

# Biofilms in Pediatric Respiratory and Related Infections

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Bacteria can grow as free-floating, planktonic bacteria or complex communities called biofilms. Biofilms promote bacterial growth and diversity and offer bacteria unique environments, including aerobic and anaerobic layers, that facilitate resistance to antimicrobial therapies. Respiratory and related structures provide ideal environments for the development of bacterial biofilms, which predispose patients to recurrent and chronic infections. Biofilms are important for the persistence of chronic rhinosinusitis, pulmonary infections in cystic fibrosis, chronic otitis media, and device-related infections. Antimicrobial therapy that is proven effective against planktonic bacteria is often insufficiently effective against the defenses of biofilms. Furthermore, biofilms modify themselves following exposure to antimicrobial therapy, thus developing increased resistance. Understanding the nature of biofilms in common pediatric infections is essential to comprehending the expected course of bacterial illness and identifying treatments that are most likely to be beneficial against more resistant biofilms.

## Introduction

Respiratory infections are common in pediatrics; the three most common pediatric diagnoses from outpatient visits are general examination, otitis media (OM), and upper respiratory infection [1]. Respiratory infections are common in all pediatric age groups, including the youngest patients. For example, a population-based survey reported that healthy infants experienced acute respiratory symptoms for an average of 3.5 months during their first year of life, with the average infant experiencing six episodes of acute respiratory illness and six episodes of simple rhinitis during that year [2]. Furthermore, the Asthma and Allergy Foundation of America reported that allergy is the third most common

chronic disease in children [3], and children with allergies are predisposed to developing upper respiratory tract infections, rhinosinusitis, and OM [4].

Bacterial biofilm development is increasingly recognized as a major factor in the development of recurring or chronic infections of respiratory and related structures in adult and pediatric patients. The unique characteristics of biofilms help to explain their persistence and antibiotic resistance. Appreciating the role of biofilms in the development and perpetuation of common pediatric respiratory and related illness is essential to understanding the expected clinical course of common pediatric infections and designing effective treatment options.

## Understanding Biofilms

Bacteria can exist in two forms: free-floating, individual planktonic bacteria or biofilms. Biofilms are complex communities made up of an extracellular glycocalyx matrix that often houses several species of bacteria and fungi. The biofilm creates a protective living environment for bacteria. Microcolonies of bacteria within the biofilm are separated by water channels that facilitate growth by allowing diffusion of nutrients and communication between distant layers within the biofilm community. Bacteria can develop within the biofilm, with the biofilm releasing free-swimming planktonic bacteria to seed new areas.

Biofilms are formed through several steps (Fig. 1). First, planktonic bacteria reversibly attach to an acceptable moist surface, where they divide and begin to form a colony. Later, bacterial attachment becomes irreversible as a glycocalyx matrix of water and macromolecules (including exopolysaccharides, proteins, and nucleic acids) forms a stable and protective dynamic structure for the embedded bacteria. As more bacteria aggregate into a microcolony, the production of signaling molecules increases until a threshold level is reached, activating a “quorum-sensing” mechanism that biofilm bacteria use to coordinate their activities, including glycocalyx production, bacteria division rate, and gene regulation. The fully developed biofilm is a complex community with integration of communication and development within the population. Superficial bacteria shed to seed new locations, promoting biofilm growth and perpetuation.

