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An Overview of Infections in Cystic Fibrosis Airways and the Role of Environmental Conditions on *Pseudomonas aeruginosa* Biofilm Formation and Viability

Cameron T. McDaniel, Warunya Panmanee and Daniel J. Hassett

Additional information is available at the end of the chapter

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Abstract

In this chapter, the authors review a major complication associated with cystic fibrosis (CF), problematic bacterial infections of the lungs. Infection by organisms such as *Staphylococcus aureus*, *Burkholderia cepacia* complex, and *Pseudomonas aeruginosa* (a major player in CF related infections) results in complications due to increased inflammation and production of virulence factors produced by the bacteria. In addition to these more canonical organisms associated with CF infection, emerging-bacterial species have been found in the CF, including anaerobes that have only within the past 5-10 years have been reported to exist in the lungs. *P. aeruginosa* has long been a cause of devastating infections, and is often seen as the “hallmark” organism associated with the disease. The authors describe the *P. aeruginosa* infection, including its conversion to a mucoid phenotype, as well as its ability to utilize the thicker airway surface layer associated with CF to grow in “mode two biofilms.” Finally, the authors discuss treatments for bacterial infections, and some of the new advances that offer hope for treatment of CF symptoms and infections by multi-drug resistant organisms. Among these new treatments is the application of acidified nitrite, a non-antibiotic treatment that has been found to be effective at killing nonmucoid and mucoid variants of *P. aeruginosa*.

Keywords: Cystic Fibrosis, Infections, Bacteria, *Pseudomonas aeruginosa*, Biofilm, Acidified Nitrite

1. Introduction

Cystic fibrosis (CF) is the second most common genetic disease in the United States, second only to sickle cell anemia. A mutation in the associated gene, cystic fibrosis transmembrane regulator (CFTR), results in the clinical symptoms seen for the disease. While the disease itself is devastating, it does not usually result in the immediate death of the patient. Rather, the bodily conditions that the CFTR mutation creates, especially in the lungs, results in the acquisition of problematic bacterial biofilm infections that remain in the thick, inspissated mucus layer for the remainder of the patient's life. This unique niche provides many complex nutrients that the bacteria utilize and offers an environment that is protected against the host's innate immune system. While only a few bacterial species have generally been thought of as dominating the CF lung, it has recently been revealed that there are many species inhabiting the lung, and that these species vary from normal flora to even obligate anaerobes [1]. While the exact role of all these bacterial species in the lung has not yet been determined, the clinical picture is becoming clearer.

When the genetic cause of CF was first identified in 1989 [2], the average lifespan of a CF patient was approximately 15 years of age. Since then, it has increased to roughly 40 [3, 4]. This is due to the tremendous efforts that have been made in determining new and improved means of treating patients suffering from CF, including addressing physiological parameters and methods of treating bacterial infections.

This chapter will focus on the bacterial infections that develop during CF and the conditions of the lung that make it so favorable for bacterial infection. We will discuss some of the major bacterial species that contribute to the morbidity of the disease, and focus on perhaps the largest contributor to airway infection, *Pseudomonas aeruginosa* (PA). In addition, we will define some of the niches that the bacteria inhabit within the lung, and discuss treatment options for infected individuals.

2. Overview of CF and the role of CFTR in the lung

2.1. CFTR: Role and function

The function of CFTR in the lungs has been established previously, supporting the fact that its absence or modification (depending on the mutation) leads to the dangerous symptoms that are diagnostic hallmarks in CF patients. Although some canonically think of CFTR as only being influential in the development of the lung problems, CFTR is actually expressed in many areas of the body, including the liver, intestines, pancreas, skin, and reproductive organs (Figure 1). In all these cases, a defect in CFTR can cause many problems for the affected organ. The reason is due to the large role the transporter plays in the maintenance of osmotically balanced fluids in these tissues. CFTR is an anion channel found in the apical membrane of epithelial cells, primarily responsible for pumping chloride ions into the fluids surrounding the epithelial cells, and allowing for the passage of water from the epithelial cells into the fluid

layers lining the cells (e.g, the pericilliary layer (PCL) of the airways) [5, 6, 7]. This allows the fluids to maintain their function, which, in the lung, is usually to facilitate clearance of opportunistic pathogens and cellular debris from the area or to transport the fluid to a different area (as is the case in the reproductive system). The transporter itself is ATP-driven, and is activated due to rising cAMP levels in the cells [7].

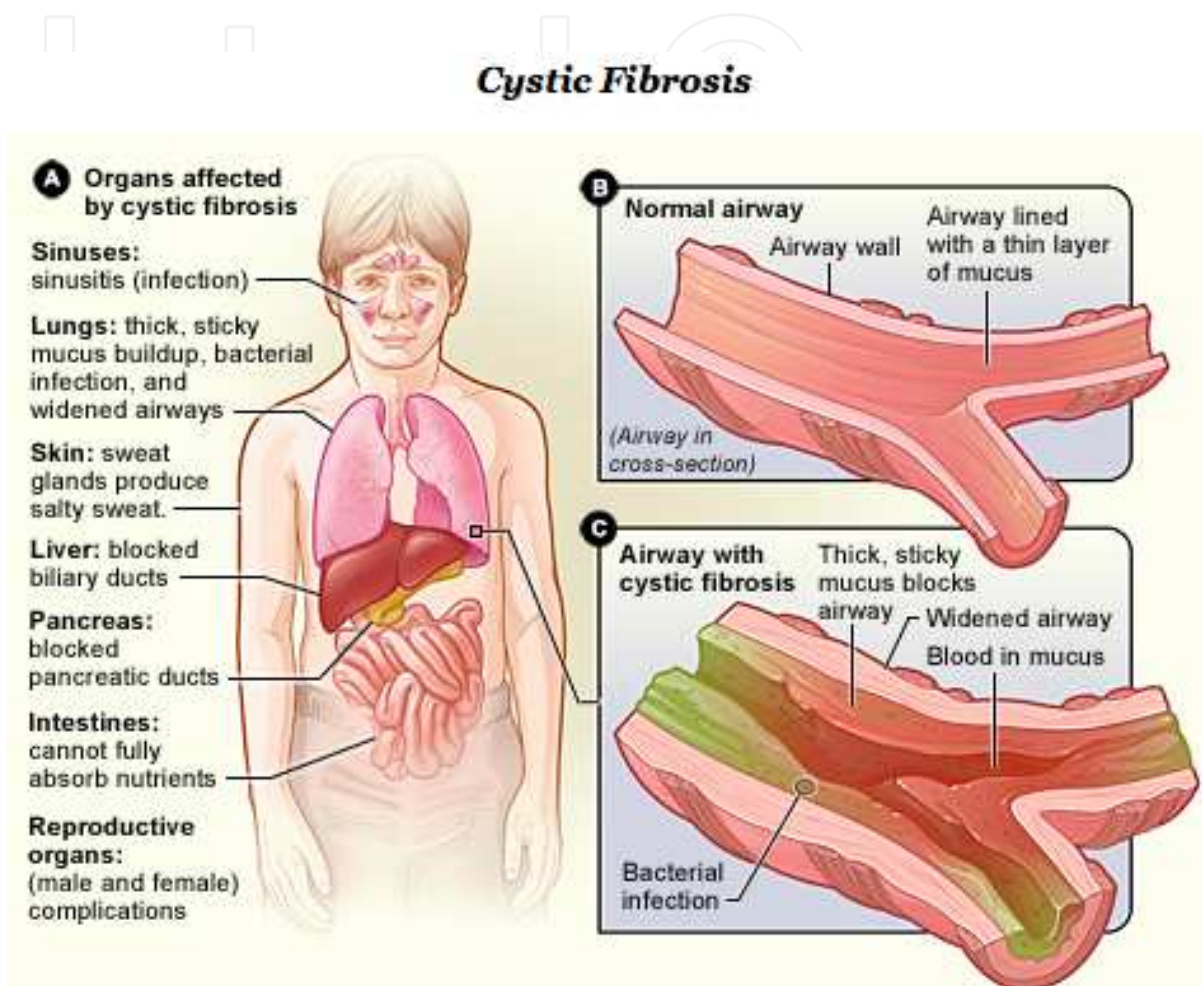


Figure 1. Organs affected by CF and its effect on airways.

A) An overview of the organs affected by CF, with a brief description of the complications associated with it. B) A normal airway depicting open passages. C) A CF airway depicting the buildup of mucus, inflammation, and bacterial infection that will lead to further complications. (Source: National Heart, Lung, and Blood Institute; National Institutes of Health; U.S. Department of Health and Human Services. [8])

In cases where the CFTR is not active or only partially active, the consequences can be quite severe. This is due to a myriad of potential mutations in the CFTR gene. These mutations have been categorized into classes, as previously reviewed by Rowntree and Harris [9] and summated by the Cystic Fibrosis Foundation. As shown in Table 1, these classes focus on the means by which the CFTR is rendered dysfunctional, such as mutations affecting protein

maturation (Class II) or those leading to dysregulation of Cl⁻ conductance (Class IV) [9, 4]. The most common mutation is a deletion of a phenylalanine residue at position 508 of the protein, referred to as homozygous recessive $\Delta F508$ [7]. Although the exact reasoning of why this mutation was clinically deleterious was at first a mystery, it has come to be discovered that this leads to a misfolding of the channel, causing it to never reach the cell membrane and instead be destroyed in the Golgi apparatus. Although one could surmise that the loss of CFTR alone is not enough to cause harm and the body could compensate for this loss by redundant channels, it is also suspected that CFTR can help mediate the activation and use of other channels in the membrane. Thus, its loss may be far more reaching than simply its anion channeling properties. With this loss of function, the surrounding fluid begins to become osmotically imbalanced and often viscous and impervious to other ions [6]. With no new water coming into the fluids from the epithelial cells, the fluid layer begins to thicken, eventually forming mucus plugs in the respective organ. As such, the associated ducts are no longer able to perform their proper functions.

CFTR Mutations and Their Functional Effects		
Class	Impact on CFTR Protein	Examples of Mutations
Class I	No functional CFTR protein created	G542X, W1282X, R553X, 621+1G->T, 1717-1G->A
Class II	CFTR protein is created, but misfolded, keeping it from reaching the cell surface	F508del, N1303K, I507del, G85E, R560T
Class III	CFTR protein is created and reaches cell surface, but does not function properly	G551D, S549N, V520F, L1077P, G1244E
Class IV	The opening in the CFTR protein ion channel is faulty	R117H, D1152H, R347P, R334W, L206W
Class V	CFTR protein is created in insufficient quantities	3849+10kbC->T, 2789+5G->A, A455E, 3272-26A->G, 3120G->A

Table 1. Classifications of CFTR mutations and their impact on the protein.

