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# Current and Novel Antimicrobial Approaches

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## Abstract

Antibiotics are the mainstay of treatment for pulmonary disease in CF. Along with improved airway clearance techniques and nutrition, the availability (and aggressive use) of effective antimicrobials has been a major contributing factor in the improved health and prognosis of patients today. However, significant problems still exist: bacteria develop resistance, either via genetic mutation or biofilm formation; the huge numbers of antibiotics administered to patients pose a burden on them, both in terms of time and toxicity; and most of the current strategies treat rather than prevent infection. For all these reasons, new approaches would be highly desirable. This chapter will review recent developments in conventional antimicrobial approaches and go on to highlight some of the research areas with promise.

## Conventional Antimicrobial Treatment

Antibiotics are used in four distinct situations in patients with CF: (a) long-term treatment for *Staphylococcus aureus*, either prophylactically or after infection, (b) in an attempt to eradicate early *Pseudomonas aeruginosa* infection, (c) as suppressive therapy in chronic pseudomonal infection and (d) as acute treatment for respiratory exacerbations. For each of these scenarios, there remain unanswered questions, with centres making many clinical management decisions in the absence of clear-cut evidence. Some of these are highlighted in table 1. The background to conventional treatment regimen has been well reviewed [1],

**Table 1.** Areas of uncertainty in conventional clinical management

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Anti-staphylococcal drugs
Should anti-staphylococcal prophylactic antibiotics be initiated from diagnosis?
Is the risk of <i>P. aeruginosa</i> increased with all long-term anti-staphylococcal antibiotics, or only cephalosporins?
What is the optimal treatment regimen and when should treatment be stopped in an uninfected child?
Eradication of early <i>P. aeruginosa</i> infection
What is the optimal drug regimen and duration of administration?
Chronic suppressive anti-pseudomonal therapy
Is there a superior nebulized antibiotic?
Will the development of aminoglycoside resistance matter in the longer-term?
Treatment of multiresistant organisms
Would the availability of synergy testing improve outcome?

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and this chapter will largely focus on advances over the last 5–8 years.

### *Anti-Staphylococcal Antibiotics*

As discussed in previous chapters, *S. aureus* is often the first bacterium to be cultured from the respiratory tract in infants and children with CF. Several studies have addressed the benefits and risks of the early initiation of anti-staphylococcal prophylactic antibiotics, some of which were included in a systematic review in 2003 [2]. A major problem with interpretation of the data is the extreme heterogeneity in study design, indications for starting drugs (at

the time of diagnosis or only after detection of the pathogen), choice of drug and outcome measures. Conclusions on the benefit of prophylaxis could not be reached from this meta-analysis, and although treatment did reduce the rate of positive cultures, clinical benefit could not be determined with certainty in those already infected with the organism. More recently, a large retrospective review of German patients on the European database was reported by Ratjen et al. [3]. Of 693 children who were *P. aeruginosa* negative, 48.2 and 40.4% had received anti-staphylococcal treatment, either continuously or intermittently, respectively. Continuous treatment reduced the risk of *S. aureus* culture positivity, but worryingly, appeared to be associated with an increased frequency of *P. aeruginosa* infection. Continuous treatment did not confer clinical benefit over intermittent use. Importantly, unlike in the UK, where flucloxacillin is the drug used most commonly, almost half the patients had been treated with an oral cephalosporin, and only 4.6% with flucloxacillin. Another study has raised concerns over the use of cephalosporins in this context, pseudomonal infection being more common in children receiving long-term cephalexin as part of a randomized, placebo-controlled trial [4]. This may be a concern only for this group of drugs therefore, although insufficient data are available on other drugs. Several reports have been published recently of linezolid, a new, potent anti-staphylococcal antibiotic, which has been used with some success for methicillin-resistant *Staphylococcus aureus* (MRSA) [5, 6].

#### *Eradication of Early P. aeruginosa Infection*

Initial infecting strains of *P. aeruginosa* are usually non-mucoid, antibiotic susceptible and thus more amenable to treatment than their mucoid counterparts, which appear with chronic infection and form biofilms offering further protection against clearance (see chapter 18). There are accumulating data in support of the benefits of early detection and aggressive treatment, to prevent or delay the onset of chronic infection. Recent data demonstrating significant deterioration in lung function after the bacteria have become mucoid [7] lend further support to attempts to eradicate early. Pioneered by the Danish clinics following the success of a small randomized, controlled trial in the early 1990s [8], many studies since have been uncontrolled, limiting interpretation of the data. The same group has recently reported longer-term results, demonstrating that their strategy of combined oral ciprofloxacin and nebulized colomycin treatment prevented or delayed chronic infection in over 75% of patients during the 3.5-year follow-up [9]. Eradication has also been reported with a combination of intravenous and nebulized agents [10]. The duration of

