimethate sodium and nebulised

Aztreonam lysine to standard dual therapy is already underway (NCT02894684). Combinations of IV, oral and nebulised antibiotics may be more common, with the risk: benefit profile of differing regimes weighed for each individual. The US Standardised Treatment Of Pulmonary Exacerbations (STOP) completed an observational study of response to IV therapy for PEx, using the results to design an RCT comparing IV antibiotic durations, currently recruiting across the US (NCT02781610).

The high levels of resistance seen across the Bcc, necessarily lead to complex antibiotic regimes, comprising dual or triple IV antibiotics, frequently with an additional oral or nebulised agent. Whilst this reduces alternative options, it is critical the clinical review considers whether other factors may have led to clinical decline to prevent unnecessary courses, and that length of treatment is determined by individual response. Even in a multi-resistant organism, like *B.cenocepacia*, the principles of antimicrobial stewardship still apply.

For emerging pathogens, the evidence on optimal antibiotic regimes is less clear. Despite their increasing prevalence, and likely clinical impact, no formal guidance on management during PEx exists. *H.influenzae* has greater antibiotic susceptibility, with lower rates of multi-drug resistance than other Gram negative bacteria in CF [19]. Increased antibiotic pressure over a longer lifespan, with the presence of hypermutator strains, potential for biofilm formation [99], and greater resistance in persistent strains, particularly to Ciprofloxacin, could increase pathogenicity in the future [100]. Despite being an independent risk factor for PEx development, no randomised trials of PEx treatment for *S.maltophilia* exist [20,49]. In one retrospective cohort study, the number of days of treatment given for *S.maltophilia* had no effect on ppFEV₁ recovery or time to next exacerbation [101]. *Ralstonia* spp. have significant resistance across antibiotic classes, including beta-lactams and aminoglycosides, with further inducible resistance to antibiotics in vivo [28]. This limits antibiotic options, in a similar fashion to Bcc. At a minimum, dual therapy is suggested, with combinations of Co-Trimoxazole, Ciprofloxacin, Piperacillin-Tazobactam or Tigecycline potentially the most effective [28].

Figure 4. A decision tree approach to antimicrobial stewardship when considering IV antibiotic treatment for a person with CF. At all stages alternatives to IV antibiotics can be considered, or antibiotic exposure minimised to reduce the risks of adverse effects and development of antibiotic resistance. (Abbreviations: Pseudomonas aeruginosa (Pa); Burkholderia cepacia complex (Bcc); Stenotrophomonas Maltophilia (STM); Achromobacter species (Ach spp.); Haemophilus Influenzae (HI))

6. Adherence, Resistance and antibiotic de-prescribing

Reducing the development of antibiotic resistance through effective antimicrobial stewardship is a global health priority [69]. Core principles are reducing inappropriate antibiotic prescribing, effective targeting of antibiotic therapy, in form and duration, aiming to limit the development of antibiotic resistance whilst optimising clinical outcomes [69]. Antimicrobial stewardship strategies can play a role in CF care. Key to this may be reducing inappropriate antibiotic prescribing, optimising therapy for PEx and de-escalation of chronic antibiotic therapy [69]. Understanding the impact non-adherence to chronic therapies has on the need for “rescue” antibiotic therapy may play a critical role [102]. Non-adherence to chronic therapies is associated with clinical decline, increased need for additional antibiotics, and hospital admission [83,102]. If non-adherence is identified, strategies to promote adherence may result in a reduction in additional antibiotic prescribing [102]. A UK

nationwide RCT to assess this approach, CF Health Hub, has completed its intervention period, with full results awaited (IRAS 89701). The investigators propose that if this approach proves positive, embedding adherence monitoring and intervention into routine clinical care could result in a significant reduction in “rescue” antibiotic use within CF [102].

Identifying non-adherence could also prove positive in reducing antimicrobial resistance. Failure to adhere to antibiotics as prescribed increases resistance rates, and sporadic use of inhaled or oral antibiotics in CF could also generate resistance [69]. If regular adherence cannot be established, then de-prescribing antibiotics could be considered, both to reduce the risk of resistance and to ensure more appropriate escalation of therapy in the event of clinical deterioration. Equally in prolonged clinical stability, such as that generated by long-term CFTR modulating therapy [103], it is not known if continuing long-term antibiotic treatment remains beneficial. With evidence of slower lung function decline, significant reductions in PEx, and reduced bacterial growth in response to CFTR modulators, PWCF may wish to reduce their treatment burden [103-105]. Whilst this is a key aim of CF care, as yet no evidence exists to confirm which therapies, including antibiotics, could be safely stopped, or in what order therapies could be de-prescribed in this scenario.