Although there are numerous mutations that have been associated with CF, they can generally be broken down into five classes based on the way this mutation affects CFTR. This table briefly summarizes these classes and provides an example of each mutation [4].

2.2. The CF lung

Within the CF lung, the loss of functional CFTR is quite dramatic, and usually leads to the canonical respiratory symptoms associated with CF lung disease. A healthy functioning lung will have a thin, hydrated periciliary mucus layer lining the airway. This mucus rests above

the cilia of the epithelial cells in a biphasic layer [7]. The top layer is slightly more viscous than the bottom layer and serves to trap bacteria and particles that enter the lung. The bottom layer, referred to as the PCL, is much more fluid, and allows for the cilia to beat within it, pushing the entire mucus layer up the lung for expectoration [7, 10]. Through this mechanism, the lungs can clear bacteria and debris that has been inhaled or otherwise entered the airway passages. The PCL is kept hydrated by the action of the CFTR and other ion channels present in the epithelial cells lining the airway that also maintain the osmotic balance.

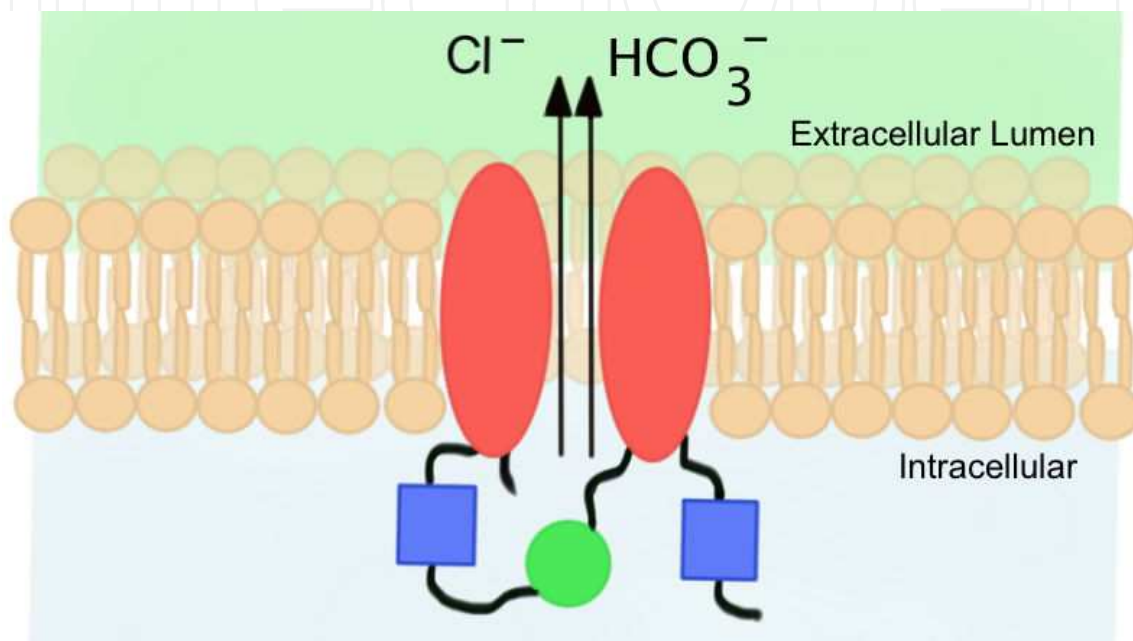


Figure 2. A model of the CFTR in the apical membrane of lung epithelia.

The CFTR, embedded in the apical membrane of epithelial cells, serves to transport anions, specifically chloride and bicarbonate, into the lumen of the associated organ. The CFTR is composed of several domains, including two transmembrane domains (red ovals), two nucleotide binding domains (blue, squares), and a regulatory domain that controls the opening of the transporter. The nucleotide binding domains use ATP to provide energy for the transport of the anions into the lumen bordering the cell.

However, in a case such as CF, where the CFTR is functionally absent and proper ion transport is lacking, the mucus layer begins to thicken. This is believed to be due to the primary transport of Cl^- [11, 6, 7] and the secondary transport of HCO_3^- by CFTR (Figure 2). Recent studies have shown that the ability of CFTR to transport HCO_3^- is very important, as it seems to play a large role in the regulation of the mucin folding [7]. Mucin, the primary protein component of airway mucus, is a long chain-like, repetitive peptide that is heavily O- and N-glycosylated. At the C- and N-terminal regions, the protein is rich in cysteine residues, which can lead to intermolecular disulfide bridges. These disulfide bridges will link the chains together, creating a larger oligomer. It is suspected that in a more acidic environment, the mucin molecules contract, causing the overall density of the mucus to increase. This causes impermeability issues [6] that

can be devastating to the patient. In addition to the effects that decreased HCO_3^- levels have on the density of the mucus, the general inability to transport anions across the apical membrane also affects the airway mucus layer. The PCL that lines the cilia of the epithelial cells is very sensitive to changes in water concentration. When the cells are not exporting significant amounts of ions, the PCL will then lose water as a sequela, resulting in it becoming denser and reducing the effectiveness of ciliary beating. This leads to an overall larger amount of material that cannot be cleared from the airway, causing a buildup or mucus plug.

In addition to the buildup of mucus as the disease progresses, the patient will experience several other symptoms as well. Commonly, the bronchi become inflamed, caused by an overreactive response from the immune system due to both mucus buildup (containing a plethora of bacterial components such as virulence factors, DNA, and cell debris from lysed bacteria or airway epithelial cells) and a potential infection. While the infections will be covered in more detail later in this chapter, bacteria such as *Pseudomonas aeruginosa* (PA), *H. influenzae*, and *S. aureus* have been found to infect CF patients early in life (roughly 1 year of age) [1], and even if eradicated once, will often arise from a re-infection later in life. PA is the organism most commonly associated with a decline in the clinical course of CF patients, as its ability to form biofilms and convert to a mucoid phenotype often provides a large level of resistance to antibiotics and other disease treatments.

Interestingly, the CF lung will also develop an oxygen gradient in its luminal mucus [12, 13]. As mentioned earlier, the increased density of the mucus makes it more difficult for oxygen to diffuse across it freely and into the blood. While this has consequences for the overall health of the individual, it also has implications for growth of bacteria enmeshed within it. The oxygen gradient is severe enough that the basal layer of the mucus could be termed microaerobic, or in more severe cases, anaerobic. This leads to the growth and development of bacteria that would normally not be found in the lung, and eventually to the growth of the mucoid form of PA. This will be covered in more depth later in this chapter.

2.3. Pathology of the CF lung

As might be expected, the buildup of thick mucus in the CF lungs often has severe clinical implications. The significant reduction or loss of mucus clearance often results in infection and leads to inflammation due to the dramatic ~1,500-fold increase in airway neutrophils [14]. Coupling the inflammation and buildup of mucus, it is not surprising that the overall lung capacity of CF patients decreases dramatically throughout life. This can be tested using a series of pulmonary function tests (PFTs) [15]. These often involve a spirometry test, which is a measure of the forced efflux volume in one second (FEV1), or how much air the patient can forcefully exhale in one second. This is a hallmark test for overall lung volume and strength. Clinically it has been shown that the FEV1 of a CF patient will be approximately 10% below the expected for a healthy individual of the same age [16].

In older patients, the prolonged effects of the disease often lead to chronic infections. These invading organisms can then be cultured and analyzed to determine the best treatment strategy. While we will be covering the type of infections further in this chapter, it is important to note that clinically, this also affects the patient in other ways, primarily leading to inflam-

mation of the respiratory system and decreased airway capacity [17]. This inflammation is often brought on by increased neutrophil accumulation in the lungs, which not only serve to act as a preliminary means of immune defense, but also to recruit macrophages. These neutrophils and macrophages will phagocytose bacteria and dead immune cells, but also produce pro-inflammatory cytokines that exacerbate the inflammatory process. These cytokines not only attract other immune cells, but also serve as a trigger for the release of proteases and elastases by the immune cells. Normally, these help eradicate the bacteria that triggered this response, but their over-production in the CF lung can actually damage epithelial cells, leading to the fibrotic nature of cells associated with this disease [18].

3. Bacterial infections

While CF airway disease is based on the genetic mutation of the CFTR gene, this is not usually what leads to the morbidity and mortality associated with the disease. Rather, an infectious agent that grows under the physiological conditions created by this mutation will lead to detrimental symptoms and eventual death. In the lungs, the buildup of thick mucus leads to decreased clearance of bacteria and provides a nutrient rich medium with which they can grow and even thrive. This mucus becomes colonized relatively easily with potentially several different species of bacteria at once [19, 16, 20]. Considering that infection is the major source of morbidity and mortality for CF patients, much research has focused on this aspect of the disease and different means by which to eradicate it. However, time has shown that this is not quite as easy as hoped, but the advancements of alternate treatments are helping this issue.

Bacterial colonization of the lungs of CF patients has been known for many decades. However, our understanding of what bacteria colonize the lungs has evolved dramatically. Early research identified that there were several species of bacteria that could colonize the lungs easily, and were often found associated with CF patients. By far, the most common (and most linked to severe progression of the disease) was *PA*. This gram-negative bacterium is often considered an opportunistic pathogen, existing naturally in soil, water, and in some cases, as part of the flora of human skin. However, in cases where the bacteria come into contact with immune-deficient or severely compromised tissues, they can grow quite prolifically. A common example is found with burn wounds, where *PA* can colonize the exposed flesh and lead to the need for aggressive antibiotic treatment. This type of infection is often associated with a distinct "smell of grapes" and the potential for a green-blue color to develop, indicative of several of the toxins that *PA* produces such as the blue phenazine antibiotic, pyocyanin. There are additional phenotypes that are seen with lung colonizing *PA*, the most dramatic of which was found in 1964 by Doggett et al. [21]. In that work, the authors noted that the multiple strains of *PA* that they could isolate on agar from CF patients all had a similar, mucoid phenotype. Although, at the time, the significance of this was not known, this mucoid phenotype is tightly linked to the overall progression of CF lung disease. It is indicative of the organism's ability to create a biofilm, a highly resistant matrix of exopolysaccharide and bacteria that allows survival in harsh environments. We will cover the details of biofilms

further into this chapter, but it is important to note the significance they play in CF disease progression.