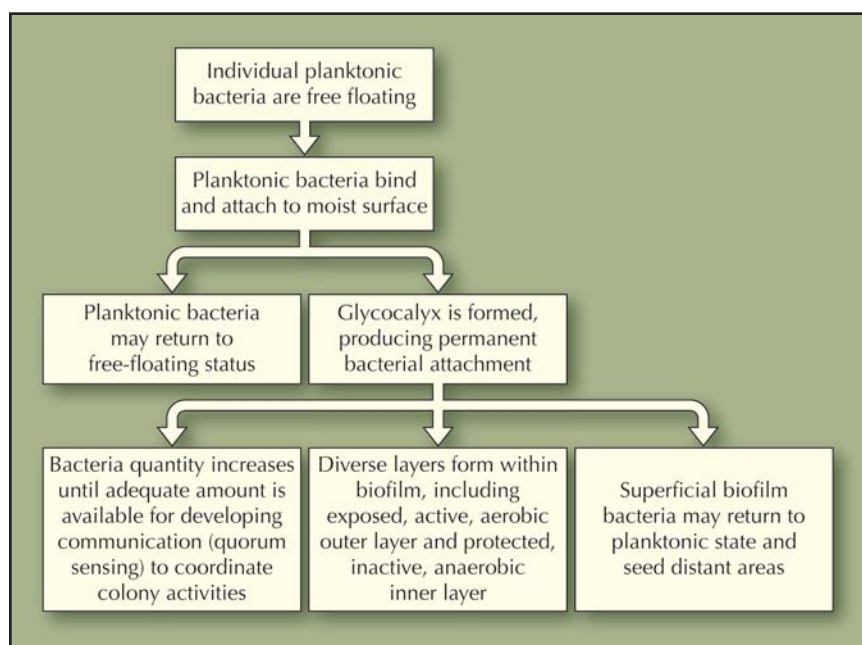


**Figure 1.** The development of biofilms. The ready conversion of planktonic bacteria to complex biofilm communities facilitates the development of bacterial diversity as well as infection persistence and recurrence.

Biofilms offer a variety of important survival advantages over planktonic bacteria. The complex structure within biofilms creates discrete cell layers with different living conditions. Metabolically active outer biofilm bacteria layers are exposed to higher concentrations of oxygen and nutrients; these active outer layers enclose an inactive, interior anaerobic cell layer. Biofilms are more resistant to antibiotics and can tolerate minimal inhibitory concentrations 10 to 1000 times higher than free-floating, planktonic bacteria [5]. Key antigens and ligands can become hidden within biofilm, effectively masking target sites to which antibiotics might bind. Antibiotics such as  $\beta$ -lactams, which target metabolically active cells, can only eradicate bacteria from the active outer layers of the biofilm. Deeper biofilm layers are better protected from a variety of antibacterial methods, including host immune mechanisms, detergents, and antimicrobial exposure [6,7]. Furthermore, biofilm water channels provide bacteria with ready access to nutrients and waste removal and also facilitate communication within and between biofilm layers. Genetic material can also be exchanged within the biofilm, increasing genetic diversity and thus improving chances for survival. For example, hypermutable organisms are better able to adapt to new pathologic niches, with hypermutable *Pseudomonas aeruginosa* typically identified in the airways of patients with cystic fibrosis (CF) during the later stages of infection [8]. These biofilm features can result in persistent and resistant infections.

### Biofilms in Pediatric Disease

The resistant and resilient nature of biofilms makes them important factors for recurrent and chronic infections in all age groups, including pediatric patients. Biofilms prefer moist environments, which makes respiratory pathways and related mucosal surfaces particularly attractive for

their development. Biofilms affect a variety of common pediatric infections, including those involving respiratory and related structures. Furthermore, biofilm-related upper airway infections may promote lower pulmonary infections and aggravate other chronic pulmonary conditions (eg, asthma) [9,10].

Oral structures such as the adenoids and tonsils may develop infections and serve as reservoirs for biofilms that seed upper respiratory infections. Chronic adenoiditis is recognized as a possible cause of chronic OM, with biofilms identified using confocal laser scanning microscopy in 22 of 39 samples (54%) of adenoid tissue from children with chronic OM [11]. A small study evaluating adenoids from 18 pediatric patients undergoing surgical treatment for recurrent upper airway infections with chronic effusive OM identified biofilms in each specimen; these biofilms were postulated to have acted as reservoirs of resistant bacteria [12]. In a larger study, bacteria were identified in 79% of adenoids removed from 410 children less than 14 years of age, most commonly *Haemophilus influenzae* (28.5%), *Streptococcus pneumoniae* (21.7%), *Streptococcus pyogenes* (21.0%), and *Staphylococcus aureus* (15.6%) [13]. Not only were pathogenic bacteria present, but the bacterial isolation rate increased significantly according to the grade of sinusitis identified. Adenoidal biofilms appear to be directly linked to infections, as biofilms are typically identified on adenoidal tissue removed from pediatric patients with chronic OM or chronic rhinosinusitis (CRS), but not from adenoids from pediatric patients with obstructive sleep apnea [14,15••]. The possible role of biofilm bacterial reservoirs in the tonsils in upper respiratory disease has been less well studied. In two uncontrolled studies, biofilms were also identified in 17 of the 24 tonsil samples from chronic tonsillitis patients in one study [11] and 11 of the 15 infected tonsils and 3 of the 4 hypertrophic tonsils in the other study [16].

