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MINI-REVIEW

The cystic fibrosis microbiome in an ecological perspective and its impact in antibiotic therapy

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Abstract The recent focus on the cystic fibrosis (CF) complex microbiome has led to the recognition that the microbes can interact between them and with the host immune system, affecting the disease progression and treatment routes. Although the main focus remains on the interactions between traditional pathogens, growing evidence supports the contribution and the role of emergent species. Understanding the mechanisms and the biological effects involved in polymicrobial interactions may be the key to improve effective therapies and also to define new strategies for disease control. This review focuses on the interactions between microbe–microbe and host–microbe, from an ecological point of view, discussing their impact on CF disease progression. There are increasing indications that these interactions impact the success of antimicrobial therapy. Consequently, a new approach where therapy is personalized to patients by taking into account their individual CF microbiome is suggested.

Keywords Cystic fibrosis · Ecological perspective · Microbe–microbe interactions · Microbe–host interactions · Polymicrobial biofilms · Antibiotic therapy

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Introduction

Cystic fibrosis (CF) is a common lethal disease affecting nearly 70,000 people around the world. It is characterized by the build-up of thick mucus overlying lung epithelial cells, wherein persistent cycles of chronic infection and inflammation occur (Gibson et al. 2003; Goss and Burns 2007). The CF airways provide microorganisms with heterogeneous microenvironments containing varying levels of oxygen, pH, nutrients, and antibiotics. This heterogeneity contributes largely for the proliferation of a phylogenetically diverse ecosystem, influencing the consortia of microbes able to occupy it (Yang et al. 2011a).

A complex microbiome has been previously described in the context of CF (e.g., Lopes et al. (2014a)). This microbiome encompasses species that are believed to be clinically significant and species thought to be bystanders, i.e., microorganisms for which no direct evidence exists to support their impact in the disease. *Pseudomonas aeruginosa* is recognized as the most significant and the most commonly isolated pathogen in CF infections, worsening CF pulmonary status due to chronic infections and being responsible for higher fatality rates (Winstanley and Fothergill 2009). In addition, a small number of other pathogens, such as *Staphylococcus aureus*, *Haemophilus influenzae*, and the *Burkholderia cepacia* complex, have been also documented as having repercussions in disease progression (Alexander and Hudson 2001; Lambert 2002; Lyczak et al. 2002; Yang et al. 2006; Starner et al. 2006; Treggiari et al. 2007; Drevinek and Mahenthiralingam 2010; Kahl 2010; Hauser et al. 2011; Høiby et al. 2011; Huang and Lynch 2011). Novel molecular technologies have more recently detected and identified a diverse microbial community inhabiting CF lungs (*Inquilinus limosus* and *Dolosigranulum pigrum*, etc.) of unexplored relevance in CF disease (Coenye et al. 2002; Bittar et al. 2008).

The multispecies microbiome composition in CF is constantly shaped by selective pressures exerted by the niche characteristics at sites of infection. It is increasingly recognized that the properties of such communities may be distinct from those of their individual members, due in large part to interspecies interactions shaping behavior (Faust et al. 2012; Korgaonkar et al. 2013). This may, in part, explain the lack of response to conventional therapeutic regimens that primarily target single causative agents instead of all members in the community.

Nonetheless, the precise ways under which the many different organisms interact within the CF airways and how these interactions influence the behavior of the individual species, the activities of the polymicrobial communities, and the relationship between host and microbes are poorly understood questions. Some studies have highlighted the potentially important roles of such interspecies interactions in disease phenotype and clinical outcome of CF infections (e.g., Amin et al. 2010; Chattoraj et al. 2010; Bragonzi et al. 2012; Twomey et al. 2012; Lopes et al. 2012). Such studies suggest that both synergistic and antagonistic interactions in mixed-species infections can impact microbial virulence and antibiotic resistance, which most likely will have clinical effects on disease severity and responses to therapy. Consequently, it is pertinent that therapeutic/prophylactic strategies should not be limited, and/or focused only, on the major pathogenic members that are able to directly cause disease but also on polymicrobial communities that involve complex interactions among members of the CF microbiota.