In addition to *PA*, three other major strains of bacteria were found associated with the CF lung. The first of these is *Staphylococcus aureus*, a gram-positive organism that is part of the normal skin flora. Often, *S. aureus* is found colonizing the nares of hosts, where it is hypothesized that it can travel to the lungs. It is often the first bacterium identified in CF patients, potentially due to a lack of proper immune defense within the newborns diagnosed with CF [22, 23]. It often resides in the lungs for a prolonged period, although it can be eradicated with proper antibiotic treatment. Similar to *PA* acquiring a mucoid variant, *S. aureus* can also acquire a different phenotype. Small colony variants have been seen associated with lung cultures, which have shown decreased expression of many of the virulence factors associated with *S. aureus* [24]. This could indicate that the bacteria are attempting to avoid the host immune system, allowing survival in the lungs for a longer duration. What potentially worsens the overall clinical course is the acquisition of MRSA in the lungs [23]. Although it is more rare than its antibiotic-sensitive counterpart, it is possible to acquire this strain nosocomially, or even develop the strain independently through the constant use of β -lactam antibiotics to treat existing methicillin-sensitive *S. aureus* (MSSA) infections. Interestingly, it has been found that in situations where a patient is infected with both *S. aureus* and *PA*, treatment of one can lead to the prominence of the other [25]. When patients were given anti-staphylococcal antibiotics, it was found that *PA* infection could develop and fill the niche that the eradicated *S. aureus* no longer occupies. This is another unfortunate side effect of a *S. aureus* infection, that not only can its treatment lead to a potential development of MRSA but it can also cause an exacerbation of any existing *PA* infection. This is of great clinical importance to note for younger patients who have not yet been colonized with *PA*.

The second major strain historically found in addition to *PA* is *Haemophilus influenzae*. This bacterium is similar to *S. aureus* in that it is a normal commensal organism of the nasopharynx region, but can actually be found in normal lungs. As an individual progresses through life, the chances of being colonized with *H. influenzae* increases, rising from 20% in infants, to 50% of children, to more than 75% of adults [26]. This has led some to believe that *H. influenzae* is one of the earliest CF pathogens, and that it modifies the lung in a manner that allows for subsequent *PA* infection [27]. The forms of *H. influenzae* that can colonize an individual vary, depending on the presence or absence of a capsule around the bacterium. When a capsule is absent, the bacterium is referred to as non-typeable *H. influenzae* (NTHi), and this form is most associated with respiratory infections [26]. Within the CF lungs, the bacteria attempt to attach to the epithelial cells underlying the PCL. Once attached, the cells will trigger innate immune responses, which can lead to initial inflammation processes [27]. After this, NTHi will try to evade the immune response, using a series of enzymes to destroy antibodies, and its ability to create microcolonies, and to invade the epithelial cells [26]. For this reason especially, NTHi is an important pathogen to be aware of for treatment strategies.

The last of the historically major three bacteria associated with CF, *Burkholderia cepacia* is often considered the most dangerous lung pathogen requiring rapid and rigorous medical attention. Although first identified as an individual bacterial species related to *Pseudomonas*, it has since

been reclassified as a complex of genomovars (*Burkholderia cepacia* Complex, BCC), which have similar characteristics [28]. BCC is generally considered one of the main causes of pneumonia in immunocompromised individuals, which includes CF patients. BCC has been found to co-infect individuals with other CF associated bacteria, such as *PA* [29]. The complex is also known to produce virulent factors, such as elastases and gelatinases, which can weaken cell-to-cell interactions [29]. In addition, BCC fills a unique niche in the CF lung by being able to invade macrophages that have made it to the lung. These tactics caused the bacterium to be of great concern when it was first identified in CF patients in 1984, but since then, epidemiological and clinical studies have greatly advanced treatment [28].

Several other genera of microbes, including *Achromobacter*, *Pandorea*, *Alcaligenes*, *Stenotrophomonas*, and *Ralstonia*, have also begun to be found in CF sputum in the past 20 years. These bacteria are quite often associated with more advanced stages of the disease, indicating that they may need the fertile ground of the CF lung to which they become accustomed before they can thrive [19, 16]. *Alcaligenes* and *Stenotrophomonas* are increasingly found with a *PA* or BCC infection [19]. In addition, several fungal pathogens have been associated with CF as well, such as *Aspergillus fumigatus* and *Candida albicans*. While these fungi have not been the primary focus of CF microbial treatment, recent evidence suggests that they can, in fact, become a problem for CF patients. They have been associated with an increase in inflammation, as well as a possibly more severe disease progression. Both host and fungal factors are equally important in determining if the fungus will become pathogenic [30].

Although each of these pathogens has been studied as a single organism in the CF pathology, limited efforts have been made into looking at the interaction between the bacteria. Considering that more often than not multiple genera of bacteria are found in the CF lung, this is an important aspect to consider. Research that has been studying this topic has focused generally on the interaction between *PA* and another species of bacteria, as this is the most common pathogen associated with CF. For instance, work has been conducted to elucidate the relationship and interactions between *PA* and BCC. In these cases, initial reports showed that BCC and *PA* formed communal biofilms, where both microbes could be detected in a “biofilm-like” structure. They share similar quorum sensing molecules, and together could be promoting the growth of one another. However, further investigation began to see that these bacteria did not necessarily occupy the same biofilm structures [29]. In some cases, it could occur, and could be recapitulated in vitro. However, in other situations, the bacteria did not cooperate. It seemed that *PA* was producing a secreted chemical that prevented the growth of BCC nearby, which was suspected to be pyocyanin [29]. This suggests that all of the relationships in CF may not be symbiotic or even mutually beneficial. Perhaps it depends on the timeline of when the infections are established. However, more work is needed to determine the actual relationship between these bacteria. The entire web of bacterial networks in the CF lung is not trivial, but deciphering how it works could potentially allow for more effective treatment of lung infections.

While this paradigm of “the big three” bacteria for CF persisted for several decades, along with the recent knowledge that several other species could infect the CF lung, a paradigm shift occurred around 2008, when it was discovered that there were obligate anaerobes present in

the lungs of older CF patients [31]. Included in this group of bacteria were the genera *Prevotella*, *Veillonella*, *Propionibacterium*, and *Actinomyces* [31]. This indicated that at least part of the lung must become anaerobic during the disease, which added to the notion that the CF lung has a vast array of micro-environments within it, each of which can be colonized by different bacterial species. It also corroborated the evidence of anaerobic niches being produced in the mucus plugs, which to this point had only been associated with bacteria converting to anaerobic growth.

With the discovery of the aforementioned anaerobic bacteria, it became clear that there was most likely a temporal aspect of CF infections as well. Clinical evidence has shown that depending on the age of the patient, certain bacteria are more likely to be cultured with their sputum (Figure 3). Early in the patient's life, from birth until around ten years of age, it is common for patients to test positive for *S. aureus*; but later in life this infection seems to become less likely [25]. *PA* can also be acquired early in life, however, much effort is often put into trying to eliminate this infection. As a result, a patient may test positive for *PA* several times throughout their life, even if the infections are not chronic. However, often by the patient's teenage years, *PA* has established a persistent infection and may have already converted to its mucoid form [32], leading to 73% of adult CF patients harboring such an infection [33]. In contrast to the "major" bacteria of CF, the newly discovered anaerobic bacteria generally do not infect a patient until later in life. This is associated with the development of more anaerobic niches in the lung, which is indicative of mucus buildup and thickening. This occurs later in life as the detrimental effects of a defective CFTR build and the inflammation worsens. Although treatment would still depend on cultures from the patients, establishing a general timeline is beneficial for physicians who need to create a treatment plan for their patients, and could allow for proactive treatment as the patient enters different phases of life.

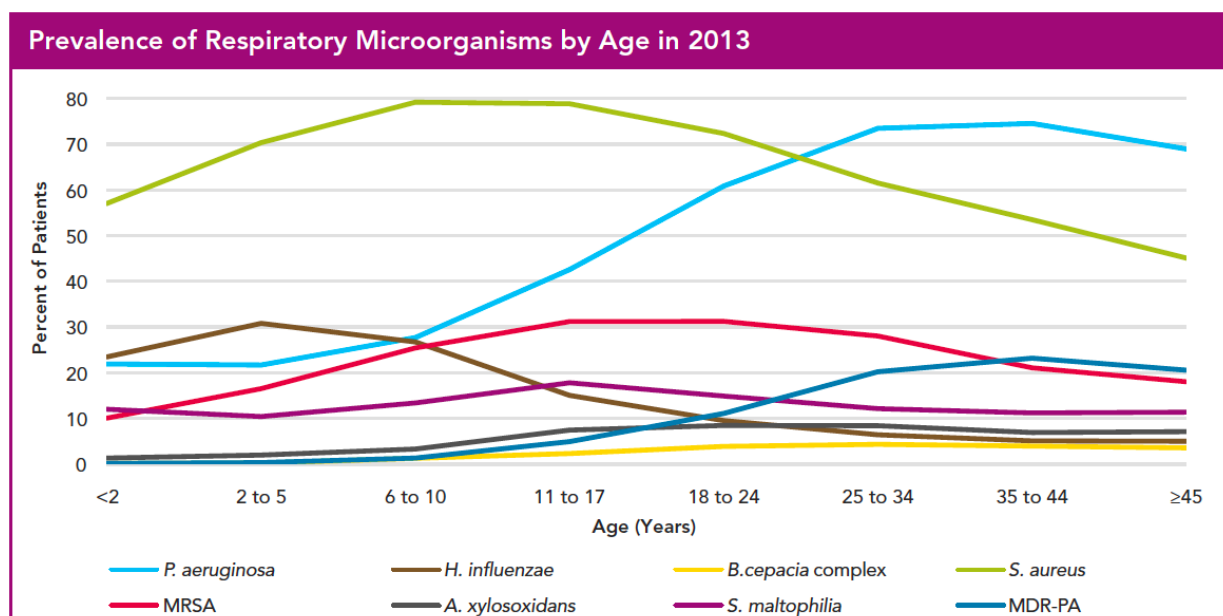


Figure 3. Prevalence of respiratory microorganisms by age in 2013.

As a CF patient progresses through life, the likelihood of culturing positive for a particular microorganism changes. This is due mostly to the changing environment in the CF lungs. This graph depicts the percentage of patients registered in the Cystic Fibrosis Foundation patient registry who tested positive for a particular bacterium, separated by age [4].