### Rhinosinusitis

CRS is an inflammatory condition of the paranasal sinuses that features symptoms of nasal congestion, post-nasal drip, and malaise lasting for more than 3 months. Damaged mucosa provides excellent substrate for biofilm development [17]. Biofilms associated with CRS are typically polymicrobial, most commonly including *S. aureus*, *P. aeruginosa*, coagulase-negative staphylococci, *S. pneumoniae*, *Moraxella catarrhalis*, and *H. influenzae* [17]. Fungi are also frequently included. Biofilms generally develop in most tissue samples from patients with chronic sinusitis [18•]. For example, in one study, mucosal specimens were evaluated from surgical endoscopic samples from 12 patients with CRS and six control patients with obstructive sleep apnea [19]. Bacterial biofilms were identified in 83% of the samples collected from patients with CRS but none of the controls.

Deficiencies in the immune system have been postulated to predispose individuals to biofilm infections, such as CRS [20]. In an interesting study of adult CRS patients, nasal mucosal expression of lactoferrin was evaluated [20]. Lactoferrin is a glycoprotein with important antimicrobial properties that is found at high concentrations on mucosal surfaces. In this study evaluating biofilm growth and lactoferrin in 41 CRS patients and 21 healthy controls, lactoferrin expression was downregulated in CRS patients, with the greatest reduction evident in biofilm-positive CRS patients. This study could not determine whether the reduction in lactoferrin expression predisposed patients to biofilm infection or whether it occurred as a consequence of changes precipitated by the establishment of biofilms.

### Cystic fibrosis

CF is an autosomal recessive genetic disease with a mutation in the CF transmembrane conductance regulator (CFTR) protein that leads to abnormal electrolyte transport and the accumulation of viscous secretions in the respiratory, digestive, and reproductive tracts. In the lung, secretion buildup leads to airway obstruction and subsequent infection and inflammation, with infections most commonly caused by *H. influenzae* and *P. aeruginosa* [21]. Airway damage is the major cause of morbidity and mortality in CF patients [22].

*Pseudomonas aeruginosa* biofilm formation in the lung of CF patients is difficult to treat with the current antimicrobial therapy and is also resistant to the host's immune system. The viscous secretions in the lungs of CF patients create an anaerobic environment that favors the conversion of *P. aeruginosa* from the free-floating planktonic bacteria to biofilm [23]. Furthermore, active seeding dispersal of *P. aeruginosa* may facilitate infection chronicity and diversity in CF [24]. An analysis of CF-related biofilm linked bacteriophage activity in maturing biofilms to seeding dispersal [24]. Bacterial variation was highest for CF strains with the most cell death and seeding dispersal. Interestingly, iron level plays an important role in *P. aeruginosa* infection in CF patients. *P. aeruginosa*

requires iron as an essential co-factor for major metabolic pathways. The mutated *CFTR* gene causes airway epithelial cells to release more iron into the extracellular space, promoting *P. aeruginosa* biofilm growth [25].

### Otitis media

OM is most commonly caused by *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *P. aeruginosa* [26]. *P. aeruginosa* is a common cause of OM in pediatric patients 6 years of age or older [27]. The nasopharynx and surrounding tissues may act as important reservoirs of resistant bacterial biofilm, resulting in recurrent or chronic OM. In one sample, pathogenic bacteria were identified in the nasopharynx in nearly 75% of healthy adults and those suffering from common cold symptoms [28]. The most commonly identified pathogens were *S. pneumoniae* (45%), *M. catarrhalis* (33%), and *H. influenzae* (30%). A small pediatric study testing bilateral nasopharyngeal samples from each nostril in 15 children with acute OM identified *S. pneumoniae* in 67% of children, *M. catarrhalis* in 47%, and *H. influenzae* in 27% [29]. A larger pediatric study culturing isolates from the nasopharynx and middle ear in children with acute OM ( $n = 128$ ) reported bacteria in 76% in the nasopharynx and 78% in the middle ear, most frequently *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* [30].