7. Non-microbiological approaches

Traditionally bacterial colonisation was considered the major driver for inflammation in the CF lung. It is now clear that lung damage starts very early in the disease course, with dysregulated inflammatory pathways potentially preceding chronic microbial growth [6]. The pathophysiology of inflammation in the CF lung is complex, with a plethora of interlinked inflammatory pathways, and the additional influences of CF genotype, modifying polymorphisms and alternative ion channels [6]. The inflammatory pathways present in CF incorporate innate and adaptive immune responses, dysregulation of anti-inflammatory responses, and upregulation of pro-inflammatory factors [6].

A complex interplay between inflammatory changes and the bacterial ecosystem is recognised. It is hypothesised that subtle changes in microbial ecosystems may have much greater effects on lung inflammation, and therefore clinical symptoms [18]. Long and short term antibiotic treatment may have beneficial effects on inflammation [70], although understanding of the mechanisms underlying this are limited. Therefore an alternative approach to acute and chronic microbial management could combine antibiotic therapy, with therapies targeted at reducing lung inflammation. The use of Azithromycin exemplifies this approach, with its benefits postulated to be due to anti-inflammatory effects, rather than anti-microbial efficacy [70,71].

The intertwining of inflammation with infection makes therapeutic anti-inflammatory interventions challenging [106,107]. Increased infection rates, as seen in trials of corticosteroids and leukotriene B4 receptor antagonists remain a concern [106]. Trials of anti-inflammatories, aimed at multiple targets, have demonstrated variable therapeutic results [106]. Length of treatment may be important, with the prolonged and intense inflammatory response present in the CF lung unlikely to improve significantly after the short treatment durations present in most trials [107]. Two anti-inflammatories (Lenabasum (NCT02465450) and Acebilustat (NCT02443688)) have shown sufficient signal of safety and potential clinical improvement in phase 2 trials to proceed to larger phase 2/3 studies. Both are using a reduction in exacerbation frequency as evidence of efficacy, focusing on the complex interplay between inflammation and infection in driving PEx frequency (NCT03451045).

CFTR modulators have changed the face of CF care, with single, double and triple combinations now licensed, or in clinical trials [2]. These small molecules are capable of modulating the cellular effects of the underlying genetic defect, improving CFTR function and salt/water transport at cell surfaces [2]. All have shown evidence on improved clinical outcomes, of varying magnitudes, dependent on the modulator type, and genetic defect [1,2]. Along with greater stability, improved lung function, reduced PEx and better nutritional status, CFTR modulation has been shown to affect microbial growth [9] (Table 5). In-vitro studies have shown direct antimicrobial effects from Ivacaftor [104]. Chronic growth of Pa is reduced in those on long-term CFTR modulation [104]. Acquisition of new Pa is also delayed in those treated with Ivacaftor [104] or Lumacaftor/Ivacaftor [105]. However, CFTR modulation appears to have less effect on new acquisition or chronic growth of Bcc, and the impact on Pa may not persist over a longer lifespan [104]. It is postulated that interventions to reduce inflammation and prevent the development of progressive lung disease, including anti-inflammatory agents and CFTR modulators, may be of most benefit if initiated early in disease [6,108]. If started early, improved CFTR function and lower levels of inflammation may reduce new bacterial acquisition, forming another method to “prevent” future bacterial infection. However, the evidence in this area is so far limited, and further data is required to fully assess the impact of CFTR modulation on bacterial growth in the long-term.