Given the presence of anaerobic bacteria, this indicates that current antibiotic regimens for CF patients may have to be revisited. Normal antibiotic treatments include tobramycin, kanamycin, and several other antibiotics of the aminoglycoside class. Those in this class are generally ineffective against anaerobic bacteria due to their mechanism of entry. Aminoglycosides rely on the ability of an organism to respire, using either nitrate or oxygen as the terminal oxygen acceptor [34]. For fermenting bacteria, such as *Actinomyces* [35] and *Prevotella* [36], that means that this class of antibiotics is ineffective. In the case of *PA*, the organism exists as a facultative anaerobe that does use nitrate as a terminal oxygen acceptor. Although that should allow for these antibiotics to work, reports are mixed on the efficacy of the treatment alone, noting the ability for the bacteria to resist the action through a phenomenon termed “impermeability” [37]. It has been observed in multiple studies that CF-related *PA* gains this ability, characterized by a lack of uptake [37]. This may be related to the innate ability of *PA* to produce protective biofilms in its mucoid state, a mechanism already shown to be protective against antibiotic treatment. It may also be due to the action of certain genes involved in the biofilm formation, as one study found that biofilms upregulated *tolA*, whose gene product can alter LPS structure in such a way as to make it more resistant to aminoglycoside recognition, and bacteria which did not produce as much of this protein were far more susceptible to aminoglycosides [38].

4. The major contributor: *Pseudomonas aeruginosa*

Perhaps the most problematic and dangerous of the bacteria associated with CF is *PA*. *PA* is a gram-negative opportunistic pathogen that can sometimes be found as part of the normal human skin flora. However, in cases where the immune system is compromised in some way, the bacteria finds a way to infect a host, which can lead to severe complications. It is also perhaps the most deadly bacteria to be associated with CF, as a patient's life expectancy decreases by roughly 7 years if the patient cultures positive for *PA* [37]. The reasoning for this is that *PA* produces a large number of virulence factors that stimulate inflammation in the airway, and often converts to a mucoid phenotype that allows it to escape a number of host defenses and the use of many antibiotics.

4.1. The role of quorum sensing in *PA* infections

To control the expression of its virulence factors, *PA* uses a bacterial “communication” system known as quorum sensing. This is not unique to *PA*, and has been shown to exist in a number of other bacteria, including *E. coli*, *S. pyogenes*, and *S. aureus* (another CF associated bacteria) [39]. However, much research has been conducted into the complex regulatory pathways associated with this system in *PA*, which controls not only the means by which the bacteria move in relation to each other, but also the expression of virulence factors that can affect the

patient and the ability to convert into a biofilm [39]. Quorum sensing in *PA* is accomplished by at least two regulatory pathways, the *las* and *rhl* systems [40]. The *las* system is hierarchically first for sensing other molecules. The system is composed of two primary genes, *lasR* and *lasI*, respectively. The transcriptional activator gene *lasR*, responds to environmental cues to produce the transcription factor LasR. LasR on its own, however, cannot properly interact with any genes. It needs a cofactor, specifically the quorum sensing autoinducer 3-oxo-C₁₂-HSL (PAI-1) produced by the *lasI* gene product. This molecule can be taken up from other nearby bacteria that have released it into the supernatant and especially in biofilms [41]. When PAI-1 enters the bacteria, LasR forms a complex that is then able to act as a transcription factor. It has two major targets in the system, *lasI* and *rhlR*. Importantly, by activating *lasI*, the system begins an autoinduction loop that will maintain it in the cell and allow for the spread of signal to other bacteria. The gene encodes an enzyme, LasI, which is able to produce the 3-oxo-C₁₂-HSL necessary to activate LasR. More active LasR results in the production of more LasI and PAI-1, amplifying the signaling effect.

However, the system looping and increasing would mean nothing if there was not an output somewhere. This output happens to be the second major system for quorum sensing, the *rhl* system. This system behaves very similarly to the *las* system. After activation by the LasR-PAI-1 complex, the RhlR transcription factor is produced. This requires a different signaling molecule, C₄-HSL (PAI-2), with which to form a complex. Once the initial signal is received, the complex is formed and activates RhlI, which produces more PAI-2, and this, in turn, perpetuates this signaling process. At the end of this process, the bacteria result in having a buildup of RhlR-PAI-2 complex, which can help upregulate transcription of a number of virulence factors, including *lasA* and *lasB* (elastase related enzymes necessary for invasion) [42, 40], *xcpP* and *xcpR* (components of the type II secretion system), and even the stationary phase sigma factor gene *rpoS*, a gene linked to antibiotic resistance in *PA* [40]. However, one of the most important secreted factors dependent on the *rhl* system is the production of rhamnolipids, for which the system was named. These are important virulence factors that *PA* produces that can lead to inflammation and associated immune responses.

Another important output of the *las* system is the *Pseudomonas* quinolone signal (PQS) regulon. The PQS regulon is responsible for some of the phenotypic changes seen with *PA* once it begins to grow in communities [39, 40]. The regulon itself requires the induction of *pqsR*, the regulatory transcription factor for the rest of the genes. Once this is active, the transcription factor will homodimerize to activate the rest of the *pqs* genes, as well as the induction of phenazine genes (*phnAB*). Together, these will allow for the production of HHQ (2-heptyl-4-quinolone, a precursor to PQS) and PQS signals that will not only allow for the autoinduction of the pathway, but also for the eventual production of pyocyanin and pyoverdine, important siderophores that also lead to the green color that is often seen with *PA*-laden CF sputum. In addition, this activates the production of Hydrogen Cyanide (HCN), an important metabolite for *PA* as well as a potential toxic agent for the patient and other bacteria.

4.2. Alginate production and the conversion to mucoidy

Although these quorum-sensing pathways are important for the virulence associated with *PA*, it is also important to note that this is not the only system involved in its infection of a CF

patient. Perhaps more important for the chronic infection of patients are the genes and pathways involved in the conversion of *PA* from its normal phenotype to its mucoid form. This mucoid phenotype has been known to be associated with CF infection, especially during chronic infection of patients [43, 31]. It is characterized by the general loss of motility for the bacteria [44], and the overproduction of the viscous exopolysaccharide called alginate. Alginate surrounds the bacteria and creates a material that seems quite like mucus, hence the appropriate coining of the term “mucoid phenotype.” Mucoid *PA* has also been associated with the production of biofilms and is involved in general resistance to several types of antibiotics and environmental stresses, such as desiccation and nutrient depletion. However, this conversion is a complicated procedure that results in a significantly altered regulatory network of several genes associated with alginate production.

The most important gene involved in the process of alginate production is *algU* (*algT*), a major component of the 12-gene operon that results in the production of alginate. AlgU is a extra-cytoplasmic sigma factor that is expressed at a low level in nonmucoid bacteria, and this basal level of protein is sequestered by the membrane spanning anti-sigma factor, MucA (Figure 4). This interaction is very important for the eventual conversion to the mucoid state. When MucA normally sequesters AlgU in its nonmucoid state, the bacteria do not produce any significant amounts of alginate. However, under conditions of stress (oxidative, osmotic, heat shock), the bacteria will eliminate MucA through one of two means [45]. The first is the proteolytic destruction of MucA. In conditions where the bacteria are stressed due to nutrient depletion (iron, carbon, or phosphorus), desiccation, or antibiotics, the bacteria can trigger the production of a protease that targets MucA. Upon the degradation of this protein, the bound AlgU is released into the cell to act as a sigma factor ($\sigma^{E,22}$), in combination with several other proteins, to upregulate the operon responsible for alginate production, including most of the *alg* genes, such as *algD*, *algC*, and many others. Once this is accomplished, the cell will produce and secrete alginate, allowing for expression of the mucoid phenotype.

Under normal growth conditions in *PA* (Panel A), MucA (Blue) interacts with periplasmic MucB (Green) to allow for cytosolic sequestering of AlgU (Red). This prevents AlgU from acting as a transcription factor, effectively keeping mucoid genes inactivated. However, in times of stress, *PA* can either inactivate MucA through proteolysis (Panel B) or hypermutation (Panel C). In these cases, MucA is no longer able to interact with the periplasmic MucB, and the result is the loss of sequestered AlgU, allowing it to now activate genes in the cell, including AlgD and related mucoidy genes. Adapted from Hassett et al., 2009 [46].

However, in cases where constitutive mucoidy is found, such as in CF patients chronically infected with *PA*, the phenotype is most often caused by a mutation within the *mucA* gene. This is suspected to still be an outcome of environmental stress (as the CF lung is often lacking water and may contain innate immune defenses). However, the result is not a temporary upregulation of alginate related genes, but rather, a hypermutable phenotype that will usually allow for mutations within the *mucA* gene to occur [45]. This hypermutability stems from the mutation of several genes associated with DNA repair, including the DNA mismatch repair system (MMR) and the deoxyguanine repair system (DO) [45], although errors from the DNA polymerase IV could also lead to these mutations. The most common mutation found associ-