eradication was between 2 months and 2 years, and interestingly, the majority of patients presented with a genetically distinct organism. Gibson et al. [11] have reported a significant rate of eradication with inhaled tobramycin, which led to early termination of their trial. After 28 days treatment, 0/8 children had positive *P. aeruginosa* cultures from broncho-alveolar lavage (BAL), compared with clearance in only 1/13 of the placebo group. A multicentre study of the use of tobramycin for inhalation (TOBI™) has started in Europe. It seems clear despite the limited amount of data from randomized, controlled trials that eradication can be successfully achieved after early infection. What remains less clear is the best type of therapy and the duration for which it should be administered.

#### *Chronic Suppressive Therapy*

Nebulized antibiotics, most commonly the polymixin, colomycin, have been used in this context for many years in European centres. Although individual studies were small, a published meta-analysis confirmed benefit with very few side effects [12] and the approach has been the subject of a recent Cochrane Review [13]. Recent trials have demonstrated clinical benefit with long-term nebulized tobramycin, although the induction of resistance means that the drug is best used only on alternate months [14]. Resistance does not seem to translate into lack of clinical efficacy, although whether this will remain so in the longer term is unknown. Systemic absorption is low, limiting the risk of toxicity. No good trial has compared different nebulized drugs. One study compared colomycin with tobramycin over 1 month (not a complete month on, month off TOBI cycle), demonstrating a significant improvement in lung function with the latter drug only [15]. However, all patients were already on colomycin, which is likely to have affected the results. A retrospective study showing that inhaled tobramycin was associated with an increased risk of death was almost certainly influenced by patient selection, the more severely affected patients receiving treatment [16]. One significant disadvantage of the nebulized route is the time taken to administer treatment, which can be up to an hour daily. Alternatives to inhaled antibiotics include the long-term use of macrolides (see chapter 25) and regular 3-monthly courses of intravenous antibiotics. These approaches can be combined. The benefit of the latter approach has not been demonstrated for the majority of patients [17]

#### *Acute Treatment for Pulmonary Exacerbations*

Intermittent treatment for exacerbations is most commonly administered as either short courses of oral or

intravenous antibiotics. Decisions must be guided by microbiological sensitivities, although in vitro susceptibility testing does not always predict clinical response [18].

In general, two intravenous antibiotics with different mechanisms of action (e.g. a  $\beta$ -lactam and an aminoglycoside) will be used in combination. There are few recent advances or changes to conventional management, which has been recently reviewed [1]. However, one such change is to once-daily high dose (as opposed to three times daily) intravenous aminoglycosides [19–21]. This regimen achieves a higher peak (upon which bacterial killing is dependent) whilst providing a longer period for the post-antibiotic effects of the drug [22]. There does not appear to be an increased risk of toxicity, although levels must be monitored routinely. Multi-resistant organisms, such as *Burkholderia cepacia*, can pose a serious management problem. Synergy testing may reveal a combination of useful antibiotics, despite lack of in vitro sensitivity to each of the agents individually, and may be helpful in certain cases [23]. However, this is not widely available and the impact of such a service on clinical outcomes has not been determined.

## Novel Treatments

It will be clear from the above that there are significant limitations to current anti-infective approaches: the development of resistance to conventional antibiotics; the reduced efficacy of most drugs on chronic, mucoid *P. aeruginosa* growing in biofilms; the burden of treatment to the patient; and the risk of toxicity with certain intravenous antibiotics, which necessitates invasive monitoring. Furthermore, there are no treatments that have conclusively been shown to prevent initial infection, in particular with *P. aeruginosa*. Clearly, new strategies are needed. The next section of this chapter will discuss the possible future approaches to preventing and treating infection in the CF lung, some of which may eventually become clinically useful. Much research is beginning to focus on genomic- and proteomic-based strategies [24–26] made possible by the recent mapping of the *Pseudomonas* genome ([www.pseudomonas.com](http://www.pseudomonas.com)).

