Title: Diagnosis and treatment of biofilm infections in children

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Article word count: 2276

# Structured Abstract

Purpose of review: Biofilm associated infections cause difficulties in the management of childhood chronic infections and other diseases, due to the invasive nature of interventions which are often necessary for definitive management. Despite their importance, there are challenges in diagnosing biofilm infections and gaps in clinicians’ understanding regarding the significance of biofilms.

Recent findings: Many chronic infections associated with biofilms remain difficult or impossible to eradicate with conventional therapy. Surgical intervention, implant removal or long term intermittent or suppressive antimicrobial therapy may be required. There are still significant challenges in detecting biofilms which presents a barrier in clinical practice and research. Novel therapies to disrupt biofilms are currently under investigation, which may help reduce the impact of antimicrobial resistance.

Summary: Biofilm associated infection should be considered wherever there is clinical concern for an infection affecting prosthetic material, where there is a predisposing condition such as suppurative lung disease; or in the setting of chronic or relapsing infections which may be culture negative. New diagnostic methods for detecting biofilms are a research priority for both clinical diagnosis and the ability to conduct high quality clinical trials of novel anti-biofilm interventions.

Keywords: Biofilms, Paediatrics, Cystic Fibrosis, CLABSI, Antimicrobial resistance

# Introduction

This review will introduce fundamental concepts about the nature and significance of bacterial biofilms, their role in childhood disease, and how they can be diagnosed and treated. The focus is on recent developments in the understanding of the role of biofilms in chronic childhood infections, current barriers to the effective diagnosis and clinical research of biofilms, and novel antibiofilm treatments currently under investigation.

# What is a biofilm?

A biofilm is a complex microbial community which may be formed from single, or multiple species of microbes, encased in an extra-cellular polymeric substance (EPS) comprised of material from both the pathogen and the host 1. The EPS is formed from polysaccharides, proteins, lipids and nucleic acids, with polymers forming a scaffold holding its structure in place 2. The life cycle of a biofilm includes several parts. Planktonic cells attach to a surface within the body (which may be host or prosthetic material), at first in a reversible manner. This association then becomes irreversible, leading to colony formation on the surface3. Complex signalling and quorum sensing takes place between bacteria, leading to maturation and stabilisation of a biofilm. Following this, there are episodes of discrete dispersal, where by microbes residing on the outer surface of the biofilm are released, leading to colonisation of adjacent or distant surfaces and, “metastasis”, of infection 4**.**

Microbes within biofilms are physiologically and metabolically different from their planktonic (“free-living”) counterparts. The existence of a biofilm confers many survival advantages, including resistance to host immune response and in particular to antimicrobial therapy. This is through a number of mechanisms including;

1. EPS providing a physical barrier against antimicrobials, cellular and humoral immunity.
2. Metabolic quiescence, making antimicrobials ineffective due to formation of, “persister cells”.
3. Facilitating survival of resistant phenotypes by exposure to sub-lethal concentrations of antimicrobials.
4. Horizontal gene transfer propagating further antibiotic resistance 5.

These factors combined make biofilm infections highly recalcitrant to standard antimicrobial therapy, and can make some infections impossible to eradicate completely. Biofilm disease therefore presents an important problem in paediatric or adult medicine.

# Biofilm infections in children

Biofilms are responsible for a significant number of infections in children, in particular chronic infections which are often refractory to conventional therapies. This is particularly problematic because traditional methods of definitive management of biofilm infections commonly involve prolonged, intensive antibiotic therapy, surgical interventions, or removal of indwelling devices. All of these interventions are more complicated and pose unique problems when dealing with young children.

## Cystic fibrosis and suppurative lung disease

One of the most well understood conditions involving biofilms in children is cystic fibrosis (CF). Other similar conditions include Primary Ciliary Dyskinesia (PCD) and non-CF bronchiectasis. All are characterised by defects in the innate immune function of the lungs, leading to an accumulation of hyperviscous mucous and subsequent infection 6. Lung infections in CF are commonly cause by *Staphylococcus aureus* earlier in childhood, and later characterised by infection with *Pseudomonas aeruginosa* 7**.** Infections with mucoid strains of *P. aeruginosa* in particular are known to form biofilms 8 9 10, which once established become impossible to eradicate 11. The presence of chronic infection leads to a pronounced antibody response and persistent inflammation, characterised by a proliferation of polymorphonuclear leukocytes and surrounding lung tissue damage, which is ultimately responsible for the majority of the accompanying morbidity and early mortality associated with the disease 12. New drugs which modulate the Cystic Fibrosis Transmembrane Receptor (CFTR) protein have been shown to delay, but not prevent infection with these pathogens13, suggesting that biofilm associated infections will remain a persistent problem despite advances in this field.