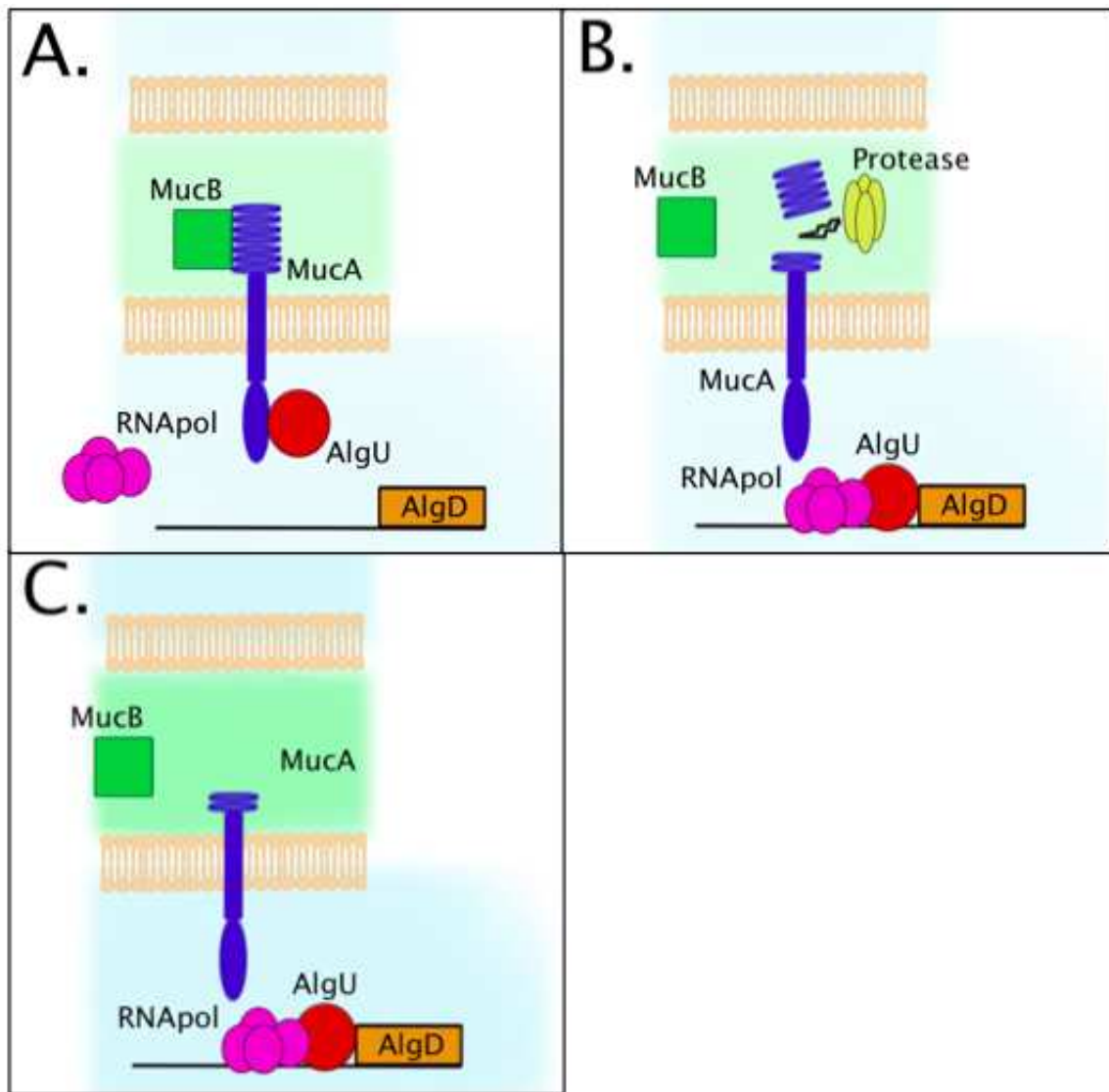


Figure 4. MucA interactions with AlgU.

ated with this is in *mucA*. One such mutation, *mucA22* (a deletion of 5 G residues from bases 431–436), is commonly found, although several others have been associated with this phenotype. Because of these base deletions, the protein is truncated to 15.8 kDa, where it has lost its binding domain for AlgU. As a result, AlgU is constitutively active in the bacteria, resulting in a constant mucoid phenotype in vivo. A mucoid phenotype has also been associated with mutations in other *muc* genes, including *mucB*, *mucC*, and *mucD*, however, these are not as frequent.

Once the patient acquires mucoid *PA*, their clinical course usually diminishes dramatically. This conversion is often a result of chronic *PA* infection, which may not cause a drastic change in health immediately, but will cause chronic inflammation and lead to decreased lung function. Currently, much of the effort for treating CF infections is focused on dealing with biofilm associated bacteria, which includes the mucoid *PA*.

4.3. PA biofilm formation in CF patients

Herein, we will focus on the biofilm formation of *PA* on the biotic or abiotic surface, in vitro. There are five steps for biofilm development on this kind of surface (Figure 5). This process begins when (Step 1) the planktonic or free-living bacteria (Step 2) attach to the surface as show in Figure 5. Several genes and factors are required for this initial step of biofilm formation such as the expression of flagella, type IV pili, Cup fimbria, and the activation of *sadB* [47]. Next, (Step 3) cells begin to produce the extracellular matrix components that facilitate the bacteria joining together and forming microcolonies. The matrix contains polysaccharides, proteins and eDNA, which plays a role in the up to 1,000-fold increase in antibiotic resistance of the biofilms compared to planktonic bacteria cells [48, 49]. Then, (Step 4) the microcolonies will progress and mature into a thicker biofilm (maturation), thus resulting in the development of a gradient of oxygen in the biofilm. Here, the surface has a higher oxygen concentration than in the deeper part of the biofilm. In this case, the biofilm is composed of aerobic, micro-aerobic, and anaerobic environments. The factors that also play an important role in this maturation step are involved cell motility. For example, the creation of the mushroom cap-like structure (biofilm maturation) required *pilA* (a pilus component). This also involves parts of the quorum sensing system as it has been shown that a *lasI* mutant will form a flat and thin biofilm while *rhlI* or *pqsA* mutant form microcolonies lacking the mushroom caps [48]. Several genes have been reported to be involved in the biofilm development such as the response regulator of the GacA/GacS two-component regulatory system (*gacA*) that is involved with the extracellular matrix components, a sigma factor (*rpoS*) that regulates a number of genes in the stationary phase that overlap with genes regulated by quorum sensing, the alginate biosynthesis regulator (*algR*) that is involved in type IV-mediated twitching motility, and a regulator of exopolysaccharide and Type III secretion (*retS*) [48, 50, 51, 52]. Finally, (Step 5) biofilm dispersion will occur where the biofilm components have been broken down or modified to release the surface proteins resulting in changes of the biofilm structure and also the forming of a new biofilm [47, 53]. Many genes have been studied in regard to biofilm dispersion. One such gene is *rhlA*, one of the genes required for rhamnolipid biosynthesis, which has been reported to be required for the dispersion of the bacteria from the center of the mushroom structure [54]. Another gene involved is the biofilm dispersion locus (*bdlA*), which studies have shown may be a link between sensing environment cues, c-di-GMP levels and detachment of the biofilm [52].

In CF patients, 65%–80% of all microbial infections are biofilm related [48]. The CF lung provides a suitable environment for *PA* to infect and form a biofilm. The normal airway epithelia cells are hydrated by a mucus that contains a mixture of electrolytes, glycoproteins such as mucins, protein, and lipids. The failure to elucidate free-living *PA* by the inflammatory system combined with the bacterial defense mechanisms such as oxidative stress in a CF patient results in a vicious environment [55, 56]. This environment can be oxygen depleted as well, causing it to be considered micro-aerobic or anaerobic [5]. Several studies have supported the ability of *PA* to form a biofilm in the CF lung, such as one that used a microscopic study of CF sputum, showing an increased resistance to antibiotics and phagocytosis because of the higher production of EPS in the biofilm [46]. This is a common reason to initiate biofilm development, as *PA* biofilms resist phagocytosis by immune cells and are also more resistant

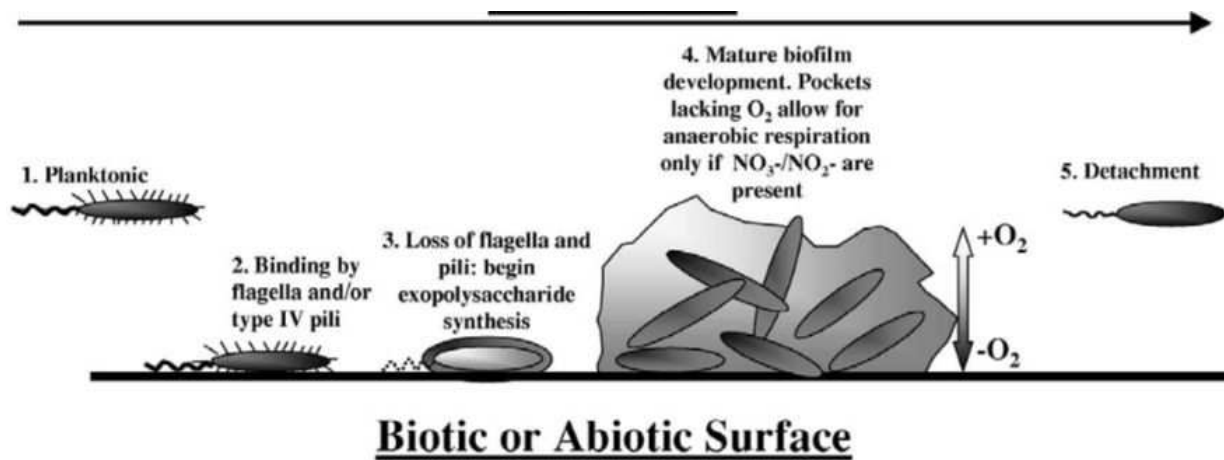


Figure 5. The five steps of *Pseudomonas* biofilm formation on a biotic or abiotic surface (in vitro).

to antibiotics than a free-living cell [55]. To form a biofilm in the CF lung, *PA* must penetrate the mucus layer and enter into a hypoxic zone, where it can then form a biofilm with a quick transition from aerobic to anaerobic metabolism by using the alternative electron acceptors in the mucus such as nitrate (NO_3^-), nitrite (NO_2^-) or nitrous oxide (N_2O) through the respiratory process known as “denitrification” to support anaerobic growth. The ability of *PA* to be able to grow in the anaerobic condition is an important factor for the formation of biofilms in the CF patient. *PA* also can use alginate as an energy source for anaerobic growth [48]. Finally, these macrocolonies of *PA* are formed during a chronic infection of CF [53]. The summary of the biofilm formation in CF patients is shown in Figure 6. Some of the important genes that are involved in the denitrification system are nitrate reductase (*nar*), nitrite reductase (*nir*), nitric oxide reductase (*nor*) and nitrous oxide reductase (*nos*). These genes are under tight regulation in the chromosome. One gene that has been found to regulate this process, *oprF*, is particularly important. It has been found that antibodies against this protein are elevated in CF patients, suggesting that it is upregulated in the bacteria. In addition, an *oprF* null mutant formed poor anaerobic biofilms due to no NIR activity [53]. Quorum sensing (QS) also regulates the genes in the denitrification system. One study showed that *rhl* was required for an optimum balance of the denitrification pathway [13].

4.4. Genetic alterations during *PA* infection

In addition to the changes that are phenotypically eminent such as during mucoid conversion, it is also important to note that additional genetic regulation is occurring in this environment. As mentioned earlier, the CF lung is not a hospitable environment for bacteria, due especially to the dessicated mucus layer, yet it is quite nutrient-rich. If the bacteria can benefit from this, then they will be able to survive and flourish in the lung. In order to do this, the bacteria must undergo several layers of genetic regulation in order to activate specific shunt pathways that will allow them to use the nutrients provided. In the case of *PA*, this has been studied extensively, but has required significant efforts to unravel, notably the creation of various synthetic media that represents CF lung ASL [57] as close as anything else to date.