In a recently published review (Lopes et al. 2014a). we described the diversity of the CF microbiome and provided a few examples of possible interactions between different microorganisms. In here, we consider the collective microbiome as a potential pathogenic entity in itself, describe in detail the social behavior within CF communities, highlighting the interactions established among microbes and between microbes and their host in the context of CF and analyzing whether a particular community causes or worsens disease, in a manner analogous to individual pathogens. We conclude that the relationship between a microbial community and disease is better understood from an ecological perspective and can improve clinical understanding, ultimately providing guidelines for an effective treatment and chronic infection suppression.

Relevant aspects of CF—pathogenesis, reduced-oxygen environment, and microbial colonization

CF is a human genetic disorder that results from mutations in the CF transmembrane conductance regulator (CFTR) gene. The most prevalent of those mutations (ΔF508) is the deletion of three nucleotides at the position 508 of the CFTR protein sequence (Lopes et al. 2014a). The CFTR protein acts as a channel for the chloride and sodium ions transport across the cell membranes. Therefore, a dysfunctional CFTR protein leads to the absence or a decreased chloride secretion, resulting in an intracellular accumulation of those ions and ultimately to the depletion of chloride, sodium, and water from the airway lumen. This causes abnormal thick and viscous secretions and impairs mucociliary clearance in CF airways (Rowe et al. 2005; Davis 2006; Farrell et al. 2008).

The clinical manifestations of CF are quite variable, affecting individuals throughout their entire life. CF-affected individuals typically have a lifespan of approximately 30–40 years (Castellani et al. 2008). It is well established that the greatest contributor to the morbidity and/or mortality is the failure in lung function that generally occurs in older patients, caused by the build-up of mucus that clogs the airways and leads to persistent colonization by different microorganisms (frequently bacterial species). Hence, recurrent cycles of infections and inflammation lead to progressive airway and lung damage, respiratory failure and eventually death (Fig. 1) (Nixon et al. 2001; De Boeck et al. 2006; Boucher 2007).

The existence of steep oxygen gradients within the CF airway mucus is well-known, with zones ranging from aerobic (in the top layers) to completely anaerobic (deeper mucus layers) (Fig. 2 steps 1–3) (Worlitzsch et al. 2002).

Typically, the airway epithelial cells have a thin and hydrated mucus layer, located on top of the periciliary liquid layer (PCL), which enables an efficient mucociliary clearance (Worlitzsch et al. 2002; Hassett et al. 2002; Boucher 2004). A normal rate of epithelial O_2 consumption (Qo_2) produces no O_2 gradients within the thin airway surface liquid (ASL). In CF, the airway epithelium absorbs the sodium (Na^+) and chloride (Cl^-) ions and water from the lumen, depletes the PCL and slow down or even stop the mucus transport. The increased O_2 consumption associated with accelerated CF ion transport does not generate gradients in the thin biofilm of ASL, but the persistent mucus hyper secretion leads to the production of luminal mucus plugs, hence increasing the mucus layer on the epithelial cells and generating steep oxygen gradients, with zones ranging from aerobic (generally located at the top) and microaerobic and/or even completely anaerobic (located in the deeper layers) (Worlitzsch et al. 2002; Hassett et al. 2002; Boucher 2004).

Patients suffering from CF are prone to develop severe biofilm-related infections that are thought to contribute greatly to the emergence and dissemination of antibiotic resistance (Høiby et al. 2010a). The biofilm formation represents a protective mode of growth that allows microorganisms to survive in hostile environments and disperse by seeding cells to colonize new niches under desirable conditions (Wei and Ma 2013). *P. aeruginosa* persists in the CF airways due to its ability to form biofilms, being considered the key CF pathogen (Hassett et al. 2010). *P. aeruginosa* presents a notorious

