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Biofilm Formations in Pediatric Respiratory Tract Infection Part 2: Mucosal Biofilm Formation by Respiratory Pathogens and Current and Future Therapeutic Strategies to Inhibit Biofilm Formation or Eradicate Established Biofilm

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Abstract

Purpose of Review The purpose of this review is to discuss the unique pathways of biofilm formation utilized by respiratory pathogens and current and future therapeutic strategies to inhibit biofilm formation or eradicate established biofilm in the context of these pathogens. Both nonselective and selective strategies for inhibiting biofilm formation or disrupting established biofilm are discussed.

Recent Findings Numerous strategies are being actively pursued to inhibit biofilm formation or eradicate established biofilm in respiratory pathogens. These can be broadly categorized by the stage of biofilm formation (adhesion, extracellular polysaccharide synthesis or structure, EPS, and matrix degradation) that they target and by their selectivity or lack thereof for specific biofilm pathogens. Nonselective inhibitors of adhesion include *N*-acetylcysteine and artificial surfactants and biosurfactants. Selective inhibitors of adhesion include mannosides that target host-EPS interactions, EPS-targeted antibodies, and other inhibitors of bacterial adhesion. Nonselective inhibitors of EPS synthesis and structure include cyclic di-GMP and cyclic di-AMP—through disruption of glucan-producing exoenzymes. Selective inhibitors of EPS synthesis and structure include antibodies that target proteins essential for biofilm structure (such as DNABII proteins and type IV pilin protein in NTHi) or antibodies that target critical molecules in biofilm formation (such as DNA adenine methyltransferase in *Streptococcus pneumoniae*). Nonselective agents for EPS or biofilm matrix degradation include peptidoglycan hydrolases that enzymatically degrade bacterial cell wall peptidoglycan and DNase, which degrades extracellular DNA from neutrophils and microorganism-derived DNA. Selective agents for EPS or biofilm matrix degradation include exopolysaccharide-degrading enzymes, such as glycoside hydrolases active against *Staphylococcus aureus* or exopolysaccharide-degrading enzymes that target Psl and Pel from *Pseudomonas aeruginosa*.

Summary Current strategies toward inhibiting biofilm formation or disrupting established biofilm represent an exciting new approach toward treatment of chronic infectious diseases. Application of these strategies toward treatment of pediatric respiratory tract infections also offers promise of a better understanding of the significance of mucosal biofilm in the pathogenesis of these conditions.

Keywords Mucosal biofilm · Pediatric respiratory · Respiratory tract infection · Antibiotic resistance · Extracellular

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Introduction

While the importance of biofilm to certain chronic pediatric respiratory tract conditions, such as cystic fibrosis (CF) and non-CF bronchiectasis, would appear self-evident, the importance of biofilm in other pediatric respiratory tract conditions remains somewhat controversial. Part of the uncertainty regarding biofilm significance centers around the fact that current modalities of treatment to combat biofilm have limited evidence of efficacy. There is a clear need for more effective

and targeted strategies for controlling or eradicating biofilm. The purpose of this review is to discuss the unique pathways of biofilm formation utilized by respiratory pathogens and current and future therapeutic strategies to inhibit biofilm formation or eradicate established biofilm in the context of these pathogens. Both nonselective and selective strategies for combatting biofilm are discussed.

Mucosal Biofilm Formation by Respiratory Pathogens

There are distinctly different genetically programmed mechanisms of biofilm formation in different genera of bacteria. This section will highlight the distinct features of biofilm formation in the most widely studied respiratory pathogens.

Nontypeable *Haemophilus influenzae* Biofilm

Nontypeable *Haemophilus influenzae* (NTHi) is the most prevalent bacterial pathogen in chronic and recurrent otitis media, and this organism is also associated with chronic rhinosinusitis and exacerbations of chronic obstructive pulmonary disease (COPD) and cystic fibrosis. It has the capacity to cause chronic and recurrent infections, and this capacity has been linked to its ability to form biofilm [1••]. NTHi exists as a commensal in the human nasopharynx [1••], although the relationship between NTHi colonization and biofilm formation is not entirely clear [2]. It has been discovered that the type IV pilus (Tfp), a critical adhesin, participates in multiple important biological functions in NTHi, including adherence, competence, twitching motility, and biofilm formation [3•, 4•, 5]. The type IV pilus expressed by NTHi is composed of multiple PilA subunits [1••]. Juricek et al. showed that Tfp is required for long-term persistence of NTHi in the chinchilla nasopharynx (NP) [4•]. Mokzran et al. showed that expression of Tfp is required for NTHi biofilm formation at 34 °C and 37 °C [1•]. In these studies, they examined biofilm formation at 34 °C, because 34 °C is the average temperature of human NP [6]. They further showed that NTHi biofilms formed on polarized epithelial cells demonstrated enhanced pilA expression at 34 °C relative to 37 °C.