### Immunization

*Active Immunization.* One of the paradoxes of CF pathophysiology is that despite the exuberant immune response mounted by CF patients upon infection with *P. aeruginosa*, it is insufficient to clear the infection. One suggestion is that this reflects low affinity or effector functions of natural

antibodies to various bacterial components. Advocates of active immunization argue that vaccines (particularly conjugates) could generate more effective immune responses and prevent early infection. The subject has been the focus of a recent Cochrane Review [27]. Preclinical animal studies have demonstrated enhanced clearance of *P. aeruginosa* from the lungs of mice and increased survival following immunization against a variety of epitopes, in particular components of lipopolysaccharide (LPS) [reviewed in ref. 28]. The earliest human studies involved vaccines with unacceptable side effects; the progress from these to more recent studies has been well reviewed [29], and the safety concerns appear, at least in part, to have been resolved in human studies [30, 31]. In targeting LPS, one problem is the wide variation between strains of the O-antigen, which would lead to a narrow spectrum of protection. In an attempt to combat this, Lang et al. [32] developed a conjugate vaccine comprising an octavalent O-polysaccharide and an exotoxin A. They reported short-term safety and immunogenicity of the vaccine in CF children without prior *P. aeruginosa* infection. Over the next 10 years, the patients have received annual immunization, and have recently been compared with controls, who have received otherwise identical management, although this follow-up was not conducted in a blinded or placebo-controlled fashion. Fewer immunized patients were infected, and in fewer of these had the organism converted to a mucoid phenotype, leading to better preservation of lung function. The vaccine has since been granted Orphan Drug status and is being evaluated in a large multicentre European trial ([http://www.bernabiotech.com/news/archive/news\\_full/id.25/](http://www.bernabiotech.com/news/archive/news_full/id.25/)).

*Passive Immunization.* Kollberg et al. [33] administered IgY (derived from the yolks of *P. aeruginosa*-immunized hens) as a topical gargle in a small, open-label study. CF patients had their first isolate of *P. aeruginosa* eradicated with conventional antibiotics, after which the IgY was administered. The treatment was well tolerated, and compared to a non-treated group, a second positive culture was significantly delayed. A similar approach has been employed for other infective diseases, such as gingivitis and enteric infections, although, clearly larger, controlled studies will be required before benefit is confirmed in CF. Monoclonal antibodies derived from transgenic mice are another route under investigation, conferring complete protection from fatal infection in neutropenic mice [34].

### Preventing Adherence

Although organisms such as *P. aeruginosa* have been shown to adhere in increased numbers to CF epithelia [35],

the relevance of this observation to disease pathogenesis is unclear, and it is in fact unclear whether the bacteria adhere to the mucosa or the mucus in the lumen. Strategies aimed directly at the cell surface receptor, a disaccharide moiety contained within asialylated glycolipids, are unlikely to be successful; *in vitro* studies have demonstrated a prompt immune response to ligation of this receptor with antibody as well as bacterial components [36]. Non-specific strategies may, however, be more applicable. Sajjan et al. [37] demonstrated that either dextran or xylitol significantly decreased the adherence of *B. cepacia* to explanted tracheas, possibly by removing or thinning surface mucus. The former agent has also been shown to improve mucus clearance [38], which could be an additional clinical benefit. Heparin, which has been administered topically to the human airway, may also possess this ability [39], in addition to its postulated anti-inflammatory or anti-allergic properties [40]. As discussed earlier in this book, the mechanism of action of the macrolide group of antibiotics in CF is incompletely understood. Baumann et al. [41] have reported decreased adherence of *P. aeruginosa* to the buccal epithelium after treatment with azithromycin, which may be relevant in the early stages of oropharyngeal infection.

#### *Anti-Biofilm Strategies*

As discussed in chapter 18, biofilm formation is a survival strategy employed by many bacteria, most importantly, in the context of the CF airway *P. aeruginosa*. In addition to the physical barrier posed by the biofilm matrix, a number of other properties render these organisms less susceptible to conventional antibiotics and many of the commonly used drugs (e.g. aminoglycosides and  $\beta$ -lactams) are poorly effective on slow-growing organisms [42]. The steps in the initiation and progression of biofilm formation are becoming clearer [43, 44], further understanding of which may lead to novel treatment approaches. Early infection occurs with low numbers of planktonic bacteria. These bacteria employ a mechanism of ‘quorum sensing’ via the production of freely diffusible compounds, acyl-homoserine lactones (AHLs), which have been detected in CF sputum [45]. The concentration of these rises with increases in bacterial numbers, and at high concentration induce changes in gene expression leading to a switch from a planktonic to a biofilm mode of growth. Bacteria, which have previously been motile, become sessile and dormant. Synthetic furanones, compounds able to inhibit AHLs, were highly protective in a murine model of *P. aeruginosa* pneumonia [46]. Rogan et al. [47] demonstrated that lactoferrin, a component of the innate immune system present in the lung,

prevents biofilm formation by enhancing ‘twitching motility’ and preventing the formation of the clusters required in the early stage of biofilm development. It achieves this by chelating iron, leading to the suggestion that this molecule or other iron chelators could be useful in preventing biofilm formation [48]. In the presence of mature biofilm, alternative approaches will be required. Alginate lyase [49], and the application of electrical currents [50] have both enhanced the effect of conventional antibiotics by facilitating diffusion through disrupted biofilm, although the clinical applications of the latter are unclear. Although the exact mechanism(s) by which macrolides achieve clinical benefit is unknown, they may be acting to inhibit formation of, or break down, biofilm [51].