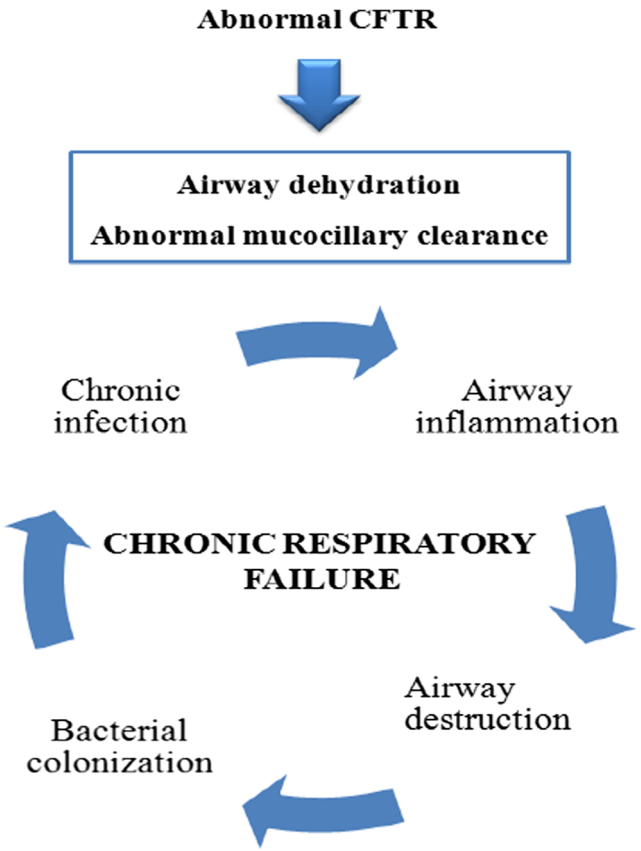

Fig. 1 Mechanism of the CF pulmonary disease. In the lungs, the defective chloride ion transport results in the decrease of the volume of the periciliary fluid, compromising the mucociliary clearance and triggering the overproduction of dehydrated and viscous mucus. This leads to the persistent colonization of bacteria in the lungs, and the physiologic consequences are persistent inflammatory responses, obstructive lung physiology, respiratory insufficiency, which ultimately results in death from chronic respiratory failure. Adapted from Kirkby et al. (2011)

ability to develop resilient biofilms in the form of "bacterial aggregates" within the CF mucus (Fig. 2, steps 4–6) (Worlitzsch et al. 2002; Hassett et al. 2002; Boucher 2004; Wei and Ma 2013). The persistence of chronic *P. aeruginosa* lung infections in CF patients is due to biofilm-growing mucoid strains, protected by alginate overproduction (Høiby et al. 2010b). The persistence of these biofilms into the CF airway mucus often leads to a high tolerance to many antibiotics (Borriello et al. 2004). Conventional resistance mechanisms, such as the presence of a chromosomal β-lactamase, upregulated efflux pumps, and mutations of antibiotic target molecules in the bacteria, have also contributed to the adaptation of *P. aeruginosa* biofilms to the CF environment (Høiby et al. 2010b).

Although *P. aeruginosa* prefers oxygen respiration as the highest energy-yielding process for growth, it can survive in the mucus anaerobic zones (Hassett et al. 2002). The ability of this bacterium to adapt to the oxygen-limited environments is associated with a drastic physiological change in *P. aeruginosa* (e.g., increased alginate production; alterations in the outer membrane; biofilm development), which contributes to an increased antibiotic tolerance (Schobert and Jahn 2010). The alginate produced by the biofilm bacteria in CF lung infections also provides a physical barrier to host defense systems (Worlitzsch et al. 2002; Hassett et al. 2002; Boucher 2004).

The complex CF microbiome

Traditional CF microbiology

As stated above, the infections in the CF airway are frequently polymicrobial (Rogers et al. 2004; Sibley et al. 2006; Bittar and Rolain 2010). The CF airways offer a favorable environment for the colonization and proliferation of a large variety of organisms, including as bacteria, fungi, and viruses, with bacterial species being the ones that are more frequently isolated (Guss et al. 2011).

