



## Complex ecological approach to cystic fibrosis respiratory infections

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### Abstract

Cystic fibrosis (CF) is one of the most common genetic disorders; resulting in a wide variety of complications, including respiratory infections. Such infections are often ineffectively treated within the framework of a classical paradigm of the infectious process. However, little attention is paid to the unique microecological conditions that are formed in CF respiratory tract. This study aimed to exploring the microecological conditions and to finding out how they may influence the pathogenesis of CF infections. These conditions emerge under the influence of local disruptions in the respiratory functions; inflammatory processes, and complicated relations of the individual microorganisms between each other and between the human bodies as their ecological substrates. As a result, microorganisms that are usually safe for the healthy people become extremely dangerous for CF patients; having adapted to a new ecological niche and having got definite resource advantage, which is attributed to the respiratory tract tissue destruction. Additionally, it is still unknown; how do the anaerobic microbes contribute to CF infections, and whether they collaborate with the traditional CF pathogens or compete with them. In this article, we are analyzing CF respiratory infections from the ecological perspective and proposing in our opinion a more comprehensive picture of their pathogenesis.

**Keywords:** Cystic fibrosis, Respiratory microbiota, Bacterial communities, Bacterial successions

### 1. Introduction

Cystic fibrosis (CF) is one of the most common genetic disorders around the world, which is being transmitted through an autosomal recessive type. The cause of this disorder is a mutation in the CFTR (cystic fibrosis trans-membrane conduction regulator)

gene, which encodes for the trans-epithelial chloride-ion transporter. As a result of this genetic defect, secretion of the chloride-ions is disrupted. Accumulation of these ions in the cell and the water molecules associated with them, lead to the secretion



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of mucus with an increased viscosity. This in turn leads to disruptions in the functions of the respiratory tract; pancreas, liver, and the reproductive system ([De Boeck, 2020](#); [Chen \*et al.\*, 2021](#); [Lopez-Valdez \*et al.\*, 2021](#)).

Despite the fact that CF is a multi-organ disease; however the respiratory disorders are the most severe ones. As a rule, they represent a main reason for the high mortality rate among CF patients, which is attributed to the emergence of various chronic infections. These infections are most often caused by such several bacterial spp.; mainly *Pseudomonas aeruginosa*; *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, *Burkholderia cepacia* complex (BCC), and non-tuberculosis mycobacteria ([Blanchard and Waters, 2019](#); [Cuthbertson \*et al.\*, 2020](#)).

The typical respiratory manifestations of CF include productive cough; increased sputum secretion, dyspnea, general malaise, and loss of weight. Over time, the increase of chronic inflammation leads to destruction of the lower respiratory tract (LRT) tissue and to the emergence of bronchiectasis. These cause transition of the functional disruptions in the lungs into an irreversible form ([Shteinberg \*et al.\*, 2021](#); [Polgreen and Comellas, 2022](#)).

Antibiotic therapy of the chronic infections emerging in CF patients is often ineffective. This is attributed to the fact that the traditional antibacterial treatment is based on classical paradigm of the infectious process. The main principle of this view implies the presence of a specific pathogenic microorganism in the human, which has a harmful impact. In CF patients, the pathogenesis of infections is much more complex and multifactorial.

Nowadays, a lot of data has been accumulated concerning disadvantages of the existing antibacterial therapies of the chronic infections in CF. It is widely known that one of the most common CF pathogens is *P. aeruginosa*, which has an outstanding ability to acquire resistance to a wide range of modern

antibiotics ([Pang \*et al.\*, 2019](#); [Hurley \*et al.\*, 2020](#)). In 2014, [Chmiel \*et al.\*, \(2014\)](#) revealed the resistance mechanisms of the traditional CF pathogens to such groups of antibiotics; mainly the  $\beta$ -lactams; aminoglycosides, and fluoroquinolones. The authors also reported about importance of the combined use of oral and inhaled antibiotics; lack of data on the significance of 'non-traditional' pathogens in CF, and how antibiotic therapy can affect these microorganisms and what it can lead to.

A previous study reported by [Goss, \(2019\)](#) that the choice of an antibiotic for the treatment of CF remains a serious issue, as there is no consensus on the appropriate duration of therapy. Later, [Frost \*et al.\*, \(2021\)](#) revealed the absence of an unequivocally effective treatment strategy for the infections caused by BCC. Recently, [Marner \*et al.\*, \(2023\)](#) reported that there is evidence about resistance of CF pathogens to several new antibiotics, including ceftazidime-avibactam; ceftolozane-tazobactam, and cefiderocol.

In CF, as a result of the respiratory tract disfunction; tissue destruction, and chronic inflammation, a unique microecological environment emerges. Such environment differs from the one that the respiratory microbiota occupies under the normal conditions. In this case, microorganisms that are not usually considered as dangerous to the healthy people can be involved in the infectious process, which may be attributed to both of adaptations to the altered environment and obtaining a definite resource advantage.

