Staphylococcus aureus in cystic fibrosis: pivotal role or bit part actor?

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Abstract

Purpose of review: describe why this review is timely and relevant.
The cystic fibrosis lung has long been appreciated as a competitive niche for complex interactions between bacterial species. The individual relationships between effects on the host, and thereafter clinical outcomes, has been poorly understood. We aim to describe the role of *Staphyloccus aureus*, one of the most commonly encountered bacteria cultured from the respiratory tracts of people with CF, and it's complex interplay with other organisms, with particular attention to *Pseudomonas aeruginosa*.

Recent findings: describe the main themes in the literature covered by the article.
We describe the challenges posed in understanding the role that *S. aureus* plays in the CF lung, including the difficulties in interpreting culture results depending upon sampling technique, relationships with *P. aeruginosa* and the rest of the microbiome, as well as discussing the relative merits and potential harms of antibiotic prophylaxis. Finally, we describe the particular challenge of methicillin-resistant *S. aureus*.

Summary: describe the implications of the findings for clinical practice or research.
We describe research underway that will address the long-held contentious issues of antibiotic prophylaxis. We also describe the emerging research interest in determining whether, at differences phases in the evolution of CF airways infection, *S. aureus* infection can have both harmful and protective effects for the host.

Keywords:
Staphylococcus aureus and MRSA
Interbacterial relationships
Respiratory sampling
Antibiotic prophylaxis

Introduction
Shortly after cystic fibrosis (CF) was first described, by Dorothy Anderson, *Staphylococcus aureus* was recognised to be an important respiratory pathogen. Dorothy Anderson[1] remarked that:

"The bronchial secretion is viscous and may be abundant… Cultures taken early in the course of the disease grow Staph. aureus hemolyticus in nearly every case… A mixed flora is sometimes present in cases with a chronic bronchiectasis, with pyocyaneus as a common associate of the staphylococcus."

The term "pyocyaneus" was often used, at the time, to refer to *Pseudomonas aeruginosa*. Hence, since CF care was in its infancy, it has been recognised that *S. aureus* is a common pathogen, contributing to CF lung disease and that this organism is associated with *P. aeruginosa* infection. So, almost 70 years later, do these propositions still hold true and what are the practical implications for antibiotic treatment? The purpose of this article is to provide a critical review of literature published in the last few years, which describes the role of *S. aureus* and how the organism might interact with other players in the microbiological drama which unfolds in the CF airway. Earlier literature will also be cited, when relevant.

What specimen to send for microbiological testing?
In paediatric practice, many patients have minimal bronchiectasis and so do not produce sputum. Young children who do produce sputum may not expectorate it. In this age group, other approaches must be taken to obtain a respiratory specimen. The most commonly used is the oropharyngeal swab. Sometimes the child is asked to cough as the swab is taken ("cough swab") in an attempt to improve the yield of true lower respiratory organisms. The diagnostic accuracy of oropharyngeal swabs has been compared with the "gold standard" of bronchoalveolar lavage (BAL). In a study which included 119 children under 18 months, with paired oropharyngeal and BAL samples[2], Rosenfeld and colleagues found that the positive predictive value (PPV) of oropharyngeal swabs was poor for both *S. aureus* (64%) and *P. aeruginosa* (44%). The PPV is the likelihood of a positive oropharyngeal swab indicating true lower respiratory infection. The negative predictive value (the likelihood of a negative oropharyngeal swab indicating the absence of lower respiratory infection) was better - *S. aureus* (88%) and *P. aeruginosa* (95%). Similar negative predictive values have been found when the oropharyngeal sample has been collected using suction[3]*. A negative oropharyngeal sample may therefore help rule out significant lower respiratory infection.

Bronchoscopy and BAL require a general anaesthetic, in most cases. Is there a way to improve diagnostic yield without having to resort to this procedure? A recent paper by Ronchetti *et al* [4]** compared sputum induction in children (using nebulised 7% sodium chloride) with cough swab, single-lobe, two-lobe, and six-lobe BAL. They recruited 124 children (6 months - 18 years) and found sputum induction had a high technical success rate (84% of procedures yielded mucoid sputum) – though oral suction was usually required in the children under 6 years. The induced sputum sample was more likely to be pathogen positive than the cough swab, whether or not the child was symptomatic. Sputum induction was comparable to the 2 lobe BAL, with 69% of pathogens recovered from the induced sputum *vs.* 72% on two-lobe BAL. The results of induced sputum in young children are therefore promising,

although agreed standard operating procedures will be needed, if the technical success rates of the procedure are to be reproduced in other paediatric CF centres.