Traditionally, the detection and identification of microbial species has relied on culture-based studies, using sputum or bronchial alveolar lavage samples for microbial detection and identification (Price et al. 2013). These techniques allow identification of several key microbial species that contribute to CF lung infection and disease progression, beginning early in life with *S. aureus* and *H. influenzae* and culminating in chronic infections caused by *P. aeruginosa* or *B. cepacia complex* species (Table 1) (Razvi et al. 2009; Price et al. 2013).

S. aureus, one of the first pathogens isolated from CF samples, is the most prevalent pathogen in children and adolescents; however, 40 % of adult patients still remain colonized (Kahl 2010). *S. aureus* has the ability to cause chronic infection (Alexander and Hudson 2001; Kahl 2010; Hauser et al. 2011). *H. influenzae* is also involved in chronic lung infections in CF pediatric patients, forming structures consistent with biofilms even before the onset of clinical signs or symptoms of lung disease (Starner et al. 2006). *B. cepacia complex* is a group of 18 *Burkholderia* species infecting 2 to 8 % of patients with CF, with some of them (*B. cenocepacia*, *B. multivorans*, *B. cepacia*, *and B. dolosa*) being highly transmissible, presenting pathogenic potential and very high resistance to antibiotic therapy (Yang et al. 2006; Lynch 2009; Drevinek and Mahenthiralingam 2010).

Approximately 50 % of CF patients are colonized with *P. aeruginosa* (Government 2013). which remains the most common pathogen isolated from CF sputum, being more prevalent in adults (Folkesson et al. 2012). The presence of *P. aeruginosa* in CF airways is highly associated with poor lung function, morbidity, and mortality of patients. After colonization with *P. aeruginosa*, consecutive episodes of recolonization frequently occur, resulting in a chronic infection that