Table 5. A summary of the anti-microbial effects of currently licensed CFTR modulators, and CFTR modulators/anti-inflammatory compounds in phase 2/3 trials. Both CFTR modulators and anti-inflammatory compounds lead to a reduction in pulmonary exacerbations, likely generated by multifactorial mechanisms. CFTR modulators, particularly Ivacaftor, may have positive effects on Pa acquisition, eradication and chronic growth. Evidence on their effect on other bacteria is less, and requires further study. Anti-inflammatory agents have potential to have positive anti-microbial effects but also require further study [9,104,105,109-119]

7.1 Other “Anti-Infective” agents

Nitric Oxide (NO) is an anti-inflammatory compound, with some anti-bacterial properties. A randomised, placebo controlled phase 2 trial of inhaled NO is underway in the US. It investigates the effect of NO on lung function, and bacterial density in adults with CF chronically colonised with *S.aureus*, Pa and *S.maltophilia* (NCT02498535). NO also has anti-biofilm properties which could impact bacterial growth, and are under investigation (PMID 28750737). Hypothiocyanite and lactoferrin are molecules involved in inflammatory signalling and host bacterial defence mechanisms. Levels of these molecules are low in the airway surface liquid of PWCF, contributing to the development of chronic bacterial growth in the CF lung. A phase 1 study of an inhalational compound (ALX-009), combining these two molecules, is exploring the effect on chronic bacterial growth in healthy volunteers, PWCF and people with non-CF bronchiectasis (NCT02598999). Chronic bacterial growth in the CF lung is facilitated by biofilms generated by bacteria. An inhaled dry powder, Alginate Oligosaccharide (OligoG), derived from seaweed, has been shown to improve mucus clearability and disrupt biofilm formation. One European Phase IIb study is complete (NCT02157922), with full results awaited, and a second phase IIb study is currently recruiting in Australia (NCT03822455). An inhaled glycopolymer (SNSP113) has been designed to disturb and disrupt biofilm formation. With evidence of safety in a phase 1a healthy volunteer study, a phase 1B study in PWCF is planned (NCT03309358).

Gallium disrupts iron dependent cellular processes, and has demonstrable antibacterial effects against Pa in vitro. A phase two study of IV gallium nitrate in PWCF chronically colonised with Pa, demonstrated safety, but failed to meet its primary endpoint of a $\geq 5\%$ increase in ppFEV₁ compared to placebo. A phase 1 study of inhaled gallium citrate (AR-501) is underway in healthy volunteers (NCT03669614).

8. Conclusion

Our understanding of the microbial environment within the CF lung is changing, driven by advances in diagnostic methods, therapeutic advances and an ageing, enlarging CF population. Gram negative bacteria remain significant pathogens, but with stringent infection control, focused eradication, and advancing therapeutic options, the traditional dominance of Pa and Bcc is being supplanted by the emergence of a wider spectrum of Gram negative bacteria. Evidence on the clinical effects of these emerging Gram negatives is limited, but enlarging. Further research is needed to understand the clinical impact of these emerging pathogens, and to optimise therapeutic options.

Therapeutic approaches to prevention, eradication and management of acute and chronic infection must evolve to reflect this changing microbial landscape. Effective eradication strategies for Bcc, and other Gram negatives require investigation, to match the benefits shown by effective Pa eradication. Acute and chronic antibiotic therapy must be personalised to promote optimal individual response, taking into account poly-microbial interactions, and the long-term effects of anti-microbial treatment. Despite the significant resistance patterns of many Gram negative bacteria, antimicrobial stewardship must play a larger role in CF care, with a focus on targeted therapy and a reduction in antibiotic harm over a longer lifespan. Reducing rescue therapy related to non-adherence, promoting effective chronic therapies and assessing the risk: benefit profile of antibiotic therapy must become core pillars of future management of Gram negative bacteria, and antibiotic prescribing in CF.

Expert Opinion

The microbial landscape of cystic fibrosis (CF) is changing, reflecting advances in microbial detection, treatment options and an increasingly heterogeneous, ageing population. Gram negative organisms, particularly *Pseudomonas aeruginosa* (Pa) and Burkholderia cepacia complex (Bcc) are key players in the polymicrobial ecosystem of the CF lung. However, the clinical impact of emerging Gram negative pathogens such as *Stenotrophomonas maltophilia* and Achromobacter species is less well understood. Further research to understand disease trajectories and optimise therapy targeted at these pathogens is required.