This fact establishes a necessity for a new approach to study the pathogenesis of infections in CF; mainly from a microecological perspective. It is crucial to understand the conditions in which the microbiota enters the respiratory tract in CF patients, and which relationships are formed between microbiota and the human body. So far, there are already several scientific publications, in which the authors made several attempts to propose an ecological approach to this issue ([Magalhaes \*et al.\*, 2016](#); [Bevivino \*et al.\*, 2019](#)). However, in these previous

works, as a rule, only the pathogenesis of CF itself and the relationship with the individual microorganisms are explored; without reference to the human body as an ecological environment for these microorganisms. As a result, at the actual moment, there is no complete picture of the chronic infections in CF.

In this article, the emergence of infections in CF was explored from several positions, including the physico-chemical properties of the CF respiratory tract; the effect of antibiotic therapy, and the relationship between the "microbe-microbe" and "microbe-host" systems. Afterwards, data on the stages of respiratory microbiota assembly that are typical for CF patients have been provided; considering the above-mentioned features of microecology.

The objectives of this study were to investigating the microecological conditions of CF respiratory tract and to studying how these conditions may impact the pathogenesis of CF respiratory infections.

## 2. Processes, affecting the initial stages of the respiratory microbiota assembly in CF

If we considered assembly of the respiratory microbiota in CF from an ecological view; first of all it is necessary to understand the processes affecting the initial stages of this assembly. These processes include, on one hand, primary settlement of the respiratory tract as an ecological niche, and on the other hand, the formation of certain physico-chemical conditions (*i.e.*, pH and oxygen saturation), which establish stable microbial communities.

Oropharynx is widely regarded as the source of bacterial colonization of LRT. The predominant contribution of the oropharyngeal microbiota to colonization of LRT is associated with high salivation ([Whiteson \*et al.\*, 2014](#); [Venkataraman \*et al.\*, 2015](#)). The nasal sinuses also play a significant role in colonization of the described locus. Their contribution to the colonization is large especially in the cases of rhinosinusitis in CF patients. A recent study conducted by [Armbruster \*et al.\*, \(2022\)](#) has associated pathogens of the chronic infections in the nasal sinuses

(*i.e.*, *P. aeruginosa* and *Staphylococcus aureus*) with further emergence of infections in the lungs.

In general, occupation of an ecological niche (*i.e.*, respiratory tract) occurs during two mutually directed processes; mainly immigration and elimination. The first process includes the inhalation and aspiration of microbes, while the second process includes a mucociliary clearance. Therefore, composition of the primary microbial communities is determined by the balance between immigration and elimination ([Yagi \*et al.\*, 2021](#); [Chen \*et al.\*, 2023](#)).

In CF airways, this balance is disrupted. Initially, the respiratory tract represents a complex and branched system. This environment may become more heterogeneous, due to LRT tissue destruction during emergence of the inflammation and bronchiectasis, in addition to the production of biofilms by some microbial species ([Turcios, 2020](#); [Jurado-Martin \*et al.\*, 2021](#)). All these pre-described factors cause local disruptions in the microbial immigration and elimination. In turn, they lead to the emergence of heterogeneous ecological niches that can be occupied by the various microorganisms. It is important also to consider secretion of the viscous mucus, which creates favorable conditions for colonization and disrupts the functions of mucociliary clearance ([Bergeron and Cantin, 2019](#); [Esther \*et al.\*, 2019](#)).

After occupation of LRT, the microorganisms are exposed to various local factors. A significant process affecting the structure of the microbial communities in CF is the change in pH, which is associated with reduced secretion of bicarbonate ions into the lumen of the respiratory tract ([Cantin \*et al.\*, 2015](#)). The role of such process can be explained by several facts. For example, low pH causes deactivation of the cationic antimicrobial peptides, which are innate immune defense factors secreted by the epithelium and leukocytes ([Shah \*et al.\*, 2016](#)). In addition, acidification of the LRT contributes to hyperproduction of mucus; disruption of its excretions, and disruption of the mucociliary clearance ([Hoegger \*et al.\*, 2014](#)). Moreover, such acidification of the

respiratory tract is also able to activate the cysteine proteases, such as cathepsins B, L, and S, which in turn are able to destruct the immune factors ([Malhotra et al., 2019](#)). All these factors prevent the microorganisms from being eliminated by the immune agents.

In addition, a reduced pH represents a condition that is physiologically more suitable for certain groups of microorganisms ([Quinn et al., 2015](#); [Massip-Copiz and Santa-Coloma, 2018](#)). There is an evidence of direct correlation between pH and functional properties of the microbiota. In the previous study conducted by [Quinn et al., \(2018\)](#), the authors reported a positive correlation between microorganisms using fermentation for energy production and low pH values.

Another important factor affecting the assembly of bacterial communities is the concentration of oxygen in the respiratory tract. Despite high concentrations of oxygen exist in this locus; however the respiratory microbiota includes a wide representation of anaerobic microorganisms. This can be caused by mucus, which prevents oxygen from diffusion into the deeper LRT tissue layers ([Vieira et al., 2022](#)).