The DNABII family of nucleoid-associated proteins (NAP), which includes integration host factor (IHF), represents a prominent group of bacterial gene regulators [7]. This family of proteins was found to be uniquely and critically involved in the structural integrity of the biofilms formed by NTHi [8•].

Streptococcus pneumoniae Biofilm

Streptococcus pneumoniae also exists as a commensal in the human upper respiratory tract (nasopharynx) where it grows at a lower than bodily temperature (32–34 °C) under a partial pressure of oxygen close to atmospheric pressure and with limited nutrients [9••]. During colonization of the nasopharynx, pneumococci organize into collaborating multicellular biofilm communities attached to the mucosal epithelium [10, 11], and this biofilm facilitates persistence of colonization [9••]. Bacterial colonization with production of biofilm represents the primary mode of pneumococcal growth during colonization, recurrent otitis media, and the early stages of invasive disease [12, 13]. Early *S. pneumoniae* biofilm formation was found to be regulated by two quorum-sensing systems, Com and LuxS/AI-2 [14•]. The enzyme DNA adenine methyltransferase (Dam) in *S. pneumoniae* is involved in the biosynthesis of quorum sensing molecules that regulate competence and biofilm formation [15•].

Natural DNA transformation is a gene transfer mechanism in which bacteria take up naked DNA from their environment and stably integrate it in their genome [16]. The proteins required for this process are conserved between species and are produced during a specific physiological state known as "competence" [16]. Marks et al. demonstrated that there is a high rate genetic exchange in *S. pneumoniae* under conditions associated with nasopharyngeal biofilm [9••]. In this process, they demonstrated that genetic exchange during dual-strain carriage in vivo is extremely efficient and roughly 10 million-fold higher than that during septic infection. Important factors promoting this high transformation efficiency include the lower temperature of the nasopharynx (32–34 °C), limited nutrient availability, and interactions with epithelial cells. These conditions induce constitutive upregulation of competence genes and downregulation of the polysaccharide capsule that promotes DNA transformation.

In their in vitro single strain *S. pneumoniae* biofilm experiment, [9••] found that the transformation efficiency of exogenous DNA was comparably high at 34 °C and 37 °C. In an in vitro dual strain experiment, they found that dual strains of *S. pneumoniae* showed somewhat impaired biofilm formation and no transformants at 37 °C, whereas the dual strains formed biofilm with high transformation efficiency at 34 °C. These data suggest that nasopharyngeal temperature plays an important role both for the ability of the bacteria to organize into biofilm and for their ability to exchange genetic material [9••].

The implications of horizontal gene transfer for *S. pneumoniae* evolution and adaptation include the rapid emergence and spread of antibiotic resistance and capsular switching [17]. Spontaneous gene uptake and transformation of *S. pneumoniae* strains have previously been reported to occur at extremely low frequencies in vivo, but most previous studies examining this phenomenon did so in the context of sepsis or other active infection [9••]. In contrast, epidemiologic evidence suggests that a high level of resistance selection occurs mainly in *S. pneumoniae*-colonized children, where nasopharyngeal carriage and antibiotic use favor selection of drug-resistant strains [9••].

Pseudomonas aeruginosa Biofilm

Pseudomonas aeruginosa produces three different exopolysaccharides that can potentially be components of the biofilm matrix, including alginate, Psl, and Pel. Alginate, which is comprised primarily of mannuronic and guluronic acid residues, is often overexpressed in *P. aeruginosa* strains isolated from chronically infected cystic fibrosis patients and accounts for mucoid *P. aeruginosa*. The conversion from nonmucoid to mucoid phenotype is due to the production of alginate [18•]. However, alginate is not required for biofilm formation by nonmucoid strains of *P. aeruginosa* which are involved in the initial colonization in CF patients [19].