To reflect this changing landscape, microbial detection methods require review to ensure timely identification. Consideration to inclusion of non-culture based detection in screening, and monitoring of respiratory specimens must be made, whether this be at a local, regional or reference laboratory level. Stringent infection control and aggressive early eradication have contributed to reductions in the prevalence of Pa and Bcc. Similar approaches may be required for other emergent Gram negatives. If chronic bacterial growth in the respiratory tract is established, individually tailored antibiotic approaches should be employed alongside established antimicrobial stewardship principles. With a plethora of inhaled antibiotic options available, and more in the pipeline, study of

the comparative efficacy of combination therapy is urgently needed so that chronic suppressive therapy can be individualised. Outcomes should be driven by clinical response and tolerability rather than a generalised pyramid of anti-microbial escalation, in part driven by cost and licensing restrictions.

With changes in our understanding of the drivers for pulmonary exacerbations (PEx) in CF, and bacterial response to antibiotic therapy, a change in our approach to PEx management is needed. Avoidance of inappropriate antibiotic prescribing in scenarios where clinical deterioration may be driven by other factors, including non-adherence to therapies must be given high priority. With limited evidence to guide current PEx antibiotic regimes, varying trajectories of response, and the risks of cumulative antibiotic exposure over a longer lifespan, alternative approaches to intravenous (IV) therapy must be considered. Regimes may need to be more targeted, vary in duration, and combine intravenous with oral or nebulised antibiotics to ensure clinical effectiveness. Studies investigating the efficacy of such regimes are urgently needed to guide future therapy for PEx.

Assessment of the risk: benefit profile of antibiotic therapy must become core to the future management of Gram negative bacteria and antibiotic prescribing. This must increasingly take into account poly-microbial interactivity and community dynamics, which are critical to current and future antibiotic response. Alternatives to traditional antibiotics may play a greater role, with anti-inflammatories, molecules to promote host defence and biofilm disrupters already in the pipeline. Combining these with antibiotics may help reduce antibiotic resistance, and target the inexorable interplay between inflammation and infection within the CF lung. With increasing stability, and wider access to CFTR modulators, de-prescribing of antibiotics may become more prevalent. In the future, prolonged stability may lead to further alteration in the microbial landscape. With a predicted increase in the CF population of 78% by 2025, and infants born today predicted to have an almost “normal” lifespan, playing the long game will become critical to the management of Gram negative infection in CF in the future.

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Figure 1.

Inhaled Antibiotic	Delivery	Dose	Potential antimicrobial cover						Adverse effects*	Mechanism of action	Cost per month**	Current status/ License	Brand/Trade names
			Pa	Bcc	STM	Ach Spp	Other GNB	Gram positive					
Colomycin Inhalation Solution (CIS)	Jet or mesh nebuliser	1-2 MU twice daily	✓	✗	✓	✓	✓	✗	Neurological side effects/ toxicity Bronchospasm	Damage bacterial cell membrane	£32.40 (generic) £204.00 (Promixin)	Licensed	Colomycin™ Promixin™
Colomycin Inhalation Powder (CIP)	DPI device	1.6 million IU twice daily	✓	✗	✓	✓	✓	✗	Neurotoxicity Cough Bronchospasm	Damage bacterial cell membrane	£968.80	Licensed	Colobreathe™
Tobramycin Inhalation Solution (TIS)	Jet or mesh nebuliser	300mg twice daily	✓	✗	✗	✗	✓	✓	Ototoxicity Nephro-toxicity Cough Bronchospasm	Bactericidal via altered bacterial cell membrane permeability	£780.00 (Tymbrineb™) £1187.00 (Bramitob™) £1305.92 (TOBI™/ Vantobra™)	Licensed	Bramitob™ TOBI™ Vantobra™ Tymbrineb™
Tobramycin Inhalation Powder (TIP)	DPI device	112mg twice daily	✓	✗	✗	✗	✓	✓	Ototoxicity Nephrotoxicity Cough Bronchospasm	Bactericidal via altered bacterial cell membrane permeability	£1790.00	Licensed	TOBI Podhaler™
Aztreonam Lysine solution	Mesh E-flow nebuliser	75mg thrice daily	✓	✓	✗	✗	✓	✗	Cough Bronchospasm Rash Arthralgia Pyrexia	Binds to bacterial penicillin-binding proteins leading to inhibition of bacterial cell wall synthesis	£2181.53	Licensed	AZLI™ Cayston™