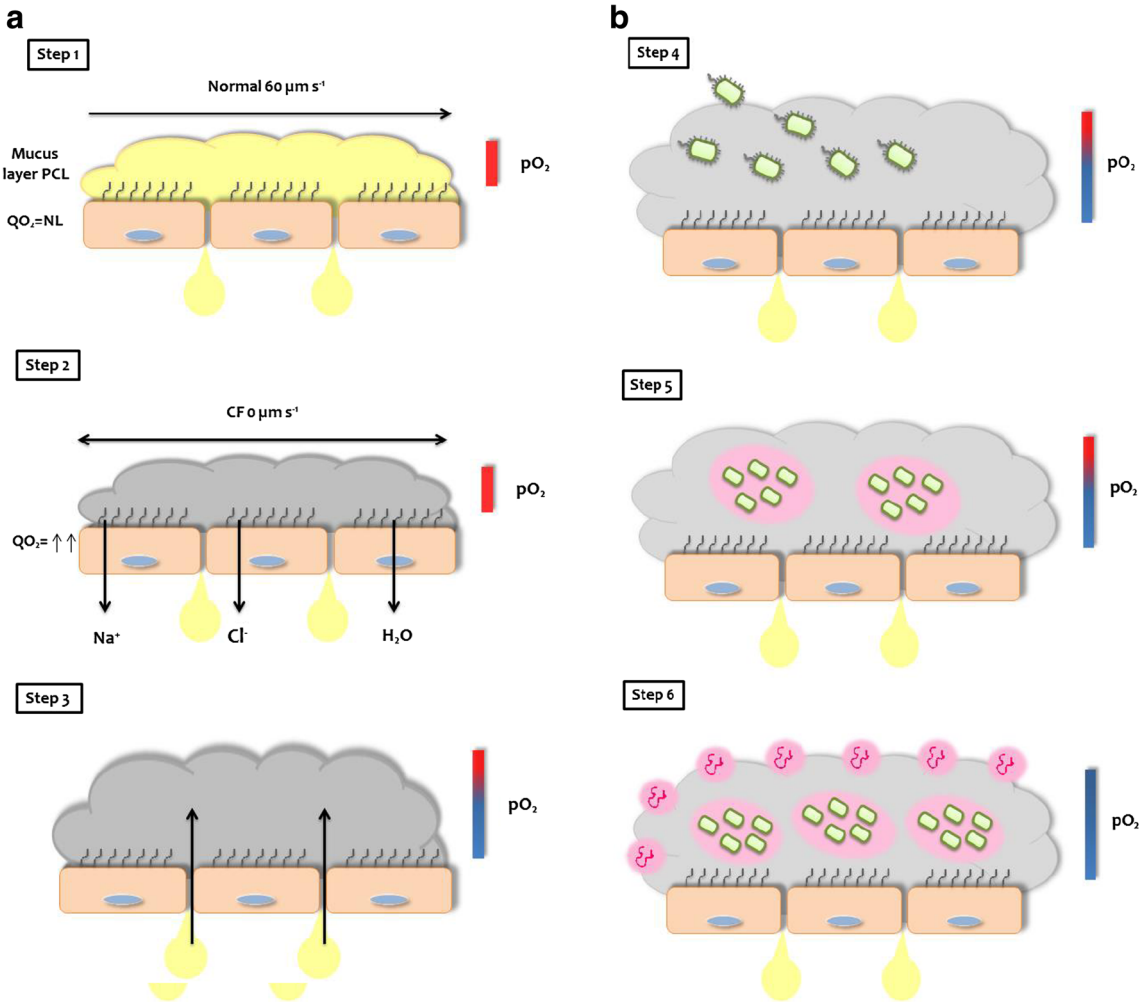

Fig. 2 **a** Alterations in mucus of normal epithelial airway cells (Step 1 to 3): (*Step 1*) On a normal airway epithelia, a thin mucus layer (*yellow*) resides on top of the PCL (*clear*). The presence of the low-viscosity PCL facilitates efficient mucociliary clearance (denoted by *black arrow*). A normal rate of epithelial O_2 consumption (Qo_2; left) produces no O_2 gradients within this thin ASL (denoted by the *red bar*). (*Step 2*) Excessive CF volume depletion (denoted by *vertical arrows*) removes the PCL, mucus becomes adherent to epithelial surfaces, and mucus transport slows/stops (*bidirectional black arrow*). The raised O_2 consumption (*left*) associated with accelerated CF ion transport does not generate gradients in thin films of ASL. (*Step 3*) Persistent mucus hypersecretion (denoted as mucus secretory gland; *gray*) with time increases the height of luminal mucus masses/plugs. The raised CF epithelial Qo_2 generates steep hypoxic gradients (*blue* color in bar) in thickened mucus masses. **b** Schematic model for *P. aeruginosa* biofilm in the CF mucus (Step 4 to 6): (*Step 4*) *P. aeruginosa* are deposited on the thickened mucus surfaces and can penetrate the mucus actively (e.g., by inhalation, flagellum- or pili-dependent motility) and/or passively (due to mucus turbulence) into the CF mucus. (*Step 5*) Afterward, *P. aeruginosa* start to develop bacterial aggregates (the biofilms), which are protected by an alginate capsule. In this step, the consumption of O_2 is drastically increased by the bacterial cells, and hypoxic and/or anaerobic pockets are formed. (*Step 6*) In the final stages, where O2 is almost depleted, the bacterial aggregates become highly resistant to the neutrophils and antibiotics, setting the stage for persistent chronic infection (Worlitzsch et al. 2002; Hassett et al. 2002; Boucher 2004)

can persist for years or even never being eradicated in CF patient lungs (Sousa and Pereira 2014).

Emergent CF microbiology

In addition to the bacterial species documented as CF pathogens, recent molecular methodologies have documented complex microbial ecosystems in CF samples, with a wide array of uncommon microorganisms coexisting with traditional pathogens acting collectively to facilitate disease progression (Peters et al. 2012). Figure 3 discloses all the genera of microorganisms recovered so far from the respiratory tracts of patients with CF. The analysis of this figure highlight that almost 120 genera were recovered from CF airways, with a clear dominance of bacterial genera and only 7 genera of viruses detected. These microorganisms include bacteria (e.g., *I. limosus*, *D. pigrum*, *Stenotrophomonas maltophilia*), fungi (e.g., *Aspergillus fumigatus*, *Candida albicans*), and viruses (e.g., rhinovirus, adenovirus, influenza). In addition, Worlitzsch and colleagues (2009). identified in a cross-