Both Psl and Pel play important roles in biofilm formation [20•]. The *pel* gene cluster is involved in the production of a glucose-rich matrix, whereas the *psl* gene cluster is involved in the production of a mannose-rich matrix material [21•]. As a result, *Pseudomonas aeruginosa* is capable of producing biofilm of two distinct carbohydrate-rich matrix materials [21•]. Psl is critically important during the primary attachment of sessile cells to biotic and abiotic substrates and was shown to directly activate NFkB in an airway epithelial cell line resulting in production of interleukin-8 chemokine [22]. The Psl polysaccharide distributes helically around the cell surface and enhances the cross-linking and elasticity of the matrix thereby promoting the establishment of microcolonies [23]. Pel is a cationic exopolysaccharide important in the establishment of solid surface-associated *P. aeruginosa* biofilms [24]. Pel also enhances the specific resistance of biofilms against antibiotics such as aminoglycosides [23].

Staphylococcus aureus Biofilm

It has been shown that nasal carriers of *Staphylococcus aureus* have an increased risk of acquiring an infection with this pathogen [25].

Two-component systems (TCSs) are signal transduction regulatory pathways in certain bacteria composed of a sensing module, a histidine kinase, and its cognate transcription regulator [26•]. Dubrac et al. found that a two-component system which they named WalK/WalR (YycG/YycF) is a critical signal transduction pathway in *S. aureus* functioning in the control of cell wall metabolism and biofilm formation [26•].

Kim et al. used solid-state nuclear magnetic resonance (NMR) imaging and radioisotope amino acid labeling to examine the structure of *S. aureus* in planktonic versus early and mature biofilm cells [27]. The cell wall of *S. aureus* has approximately 20 layers of peptidoglycan with interpeptide bridges consisting of 5 glycine molecules (pentaglycine bridges). In planktonic cells, they found that the peptidoglycan cell wall has essentially no surface proteins, whereas in a mature biofilm, the peptidoglycan cell wall is extensively decorated with covalently attached proteins [27]. The surface proteins are linked covalently to the cell wall and promote cell-cell adhesion.

In the study by Iwase et al. [28••], an epidemiologic study of college students was done to examine the relationship between the nasal colonization with inhibitory *Staphylococcus epidermidis* and that of *S. aureus*. In the presence of inhibitory *S. epidermidis*, they found that the detection rate of nasal *S. aureus* carriage was significantly reduced. In this same study, they nasally administered an Esp-producing strain of *S. epidermidis*, purified Esp serine protease, or a non-Esp-producing *S. epidermidis* strain to a subgroup of volunteer college students who were persistently nasal colonized with *S. aureus*. In these students, they found that treatment with either the Esp-producing strain of *S. epidermidis* or purified Esp resulted in a progressive reduction in nasal *S. aureus* carriage rate. An Esp-negative strain of *S. epidermidis* did not have this effect [28••]. They further found that purified Esp alone demonstrated no bactericidal activity and that human beta defensin (hBD2) had low-level bactericidal activity towards *S. aureus* in biofilms, but the combination of purified Esp with hBD2 effectively killed *S. aureus* in biofilms [28••].

Brady et al. identified certain cell wall and membrane-associated proteins that are immunogenic within *S. aureus* biofilm infection and whose genes are upregulated during biofilm growth [29]. Four of these proteins, including a glucosaminidase, an ABC transporter lipoprotein, a conserved hypothetical protein, and a conserved lipoprotein, were found to be upregulated in *S. aureus* biofilm in vitro and in vivo [30]. These showed heterogeneous production within *S. aureus* biofilm. Based upon this, a quadrivalent vaccine was generated against these four proteins and found to inhibit biofilm formation when combined with vancomycin in 87.5% of experimental rabbits in a chronic osteomyelitis model, whereas biofilm was not eradicated nearly as well in vaccine-alone or vancomycin-alone treated animals [30]. This study suggests that targeting *S. aureus* biofilm-associated proteins may be a viable vaccination strategy.

Role of Anaerobiosis in Mucosal Biofilm Formation

Although a search of the literature failed to reveal articles directly implicating anaerobic bacteria in biofilm formation in pediatric respiratory tract infections, two studies demonstrate a role for anaerobiosis in mucosal biofilm formation.

Osgood et al. studied the ability of nontypeable *Haemophilus influenzae* (NTHi) strains from children to form biofilms in vitro under conditions presumed to exist in the middle ear during acute otitis media (AOM), recurrent acute otitis media (rAOM), or otitis media with effusion (OME) [31]. They studied biofilm formation in NTHi isolates under aerobic, microaerophilic, and anaerobic conditions and over a pH range of 4.5–10. They found that the pH of middle ear fluid collected from AOM, rAOM, and OME was similar with

a mean of 8.0 and that NTHi biofilms also formed optimally at pH 8.0. Furthermore, biofilms failed to form under aerobic conditions but formed under microaerophilic and anaerobic conditions, conditions felt likely to exist during rAOM and OME.