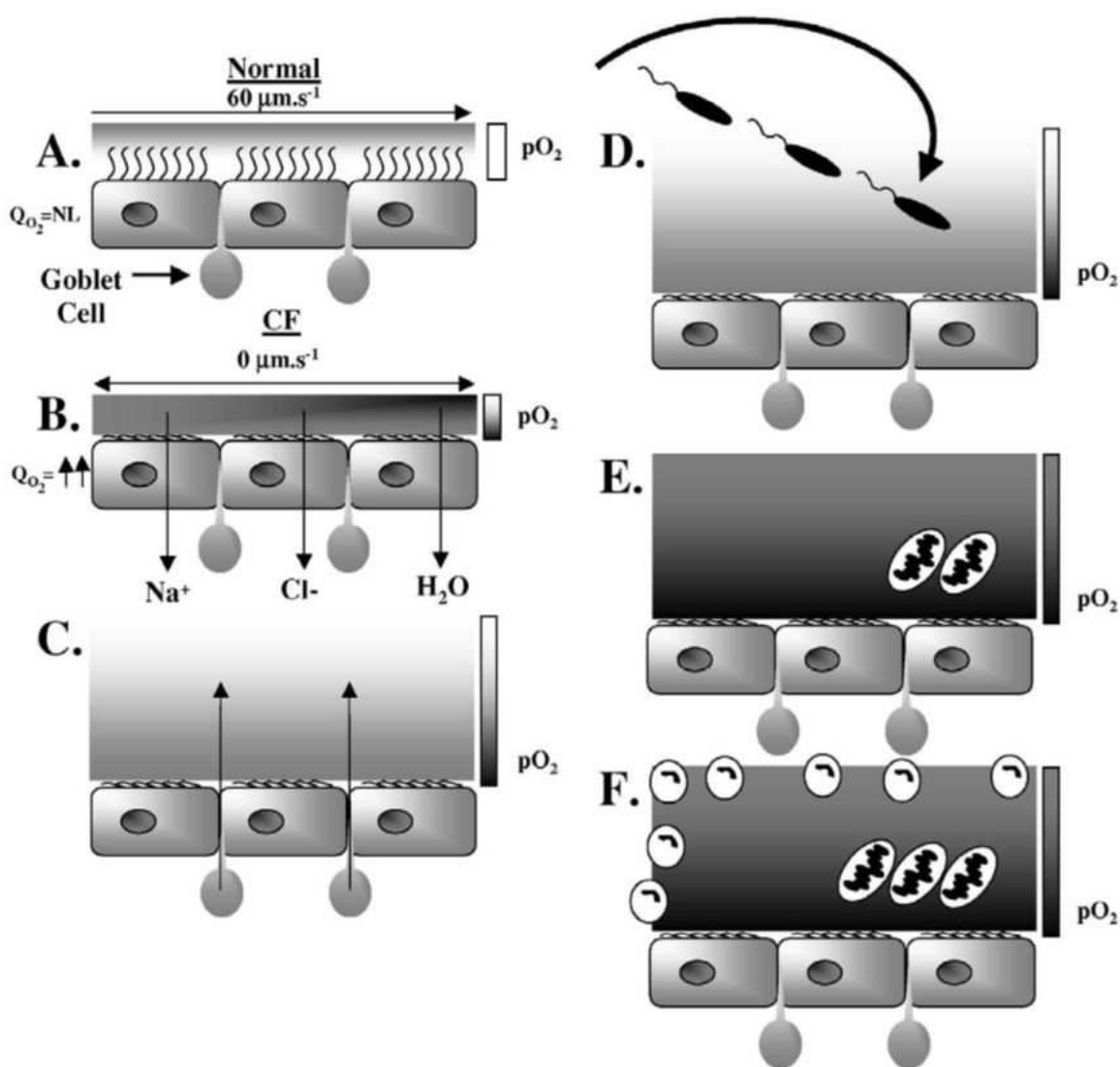


Figure 6. *PA* biofilm formation in the CF lung. A shows the normal epithelia cells. B–F depict CF airway epithelia. B–C show the mucus formation as mentioned previously in the CF lung section of this chapter, resulting in the hypoxic zones in the CF lung. D–F illustrate the free-living cell bacteria moving into the hypoxic area (D). E shows the bacteria adjusting themselves from an aerobic to anaerobic condition and forming macrocolony, biofilm, in this environment. F shows the increasing amount of macrocolonies.

Early gene expression analyses showed several important changes in expression levels, but suffered from lack of application. Some of the first analyses were of general biofilm gene expression, which were important as a seminal work. For example, Whiteley et al. performed a transcriptional profiling analysis in 2001 that examined gene expression by *PA* biofilms grown on granite pebbles [38]. They discovered that only a small subset of genes showed any change in expression (73 genes), roughly splitting 50:50 into up- and downregulated genes. This was quite interesting, as considering the relatively large change in phenotype between normal *PA* and biofilm structure, it would seem that more changes would have had to occur. In addition, several genes promoting the resistance to certain antibiotics were upregulated, and when they treated the biofilms with antibiotics, a different gene expression profile was

seen. This all suggested that biofilm bacteria might be able to respond better to antibiotics and environmental stresses than planktonic cells, as the biofilm is not only more inherently resistant, but the bacteria interior to the film experience a lower concentration of the stressor, allowing for genetic regulations and responses [38]. Following this paper, more attempts to analyze biofilm-related genes ensued. These studies involved mostly environmental biofilms, and not those associated with human infection. Eventually, as the prevalence of biofilms in disease became better known, it became important to discover if these changes in biofilm gene regulation were able to be transitioned over to this situation.

In this regard, several attempts to examine human-associated biofilm infections occurred, but it had to be verified that the gene expression difference was reliable, and not an artifact of the harvesting procedure. Some studies did well with this issue, collecting *PA* from patients and analyzing both that sample and in vitro grown cells. These differences did seem quite significant, showing changes between the two samples that suggested a decrease in the regulatory machinery for biofilm cells rather than an upregulation of many of the biofilm associated genes (such as *muc*, *alg*, *rhl*, *hcn*, and *plc*) [58]. This was novel, as the biofilm conversion seemed much more complicated than simply via a dysregulatory pathway.

Other genes that were found to be changed in the CF lung associated samples seemed to actually be associated with the acquisition of nutrients in the surrounding area. For example, one such study found that there was an increase in genes associated with arginine metabolism and the glyoxylate shunt (a process found often with the β -oxidation of lipids to acetyl-CoA) [59]. This seems to indicate that the CF lung has a higher available amount of arginine and free lipids for degradation, potentially linked to the death of epithelial cells in the lung. This same study also found the cells generally change to a sessile state, where many of the genes associated with motility and chemotaxis are downregulated. This indicates that the cells initially colonizing the CF lung are able to prosper enough that the organisms can rapidly adapt to such conditions. This same down-regulation of motility genes is often seen with the conversion to a biofilm mode of growth. Once the bacteria have shifted to the mucoid state and are able to survive the thick, dessicated mucus, then they will be able to thrive in the lung. This can help explain why *PA* is often such a major problem for the CF patients.

5. Treatment strategies for CF

Considering the relative abundance of CF cases (~70,000 world-wide), it is important that efforts are put forward into treating the symptoms that arise from the disease [60]. These symptoms can be very detrimental, especially in the case of serious bacterial infections and ensuing exacerbations. Initially, the treatments that were available were only focused on chest physical therapy for the buildup of airway mucus, but those efforts have evolved as current research has illuminated more on the pathophysiology of CF lung disease. An example of advanced treatment with continuing research was the initial attempts to provide gene therapy for CF. With the idea that complementing the mutated CFTR with wild-type CFTR would allow for functional ion transport, efforts were made to transfect cells with a functional copy

of the gene using either a liposomal or viral vector. However, this eventually ended in failure, as the complementation strategy was unsuccessful and in some cases harmful. Specifically, adenovirus complementation caused a hyper-immune response, and DNA liposome transfection could not properly deliver the DNA to the nucleus [61]. Recent attempts are examining whether transfecting cells with mRNA coding for functional CFTR could be beneficial. However, this still requires much more research. With that in mind, treatment falls primarily into two main camps; the removal of the desiccated mucus, and the treatment of bacterial infections.

5.1. Treatment of mucus buildup

Even without the involvement of pathogens in the mucus plugs that develop in the CF lung, the plugs themselves can cause serious effects on the patients, specifically in their quality of life. As mentioned previously, these plugs develop from a lack of clearance of mucus associated with the pericilliary layer, resulting in a continually developing blockage and a decreased airflow that reaches the respiratory zone of the lungs. With reduced oxygen levels, the patients begin to suffer and can eventually become cyanotic. However, this is one of the more apparent symptoms of CF, and has been a focus since the disease was first identified. Here, the primary mode of treatment has been physical percussion of the patient. This is termed as chest physical therapy (CPT) and has progressed much since it was initially developed. This treatment initially involved pounding on the patient's back in such a manner as to dislodge mucus that was clinging to the bronchi [62]. After dislodging the mucus, the patient would then cough up any that was loosened, but this process in general was quite painful and not desired by patients, even when it was successful. In an effort to make this a more effective procedure, technology began to be introduced into the process. Initially, a device similar to a back-pack, was created that performed the percussion automatically, rather than rely on the doctor or medical professional. Although this is effective in providing the same amount of force each time, the percussion itself was not eliminated from the process.

Following this, different methods were employed to physically break up the mucus. These have become more advanced in recent decades. Most are reliant on sound waves or vibrations to loosen the mucus that allows for its eventual clearance. These methods include masks and vests that patients don that create vibrations and devices that convert the exhaled air of a patient into vibrations [62]. In all of these, the pressure and pain of physical percussion is eliminated, but the positive effects remain. Even some chemical treatments are prescribed to help loosen the mucus, such as aerosolized sodium bicarbonate that can help return the mucus layer to its normal pH and allow for better clearance. Physicians also routinely prescribe bronchodilators and anti-inflammatory medications to patients to both increase the functional airway passage and to help clear some of the mucus from the airways. In some extreme cases, where this use of medication is ineffective or does not keep up with the overall progression of the disease, the patients may have to endure a lung transplant [62]. While this is occasionally effective, it usually results in the eventual buildup of mucus in the lungs once again, resulting in further treatment or potentially even another transplant.

5.2. Treatment of bacterial infections

While the buildup of mucus within the lungs is quite problematic, it is often not what leads to the overall mortality or morbidity of CF patients. Rather, this is due to secondary infections that arise from this buildup. As stated earlier, this mucus is a rich, largely immobile niche of complex nutrients that enmesh the bacteria. In healthy lungs, the cilia are functioning properly with a thin PCL and clearing most pathogens out of the lungs. However, with the thick mucus, this is not the case, as the cilia do not have enough physical force to push it upwards. As a result, bacteria begin to infect the area. However, the precise location in the lungs where the infection resides is important for the choice of treatment methods. In general, the lungs are divided into two zones. The first is the conductive zone, which includes many of the preliminary branches of the bronchi and does not directly include the alveoli or any of the accompanying areas. The second is the respiratory zone, which includes many of the later branches of the airways and the alveoli. These differences are important due to the necessary changes for treatments. In the conductive zone, the tissue does not have quite as high a vascular exposure, but is much closer to the mouth. As such, infections in this area are often treated with aerosolized medicines. In contrast, the respiratory zone is much deeper into the lungs, but has much higher vascular exposure due to the presence of the alveoli in this zone. Treatments for this zone usually include oral or intravenous medications. While the mode of treatment is known, the actual ability to determine where the infection is occurring is dependent on a series of initial cultures from the patient, and the species present can change once treatment begins.