## Ear, nose and throat infections

Infections of the ear, nose and throat are extremely common throughout childhood, with acute otitis media (AOM) recognised as the most common cause of bacterial infection and the leading cause of antibiotic prescription 14. Recurrent AOM is common, and over 50% of cases of otitis media with effusion (OME) are thought to follow an episode of AOM. The role of biofilms in recurrent AOM and OME has been recognised for the past 20 years, initially prompted by the uncommon characteristics of OME being frequently culture negative despite the presence of pathogens on molecular processing, often unresponsive to antibiotics, and having a high rate of recurrence15. Evidence of biofilm forming phenotypes of nontypeable *H. influenza, S. pneumonia* and *M. catarrhalis* has now been demonstrated in a number of rodent and human models 16 17. Recent studies have also demonstrated the high proportion of biofilm forming strains of *H. influenza* and *S. pneumoniae* in the nasopharnyx of children with current AOM 18. This adds further weight to the theory of biofilm formation and planktonic shedding to the pathogenesis of OM and recurrent OM, with significantly increased amounts of biofilm having been previously demonstrated on the adenoid mucosa of children with recurrent OM, compared to those with obstructive sleep apnoea 19.

There is also evidence for the role of biofilms in tonsillitis, with both staphylococcal and pneumococcalbiofilms having been demonstrated in tonsillar tissue to varying degrees. Tonsilliths, soft aggregates of bacterial and cellular debris that form in the tonsillar crypts, have been described recently in more detail as actually being structurally heterogenous biofilms20. It is also known that *Streptococcus pyogenes* forms biofilms which may contribute to recurrent tonsillitis 21.

## Prosthetic device infections

Advances in biomaterials have produced implantable medical devices which have revolutionised the care of children (and adults) across many disciplines, from gastroenterology to neurosurgery. These devices however also increase risk of infection, as the presence of an abiotic material provides a surface devoid of normal immune barriers and protection to which bacteria can adhere, and subsequently colonise and form biofilms. The mechanism of bacterial adhesion to prosthetic material is complex, but is likely related to factors including the degree of hydrophobicity of cell membranes and the abiotic surface, and bacterial adhesins 22 23.

One of the most common implantable devices used in children are central venous access devices (CVADs), which provide a vital route of treatment for paediatric oncology patients, and nutrition for children with intestinal failure, among others 24. Central line associated blood stream infections (CLABSIs) are relatively common, occurring roughly every 0.8 per 1000 line days, or more commonly in-hospital up to every 3.9 per 1000 line days 25. This is extremely problematic for children who are highly dependent on their CVADs for medical care, as catheter removal may be necessary to reduce the risk of relapse, metastatic infection or persistent associated blood stream infection.

Another highly problematic device associated infection is of ventriculo-peritoneal (VP) shunts. Infection occurs at rates reported between 5% and 18% 26, with higher rates occurring in premature infants or post-haemorrhagic hydrocephalus 27. Shunts are commonly infected with coagulase negative *Staphylococci*, *S. aureus, P. aeruginosa,* all of which known to readily form biofilms on prosthetic material 28. As before, infections are highly problematic as treatment suffers a high recurrence rate and often involved removal of the shunt, with temporary insertion of an extra-ventricular drain 28.

# Diagnosing biofilms

The diagnosis of biofilms presents many challenges. At present there is no accepted standard for clinical microbiology laboratories to reliably detect biofilms in clinical samples. Due to their low level of metabolic activity, routine culture of biofilm infections often yields false negative results 17. Furthermore, routine culture and microscopy techniques cannot differentiate between planktonic and biofilm phenotypes, as traditional culture techniques will always grow bacteria in their planktonic form. Research techniques grow biofilm from such planktonic bacteria *in vitro* for future investigation, but this is slow and biofilms are not necessarily similar to the biofilm *in vivo*. More advanced molecular techniques such as polymerase chain reaction (PCR) are highly sensitive, but suffer from a higher degree of false positives, and are also unable to differentiate between biofilm and planktonic phenotypes of infection29.

Reliably diagnosing the presence of biofilms requires *in situ* visualisation of the biofilm in the sample. Advanced research methods such as fluorescent in-situ hybridisation (FISH) and confocal laser scanning microscopy (CLSM) take several days to process the sample and yield results, and are reliant on commercially available fluorescent probes 30. In clinical practice, clinicians therefore often rely on the detection of a bacteria which is known to form biofilms (such as mucoid strains of *P. aeruginosa* or *S. aureus*) and extrapolate the potential presence of a biofilm 31.

Current European (ESCMID) guidelines for the diagnosis of biofilm related infections recommend ideally visualisation through direct routine or fluorescent microscopy. However, limitations include frequent false negatives and this technique is not practical on most samples. Diagnosis otherwise relies on the clinical characteristics of infection and identification of a biofilm forming pathogen, without direct visualisation of the biofilm 32.

The difficulties with the direct detection of biofilms also greatly impacts on the ability to innovate new methods of treating or disrupting biofilms. To assess the response of interventions is prohibitively expensive, time consuming and requires expertise in cutting edge techniques such as FISH and CLSM. There is a clear need for new methods to detect and monitor biofilms, both in research and in clinical practice.