In CF, if to compare with the normal conditions, much higher oxygen gradients may emerge between the lumen of the respiratory tract and the deeper mucus layers. Emergence of such gradients is caused by local disruptions of the airway conductions; resulting from their block by large volumes of mucus. Subsequently, this can lead to increased functional heterogeneity in the bacterial community of LRT. The upper layers of mucus are occupied mainly by the aerobic microorganisms, whereas the lower ones are settled by the anaerobic ones. It should be considered that the predominant aerobic microorganisms in LRT usually include the traditional CF pathogens, such as *P. aeruginosa* ([Thornton et al., 2022](#); [Vieira et al., 2022](#)). At the same time, the number of anaerobic microorganisms in CF may decrease, compared with the normal conditions ([Lamoureux et al., 2019](#)).

Therefore, if to apply the ecological principles to CF respiratory microbiota formation, the first stages of the community assembly will be modulated by both processes of immigration and elimination (*i.e.*, stochastic processes). At later stages, assembly of the respiratory microbiota will be modulated mainly under the influence of changes in the physico-chemical parameters (*i.e.*, deterministic processes). Such changes will induce a species and functional reorganization of the primary microbial community ([Liebana et al., 2019](#); [Yuan et al., 2019](#)).

In this study, the aspects that affect the already assembled bacterial community in CF respiratory tract; modifying its species and functional composition, antibiotic therapy, and the relationship between the 'microbe-microbe' and 'microbe-host' system, will be considered.

### 3. Antibiotics as an ecological factor in CF

Antibiotics can be regarded as an ecological factor, since they are able to influence the structure of the bacterial communities; reduce their diversity, and trigger the adaptive cycles during which these communities are reorganized ([Caverly et al., 2019](#)). In addition, while examining the metabolites produced during the chronic infections, various antibiotics are often found in their composition. This can be linked with the acquired resistance of certain microorganisms of the assembled community to such remedies ([Quinn et al., 2016](#)). The ability of communities to acquire resistance after antibacterial treatment has been confirmed ([Diaz Caballero et al., 2015](#); [Clark et al., 2018](#)).

The inhaled antibiotics are effective remedies for CF treatment, since their use allows creating drug concentrations that are much higher than those recorded during oral and intravenous antibacterial treatment. However, inhalation also has certain limitations, due to the heterogeneous nature of the infected areas in CF respiratory tract. Concomitant morphological and functional changes in LRT tissues cause air retention in the individual airways, which is

the reason that the antibacterial supply may not be provided to all the infected areas ([Lenney \*et al.\*, 2011](#)).

In CF patients, efficiency of the antibiotic therapy can also be decreased by local conditions of LRT. Viscous mucosal secretions and production of biofilms by the individual microorganisms prevent the diffusion of antibiotics. Another crucial factor is the decrease in metabolic activity of the individual bacteria, which results in less susceptibility to the antibiotics. Moreover, there can be an increase in the minimal inhibitory concentrations due to the increased bacterial load (*i.e.*, inoculum effect) ([Magréault \*et al.\*, 2021](#)).

Meanwhile, the antibacterial tolerance is not caused by certain genetic determinants, but it is associated with different phenotypical features. However, the ecological principles can also be applied to the emergence of antibiotic resistance by the individual CF pathogens.

[Santi \*et al.\*, \(2021\)](#) study reported about the acquisition of resistance after a preliminary acquired tolerance in *P. aeruginosa* as an example. Initially, the individual species of this bacterium is resistant to the antibiotics only phenotypically; mainly due to the production of biofilms. This means that in the early stages of infection, these bacteria do not have any genetically determined resistance mechanisms. With antibiotic tolerance, there is no increase in the level of minimal inhibitory concentrations of these antibiotics. However, the antibiotics reach their targets in subclinical doses, which in the future may allow the bacteria to move to much a higher level of resistance.

It is important to realize that antibiotic tolerance is not specific and it can manifest itself in relation to several antibiotics; both of the systemic and inhaled ones. Subsequently, there is a possibility of emergence of resistance to several antimicrobial agents. This makes the problem of antibiotic resistance in the chronic infections associated with CF as an extremely serious issue ([Langton Hewer and Smyth, 2017](#)).

#### **4. Inter-microbial relationship in communities of CF respiratory tract**

In CF, the conditionally pathogenic microorganisms should be regarded not as a separate link in the pathological process, but as a part of a complex poly-microbial community. It is related to the fact that in CF airways; the bacteria may have other phenotypical features than they have *in vitro*. Such phenomenon has been described for *P. aeruginosa* ([Vieira \*et al.\*, 2022](#)). This fact has been also supported by the comparative studies on the *in vitro* and *in vivo* sensitivity of the traditional CF pathogens to antibiotics ([Alqahtani \*et al.\*, 2022](#)). Discrepancy between the results of these studies allows to suggesting that modern experimental models are not able to fully recreate appropriate conditions for CF bacterial communities. Only in the last few years certain attempts have been made to create *in vitro* models, which are imitating the microecological conditions of CF in the respiratory tract ([O'Brien and Welch, 2019](#); [O'Brien \*et al.\*, 2021](#)). The relationships among the individual microorganisms make a huge contribution both to the communities' assembly in the CF respiratory tract and to functioning of the already assembled community.