What is normal flora?

The historical reliance on oropharyngeal swabs, leads to difficulty in distinguishing between pathogens and normal upper airways flora in young children. One study found that, in healthy infants under 12 months, 27% of oropharyngeal cultures were positive for *S. aureus vs.* 28% in CF infants[5]. A U.S. study of healthy infants, having a routine procedure under sedation or anaesthesia, found *S. aureus* was found in oropharyngeal swabs from 48% of children[6] and a study from the Netherlands found a rate of 36%[7].

In children with CF, the prevalence of *S. aureus* in the upper respiratory tract varies greatly with country of origin. In the US, the prevalence of *S. aureus* infection, on at least on occasion over 12 months, was 71% in 2015[8]. In the UK, the figure is 30% (including both chronic and intermittent infection) for children and 35% for adults[9]. The disparity is even greater when these registry data are compared for methicillin resistant *Staphylococcus aureus* (MRSA) where the prevalence in the U.S. is 26%[8] vs. 2-3% in the U.K.[9]. The reasons for this disparity are unclear but acquisition of MRSA has been linked to a higher prevalence of MRSA in the patient's CF Centre[10*] and to environmental factors such as air pollution, due to fine particulate matter[11*].

How does *S. aureus* interact with *P. aeruginosa*?

Much recent work – both in the laboratory and in the clinic – has focused on *S. aureus* as part of an ensemble cast of other microorganisms. Of particular interest is the interaction between *S. aureus* and *P. aeruginosa*. This interaction appears to begin with conventional microbial competition. In a recent study of U.S. registry data, the presence of sensitive strains of *S. aureus* made subsequent *P. aeruginosa* infection less likely[12*].

However, there is evidence that competition may be followed by co-operation. A mouse model of chronic lung infection using bacteria embedded within agar beads, first infected mice with *S. aureus* and subsequently with *P. aeruginosa* [13]. In this model, *S. aureus* was a pathogen in its own right and formed lung abscesses. However, *S. aureus* infection also made subsequent infection with *P. aeruginosa* more likely. *P. aeruginosa* may render *S. aureus* better able to survive in an environmental niche, such as the CF lung. A recent paper by Orazi and colleagues has shown that, when these organisms are grown in co-culture, *P. aeruginosa* promotes *S. aureus* resistance to vancomycin[14*]. The investigators propose that, when the organisms grow together, *P. aeruginosa* causes *S. aureus* to shift to fermentative growth this in turn leads to decreased susceptibility to antibiotics targeting the bacterial cell wall (such as vancomycin). However, the interaction is complex. *P. aeruginosa* isolates, from CF patients, can either reduce or enhance vancomycin killing of *S. aureus*[15*]. The same is true for tobramycin killing of *S. aureus* whereas most *P. aeruginosa* isolates will inhibit killing of *S. aureus* by ciprofloxacin. *P. aeruginosa* further aids and abets *S. aureus* by encouraging the formation of *S. aureus* small colony variants[16*]. These are phenotypic forms of *S. aureus* which are not detected using conventional culture techniques; are more antibiotic resistant; can sustain chronic infection and can survive intracellularly[16*]. However, a recent report has questioned the clinical importance of small colony

variants in children[17]. In a single centre where prophylaxis was used and where lower respiratory specimens were examined carefully for small colony variants – none were found. Finally, the production of alginate by the mucoid form of *P. aeruginosa* (characteristic of chronic infection in the CF airway) inhibits pseudomonas killing of *S. aureus*[18].

Laboratory studies suggest that, when *S. aureus* returns the favour by assisting *P. aeruginosa,* then *S. aureus* makes the ultimate sacrifice. *P. aeruginosa*, like many other pathogens, needs iron. The host's innate immunity (e.g. lactoferrin) jealously guards the host's iron stores. *P. aeruginosa* acquires iron by lysing *S. aureus*[19]. Furthermore, the DNA released by dead *S. aureus* contributes to the formation of a mixed-species biofilm comprising both *P. aeruginosa* and *S. aureus*[20]. This mixed-species biofilm is also important in conferring resistance to aminoglycosides such as tobramycin[21].

What does the interaction between *S. aureus* and *P. aeruginosa* mean for those with CF?