Worlitzsch et al. [32] found that in CF patients with established lung disease, *Pseudomonas aeruginosa* was located within hypoxic mucopurulent masses in the airway lumen as spherical intraluminal microcolonies within airway mucus unattached to the epithelial surface. In vitro studies confirmed that epithelial oxygen consumption, reduced airway surface liquid volume, and mucus stasis each contribute to steep hypoxic gradients within thickened mucus on CF epithelial surfaces and that *P. aeruginosa* growth in oxygen-restricted mucosal environments can lead to frank anaerobiosis. A key observation was that motile *P. aeruginosa* deposited on CF airway surfaces penetrate into hypoxic mucus zones and respond with increased alginate production. Based on these observations, the authors proposed that hypoxic conditions are important in the formation of biofilm-like microcolonies within CF airways.

Current and Future Therapeutic Strategies to Inhibit Biofilm Formation or Eradicate Established Biofilm

Table 1 is a partial summary of many experimental strategies that have been considered for inhibiting biofilm formation or eradicating established biofilm. A more comprehensive treatise of this topic was recently published in an excellent review by Koo et al. [33••]. Specific strategies to inhibit biofilm formation or eradicate established biofilm have been devised for most of the respiratory pathogens as summarized in Table 1, but specific strategies to combat *S. pneumoniae* are lacking, although targeting the quorum-sensing systems, Com and LuxS/AI-2, would appear to be promising [14•].

Effects on Biofilm Adhesion

These include use of artificial surfactants, natural surfactants (biosurfactants), such as SPLUNC1, mannosides that target host–EPS interactions, EPS-targeted antibodies, or other inhibitors of bacterial adhesion or specific EPS adhesins [33••]. In addition, many in vitro studies have demonstrated an effect of *N*-acetylcysteine (NAC) in inhibiting biofilm formation, blocking of bacterial adhesion, disrupting preformed biofilms, or reducing bacterial viability in biofilms [34].

Strategies to Block EPS Synthesis

These include inhibitors of cyclic di-GMP and cyclic di-AMP that act through disruption of glucan-producing exoenzymes (e.g., glycosyltransferase) and using small-molecule inhibitors to interrupt EPS glucan synthesis by glucosyltransferase (Koo et al. [33••]). Other enzyme pathways involved in biofilm formation, including two-component regulatory systems and quorum-sensing and quorum-quenching systems, could also potentially be targeted [33••].

EPS Degradation

Strategies to promote EPS degradation include use of EPS degrading enzymes (including proteases, peptidoglycan hydrolases, matrix-degrading enzymes, exopolysaccharide-degrading enzymes, and degradation of extracellular DNA and neutrophil extracellular traps (NETs) (e.g., human DNase I-dornase alfa). Of these, dornase alfa is the most widely studied in human patients.

Dornase alpha (recombinant human deoxyribonuclease), which cleaves DNA released in high concentrations by degraded neutrophils as well as microorganism-derived DNA which contributes to increased mucus viscosity, reduces sputum viscosity, slows the decline in lung function, and reduces pulmonary exacerbations in cystic fibrosis (CF) patients [35]. Cimmino et al. performed a double-blind placebo-controlled trial of 24 patients with CF and chronic rhinosinusitis (CRS) in which each patient first underwent sinus surgery and then, starting 1 month after surgery, began treatment with once-daily doses of nasally nebulized dornase alfa or isotonic saline (5 mL) for 12 months [36]. In this study, the primary outcome measures were nasal-related symptoms and nasal endoscopic appearance. Both primary outcomes were improved at 24 and 48 weeks in the group receiving dornase alfa, and only at 12 weeks in the group receiving placebo. The secondary outcome measure of forced expiratory volume in 1 second (FEV1) was also significantly improved in the patients receiving dornase alfa relative to patients receiving placebo [36].

Mainz et al. examined dornase alfa versus isotonic saline alone delivered to the sinuses with a Pari-Sinus™ device (PARI Respiratory Equipment, Inc., Midlothian, VA) that delivers vibrating aerosol in 23 CF patients in a cross-over treatment study [37]. In this study, sinonasal dornase alfa treatment was associated with significant improvement in the primary nasal symptoms compared with isotonic saline alone. Additionally, this treatment but not isotonic saline was associated with a significant improvement in pulmonary function (FEF75–25). This treatment is not currently FDA-approved for use in CRS.