During bacterial communities' assembly, the primary and intermediate communities prepare the foundation for establishing a community that includes most of the common CF pathogens. This can occur by morphological transformation of LRT (*i.e.*, destruction of the tissue in a direct way and *via* stimulation of the inflammatory processes) and by changes in the physico-chemical properties of the environment *via* the production of certain metabolites. For example, *P. aeruginosa* can utilize the lactate produced by its ecological precursor; *Staphylococcus aureus* ([Khanolkar \*et al.\*, 2020](#)). Additionally, *Staphylococcus aureus* can induce the increased secretion of such virulence factors by *P. aeruginosa* such as LasB elastase. This enzyme reduces the uptake of *P. aeruginosa* by the macrophages and leads to an increased lung tissue damage ([Hotterbeekx \*et al.\*, 2017](#)). *Staphylococcus aureus* can also induce the activity of *P. aeruginosa* genes responsible for synthesis of the exopolysaccharides and increase the

mobility of these bacteria ([Tognon \*et al.\*, 2017](#); [Pallett \*et al.\*, 2019](#)). There is evidence that *Staphylococcus aureus* and *P. aeruginosa* can mutually increase the biofilm formation ([Camus \*et al.\*, 2021](#)).

In an already assembled community, *Streptococcus* spp. can produce 2,3-butanedione in response to excessive acidification of the environment. This substance can be metabolized by *P. aeruginosa*; resulting in the production of phenazines, which serve to counteract the oxidative stress. In addition, such substances stimulate *P. aeruginosa* to produce biofilms ([Quinn \*et al.\*, 2014](#)).

Several previous studies conducted by [Scoffone \*et al.\*, \(2019\)](#); [Azimi \*et al.\*, \(2020\)](#) reported that there are other examples of synergistic microbial relationships that contribute to the emergence of the infections in CF. Interactions within the community; during which the expression of virulence factors is regulated, can be summarized by the term 'Quorum sensing'. That is the ability of individual microorganisms to influencing each other's and responds to the external stimuli *via* special water-soluble signaling molecules and auto-inductors. In this way, the most typical CF processes, such as the production of biofilms and increased destruction of host tissues, can be regulated.

[Rutherford and Bassler, \(2012\)](#) added that the most common CF pathogen, *P. aeruginosa*, can use such signaling molecules as auto-inductor-2 (AI-2); 2-heptyl-4-hydroxyquinolone N-oxide (HQNO), Pseudomonas Quinolone Signal (PQS), and diffusion signaling factor (DFS). Other molecules with similar functions can also be produced by BCC-N-acyl-homoserinlactones (AHL); the SsiR signaling system, and the *Burkholderia* diffusion signaling factor (BSF) ([Deng \*et al.\*, 2009](#)).

Production of HQNO and PQS by *P. aeruginosa* can induce biofilm production by *Staphylococcus aureus* ([Fugère \*et al.\*, 2014](#)). According to [Twomey \*et al.\*, \(2012\)](#), *P. aeruginosa* has a higher level of virulence in the presence of *Stenotrophomonas maltophilia*. This is attributed to the fact that *Stenotrophomonas*

*maltophilia* produces signaling molecules, which can activate the expression of *P. aeruginosa* genes encoding for proteases and alginate. On the other hand, *Stenotrophomonas maltophilia* is more resistant to tobramycin in the presence of *P. aeruginosa* ([Pompilio \*et al.\*, 2015](#)).

A similar synergism of *P. aeruginosa* can be described in relation to BCC. Through auto-inductors, *P. aeruginosa* can stimulate the production of virulence factors of the BCC (*i.e.*, siderophores, lipases, proteases). *Burkholderia cenocepacia* is able to stimulate *P. aeruginosa* to form biofilms; in turn receiving protection from the host immune factors, due to disruption of phagocytosis and the inflammatory response ([Chattoraj \*et al.\*, 2010](#)). Several rare pathogens, including *Inquilinus limosus* and *Dolosigranulum pigrum* can modify the structure of biofilms produced by *P. aeruginosa*, thus increasing tolerance of these bacteria to the antibiotics ([Lopes \*et al.\*, 2012](#)).