# Treating biofilms

The treatment of biofilms presents many challenges. Due to their structural and behavioural properties which make them tolerant and increasingly resistant to antimicrobial therapy, bacterial biofilms require 10 - 1000 times higher concentrations of antibiotics than are necessary to treat planktonic bacteria 33. Due to the metabolic quiescence of persister cells, it is common that following a course of treatment the infection will relapse, making chronicity one of the hallmarks of biofilm associated infections. This has different consequences depending on the site of infection.

Patients with CF are monitored frequently for potential chest infections, and are treated aggressively at any sign of persistent cough or worsening lung function with regular monitoring for the presence of *P. aeruginosa*. Treatment is often with inhaled colistin or tobramycin, but may include oral ciprofloxacin 34 despite the known problem of high rates of resistance. The reason for early, aggressive therapy is that in preliminary stages of infection, it may be possible to eradicate *P. aeruginosa* and remain clear for up to 2 years. However, this has not yet been shown to impact on morbidity and mortality, and no single antibiotic strategy has been demonstrated to be best 34. Once *P. aeruginosa* infections have become established in the airway and formed biofilms, they become impossible to eradicate completely. The mainstay of treatment becomes to suppress the infection and prevent, “exacerbations”, 35 caused by biofilm dispersal triggered by concurrent viral infection or other pro-inflammatory insults 36.

Chronic or relapsing ear, nose and throat infections which fail to respond adequately to first line treatments often signify the presence of a biofilm 37. These infections require regular washings via aural toilet, and exposure to high doses of local antibiotics delivered by ear drops as systemic antibiotics do not reach high enough concentrations to clear the infection 38. In some cases the definitive management of chronic suppurative otitis media is surgical, requiring mastoidectomy (particularly in the presence of cholesteatoma) 39.

Implant associated infections in children are particularly problematic, as the child may be dependent on their implant to sustain life (such as CVADs for enteral nutrition in intestinal failure). Due to their young age and small size, removal and replacement of implanted devices is considerably more complicated and difficult in infants and young children than in older children or adults. Although many clinicians will attempt to salvage lines with prolonged courses of antibiotics, formal Infectious Disease Society of America (IDSA) guidelines for management of central line infections recommend removal of any CVADs infected with *S. aureus*, *P. aeruginosa*, fungi or mycobacteria 40, so there is a need for improved methods of salvaging CVADs which have become infected. Many different methods have been introduced to disrupt biofilms in paediatric CVADs, including 70% Ethanol 41 and Taurolidine-citrate42, which show some efficacy in reducing rates of infection, but mixed results in line salvage therapy 43. A recent randomised trial of antibiotic impregnated central venous catheters failed to reduce rates of CVAD associated infections in neonates44.

Novel treatments for disrupting biofilms are under investigation. Very low dose nitric oxide has been demonstrated *in vitro* to cause dispersal of bacteria within biofilms into planktonic forms more readily treated with antibiotics, via a mechanism of increasing bacterial phosphodiesterase activity and thereby reducing levels of cyclic-di-guanosine monophosphate, an important biofilm regulator45. A recent proof of concept clinical trial suggests that adjunctive therapy with low dose nitric oxide may improve bacterial clearance in exacerbations of CF 30. Acetic acid is a weak organic acid with antimicrobial properties which has been proposed as a treatment for prosthetic implant biofilms, although difficulties remain in finding a suitable therapeutic window46. Phage therapy has come to prominence more recently in *in vitro* studies and case reports47 due to the increasing global threat of antimicrobial resistance. There is growing evidence to suggest the ability of bacteriophages to break open biofilms, with a potential role for them to be used synergistically with antibiotics and in particular increasing their efficacy at low concentrations 48.

# Conclusion

Biofilm infections are an important cause of recalcitrant and chronic infections in childhood. There is a current lack of clinical or research techniques to readily detect and quantify biofilms, which is contributing to the slow development of novel therapies to disrupt and treat biofilm infections in children and adults. Clinicians should maintain a high level of suspicion for the presence of biofilms in the presence of risk factors such as suppurative lung diseases, chronic ENT infections and prosthetic device associated infections, or in the setting of infections which prove resistant to conventional therapies or suffer frequent relapses despite an initial clinical response.

# Key points

* Biofilm infections in children should be suspected in the presence of chronic, recalcitrant or relapsing infections
* The presence of a biofilm may necessitate more aggressive antimicrobial therapy, surgical intervention or, if applicable, prosthetic device removal
* Current diagnostic techniques have low sensitivity for detecting biofilms
* Novel interventions, including nitric oxide, are under investigation to aid the treatment of biofilm associated infections

# Acknowledgements

None

# Financial support and sponsorship

SNF and AM are supported by the NIHR Southampton Clinical Research Facility. JSW and SNF are Chief investigator and co-investigator respectively for the UK BBSRC-Innovate UK funded National Biofilm Innovation Centre.

# Conflicts of interest

None

# Drug license statement

Low dose nitric oxide is unlicensed for the treatment of Pseudomonal biofilms in cystic fibrosis

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