Another strategy involves the use of lytic phages to destroy biofilm. Lytic phages utilize exopolysaccharide depolymerases to degrade EPS [38•, 39•]. The strategy was employed in an in vitro model of *Pseudomonas* biofilm using *Pseudomonas* isolated from human subjects with and without CF. The in vitro model showed that the lytic phage reduced biofilm formation by a median of 76% [40]. It remains to be seen how lytic phage could be applied to human subjects.

Table 1 Partial summary of many experimental strategies being considered for inhibiting biofilm formation or eradicating established biofilm

Stage of biofilm targeted	Adhesion	EPS synthesis and structure	EPS and matrix degradation
	Artificial surfactants and biosurfactants (e.g., SPLUNC1)	Cyclic di-GMP and cyclic di-AMP—through disruption of glucan-producing exoenzymes (e.g., glycosyltransferase)	Purified serine protease Esp from *Staphylococcus epidermidis*—inhibits *S. aureus* biofilm in vitro and reduces nasal *S. aureus* colonization [28••].
	Mannosides – target host–EPS interactions (e.g., mannosides that target the bacterial adhesin FimH [33••].	Inhibition of EPS glucan synthesis by glucosyltransferase using small-molecule inhibitors [33••].	Endolysins – bacteriophage-encoded peptidoglycan hydrolases that enzymatically degrade bacterial cell wall peptidoglycan [44].
	EPS-targeted antibodies (e.g. monoclonal antibody against *Pseudomonas aeruginosa* Psl polysaccharide) [33••].	Antibodies against DNABII proteins (which are essential for biofilm structure in NTHi) [7, 8•].	Exopolysaccharide-degrading enzymes, such as glycoside hydrolases (e.g. α-amylase plus cellulase) – active against *S. aureus* and *P aeruginosa* biofilm [45].
	Other inhibitors of bacterial adhesion—e.g., vaccine against proteins expressed in *S. aureus* biofilm [29, 30].	Antibodies against type IV pilin protein (which is essential for biofilm structure in NTHi) [46].	Exopolysaccharide-degrading enzymes targeting Psl and Pel from *P. aeruginosa* [47].
	N-acetylcysteine [34]	Use of pyrimidinedione to inhibit DNA adenine methyltransferase (Dam) in *S. pneumoniae* thereby inhibiting the biosynthesis of quorum sensing molecules that regulate competence and biofilm formation [15•].	DNase—through degradation of extracellular DNA and neutrophil NETs (e.g., human DNase I (dornase alfa) which degrades neutrophil and microorganism-derived DNA [33••].

Other Strategies

Other strategies are also being considered, such as delivery of the antimicrobial peptide lactoferrin directly to the lungs for treatment of CF lung disease [41•]. Brotz-Oesterhelt et al. demonstrated that a new class of antibiotics—acyldepsipeptides—activates ClpP, the core unit of a major bacterial protease complex leading to proteolytic degradation, inhibition of bacterial cell division, and eventually bacterial cell death [42]. Conlon et al. showed that the acyldepsipeptide antibiotic (ADEP4) in combination with rifampicin resulted in killing of *S. aureus* in the biofilm [43].

Conclusions

The physiologic processes that govern bacterial biofilm formation are genetically programmed, and there are distinct pathways of biofilm formation in each of the most common respiratory pathogens. Interrupting these unique pathways offers promising strategies to prevent biofilm formation or to eradicate established biofilm. Current and future therapeutic strategies to combat biofilm include blocking biofilm adhesion, blocking EPS synthesis, and promoting EPS degradation. Both nonselective and selective strategies for targeting these key components of biofilm formation are being considered for application to human infectious diseases. A greater understanding of the significance of mucosal biofilm will undoubtedly emerge from human studies of these as therapeutic agents.

Compliance with Ethical Standards

Conflict of Interest Daniel L. Hamilos declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by Dr. Hamilos.

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References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1.•• Mokrzan EM, Ward MO, Bakaletz LO. Type IV pilus expression is upregulated in nontypeable Haemophilus influenzae biofilms formed at the temperature of the human nasopharynx. J Bacteriol. 2016;198(19):2619–30 **This study demonstrated that expression of type IV pilus is required for (nontypeable Haemophilus influenza NTHi) biofilm formation and that biofilm formation is more efficient at 34°C (the average temperature of the human nasopharynx) than 37°C.**