Table 1 Bacterial species most commonly associated with CF airway disease

Species	*Clinical significance*	*References*
Pseudomonas aeruginosa	Arguably the most important pathogen; presents a prevalence of 80 % at ages ≥18 years; ability to develop biofilms that protect from host responses and numerous antibiotics	Lambert (2002); Treggiari et al. (2007); Høiby et al. (2011)
Haemophilus influenzae	Most frequently isolated during infancy and/or early childhood; ability to form biofilms	Lyczak et al. (2002); Starner et al. (2006)
Staphylococcus aureus	Infects young patients, but can also be cultured from adolescents and adult patients; ability to cause chronic infection	Alexander and Hudson (2001); Kahl (2010); Hauser et al. (2011)
Burkholderia cepacia complex	Important opportunistic pathogens Ability to cause a progressive, invasive and fatal pulmonary disease known as "cepacia syndrome"	Yang et al. (2006); Drevinek and Mahenthiralingam (2010)

Adapted from Huang et al. (Huang and Lynch 2011)

sectional study of 15 genera of obligate anaerobes in 91 % of patients suffering from CF. Tunney and colleagues (2008) also reported anaerobic species within the genera *Prevotella*, *Veillonella*, *Propionibacterium*, and *Actinomyces*, which were isolated in high numbers (>64 %) in sputum samples from patients in adulthood. The high numbers of anaerobic bacteria detected in the CF airways may be a result of oxygen consumption by aerobic pathogens (such as *P. aeruginosa*) that often colonize the airways, creating a favorable niche for the proliferation of anaerobes (Worlitzsch et al. 2002; Yoon et al. 2002). Using molecular methods (16S rRNA gene clone libraries and pyrosequencing), Guss and colleagues (2011) have identified, only in 4 CF sputum samples, more than 60 bacterial genera, including facultative and obligate anaerobes, oral bacteria, and opportunistic pathogens, many of which have never before been found in the CF lung. Bittar and colleagues (2008) identified 53 different bacterial species from 25 sputum samples. Additionally, standard microbiological culture and phenotypic identification of bacteria in sputum from CF patients have been compared to molecular methods by the use of 16S rDNA amplification, cloning, and sequencing. Twenty-five sputa from CF patients were cultured yielding 33 isolates (13 species) known to be pathogens during CF. For molecular cloning, 760 clones were sequenced (7.2 ± 3.9 species/sputum), and 53 different bacterial species were identified including 16 species of anaerobes (30 %). These results indicate that the traditional culture methods are insufficient to describe the polymicrobial populations actually present in the CF lung. A recent review provides a comprehensive understanding of the great complexity of the microbiome existing in CF, detected and/or identified employing recent molecular methodologies (Lopes et al. 2014a).

Although the role of some of these emergent microorganisms in the pathogenesis of the disease and their clinical relevance remain unclear, there are already some hints about the implication of some unusual species in the pathophysiology of CF (Caraher et al. 2008; Ulrich et al. 2010; Costello et al. 2011; Sherrard et al. 2014; O'Neill et al. 2015; Pustelny et al. 2015; Benedyk et al. 2015). Further studies of this complex niche by, e.g., metagenomic analysis (Bittar et al. 2008; Price et al. 2013; Lim et al. 2013; Hauser et al. 2014; Lim et al. 2014) are currently needed to better understand these microbial communities, their implication in treatment and antibiotic resistance, their role in the development of chronic respiratory infections, and to identify their clinical significance in order to find new therapeutic targets.