Levofloxacin Inhalation Solution	Mesh E-flow nebuliser	240mg twice daily	✓	✓	✓	✓	✓	✓	Anorexia Dysgeusia Cough GI side effects Tinnitus Rash Pyrexia Arthralgia Tendinitis	Inhibition of bacterial DNA gyrase and topoisomerase IV enzymes	£2181.53	Licensed in UK, Europe and Canada	Quinsair™ Aeroquin™
Liposomal Amikacin Solution	Mesh E-flow nebuliser	560mg once daily	✓	✓	✗	✗	✓	✓	Ototoxicity Nephro-toxicity Cough Bronchospasm	Inhibits bacterial protein synthesis	Not yet determined	Treatment of NTM (US only)	Arikace™
Vancomycin inhalation powder	DPI device	30mg twice daily	✗	✗	✗	✗	✗	✓	Nephrotoxicity	Inhibits bacterial cell wall synthesis	Not yet determined	Phase 2 complete; Phase 3 underway	Aerovanc™
Ciprofloxacin DPI	DPI device	32.5mg twice daily	✓	✗	✗	✗	✓	✗	Nephrotoxicity Arthralgia Tendonitis	Inhibition of bacterial DNA gyrase and topoisomerase IV enzymes	Not applicable	Phase 2 complete, no phase 3 planned	

*Adverse effects shown are those listed as very common or common on www.medicines.org.uk/emc

**Listed UK index price per month for the National Health Service (NHS)

Table 2.

Fig 3a.

Fig. 3b

Fig4.

Therapy	Effect on PEx	Ref	Effect on P.aeruginosa (Pa)	Ref	Effect on other bacteria	Ref
CFTR Modulator/s						
Ivacaftor	Significant reduction in PEx and need for hospitalisation in clinical trials, and over 5 year long term real world follow up	[103]	Dose dependent reduction in Pa growth in vitro	[109]	Bacteriostatic effects on S.aureus, and bactericidal effects on Streptococcus spp. in vitro, synergistic with Tobramycin	[110]
			Synergistic activity with polymixin B against Pa in vitro	[111]		
			Significant reduction and delay in acquisition of new Pa in those on long-term therapy	[104, 105, 112]	Significant delay in acquisition of S.aureus and MRSA over three year follow up	[105]
			Significant reduction in Pa density; within 7 days of starting therapy. Increase in density seen after ≥210 days treatment	[9, 104, 112, 113]	Decline in total bacterial density in sputum culture and in relative abundance of BCC within first week of treatment	[9]
			Significant increase in clearance of Pa in those with chronic growth	[104 112, 113]	Reduced prevalence of S.aureus and Aspergillus Fumigatus; no effect on BCC in those on long term therapy	[104]
			Reduction in relative abundance of Pa over first year of therapy, then increase with ongoing treatment	[9]	Increase in microbial richness and diversity over first year of therapy, then decrease with ongoing treatment	[9]
Lumacaftor and Ivacaftor	Significant reduction in PEx in phase 3 trials, sustained over a 96 week open label extension	[114]	Synergistic activity with polymixin B against Pa in vitro	[111]	Significant delay in acquisition of S.aureus and MRSA over three year follow up	[105]
	Reduction in PEx frequency is independent of effect on ppFEV ₁	[115]	Significant delay in acquisition of new Pa over three year follow up	[105]		
Tezacaftor and Ivacaftor	Significant reduction in PEx rate during 24 week phase three trial	[116]	No published data available		No published data available	
Triple combinations	No effect on PEx rate in 4 week VX16-445-001 phase 2 study	[117]	No published data available		No published data available	

	No effect on PEx rate in VX16-659-101 8 week cross-over phase 2 study	[118]				
Anti-Inflammatory agents						
Lenabasum	Increase in time to, and reduction in PEx rate in phase 2a study (n=85) (NCT03451045) Larger phase 2b trial underway (n=415), with rate of PEx as primary outcome (NCT02465450)		Reduction in broncho-alveolar lavage PA density, and improved survival in Pa infected mice treated with Lenabasum compared to wild type (NACFC abstract presentation 2018)		No published data available	
Acebilustat	Increase in participants without a PEx, and reduction in risk of PEx in treatment group compared to placebo in phase 2 trial (NCT02443688)	[119]	No change in total bacterial sputum load in phase 1 trial		No change in total bacterial sputum load in phase 1 trial	

Table 5.