Once the type of treatment has been determined, the next step is determining what the effective antibiotic(s) is for the particular bacteria being targeted. This is dependent on the class of antibiotic and has to be administered in a patient-specific manner. In general, there are some that work more efficiently in certain patients than in others. For example, tobramycin, a potent aminoglycoside, has been approved by the FDA for clinical treatment of *PA* in CF patients. However, various reports have shown an impenetrability of tobramycin in biofilms, an effect that may be due to the lowered levels of oxygen within the biofilms. The ability of an antibiotic to be active on biofilm bacteria is of the utmost importance when selecting a treatment strategy [33]. As mentioned previously, biofilms generally develop more resistance to certain antibiotics due to their differential metabolism, as well as penetration issues. Those at the surface of the biofilm are generally more metabolically active, while those in the middle of the biofilm projections and those at the base are generally far less active or even inactive. Ciofu et al. [63] showed that certain antibiotics will be more or less effective in these areas depending on their mode of action. Tobramycin is highly effective on the more oxygen-rich outer layer of metabolically active bacteria, but others such as colistin are better suited at dealing with the metabolically inactive bacteria further into the biofilm. In addition, both basic and clinical scientists are focusing better on the proper physiology of bacteria for treatment. This is something that is case-dependent, and cannot necessarily be generalized.

However, the efficiency of the antibiotics for long-term treatment of *PA* infections has been worrisome, especially because of the development of multi-drug resistant *PA* (MRPA) [64]. Because of these issues, it is imperative to unravel new treatments that will work on bacteria that are growing under non-traditional conditions (e.g., anaerobic or microaerobic). That is

why certain groups are evaluating the ability of other compounds for bactericidal action. One of the more notable treatments was discovered by our group in defining the use of acidified nitrite for the treatment of mucoid *PA* infections. In 2006, we discovered that the application of acidified nitrite onto anaerobic *PA*, both planktonic and biofilm associated, virtually killed the organisms; however, the same effect was not quite seen aerobically [65]. The treatment also had to be administered at a slightly acidic pH (6.5) of the CF lung. This combination, however, was very effective. Further investigation showed that acidified nitrite was unstable and produced a toxic, bactericidal intermediate nitric oxide (NO). Although *PA* normally proceeds through the route of denitrification, this process is overloaded by an excess of NO_2^- , and subsequently NO. The enzymes involved in the detoxification process are not able to compensate for this overload, and the bacteria ultimately become poisoned. This occurs through the reaction of NO with sulfur from cysteines in the proteins in the cell, which produces S-nitrosylated proteins (S-NO) that are targeted for degradation. In addition, NO also reacts with iron/heme containing proteins leading to the formation of dinitrosyl iron complexes. Considering the simplicity and remarkable efficacy of this treatment, this is an example of what to look for in the future.

6. Future treatments

While advancements in medicine are not written in stone, there are trends that are observable that can dictate what advancements should be expected. Most of these trends are collected on the website for the CF Foundation [66], which monitors all forms of data related to CF research. In this case, they maintain a log of what is in the pipeline for research, as well as commonly available treatments for patients. In general, the medications available fall into six categories: CFTR modulators, anti-inflammatory drugs, anti-infective drugs, nutrition, mucus alteration, and airway surface restoration. Much of the research on medications has been focused on anti-inflammatory and anti-infective medications. While most of the anti-infective compounds have been modifications of available antibiotics into inhalable forms (such as aerosolized amikacin and vancomycin), the developments in anti-inflammatory medications are slightly more unique. One such example is the development of a drug that is targeting the Type III Secretion System of *PA*. This is a humanized monoclonal antibody directed against one of the protein components of the T3SS. The study states that its goal is to develop an anti-inflammatory molecule, which this antibody serves to do. By interacting with the T3SS, it will hopefully reduce any inflammation caused by sensing this molecule, and potentially reduce the amount of molecules secreted by this system. However, as they note, this is not a means to kill *PA* [66]. The antibody does not serve that purpose, so it is not able to function as an antibiotic in infected patients.

Another field that seems to be growing with potential treatments is the area of CFTR modulators. This field of treatment is focused on finding drugs that change the defective behavior of mutant CFTR protein. Since one form of mutant CFTR is produced yet does not make it to the apical membrane of the cells, some thought has gone into making it possible for the protein to make it to the surface, and hopefully in that process, fold properly to allow for a functioning

anion channel. All of these are experimental compounds, but have made it to Phase 2 clinical trials at the time of this publication [66]. The first is a “potentiator”, a compound that supposedly will be able to open a defective CFTR once it has reached the surface. While this is not meant for all forms of the disease, it is possible that this will have a great impact. Next is a “corrector” that is meant to move the folded protein to the correct location in the cell. This can help with proteins that have misfolded and are sent for degradation or are sent to a different membrane of the cell (e.g., basolateral). Finally, a synthetic signaling molecule has been developed that is meant to supplement decreased levels of S-nitrosoglutathione (an NO generator) in the cells of CF patients. This signaling molecule has been found to be decreased in these patients, and already evidence from the trial has shown that by supplementing this signal, there is increased and proper folding of the CFTR and function of the channel once it is in the membrane.

7. Conclusions

Bacterial infections of CF patients have been a cornerstone of treatment for decades, and that will not change going forward. Future efforts will be focused on finding alternative treatments that will be able to affect both planktonic and biofilm associated organisms in the lungs. This aspect of bacterial growth is most likely the key to effectively remove the organisms from the CF lung and improve the overall life expectancy for these patients. Already, great advances have been made in the last thirty years, as evidenced by not only the number of new and varying treatments, but also by the increased average life span for CF patients (40.7 years) [4]. While some research will still be focused on finding a way to directly treat the CFTR mutation, short-term research needs to be focused on discovering new and innovative treatment options.

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Author details

Cameron T. McDaniel, Warunya Panmanee and Daniel J. Hassett*

*Address all correspondence to: Daniel.Hassett@UC.Edu

University of Cincinnati, College of Medicine, Cincinnati, OH. Department of Molecular Genetics, Biochemistry, and Microbiology, USA

References

- [1] Starner TD, McCray PB Jr. Pathogenesis of early lung disease in cystic fibrosis: a window of opportunity to eradicate bacteria. *Annals of Internal Medicine*. 2005;145:816.
- [2] Riordan JR1, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science*. 1989 Sep 8;245(4922):1066-73.
- [3] MacKenzie T, Gifford AH, Sabadosa KA, Quinton HB, Knapp EA, Goss CH, et al. Longevity of patients with cystic fibrosis in 2000 to 2010 and beyond: survival analysis of the cystic fibrosis foundation patient registry. *Ann Intern Med*. 2014;161:233-241. doi:10.7326/M13-0636
- [4] Cystic Fibrosis Foundation. Cystic Fibrosis Foundation Patient Registry 2013 Annual Data Report to the Center Directors. Bethesda, Maryland.
- [5] Coakley RD, Grubb BR, Paradiso AM, et al. Abnormal surface liquid pH regulation by cultured cystic fibrosis bronchial epithelium. *PNAS*. 2003;100:16083-16088.
- [6] Gustafsson JK, Ermund A, Ambort D, et al. Bicarbonate and functional CFTR channel are required for proper mucin secretion and link cystic fibrosis with its mucus phenotype. *The Journal of Experimental Medicine*. 2012;209:1263-1272.
- [7] Livraghi A, Randell SH. Cystic fibrosis and other respiratory diseases of impaired mucus clearance. *Toxicologic Pathology*. 2007;35:116-129.
- [8] National Heart, Lung, and Blood Institute; National Institutes of Health; U.S. Department of Health and Human Services.
- [9] Rowntree RK, Harris A. The phenotypic consequences of cftr mutations. *Annals of Human Genetics*. 2003;67(5):471-485.
- [10] Tarran R, Button B, Boucher RC. Regulation of normal and cystic fibrosis airway surface liquid volume by phasic shear stress. *Annu Rev Physiol*. 2006;68:543-561.
- [11] Boucher RC. An overview of the pathogenesis of cystic fibrosis lung disease. *Adv Drug Deliv Rev*. 2002;54:1359-1371.
- [12] Worlitzsch D, Tarran R, Ulrich M, Schwab U, Cekici A, Meyer KC, Birrer P, Bellon G, Berger J, Weiss T, Botzenhart K, Yankaskas JR, Randell S, Boucher RC, Döring G. Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. *J Clin Invest*. 2002 Feb;109(3):317-25.
- [13] Yoon SS, et al. Pseudomonas aeruginosa anaerobic respiration in biofilms: relationships to cystic fibrosis pathogenesis. *Dev Cell*. 2002;3(4):593-603.

- [14] Britigan BE, Hayek MB, Doebbeling BN, Fick RB Jr. Transferring and lactoferrin undergo proteolytic cleavage in the *Pseudomonas aeruginosa*-infected lungs of patients with cystic fibrosis. *Infect Immun*. 1993;61:5049-5055.
- [15] NIH - National Heart, Blood, and Lung Institute. What are the signs and symptoms of Cystic Fibrosis? Available at: <http://www.nhlbi.nih.gov/health/health-topics/topics/cf/signs>. Accessed January 4, 2015.
- [16] Pulmonary exacerbations in cystic fibrosis with negative bacterial cultures. *Pediatric pulmonology*. 569.
- [17] Elizur A, Cannon CL, Ferkol TW. Airway inflammation in cystic fibrosis. *Chest*. 2008;133:489.
- [18] Armstrong D, Robinson L. Inflammation in the lungs of patients diagnosed with cystic fibrosis: association with Iron Deficiency. 2008;SURG 2(1).
- [19] Microbiology of airway disease in a cohort of patients with Cystic Fibrosis. *BMC Infectious Diseases*. BioMed Central 4.
- [20] Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. *Clinical Microbiology Reviews*. 2002;15:194.
- [21] Doggett RG, Harrison GM, Wallis ES. Comparison of some properties of *Pseudomonas aeruginosa* isolated from infections in persons with and without cystic fibrosis. *J. Bacteriol*. 1964;87:427-431.
- [22] Localization of *Staphylococcus aureus* in infected airways of patients with cystic fibrosis and in a cell culture model of *S. aureus* adherence. *Am J Respir Cell Mol Biol*. American Thoracic Society – AJRCMB. 83.
- [23] Christopher H, et. al. Review: *Staphylococcus aureus* and MRSA in cystic fibrosis. *Journal of Cystic Fibrosis*. 2011;10:298.
- [24] Moisan H, Brouillette E, Jacob CL, Langlois-Bégin P, Michaud S, Malouin F. Transcription of virulence factors in *Staphylococcus aureus* small-colony variants isolated from cystic fibrosis patients is influenced by SigB. *Journal of Bacteriology*. 2006;188:64-76.
- [25] Elborn JS. Treatment of *Staphylococcus aureus* in cystic fibrosis. *Thorax*. 1999;54:377-378.
- [26] King P. *Haemophilus influenzae* and the lung (*Haemophilus* and the lung). *Clinical and Translational Medicine*. 2012;1:10.
- [27] Starner TD, Zhang N, Kim GH, Apicella MA, McCray PB Jr. *Haemophilus influenzae* Forms Biofilms on Airway Epithelia. *American Journal of Respiratory and Critical Care Medicine*. 2006;174(2):213-220.