2. Langereis JD, Hermans PW. Novel concepts in nontypeable Haemophilus influenzae biofilm formation. FEMS Microbiol Lett. 2013;346(2):81–9.
3.• Jurcisek JA, Bakaletz LO. Biofilms formed by nontypeable Haemophilus influenzae in vivo contain both double-stranded DNA and type IV pilin protein. J Bacteriol. 2007;189(10):3868–75 **This paper examines the role of type IV pilus in NTHi biofilm formation.**
4.• Jurcisek JA, Bookwalter JE, Baker BD, Fernandez S, Novotny LA, Munson RS Jr, et al. The PilA protein of non-typeable Haemophilus influenzae plays a role in biofilm formation, adherence to epithelial cells and colonization of the mammalian upper respiratory tract. Mol Microbiol. 2007;65(5):1288–99 **This paper examines the role of type IV pilus in NTHi biofilm formation.**
5. Bakaletz LO, Baker BD, Jurcisek JA, Harrison A, Novotny LA, Bookwalter JE, et al. Demonstration of Type IV pilus expression and a twitching phenotype by Haemophilus influenzae. Infect Immun. 2005;73(3):1635–43.
6. Webb P. Air temperatures in respiratory tracts of resing subjects in cold. J Appl Physiol. 1951;4(5):378–82.
7. Dorman CJ. Nucleoid-associated proteins and bacterial physiology. Adv Appl Microbiol. 2009;67:47–64.
8.• Devaraj A, Buzzo J, Rocco CJ, Bakaletz LO, Goodman SD. The DNABII family of proteins is comprised of the only nucleoid associated proteins required for nontypeable Haemophilus influenzae biofilm structure. MicrobiologyOpen. 2017. **This study demonstrates the importance of the DNABII family of nucleoid-associated proteins (NAP) in the structural integrity of the biofilms formed by NTHi.**
9.•• Marks LR, Reddinger RM, Hakansson AP. High levels of genetic recombination during nasopharyngeal carriage and biofilm formation in Streptococcus pneumoniae. mBio. 2012;3(5) **This study demonstrated that there is a high rate genetic exchange in *Streptococcus pneumoniae* under conditions associated with nasopharyngeal biofilm. In this process, they demonstrated that genetic exchange during dual-strain carriage in vivo is extremely efficient and roughly 10 million-fold higher than that during septic infection. Important factors promoting this high transformation efficiency include the lower temperature of the nasopharynx (32-34°C), limited nutrient availability, and interactions with epithelial cells.**
10. Munoz-Elias EJ, Marcano J, Camilli A. Isolation of Streptococcus pneumoniae biofilm mutants and their characterization during nasopharyngeal colonization. Infect Immun. 2008;76(11):5049–61.
11. Marks LR, Parameswaran GI, Hakansson AP. Pneumococcal interactions with epithelial cells are crucial for optimal biofilm formation and colonization in vitro and in vivo. Infect Immun. 2012;80(8):2744–60.
12. Domenech M, Ramos-Sevillano E, Garcia E, Moscoso M, Yuste J. Biofilm formation avoids complement immunity and phagocytosis of Streptococcus pneumoniae. Infect Immun. 2013;81(7):2606–15.
13. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15(2):167–93.
14.• Vidal JE, Howery KE, Ludewick HP, Nava P, Klugman KP. Quorum-sensing systems LuxS/autoinducer 2 and Com regulate Streptococcus pneumoniae biofilms in a bioreactor with living cultures of human respiratory cells. Infect Immun. 2013;81(4):1341–53 **This study demonstrated that *Streptococcus pneumoniae* biofilm formation is regulated by two quorum-sensing systems, Com and LuxS/AI-2.**
15.• Yadav MK, Go YY, Chae SW, Song JJ. The small molecule DAM inhibitor, pyrimidinedione, disrupts Streptococcus pneumoniae biofilm growth in vitro. PLoS One. 2015;10(10):e0139238 **This study demonstrated that the enzyme DNA adenine methyltransferase (Dam) in *S pneumoniae* is involved in the biosynthesis of quorum sensing molecules that regulate competence and biofilm formation.**