Mutual induction of the emergence of antibiotic resistance makes a great contribution to the synergistic relationships of the microorganisms. This can occur *via* several mechanisms, including enzymatic destruction of antibiotics ( $\beta$ -lactamases of the *Prevotella* spp. protect *P. aeruginosa* from the exposure to  $\beta$ -lactam antibiotics), or inducing changes in the cell wall (modifications of *P. aeruginosa* polysaccharides in the presence of *Staphylococcus aureus*) ([Vandeplassche \*et al.\*, 2019](#)). Additionally, an increase in antibiotic resistance can occur due to various changes in metabolism of the individual species. For example, HQNO and pyocyanin produced by *P. aeruginosa* can switch the metabolism of *Staphylococcus aureus* to anaerobic metabolic pathways. This can decrease the effectiveness of certain antibiotics such as aminoglycosides, since the energy obtained during the tricarboxylic acid cycle (TAC) is often used for their absorption ([Filkins \*et al.\*, 2015](#)). Furthermore, through the production of pyocyanin, *P. aeruginosa* can increase the antibiotic resistance in *Acinetobacter baumannii*. This compound also leads to the production of reactive

oxygen species (ROS), thereby inducing the expression of catalase and superoxide dismutase, which act as protective agents against the oxidative stress ([Bhargava \*et al.\*, 2014](#)).

A previous study reported by [Schlecht \*et al.\*, \(2015\)](#) added that it is important to consider the possible interactions of bacteria with fungi. The ability of *Candida albicans* to facilitate the adhesion and invasion of *Staphylococcus aureus* has been reported. It occurs *via* the hyphae of *C. albicans*; where *Staphylococcus aureus* penetrates into the cells in parallel with the invasive *C. albicans* hyphae; the phenomenon is known as "microbial hitchhiking". On the contrary, several compounds like phenazines and siderophores may provide antifungal activity ([Santos-Fernandez \*et al.\*, 2023](#)).

The antagonistic relations within the community should also be taken into consideration. In some cases, *P. aeruginosa* may increase the vulnerability of *Staphylococcus aureus* to antibiotics rather than reduce it. This depends on the level of rhamnolipids production, which as being amphiphilic substances can increase the permeability of the cell membrane ([Sotirova \*et al.\*, 2008](#)). Sometimes *P. aeruginosa* can have a direct inhibitory effect on the growth of *Staphylococcus aureus* through the production of many substances, such as hydrogen cyanide; pyocyanin, and quinoline N-oxides ([Biswas and Götz, 2022](#)). *P. aeruginosa* may also induce lysis of the bacterial cells by producing pyocyanin, which contributes to releasing the DNA, and as a result leads to biofilms formation ([Santoro \*et al.\*, 2023](#)).

## 5. Relationship of the respiratory microbiota with the human body as an ecological substrate

The host immune system plays a significant role in the existing relationship between the human body and the bacterial communities. A lot of data has been accumulated on the origin and nature of the inflammatory processes in the respiratory tract of CF patients, as well as on the contribution of these

processes to the pathogenesis of this disease ([Roesch \*et al.\*, 2018](#); [Ghigo \*et al.\*, 2021](#)).

A recent study reported by [Ribeiro \*et al.\*, \(2023\)](#) revealed that in CF pathogenesis, it is possible to outline the so-called basal inflammation. This inflammation precedes infection, as it represents a response not to the pathogenic microorganisms, but to the specific physico-chemical conditions of CF respiratory tract. It is widely assumed that in this locus the contents of the pro-inflammatory cytokines have increased; the balances of ROS and antioxidants have disrupted, and the level of neutrophils has increased. Meanwhile, such a high pro-inflammatory activity leads to the destruction and, subsequently, to reorganization of LRT tissue. As a result, this inflammation leads to changes in assembly of the bacterial communities, since the landscape of the occupied environment has reorganized. On the other hand, proteases secreted by the neutrophils can destroy the antimicrobial peptides, leading to a decrease in the nonspecific resistance.

According to [Quinn \*et al.\*, \(2019\)](#); [Scribner \*et al.\*, \(2022\)](#), it is also crucial to consider the wide range of nutrients that the human body can provide to the microorganisms, acting as an ecological environment. Such substances include amino acids; phospholipids, DNA, and saccharides. The increased content of such nutrients is associated with several inflammatory processes; destruction of the respiratory tract cells, destruction of the protein molecules by the neutrophil proteases, and destruction of the neutrophils themselves.

In this regard, macronutrients are not the only object of interest. From the ecological perspective, it is possible to consider various trace elements. As an example, in CF, iron is an important metabolic factor that binds the human body and the microbiota as parts of one ecosystem. Iron is a valuable nutrient that is necessary for survival of the microorganisms. This is primarily attributed to the fact that iron ions play the role of cofactors in many vitally important enzymes involved in the redox reactions. It is known that in the

sputum of CF patients, the level of iron content is higher, compared with the healthy people (Reid *et al.*, 2007). Recently, Klebba *et al.*, (2021); Andrejevic *et al.*, (2023) proposed that during adaptation to the ecological conditions in CF airways, the traditional CF pathogens have acquired various adaptive mechanisms for capturing and accumulating the iron molecules. Such mechanisms include the production of siderophores; special protein molecules capable of forming soluble compounds with iron. These compounds are subsequently absorbed by the bacteria.