Ecological perspective of the CF microbiome

Microbial interactions might exist within CF polymicrobial communities, so it is not surprising that these infections are increasingly viewed as complex communities of interacting organisms, with dynamic processes key to their pathogenicity. Similarly to the relative contribution to clinical status, disease progression and efficacy of antibiotic therapy by newly identified members of a polymicrobial community, which remain to be fully explored, the know-how on the consequences of the interplay among potential pathogens and/or between them and their eukaryotic host is also pivotal for understanding and treat CF-associated infections. These interactions can be mediated by a large number of mechanisms, which encompasses interspecies signaling, metabolite exchange, and cell–cell contact and are often implicated in the modulation of microbial behavior, ultimately contributing to disease progression and clinical outcome. In addition, many types of infections are caused by biofilm-associated microorganisms (Burmølle et al. 2010). which are harder to eradicate compared with planktonic exponentially growing cells, due to several factors operating concurrently (e.g., changed structure and reduced diffusion rates of the compounds in the biofilm matrix, changed gene expression pattern, and low growth rates of the biofilm-encased cells) (Sousa and Pereira 2014).This protective effect may be further enhanced if multiple species are present within the biofilm, where the dynamics between the resident species may potentially evolve and change the volume and function of the whole biofilm both qualitatively and quantitatively (Burmølle et al. 2014). In these consortia, microorganisms frequently communicate via quorum sensing

Fig. 3 Genus of microorganisms identified in respiratory tracts of patients with CF (lung image adapted from http://lungdiseasenews.com)

(QS) complex systems, which play an important role in the social behavior, regulation of microbial population density, and expression of virulence factors (e.g., resistance genes and proteins) among members of a microbial community (Rutherford and Bassler 2012).

Although particular microbial communities may be associated with certain clinical outcomes, the heterogeneous nature of the airway environment (nutrients, as well as physiochemical characteristics, such as oxygen tension, temperature, and pH) will influence the mix of microbes that are able to occupy it, through exerting selective pressures. In addition, it is increasingly recognized that the microbes can alter the characteristics of the niche in which they grow, by influencing the behavior of other colonizing species (such as pathogenicity (Sibley et al. 2008)), or by directly interacting with the eukaryotic host (e.g., by damaging airway epithelia (King 2011) or triggering inflammation (Essilfie et al. 2012)), as well as the impact of changes in antibiotic treatment that follow clinical worsening, such as the type and intensity of antibiotic exposure (Rogers et al. 2013). For instance, Tunney et al. (2011)

have reported that substantial shifts in bacterial abundance within the microbial community can be detected following antibiotic therapy. However, Stressmann and colleagues (2012) showed that antibiotic therapy can temporarily perturb these communities, which tend to return to their pretreatment configuration following cessation of antibiotics.

The pressures affecting microbiome composition are dynamic, and the comprehensive understanding of the drives of microbial community stability is fundamental for predicting the way in which a microbiome will respond to perturbation. Microbial activity will influence the processes that select for subsequent members of the microbiome; therefore, the infection by one species can indirectly dictate microbiome composition (Rogers et al. 2013).

Hence, it becomes imperative to understand the molecular basis and the biological effect of those interplay processes within multispecies communities to help improve clinical understanding and the in-use treatment regimens, devising new targets and disease control strategies.

An extensive research in recent literature has identified studies reporting interactions among microbes and between microbes and their host in the context of CF, which is summarized in Table 2.

The interactions described within Table 2 are divided into two different categories, synergism and antagonism. Contrariwise to synergistic interactions, which represent mutual benefit to all species present, antagonistic interactions result in a negative effect for at least one species. As it is possible to observe, microorganisms can use simultaneously different mechanisms to interact with other species, which may be associated with the niche characteristics and selective pressures exerted that shaped the behavior and the way in which the species interact.

The majority of the studies found in the literature (Table 2) are carried out under in vitro conditions so that the effect of interaction in the host is only predictive. Although the predictive effects for most microbe–host interactions (most of them carried out in vivo) are considered negative, some mechanisms involve interactions that can have a predictable positive effect on the host and thereby be used as a therapeutic approach. Similarly, molecules that block key signal sensing or transduction steps in pathogens could represent lead compounds for new drugs.