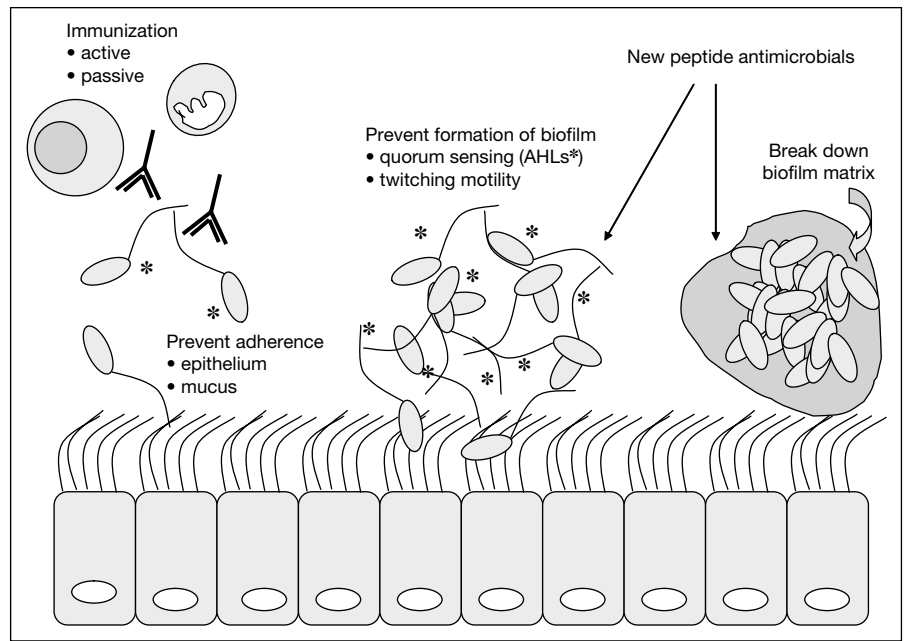
#### *Novel Antimicrobial Agents*

A full review of all microbial agents under development is outside the scope of this chapter. However, several groups of compounds merit a mention. Peptide antimicrobial agents, including defensins and cathelicidins are naturally occurring substances, produced by inflammatory cells and epithelia [52]. Although the suggestion that defensins fail to function in the CF airway because of abnormalities in salt concentration [53] is thought by most to be incorrect [54], the development of defensin-like molecules may hold some promise. They are broad spectrum and do not induce resistance, two highly desirable properties for CF infections. Analogues have reached the clinical trial stage for other conditions [55]. Histatins, similar peptides produced by salivary glands, have potent *in vitro* anti-pseudomonal activity, although certain of these are inhibited by purulent CF sputum [56]. Cathelicidins were reported to have rapid *in vitro* bactericidal properties, and importantly were also effective on multiresistant organisms such as *B. cepacia* [57].

#### *Dry-Powder Antibiotics*

Although nebulized antibiotics are effective, disadvantages include the time required for administration, maintenance of the equipment and environmental contamination. The development of dry powder formulations would overcome all of these issues. A small, proof-of-principle study has been published using a dry powder device with colistin sulphate [58]. CF patients, unlike their healthy counterparts experienced moderately severe cough and some deterioration in lung function. Colistemetate sodium, which is thought to be less irritant to the airway has also been formulated for a dry powder device and was confirmed to be safe and well tolerated in adults and children with CF [59]. Phase III trials are planned. Other antibiotics, such as

**Fig. 1.** Novel antimicrobial approaches. Investigators have considered strategies at all stages of the infective process as targets for new treatments, including prevention (vaccination and anti-adherence agents), prevention of chronic, biofilm infection (inhibiting signalling molecules or encouraging twitching motility), breakdown of biofilm matrix and novel synthetic peptides based on naturally occurring molecules, such as defensins.



aztreonam are also under development. Advances could provide significant quality of life benefits to CF patients with respect to the burden of current treatment.

Which, and how many, of the approaches mentioned above will make it through to clinically applicable treat-

ments remains to be seen. In the meantime, more well-conducted clinical research to answer the outstanding questions on basic treatment regimens and to lead to a consensus on management would be extremely useful.

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