Yu *et al.* (2016) revealed the important role of Psl (*i.e.*, one of the polysaccharides forming *P. aeruginosa* biofilms) in the accumulation of iron ions. In addition, the authors found out that high levels of iron can stimulate the production of Psl. This occurs by suppressing the synthesis of rhamnolipids and by inhibiting the ArmZ transcription factor, which activates the synthesis of alginate; another component of the biofilm. Consequently, iron acts not only as a valuable resource for the microorganisms, but also as a main factor directly leading to an increase in their virulence, since the ability of CF pathogens to persist in the human body and to acquire antibiotic resistance is associated with the formation of biofilms.

Armbruster *et al.*, (2020); Silveira *et al.*, (2021) highlighted that the increased content of mucins is typical for CF respiratory tract, which provides a resource advantage for several facultative anaerobes, such as *Streptococcus* spp. and *Rothia* spp.; capable of decomposing mucins. So far, it should be stated that the mucin decomposition's products, including amino acids and short-chain fatty acids, as well as lactic and citric acids, can be used as nutrients by the classical CF pathogens, including *P. aeruginosa*. This fact fits perfectly into the ecological picture of CF pathogenesis, since the described phenomenon is an ecological succession; during which there is a consistent change of the substrate (human body) and a shift of the dominant communities.

Guss *et al.*, (2011) revealed that the increased amount of lactate in CF respiratory tract may be

caused not only by the activity of anaerobes, but also the epithelial cells of the respiratory tract are capable of secreting lactate under the normal and the pathological conditions.

A previous study reported by Armbruster *et al.*, (2020) highlighted that in the early stages of CF, *Staphylococcus aureus* as one of the members of the pathogenic microbiota uses glucose mainly as a source of energy. The amount of this nutrient may also increase during CF disease, due to the impaired cellular glucose transport resulting from the inflammatory processes.

## 6. Concept of ecological successions and general views of the respiratory microbiota composition in CF patients

For creating a complete ecological picture of CF pathogenesis, assembly of bacterial communities typical for CF airways can be examined within the framework of an ecological succession. Ecological successions are consistent changes of one biological community by others depending on certain ecological conditions, in which such communities are functioning (Chang and Turner, 2019). Taking into account the factors affecting LRT microbiota, as well as the numerous ecological relationships that exist both within the community and between the community and the human body that were described above, it may be assumed that transfer of the ecological principles to the assembly of CF microbiota is rational. Before reviewing microbiota assembly from the ecological perspective, general views of CF microbiota composition should be considered.

Frayman *et al.*, (2019) proposed that the differences between bacterial successions in CF LRT and those in LRT of healthy humans appear already during the first months of life. Probably, these differences may be caused by the previously mentioned dis-balance of immigration and elimination. In healthy infants without any pathologies of LRT, *Streptococcus* spp. and *Neisseria* spp. predominate in the microbiota composition. On the contrary, CF newborns can have



communities that are more similar to the oropharyngeal microbiota. Such infants have higher numbers of *Staphylococcus* spp.; *Pseudomonas* spp., *Corynebacterium* spp., *Moraxella* spp., and *Haemophilus* spp., in addition to reduced representation of *Fusobacterium* spp.

In general, *Staphylococcus aureus* and *H. influenzae* are considered as the main causative agents of infections in CF children of the earliest age. Over time, the frequency of infections caused by *P. aeruginosa*; *Stenotrophomonas maltophilia*, *Achromobacter* spp., and BCC begins to increase. These microorganisms are regarded as "traditional pathogens" in CF. Their peak activity occurs in the adolescent and the young ages ([Salsgiver \*et al.\*, 2016](#)). In our opinion, application of the ecological principles to the shift in CF bacterial communities is justified, as remodeling of the environment (respiratory tract) and production of certain metabolites by the primary colonizers may prepare the basis for dominance of the traditional CF pathogens. Therefore, during activity of the 'pioneer' microorganisms, morphological changes and changes in chemical composition of the environment create selective niches for the pathogenic microorganisms ([Khanolkar \*et al.\*, 2020](#)).

The next stage of succession occurs directly under the influence of altered physical and chemical properties of the environment; reduced pH values, and oxygen saturation gradients. This succession creates favorable conditions for growth of such acid-resistant and anaerobic microorganisms; mainly *Lactobacillus* spp.; *Prevotella* spp., *Veillonella* spp., and *Granulicatella* spp., in addition to the facultative anaerobes such as *Streptococcus* spp. and *Rothia* spp. Using various types of fermentations as the final stages of catabolism, these species can maintain low pH of the environment, due to the different metabolites they produce ([Quinn \*et al.\*, 2016](#)).

## **7. Contribution of the anaerobic microorganisms in the emergence of chronic infections in CF patients**

The significance of anaerobic microorganisms in patients with CF is still extremely ambiguous and not fully understood.