A causative role for nasopharyngeal biofilms in OM was shown in an interesting animal experiment in which chinchillas free of middle ear disease were inoculated intranasally with *S. pneumoniae* isolated from a patient with recurrent OM; middle ear inflammation was detected 2 days after inoculation and peaked on day 8 [31]. Mucosal biofilms were demonstrated in the nasopharynx (83%) and middle ear (67%) of animals developing middle ear inflammation/infection, suggesting a role for biofilms in the pathogenesis of OM. The significance of these data is supported by a recent human study that evaluated biofilm formation in children with acute OM. In this sample, 84% of clinical isolates were biofilm-forming strains, with identical strains isolated from middle ear fluids and the nasopharynx [32]. Biofilm formation was significantly higher in cases in which amoxicillin failed to improve acute OM, supporting an important role for biofilms in infection persistence. The role of biofilms in chronic OM was confirmed in a study analyzing middle ear mucosa biopsies from 26 children undergoing tympanostomy tube placement for chronic or recurrent OM and eight controls undergoing cochlear implantation [33••]. Mucosal biofilms were identified using confocal laser scanning microscopy in 92% of evaluated specimens but none of the control biopsies.

### Device infections

Bacteria can form biofilms on biotic and abiotic surfaces. Compared with biotic surfaces, which have several antimicrobial mechanisms, abiotic surfaces are less resistant to bacterial attachment and the subsequent biofilm formation. Biofilms have been identified on middle ear ventilation tubes,

speech valve prostheses, tympanostomy tubes, cochlear implants, and bone-anchored hearing aid implants, resulting in device failure or recurrent infections [34,35]. Device-related biofilms in children were demonstrated in a study evaluating consecutively enrolled staphylococcal strains isolated from peripheral intravenous devices, venous blood, device insertion sites, and nasal mucosa of patients admitted to pediatric wards with peripheral intravenous devices for more than 48 hours [36]. A total of 100 invasive, 50 colonizing, and 50 commensal isolates were studied, with biofilms identified in 74% (74 of 100), 68% (34 of 50), and 32% (16 of 50), respectively. Device biofilms can develop rapidly and affect the youngest patients. For example, eight of the nine endotracheal tubes removed from neonates who were intubated for more than 12 hours (range, 13 hours–8 days) showed biofilm formation when viewed under scanning electron microscopy [37].

In addition to the risk of bacterial biofilm formation in implanted devices, the risk of fungal biofilm formation also exists. For example, fungal overgrowth was reported on cochlear implant hardware in a 26-month-old treated with antibiotics [38]. In this patient, *Candida albicans* was cultured from a middle ear effusion, with biofilm detected with scanning electron microscopy in yeast and filamentous forms on the implant.

### Targeting Biofilms When Treating Pediatric Infections

Eradication of pediatric infections requires consideration of the unique features of biofilms. For example, bacterial isolates were analyzed from sputum samples obtained from 110 CF patients with acute exacerbations [39]. Bacterial isolates were grown planktonically and as biofilms, with susceptibility to antibiotics tested for each bacterial form. Antibiotics selected for treating acute exacerbations were effective against planktonically grown bacteria in 60% of patients; however, biofilm-grown bacteria responded to antibiotics in only 22% of patients. As expected, patients treated with antibiotics to which biofilm-grown bacteria were susceptible were significantly more likely to have a decrease in sputum bacteria and length of hospitalization.

Antimicrobial therapy needs to consider unique characteristics of biofilms to maximize bacteriocidal efficacy. Macrolides provide better treatment for sinusitis than other classes of antibiotics [40]. Macrolides effectively inhibit quorum sensing for *P. aeruginosa* [41]. In one study, 3 of 12 tested antibiotics (azithromycin, ceftazidime, and ciprofloxacin) reduced quorum sensing in *P. aeruginosa*, suggesting that these may be more effective choices when the risk of biofilm production is higher [42]. In another study, azithromycin was bactericidal to in vitro *P. aeruginosa* biofilms from CF patients, although azithromycin-treated biofilms began to select for mutants that hyperproduced the multidrug efflux pump mexCD-OprJ, resulting in resistance to azithromycin, ciprofloxacin, and cefepime [43]. Although these antimicrobials became less effective as treatment, the strains selected

became hypersensitive to aminoglycosides, suggesting that aminoglycosides be considered as a preferred medication for patients who developed azithromycin resistance.