The implications of co-infection have been evaluated in a number of clinical studies. Patients with CF related diabetes (CFRD) are more likely to be coinfected with *S. aureus* and *P. aeruginosa*, than CF patients with normal glucose tolerance[22]. After adjustment for confounding, this dual infection was associated with decreased lung function and more frequent exacerbations of pulmonary symptoms. *S. aureus* small colony variants have been shown to be associated with a more rapid decline in lung function in children with CF, after adjusting for confounding variables[23]. In this clinical study, *S. aureus* small colony variants were associated with the use of trimethoprim-sulphamethoxazole and with co-infection with *P. aeruginosa.* In contrast, a clinical study by Hubert *et al* (both children and adults) suggested that the annual decline in lung function was significantly higher for MRSA-*P. aeruginosa* co-infection only and not for co-infection with sensitive strains of *S. aureus*[24]*.* Single infection with *S. aureus* is seen in younger patients with better lung function[25].

***S. aureus* and the microbiota.**

More than a decade ago, culture independent methods of bacterial identification, such as 16S rDNA amplification, showed that a much greater number of microorganisms could be demonstrated in the CF lung than was possible with conventional culture. Of particular interest was the high prevalence of anaerobes (30%)[26]. However, the discovery of such a wide range of microorganisms in this environmental niche brought new problems. The use of 16S rDNA techniques simply demonstrates the presence of bacterial DNA, not viable organisms[27] and organisms identified in sputum may have originated from the lower airway or indeed the oral cavity[28]. However, recent studies of the upper airway microbiota in young infants with CF, confirm the importance of *S. aureus* in this age group, in comparison to a group of non-CF infants[29]*.

***S. aureus:* to treat of not to treat?**

This discussion of microbiological sampling, the significance (or otherwise) of positive bacterial isolates, interaction between *S. aureus* and *P. aeruginosa* (and its clinical implications) and the place of *S. aureus* amidst a wider microbiota serves to inform the discussion of when and whether to treat *S. aureus.* This will be considered under three headings: antistaphylococcal antibiotic prophylaxis; treatment of

sensitive strains of *S. aureus ad hoc*; and the treatment of respiratory infection with MRSA. Each of these categories will be considered in turn.

Antistaphylococcal antibiotic prophylaxis

The use of prophylactic antibiotics against *S. aureus,* from diagnosis by newborn screening until 3 years, is recommended in U.K. national guidelines[30]. In sharp contrast to this approach, guidelines in the U.S. recommend that antistaphylococcal antibiotic prophylaxis should not be used[31]. The AREST CF observational study has shown that isolation of *S. aureus, de novo* at 3 years, may predispose to bronchiectasis on chest CT and reduced FEF_{25-75}, at school age[32]. It is of note, that no such effect was seen with *de novo* isolation of *P. aeruginosa*. Whilst this may provide some support for the U.K. approach, these observational data do not prove causation. A recent, registry based study[33]* has compared outcomes in the U.K. and the U.S. - where policies on the use of prophylaxis are very different. These data show that, in the first 3 years of life, initial acquisition of *S. aureus* and *P. aeruginosa* occurs significantly earlier, in the U.S. than in the U.K. A surprising finding of the same study came from a subgroup analysis of the U.K. data which showed that many U.K. children were not receiving flucloxacillin (the recommended first line prophylactic antibiotic). Furthermore, those who were prescribed flucloxacillin were more likely to acquire *P. aeruginosa* during the first 3 years (hazard ratio 2.53; 95% CI 1.71, 3.74, p<0.001) whereas there was no reduction in *S. aureus*.

More robust conclusions regarding causation can be drawn from clinical trials. The Cochrane systematic review of clinical trials of antistaphylococcal antibiotic prophylaxis[34]* has recently been updated. The review includes 4 trials with 401 randomised CF participants and shows that significantly fewer children randomised to prophylaxis had one or more isolate of *S. aureus* during the study period. There was no difference between arms in lung function, nutrition, hospital admissions, additional courses of antibiotics or adverse effects. In the first 3 years of data, there was a trend towards a lower cumulative isolation rate of *P. aeruginosa* in the prophylaxis group. However, there was a trend towards a higher rate from 4-6 years. A prospective, multicentre randomized clinical trial is currently in progress which compares continuous flucloxacillin and “as required” antibiotic therapy in infants identified by newborn screening is underway in the U.K. (ISRCTN18130649).

Treatment of sensitive strains of *S. aureus ad hoc*

Although treatment of sensitive strains of S. aureus with a 2 week course of oral antibiotics is recommended in guidelines[30] and widely practiced, there is little evidence to support this. A retrospective review[35] describes the response to *ad hoc* treatment of *S. aureus* in the lower respiratory tract in the Copenhagen CF Centre (where prophylaxis is not used). The study describes an annual rate of *S. aureus* infection of 47% in a population of 300 patients. Patients received between 2 and 6 weeks of appropriate antibiotics. In 61% of cases the organism was eradicated on follow up (53% for the longer courses) and FEV_1 improved significantly over baseline (by a median of 3.3%).