Assumptions about the possible pathogenic significance of the anaerobes began to be built a long time ago. [Tunney \*et al.\*, \(2008\)](#) claimed the isolation of a large number of anaerobic microorganisms from the sputum samples obtained from CF patients. Moreover, quantitatively some anaerobes are equal to or even superior to the traditional CF pathogens. The authors wrote about the isolation of such anaerobic microorganisms, including *Prevotella* spp.; *Actinomyces* spp., *Propionibacterium* spp., *Veillonella* spp., *Rothia* spp., and *Streptococcus* spp., as well as *Staphylococcus* spp. and *Staphylococcus saccharolyticus*. The pronounced pathogenicity of some of these isolated bacteria has been also reported. For example, *Streptococcus* spp. and *Rothia dentocariosa* may be involved in the lung tissue damage. Wide representation of the anaerobes in CF can be considered justified, since despite of the above-described emergence of oxygen gradients that in turn can explain high diversity of the aerobic pathogens; however, hypoxic conditions have emerged over time in CF respiratory tract. These were associated with vital activity of the traditional pathogens consuming oxygen.

In order to generalize the combined influence of the traditional pathogens and the anaerobic microorganisms on the chronic infection of CF, [Conrad \*et al.\*, \(2013\)](#) attempted to consider the microbiota of LRT as a complex biological community and to apply the ecological principles to CF pathogenesis. An ecosystem can be either in a state of ecological balance or in an ecological imbalance. The first condition correlates with the state of remission in CF, while the ecological imbalance correlates with exacerbations of CF infections. Based on this, the authors developed the climax-attack model (CAM). Within the framework of this model, two types of the finally assembled bacterial community of CF LRT were distinguished; mainly the climax community and the attack community. The first one is associated with

a stable clinical condition, whereas the second one correlates with various exacerbations, such as bronchiectasis; atelectasis, and fibrosis.

The climax community may include the traditional pathogens, where their presences correlate with the inflammatory processes in the respiratory tract and the morphological remodeling of LRT. Meanwhile, the attack community is represented mainly by the obligate or the facultative anaerobes. However, the authors noted that different species can enter both types of communities, since CAM implies a functional differentiation of the community, but not the structural one. Moreover, these functional differences between the communities correspond to those established by [Quinn \*et al.\*, \(2018\)](#) during their *in vitro* studies. The climax community correlates with higher pH values; higher oxygen saturation, and lower degree of fermentation and gas release processes. The inverse correlation is typical for the attack community.

[Sherrard \*et al.\*, \(2016\)](#) stated that reduced number of anaerobes was correlated with a decrease in the lung function and with an increase in the inflammatory processes. But at the same time, they also confirmed that anaerobes can act as participants in the infectious process; produce virulence factors, and behave as synergists of the traditional pathogens.

[Hampton \*et al.\*, \(2021\)](#) conducted a previous study, where they attempted to combine the most common microorganisms in CF infections into functional clusters. They noted that when CF exacerbations emerge, diversity in the community of microorganisms may increase, which does not correspond to the opinion that reduced diversity correlates with worse clinical indicators. In addition, a mild course of the infections is more often observed in patients with a more stable species composition of their microbiota, while in the more severe cases, the community undergoes changes over time. These changes correspond to CAM.

A study conducted by [Khanolkar \*et al.\*, \(2020\)](#) attempted for the first time to create a full-fledged

model of bacterial succession in the CF LRT. According to this study, the community at this locus was assembled during the following stages:

- 1) Unoccupied area during the neonatal period. In parallel, primary colonization of the body by the maternal vaginal microbiota has occurred.
- 2) Pioneer communities. Colonization of LRT by microbiota of the upper respiratory tract. Dominance of *Staphylococcus aureus*; *H. influenzae*, and *Streptococcus* spp.
- 3) Intermediate community. An increase in the amount of traditional pathogens.
- 4) Climax community. Dominance of the traditional pathogens.
- 5) A community during an ecological imbalance, which can be associated with the attack community. An increase in the amount of anaerobic microorganisms, such as *Prevotella* spp.; *Veillonella* spp., *Fusobacterium* spp., and *Lactobacillus* spp. has been detected.

Moreover, [Carmody \*et al.\*, \(2018\)](#) added that predominance of the climax community is gradually established. This fact seems logic from the point of view of general ecology, since species diversity is one of the main conditions for ecosystem stability ([Dovciak and Halpern, 2010](#)). In the case of CF, ecological conditions cause predominance of the microorganisms that receive a resource advantage; provided by the substrate on which they develop (human body). Subsequent exacerbations contribute to a microecological imbalance, which in turn leads to a decrease in the species diversity and to the emergence of a chronic infectious process. [Thornton \*et al.\*, \(2022\)](#) study reported a contradiction, and highlighted that it is necessary to distinguish between the short-term and long-term contribution of the anaerobes to the infectious processes in CF.

In the long-term perspective, increased diversity of the anaerobes may limit predominance of the

traditional pathogens. Consequently, a clinically stable condition may emerge due to the ecological competition between the anaerobes and the traditional pathogens. Over time, the traditional pathogens begin to dominate, which is reflected in deterioration of the clinical course.