### Chronic rhinosinusitis

Direct delivery of antimicrobials via nasal washes may effectively reduce biofilm associated with CRS. In in vitro studies, hydrodynamic delivery of a soap-like surfactant and a calcium ion-sequestering agent effectively reduces bacterial colony counts from biofilm isolates obtained from patients with refractory CRS [44]. Topical antibiotics offer an avenue for increasing the dose beyond what can safely be achieved with systemically administered antibiotics, with mupirocin nasal lavage shown to reduce biofilms and CRS in animal and human models [45–47].

CRS may also be eradicated with surgical removal of infected adenoids [48]. A small retrospective study recently described good resolution of CRS in 23 pediatric patients treated with a stepwise protocol that included concurrent adenoidectomy and bilateral maxillary sinus irrigation followed by long-term double oral antibiotic therapy [49].

### Cystic fibrosis

Eradication of bacteria in CF respiratory tissues is complicated by the protective nature of biofilms [50]. Regular use of azithromycin reduced *P. aeruginosa*-related pulmonary exacerbation in CF patients in a double-blind, placebo-controlled trial [51]. Interestingly, subinhibitory exposure to azithromycin, but not gentamicin, decreased biomass and maximal thickness of nontypeable *H. influenzae* biofilm [52]. Co-administration of tobramycin and clarithromycin also was shown to more effectively and synergistically reduce CF *P. aeruginosa* biofilms compared with tobramycin alone [53,54]. Monotherapy resulted in increased antimicrobial resistance to each individual therapy.

Sodium nitrite also has been postulated to offer a unique nonantibiotic treatment of CF biofilms [8]. A common *P. aeruginosa* mutation in CF patients affects the *rhl* quorum-sensing circuit, increasing its susceptibility to nitric oxide. A second common mutation, MucA anti-stigma factor, results in poor growth under exposure to acidified nitrogen dioxide. With the mutation, nitrogen dioxide is reduced to nitric oxide, which kills mucoid, MucA mutant *P. aeruginosa*, but not the MucA<sup>+</sup> organisms. Iron restriction provides another novel therapy for inhibition of biofilm growth [55]. Iron chelation agents deferoxamine and deferrioxamine added to tobramycin effectively treat established *P. aeruginosa* biofilm and prevent the formation of new biofilms on CF airway cells [56].

### Otitis media

In vitro, fluoroquinolones have been shown to be more effective against OM from nontypeable *H. influenzae* than  $\beta$ -lactams, cephalosporin, and macrolides [57]; however, in vivo data are lacking. Subinhibitory concentrations of macrolide clarithromycin inhibit biofilm formation by interfering with the twitching mobility of *P. aeruginosa*

**Table 1. Role of biofilms in common pediatric infections**

|                                                                                                        |
|--------------------------------------------------------------------------------------------------------|
| Establish reservoirs of pathogenic bacteria that colonize moist surfaces                               |
| Adenoids                                                                                               |
| Nasopharynx                                                                                            |
| Tonsils                                                                                                |
| Produce recalcitrant, recurrent, and chronic infections                                                |
| Chronic rhinosinusitis                                                                                 |
| Cystic fibrosis-related respiratory infections                                                         |
| Otitis media                                                                                           |
| Device-related infections                                                                              |
| Biofilm morphology and physiology resist typical antimicrobial therapy                                 |
| Inner, inactive bacteria layers are protected from antibiotics                                         |
| Quorum sensing within biofilm facilitates bacterial modifications in response to antimicrobial therapy |
| Channels within biofilm expedite nourishment and growth of bacterial colonies                          |

[58]. However, antimicrobial treatment may adversely affect susceptibility of treated bacteria. In an interesting study, bacteria in the middle ear and nasopharynx were evaluated in OM [59]. In almost half the patients (47%), the initial resistant organism in the nasopharynx replaced a susceptible organism in the middle ear within a few days of the initiation of antibiotic therapy for OM.