- [28] Mahenthiralingam E, Baldwin A, Dowson CG. 2007. Burkholderia cepacia complex bacteria: opportunistic pathogens with important natural biology. *Journal of Applied Microbiology*. 2007;104:1539-51.
- [29] Schwab U, Abdullah LH, Perlmutter OS, et al. Localization of Burkholderia cepacia complex bacteria in cystic fibrosis lungs and interactions with Pseudomonas aeruginosa in hypoxic mucus. *Infection and Immunity*. 2014;82:4729-4745.
- [30] Chotirmall SH, McElvaney NG. Fungi in the cystic fibrosis lung: bystanders or pathogens? *International Journal of Biochemistry and Cell Biology*. 2014;52: 161-173.
- [31] Tunney MM, Field TR, Moriarty TF, et al. Detection of anaerobic bacteria in high numbers in sputum from patients with cystic fibrosis. *Am J Respir Crit Care Med*. 2008;177:995e1001.
- [32] Frederiksen B, Koch C, Høiby N. Antibiotic treatment of initial colonization with Pseudomonas aeruginosa postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. *Pediatr Pulmonol*. 1997;23:330-335.
- [33] Simon RH. Cystic fibrosis: Antibiotic therapy for lung disease. Available at: <http://www.uptodate.com/contents/cystic-fibrosis-antibiotic-therapy-for-lung-disease>. Accessed January 6, 2015.
- [34] Bryan LE, Kowand SK, Van Den Elzen HM. Biosynthesis; chemistry; mechanisms of action and resistance: mechanism of aminoglycoside antibiotic resistance in anaerobic bacteria: clostridium perfringens and bacteroides fragilis. *Antimicrob. Agents Chemother*. January 1979;15(1):7-13. doi:10.1128/AAC.15.1.7.
- [35] Takahashi N, Kalfas S, Yamada T. Phosphorylating enzymes involved in glucose fermentation of Actinomyces naeslundii. *J. Bacteriology*. 1995;177(20): 5806-11.
- [36] Downes J, Sutcliffe I, Tanner ACR, Wade WG. Prevotella marshii sp. Nov. and Prevotella baroniae sp. Nov., isolated from the human oral cavity. *International Journal of Systematic and Evolutionary Microbiology*. 2005;55(4):1551-55.
- [37] Moffett KS. Pseudomonas aeruginosa in patients with cystic fibrosis. Infectious Disease and Antimicrobial Agents. <http://www.antimicrobe.org/new/b260.asp>
- [38] Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S, Greenberg EP. Gene expression in Pseudomonas aeruginosa biofilms. *Nature*. 25 October 2011;413:860-864.
- [39] Smith RS, Iglewski BH. P. aeruginosa quorum-sensing systems and virulence. *Curr Opin Microbiol*. 2003;6:56-60.
- [40] Nadal Jimenez P, Koch G, Thompson JA, Xavier KB, Cool RH, Quax WJ. The multiple signaling systems regulating virulence in Pseudomonas aeruginosa. *Microbiology and Molecular Biology Reviews*. 2012;76:46-65.

- [41] Singh, et al. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature*. 2000;407:762-4.
- [42] Cowell BA, Twining SS, Hobden JA, Kwong MSF, Fleiszig SMJ. Mutation of *lasA* and *lasB* reduces *Pseudomonas aeruginosa* invasion of epithelial cells. *Microbiology*. 2003;149:2291-2299.
- [43] Lam J, Chan R, Lam K, Costerton JW. Production of mucoid microcolonies by *Pseudomonas aeruginosa* within infected lungs in cystic fibrosis. *Infection and Immunity*. 1980;28:546-556.
- [44] ES Garrett, D Perlegas, DJ Wozniak. Negative control of flagellum synthesis in *Pseudomonas aeruginosa* is modulated by the alternative sigma factor AlgT (AlgU). *J Bacteriol*. 1999;181:7401-7404.
- [45] Okkotsu Y, Little AS, Schurr MJ. The *Pseudomonas aeruginosa* AlgZR two-component system coordinates multiple phenotypes. *Cell. Infect. Microbiol*. 2014.
- [46] Hassett DJ, Sutton MD, Schurr MJ, Herr AB, Caldwell CC, Matu JO. *Pseudomonas aeruginosa* hypoxic or anaerobic biofilm infections within cystic fibrosis airways. *Trends in Microbiology*. March 2009;17(3):130-138.
- [47] Tolker-Nielsen T. *Pseudomonas aeruginosa* biofilm infections: from molecular biofilm biology to new treatment possibilities. *Acta Pathologica Microbiologica et Immunologica Scandinavica*. December 2014;122(Supp 138):1-51.
- [48] Wagner VE, Iglewski BH. *P. aeruginosa* biofilms in CF infection. *Clinic Rev Allerg Immunol*. 2008;35:124-134.
- [49] Ishida H, Ishida Y, Kurosaka Y, Otani T, Sato K, Kobayashi H. In vitro and in vivo activities of levofloxacin against biofilm-producing *Pseudomonas aeruginosa*. *Antimicrobial Agents and Chemotherapy*. July 1998;1641-1645.
- [50] Parkins MD, Ceri H, Storey DG. *Pseudomonas aeruginosa* GacA, a factor in multi-host virulence, is also essential for biofilm formation. *Molecular Microbiology*. 2001;40(5): 1215-1226.
- [51] Schuster M, et al. The *Pseudomonas aeruginosa* RpoS regulon and its relationship to quorum sensing. *Mol Microbiol*. 2004;51(4):973-985.
- [52] Morgan R, Kohn S, Hwang SH, Hassett DJ, Sauer K. BdlA, a chemotaxis regulator essential for biofilm dispersion in *Pseudomonas aeruginosa*.
- [53] Hassett DJ, Cuppoletti J, Trapnell B, Lyman SV, Rowe JJ, Yoon SS, Hilliard GM, Parvatiyar K, Kamani MC, Wozniak DJ, Hwang SH, McDermott TR, Urs A, Ochsner. Anaerobic metabolism and quorum sensing by *Pseudomonas aeruginosa* biofilms in chronically infected cystic fibrosis airways: rethinking antibiotic treatment strategies and drug targets. *Advanced Drug Delivery Reviews*. 2002;54:1425-1443.

- [54] Boles BR, Thoendel M, Singh PK. Rhamnolipids mediate detachment of *Pseudomonas aeruginosa* from biofilms. *Mol Microbiol.* 2005;57(5):1210-1223.
- [55] Hansen C, Skov M. Evidence for the efficacy of aztreonam for inhalation solution in the management of *Pseudomonas aeruginosa* in patients with cystic fibrosis. *Therapeutic Advances in Respiratory Disease.* 2014;1-6.
- [56] Hogardt M, Heesemann J. Microevolution of *Pseudomonas aeruginosa* to a chronic pathogen of the cystic fibrosis lung. *Current Topics in Microbiology and Immunology.* 2013;358:91-118.
- [57] Fung C, Naughton S, Turnbull L, Tingpej P, Rose B, Arthur J, Hu H, Harmer C, Harbour C, Hassett DJ, Whitchurch CB, Manos J. Gene expression of *Pseudomonas aeruginosa* in a mucin-containing synthetic growth medium mimicking cystic fibrosis lung sputum. *J Med Microbiol.* Sep 2010;59(Pt 9):1089-100.
- [58] Son MS, Matthews WJ Jr, Kang Y, Nguyen DT, Hoang TT. In vivo evidence of *Pseudomonas aeruginosa* nutrient acquisition and pathogenesis in the lungs of cystic fibrosis patients. *Infect Immun.* Nov 2007;75(11):5313-24.
- [59] Hoboth C, Hoffmann R, Eichner A, Henke C, Schmoldt S, Imhof A, Heesemann J, Hogardt M. Dynamics of adaptive microevolution of hypermutable *Pseudomonas aeruginosa* during chronic pulmonary infection in patients with cystic fibrosis. *J Infect Dis.* 1 Jul 2009;200(1):118-30.
- [60] Cystic Fibrosis Foundation. About CF. <http://www.cff.org/AboutCF/>. Accessed 2-1-2015.
- [61] Conese M, Ascenzioni F, Boyd AC, et al. Gene and cell therapy for cystic fibrosis: From bench to bedside. *Journal of Cystic Fibrosis.* 2011;10(Supp 2):S114-S128.
- [62] NIH - National Heart, Blood, and Lung Institute. How is Cystic Fibrosis treated? Available at: <http://www.nhlbi.nih.gov/health/health-topics/topics/cf/treatment>. Accessed January 4, 2015.
- [63] Ciofu O, Tolker-Nielsen T, Jensen PØ, Wang H, Høiby N. Antimicrobial resistance, respiratory tract infections and role of biofilms in lung infections in cystic fibrosis patients. *Adv Drug Deliv Rev.*
- [64] Luna RA, Millecker LA, Webb CR, et al. Molecular epidemiological surveillance of multidrug-resistant *Pseudomonas aeruginosa* isolates in a pediatric population of patients with cystic fibrosis and determination of risk factors for infection with the houston-1 strain. *Journal of Clinical Microbiology.* 2013;51:1237-1240.
- [65] Yoon, SS, Coakley R, Lau GW, et al. Anaerobic killing of mucoid *Pseudomonas aeruginosa* by acidified nitrite derivatives under cystic fibrosis airway conditions. *Journal of Clinical Investigation.* 2006; 116: 436-446.

[66] Cystic Fibrosis Foundation. Drug Development Pipeline. Available at: <http://www.cff.org/research/DrugDevelopmentPipeline/>. Accessed January 5, 2015.

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