In the short-term perspective, deterioration of the clinical condition may be associated with the individual anaerobes and specific mechanisms. For example, *Prevotella* spp. can produce proteases that decompose the host defense factors. The sharpest increase in the number of anaerobes, including those that can carry any pathogenic potential, occurs during the transition from the climax community to the attack community. This may lead to a false-positive correlation between biodiversity and the frequency of exacerbations. And this in turn could be the reason for such contradiction that [Hampton \*et al.\*, \(2021\)](#) also came to in their study regarding increased diversity during exacerbations.

## 8. Successional model of the chronic infection in CF

The unique microecological environment, which is formed in the LRT in CF patients, causes an atypical pattern of successional processes from the earliest stages of life.

[Muhlebach \*et al.\*, \(2018\)](#) reported that the oropharyngeal microbiota plays a key role in the first stages of assembly of the bacterial community typical for CF. Dominance of these species is caused by disruption in the elimination process; resulting from dysfunctionality of the mucociliary clearance. At the same time, favorable conditions for immigration to LRT remain in the oropharynx. The oropharyngeal communities that occupy a new ecological niche have gained a resource advantage due to the increased level of mucins. They also contribute to the emergence of inflammatory processes.

The inflammation and decomposition of mucins prepare the substrate for development of the next community traditional pathogens. The destructive

changes in LRT in addition to a large variety of products of mucin metabolism provide a resource advantage for this group of microorganisms. Over time, the traditional pathogens become also associated with a high level of structural changes in the tissues of the respiratory tract and with worse indicators of lung function.

During metabolic activity of the traditional pathogens, hypoxic conditions and low pH values emerge in the respiratory tract, which cause the predominance of anaerobic microorganisms in the communities' structure. The anaerobes can cause exacerbation of CF, partially *via* stimulation of synthesis and secretion of several virulence factors by the traditional pathogens; as in the case with 2,3-butadione and phenazines. Over time, the microecological imbalance is eliminated, due to the fact that the traditional pathogens can regulate the ecological conditions. This occurs *via* the production of ammonia, which stabilizes the pH of the environment ([Khanolkar \*et al.\*, 2020](#)). Afterwards, the traditional pathogens begin to dominate once again, until they cause another exacerbation.

Therefore, if to generalize the debates about significance of the anaerobes for chronic infections in CF, it is possible to report that the traditional pathogens have a higher pathogenic potential. This may be attributed to the fact that they have a widest range of virulence factors and the most pronounced resource advantages. Furthermore, they have high correlation with deterioration of the lung function on the background of the structural changes induced in LRT.

A previous study revealed that the anaerobes represent a kind of 'trigger' that forces the traditional pathogens to manifest their pathogenicity. Most probably, over time, the classical pathogenic bacteria change their environment and adapt to it so much, thus the anaerobes gradually do not play any role. As a consequence, the traditional pathogens begin to dominate in the older CF patients. Such specific adaptive cycles are also found in the bacterial

communities in nature, and as a result of these cycles, the communities can acquire resistance to certain external factors ([Shabarova \*et al.\*, 2021](#)).

## Conclusion

Making a conclusion, it must be stated that the rational therapeutic strategies for CF should be aimed not only to eradicating the pathogenic microorganisms, but also to maintaining microecological balance in LRT of CF patients. This can only be achieved by complex approaches for each patient. These approaches should include a wide microbiological examination of the individual patients; monitoring of their clinical conditions and lung functions, purification of the respiratory tract from the excess of mucus, and rational antibiotic therapy and oxygen therapy. It is necessary to realize that the careless use of antibiotics, due to the numerous mechanisms of tolerance and resistance of microorganisms to them, can only aggravate the existing ecological imbalance in the respiratory tract. Theoretically, this can happen *via* eradicating the ecological competitors of the resistant CF pathogens. Additionally, at the moment it is impossible to say unequivocally how much of resource advantage of the supplemental oxygenation can be supplied to the traditional pathogens. Probably, in the treatment of chronic infections in CF, it is vitally important not only to eliminate the traditional pathogens, but also to restrain the growth of the anaerobic microorganisms, thereby breaking the vicious circles of the periodic exacerbations. In fact, these exacerbations turn the infections into chronic forms and lead to structural changes in LRT, which gradually leads to an irreversible decrease in the lung function. The authors are convinced that it is necessary to carry out several studies in the future, which are dedicated to the contribution of the anaerobic microorganisms to the emergence of infections in CF.

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## Conflict of interest

The authors declare that they do not have conflict of interests.

## Ethical approval

Non-applicable

## Author's Contributions

Conceptualization, D.V., A.V. and K.A.; Data curation, D.V. and K.A.; Investigation, D.V., A.V. and K.A.; Supervision, A.V.; Validation, D.V., A.V. and K.A.; Roles/Writing - original draft, D.V. and K.A.; Writing - review & editing, D.V., A.V. and K.A.

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