In terms of chronic suppressive therapy for chronic infection with sensitive strains of *S. aureus*, a Cochrane review found no trials[36].

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Treatment of respiratory infection with MRSA

There is an emerging consensus that MRSA is an important pathogen in CF rather than simply a marker of severe disease[37]*. A recent case control study from Brazil[38] has shown that patients infected with MRSA have a greater respiratory impairment at the time of chronic infection and disease progression is more rapid in patients with MRSA compared to those with sensitive strains of *S. aureus*. CF patients with MRSA have also been shown to have worse CT appearances than those who are free of infection[39]. This consensus has led to a randomised controlled trial of MRSA eradication in CF – STAR-too[40]*. This trial randomised participants to a regimen of observation only or oral trimethoprim-sulphamethoxazole for 2 weeks. (If allergic to sulphamethoxazole, minocycline plus oral rifampicin was given.) Drug treatment was combined with nasal, skin and environmental decontamination. The primary outcome (MRSA status at day 28) was significantly better in the active treatment group (82% negative) than in controls (26% negative). However, there was a substantial recurrence of MRSA infection in both groups during follow up.

Conclusion

S. aureus has much more than a bit part in the evolution of airways infection in young patients with CF. Initially, the organism competes with other pathogens to defend its environmental niche and there is historical and laboratory evidence that *S. aureus* can cause significant lung damage while it is the dominant pathogen. In many cases, this phase of competition ends with *S. aureus* the loser. This is followed by co-existence with a new dominant pathogen – often *P. aeruginosa*. Whether antibiotic therapy can play a major part in influencing this microbiological turf war, to favour the host with CF, will be determined in part by ongoing randomised controlled trials.

- Respiratory sampling, and determining infection from normal flora, in young children with CF is challenging and poses difficulty to the treating clinician
- Complex interactions between *S. aureus* and *P. aeruginosa*, resulting in differential outcomes for bacterial survival, have been observed *in vivo*
- The relative merits and harms of approaches to early infection with *S. aureus* – prophylaxis or ad hoc, are being investigated in clinical trials
- MRSA is a harmful pathogen in CF early eradication is possible but treating chronic infection poses more of a challenge

Annotations

[3]* This study showed that the diagnostic accuracy of oropharyngeal suction was similar to “oropharyngeal swabs” although the procedure can cause distress in younger children.

[4]** The CF SPIT study demonstrated for the first time that a non-invasive technique (induced sputum with hypertonic saline) could achieve a similar diagnostic accuracy to bronchoscopy and lavage.

[10]* An important registry study, looking at the antecedents of MRSA infection. It showed that many are not amenable to change (pancreatic insufficiency and CF related diabetes) though others (such as the prevalence of MRSA at a CF centre) may be.

[11]* PM2.5 exposure was associated with a 68% increased risk of MRSA acquisition but not with an increased risk of acquiring sensitive strains of *S. aureus*, *Achromobacter xylosoxidans* or *Stenotrophomonas maltophilia*.

[12]* A large U.S. registry study which showed that *P. aeruginosa* infection was less likely to occur in patients first infected with *S. aureus*.
[14]* A laboratory study showing that *P. aeruginosa* encourages *S. aureus* to adopt the fermentative mode of growth and confers upon *S. aureus* resistance to vancomycin.
[15]* *P. aeruginosa* from CF and burn patients were cultured and supernatants added to *S. aureus* cultures to determine the effect on *S. aureus* resistance to vancomycin, ciprofloxacin and tobramycin.
[16]* Comprehensive review of *in vivo* and *in vitro* interactions between *P. aeruginosa* and *S. aureus.*
[29]* Meticulous study of the microbiota of CF and non-CF infants in the first 6 months of life.
[33]** Unique study of both U.S. and U.K. registry data showing earlier acquisition of *S. aureus* and *P. aeruginosa* in the U.S vs. the U.K. However flucloxacillin prophylaxis associated with earlier *P. aeruginosa* in the U.K.
[34]** Benchmark systematic review showing that antistaphylococcal antibiotic prophylaxis leads to fewer children having one or more isolates of *S. aureus*. No difference in other clinical outcomes.
[36]* Comprehensive review of risk factors for MRSA acquisition and treatment strategies.
[39]* Landmark randomised controlled trial, showing the microbiological effectiveness of an MRSA eradication regimen.

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