16.•• Fontaine L, Wahl A, Flechard M, Mignolet J, Hols P. Regulation of competence for natural transformation in streptococci. Infect Genet Evol. 2015;33:343–60 **This study discusses that natural DNA transformation is a gene transfer mechanism by which bacteria take up exogenous DNA from their environment and stably integrate it in their genome.**
17. Coffey TJ, Dowson CG, Daniels M, Zhou J, Martin C, Spratt BG, et al. Horizontal transfer of multiple penicillin-binding protein genes, and capsular biosynthetic genes, in natural populations of Streptococcus pneumoniae. Mol Microbiol. 1991;5(9):2255–60.
18.• Hay ID, Remminghorst U, Rehm BH. MucR, a novel membrane-associated regulator of alginate biosynthesis in Pseudomonas aeruginosa. Appl Environ Microbiol. 2009;75(4):1110–20 **This study demonstrates that the conversion from nonmucoid to mucoid phenotype and Pseudomonas aeruginosa strains is due to the production of alginate.**
19. Wozniak DJ, Wyckoff TJ, Starkey M, Keyser R, Azadi P, O'Toole GA, et al. Alginate is not a significant component of the extracellular polysaccharide matrix of PA14 and PAO1 Pseudomonas aeruginosa biofilms. Proc Natl Acad Sci U S A. 2003;100(13):7907–12.
20.• Chew SC, Kundukad B, Seviour T, van der Maarel JR, Yang L, Rice SA, et al. Dynamic remodeling of microbial biofilms by functionally distinct exopolysaccharides. mBio. 2014;5(4):e01536–14 **The study demonstrates that the exopolysaccharides Psl and Pel play important roles in *Pseudomonas aeruginosa* biofilm formation.**
21.• Friedman L, Kolter R. Two genetic loci produce distinct carbohydrate-rich structural components of the Pseudomonas aeruginosa biofilm matrix. J Bacteriol. 2004;186(14):4457–65 **This study demonstrates that the Psl and Pel exopolysaccharides of *Pseudomonas aeruginosa* give rise to distinct types of extracellular matrix thereby producing biofilm of 2 distinct carbohydrate rich matrix materials.**
22. Byrd MS, Pang B, Mishra M, Swords WE, Wozniak DJ. The Pseudomonas aeruginosa exopolysaccharide Psl facilitates surface adherence and NF-kappaB activation in A549 cells. mBio. 2010;1(3).
23. Skariyachan S, Sridhar VS, Packirisamy S, Kumargowda ST, Challapilli SB. Recent perspectives on the molecular basis of biofilm formation by Pseudomonas aeruginosa and approaches for treatment and biofilm dispersal. Folia microbiologica. 2018.
24. Wei Q, Ma LZ. Biofilm matrix and its regulation in Pseudomonas aeruginosa. Int J Mol Sci. 2013;14(10):20983–1005.
25. Wertheim HF, Melles DC, Vos MC, van Leeuwen W, van Belkum A, Verbrugh HA, et al. The role of nasal carriage in Staphylococcus aureus infections. Lancet Infect Dis. 2005;5(12):751–62.
26.• Dubrac S, Boneca IG, Poupel O, Msadek T. New insights into the WalK/WalR (YycG/YycF) essential signal transduction pathway reveal a major role in controlling cell wall metabolism and biofilm formation in *Staphylococcus aureus*. J Bacteriol. 2007;189(22):8257–69 **This study demonstrated that a two-component system which they named WalK/WalR (YycG/YycF) is a critical signal transduction pathway in *Staphylococcus aureus* functioning in the control of cell wall metabolism and biofilm formation.**
27. Kim SJ, Chang J, Rimal B, Yang H, Schaefer J. Surface proteins and the formation of biofilms by Staphylococcus aureus. Biochim Biophys Acta. 2018;1860(3):749–56.
28.•• Iwase T, Uehara Y, Shinji H, Tajima A, Seo H, Takada K, et al. *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. Nature. 2010;465(7296):346–9 **This epidemiologic study of college students examined the relationship between the nasal colonization with inhibitory *Staphylococcus epidermidis* and that of**