Tympanostomy tubes are routinely used to treat chronic OM effusion. Post and colleagues [6] theorized that the effectiveness of tympanostomy tubes results from increased oxygen tension within middle ear space with reventilation, which thus restores mucosal defenses. The tympanostomy tube itself, however, can become a target of bacteria attachment and biofilm formation. Phosphorylcholine-coated fluoroplastic tympanostomy tubes are resistant to *S. aureus* and *P. aeruginosa* biofilm formation, whereas uncoated fluoroplastic tubes develop *P. aeruginosa* biofilm, and silver oxide-impregnated tubes develop biofilm from *S. aureus* and *P. aeruginosa* [60]. Albumin coating of the tympanostomy tube may also reduce biofilm growth by preventing foreign material adherence through inhibition of fibronectin binding [61].

Preventive therapy through vaccination programs also has been proposed to reduce OM occurrence. Whereas some experts have suggested that pediatric vaccinations to minimize nasopharyngeal colonization by *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* may reduce the risk of contracting OM, a recent study that observed healthy children 6 to 36 months old for 1 year indicated that changes in colonization by one bacterial species would likely result in modifications in other species [62]. For example, these data suggested that elimination of nasopharyngeal *S. pneumoniae* and *H. influenzae* may increase colonization risk from pathogenic *S. aureus*.

## Device-related infections

Definitive treatment for established biofilm-related device infection is device removal. New approaches to treatment of device infections focus on preventing bacterial attachment to abiotic surfaces and effective cleaning of biofilm-contaminated devices. For example, *P. aeruginosa* is a pathogen commonly found in ventilated newborns. The mucolytic agent ambroxol can be used to disrupt the structure of mucoid biofilms [63]. Ambroxol reduces important production of alginate and affects the expression of biofilm genes and enzymatic activity necessary for alginate production [63]. Therefore, ambroxol is commonly used to reduce *P. aeruginosa* in newborns requiring mechanical ventilation. Several strategies have been used to prevent biofilm formation on endotracheal tubing, including selective digestive decontamination, use of antibacterial endotracheal tubes, and synchronized mucus aspiration of the distal endotracheal tube [64]. Most have been tested in adult patient samples. Endotracheal tubing cleaning may also reduce biofilm accumulation. Early success with synchronized mucus aspiration in animal studies must be demonstrated in humans [64]. Aggressive cleaning of endotracheal tubing may also eliminate biofilm that has already formed. For example, in animal studies, scraping the inside of an endotracheal tube with a mucus shaver reduces biofilms [64]. Photodynamic therapy combined with antibiotics may also eliminate *Staphylococcus* biofilms on medical implants [65].

## Conclusions

OM and upper respiratory infections are among the most common conditions treated in pediatric patients. Chronic and recurrent infections in pediatric patients are facilitated by characteristics of biofilm that enhance bacteria growth and survival. Moist mucosal surfaces in the respiratory tract and surrounding structures provide ideal conditions for biofilm growth (Table 1). Colonization of the nasopharynx and nearby structures by pathogenic bacteria is common and may provide a reservoir for the development of recalcitrant infections, such as OM and CRS. Whereas antimicrobial therapies, such as detergents and antibiotics, are typically effective against planktonic bacteria, biofilm defenses provide effective barriers against bacterial eradication from these treatments. Selection of antibiotics has shifted its focus from those that inhibit active cellular division to drugs with activities against nongrowing cells. For example, fluoroquinolones may be more effective against biofilm bacteria than  $\beta$ -lactams. However, research on combating biofilms has shown clearly that antibiotic therapy alone is generally insufficient to eradicate complex biofilms and that additional treatment is needed. The therapeutic approach will differ depending on the stage and characteristics of biofilm formation. Prevention of bacterial attachment, disruption of biofilm structure, and pharmacologic intervention all play important roles in the eradication of chronic pediatric infections caused by biofilm.

## Disclosure

No potential conflicts of interest relevant to this article were reported.

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