***Staphylococcus aureus*. In the presence of inhibitory *S epidermidis*, they found that the detection rate of nasal *S aureus* carriage was significantly reduced. In this same study, they nasally administered an Esp-producing strain of *S epidermidis*, purified Esp serine protease or a non-Esp-producing *S epidermidis* strain to a subgroup of volunteer college students who were persistently nasal colonized with *S aureus*. In these students, they found that treatment with either the Esp-producing strain of *S epidermidis* or purified Esp resulted in a progressive reduction in nasal *S aureus* carriage rate.**

29. Brady RA, Leid JG, Camper AK, Costerton JW, Shirtliff ME. Identification of Staphylococcus aureus proteins recognized by the antibody-mediated immune response to a biofilm infection. Infect Immun. 2006;74(6):3415–26.
30. Brady RA, O'May GA, Leid JG, Prior ML, Costerton JW, Shirtliff ME. Resolution of Staphylococcus aureus biofilm infection using vaccination and antibiotic treatment. Infect Immun. 2011;79(4): 1797–803.
31. Osgood R, Salamone F, Diaz A, Casey JR, Bajorski P, Pichichero ME. Effect of pH and oxygen on biofilm formation in acute otitis media associated NTHi clinical isolates. Laryngoscope. 2015;125(9):2204–8.
32. Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, et al. Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. J. Clin. Invest. 2002;109(3):317–25.
33.•• Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L. Targeting microbial biofilms: current and prospective therapeutic strategies. Nat Rev Microbiol. 2017;15(12):740–55 **This is an excellent review that discusses use of artificial surfactants, natural surfactants (biosurfactants), such as SPLUNC1, mannosides that target host–EPS interactions, EPS-targeted antibodies or other inhibitors of bacterial adhesion or specific EPS adhesins to inhibit biofilm formation.**
34. Blasi F, Page C, Rossolini GM, Pallecchi L, Matera MG, Rogliani P, et al. The effect of N-acetylcysteine on biofilms: implications for the treatment of respiratory tract infections. Respir Med. 2016;117: 190–7.
35. Pittman JE, Ferkol TW. The evolution of cystic fibrosis care. Chest. 2015;148(2):533–42.
36. Cimmino M, Nardone M, Cavaliere M, Plantulli A, Sepe A, Esposito V, et al. Dornase alfa as postoperative therapy in cystic fibrosis sinonasal disease. Arch Otolaryngol Head Neck Surg. 2005;131(12):1097–101.
37. Mainz JG, Schien C, Schiller I, Schadlich K, Koitschev A, Koitschev C, et al. Sinonasal inhalation of dornase alfa administered by vibrating aerosol to cystic fibrosis patients: a double-blind placebo-controlled cross-over trial. J Cyst Fibros. 2014;13(4):461–70.
38.• Danis-Wlodarczyk K, Olszak T, Arabski M, Wasik S, Majkowska-Skrobek G, Augustyniak D, et al. Characterization of the newly isolated lytic bacteriophages KTN6 and KT28 and their efficacy against Pseudomonas aeruginosa biofilm. PLoS One. 2015;10(5): e0127603 **This study discusses the potential use of lytic phages to destroy biofilm. Lytic phages utilize exopolysaccharide depolymerases to degrade EPS.**
39.• Parasion S, Kwiatek M, Gryko R, Mizak L, Malm A. Bacteriophages as an alternative strategy for fighting biofilm development. Pol J Microbiol. 2014;63(2):137–45 **This study discusses the potential use of lytic phages to destroy biofilm. Lytic phages utilize exopolysaccharide depolymerases to degrade EPS.**
40. Fong SA, Drilling A, Morales S, Cornet ME, Woodworth BA, Fokkens WJ, et al. Activity of bacteriophages in removing biofilms of Pseudomonas aeruginosa isolates from chronic rhinosinusitis patients. Front Cell Infect Microbiol. 2017;7:418.
41.• Marshall LJ, Oguejiofor W, Price R, Shur J. Investigation of the enhanced antimicrobial activity of combination dry powder inhaler formulations of lactoferrin. Int J Pharm. 2016;514(2):399–406 **This study discusses delivery of the antimicrobial peptide lactoferrin directly to the lungs for treatment of CF lung disease.**
42. Brotz-Oesterhelt H, Beyer D, Kroll HP, Endermann R, Ladel C, Schroeder W, et al. Dysregulation of bacterial proteolytic machinery by a new class of antibiotics. Nat Med. 2005;11(10):1082–7.
43. Conlon BP, Nakayasu ES, Fleck LE, LaFleur MD, Isabella VM, Coleman K, et al. Activated ClpP kills persisters and eradicates a chronic biofilm infection. Nature. 2013;503(7476):365–70.
44. Becker SC, Roach DR, Chauhan VS, Shen Y, Foster-Frey J, Powell AM, et al. Triple-acting lytic enzyme treatment of drug-resistant and intracellular Staphylococcus aureus. Sci Rep. 2016;6:25063.
45. Fleming D, Chahin L, Rumbaugh K. Glycoside hydrolases degrade polymicrobial bacterial biofilms in wounds. Antimicrobial agents and chemotherapy. 2017;61(2).
46. Novotny LA, Jurcisek JA, Ward MO Jr, Jordan ZB, Goodman SD, Bakaletz LO. Antibodies against the majority subunit of type IV pili disperse nontypeable Haemophilus influenzae biofilms in a LuxS-dependent manner and confer therapeutic resolution of experimental otitis media. Mol Microbiol. 2015;96(2):276–92.
47. Baker P, Hill PJ, Snarr BD, Alnabelseya N, Pestrak MJ, Lee MJ, et al. Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent Pseudomonas aeruginosa biofilms. Sci Adv. 2016;2(5):e1501632.