In any polymicrobial infection, the combined effect of two or more microbes on the disease progression can be more dramatic than any of the individuals alone and can display enhanced pathogen persistence in the infection site, increased disease severity, and increased antimicrobial resistance in a phenomenon known as polymicrobial synergism (Dalton et al. 2011; Peters et al. 2012; Murray et al. 2014). Synergistic interactions between different bacterial species allow reaping benefits that would be unattainable to them as individual cells, such as increased antibiotic tolerance, biofilm development, defense against competitors, adaptation to changing environments, increased tissue damage, and declined pulmonary infection (Jacques et al. 1998; Duan et al. 2003; Dalton et al. 2011). As examples of synergistic interactions occur in the CF context, several authors (Pilkington et al. 2011; Bragonzi et al. 2012) have demonstrated that a higher number of cells in the biofilm can be produced, which may have a great impact in antibiotic tolerance.

However, in some cases, the antagonistic interactions between organisms within a community are unavoidable due to competition for finite resources, with effects on the growth or viability of competitors (Harrison 2007). In CF, these interactions were found, for example, between *P. aeruginosa* and the fungal species *A. fumigatus* and *C. albicans*, with the small diffusive molecules secreted by *P. aeruginosa* inhibiting the biofilm formation of those fungal populations (Hogan et al. 2004; Cugini et al. 2007; Mowat et al. 2010). and between *B. cenocepacia* and *C. albicans* with QS signal produced *by B. cenocepacia* inhibiting the filament formation by *C. albicans* (Boon et al. 2008). Bacteria produce many types of diffusible molecules that can interact with other bacteria during disease. The various chemical cell-to-cell signaling mechanisms that are used by bacteria are collectively known as QS systems (Fuqua et al. 1994). a bacterial cell-to-cell communication process that involves the production, detection, and response to extracellular signaling molecules called autoinducers (AIs) (Rutherford and Bassler 2012). Some signal molecules such as autoinducer-2 (AI-2), *Pseudomonas* Quinolone Signal (PQS), 2-heptyl-4-hydroxy quinoline N-oxide (HQNO), and signal molecules of the diffusible signal factor (DSF) have been found to be produced during the infection and to influence other bacteria. For example, the ability to stimulate *S. aureus* biofilm formation was strongly associated to the production of HQNO and PQS by *P. aeruginosa* isolates (Fugère et al. 2014).

While some studies have revealed the interplay among typical CF bacteria (Hoffman et al. 2006). only few have reported the role of emergent species on lung disease chronicity (Lopes et al. 2014a, 2014b) or the interactions between those atypical microorganisms with eminent pathogens (Lopes et al. 2012). mainly when the micorganisms are encased in biofilms. Recently, Lopes et al. (2012) showed that the CF atypical bacteria *I. limosus* and *D. pigrum* could interact synergistically with *P. aeruginosa*, developing dual-species consortia with increased tolerance to several antibiotics. This suggests that previously thought clinically insignificant species may influence the behavior of individual species or even the whole microbial community. Based on these ecological interactions, it is strongly suggested to have a focus shift from an individual species to a polymicrobial community management and that modeling such multispecies interactions will help to predict

Table 2 Host–microbe and microbe–microbe interactions occurring in the context of CF and their predictive ecological effects

Microbes		*Interaction*	*Mechanism*	*Predicted ecological interaction*		*References*
				Within microbes[a]	Effect in host[b]	
Microbial – Microbial interplay	*B. cenocepacia–C. albicans*	*C. albicans* filamentation is inhibited by *B. cenocepacia*	*B. cenocepacia* QS signal, BDSF (cis-2-dodecenoic acid) inhibited the filament formation by *C. albicans*	Antagonism	+	Boon et al. (2008)
	P. aeruginosa–A. fumigatus	*A. fumigatus* biofilm formation is inhibited by direct contact with *P. aeruginosa*.	Exposure to the *P. aeruginosa* metabolites resulted in the inhibition of hyphal growth in *A. fumigatus*, decreasing biomass about 19 %. Antagonistic relationships existed between *A. fumigatus* and *P. aeruginosa*, which were influenced by the release of small diffusible extracellular molecules.	Antagonism	+	Mowat et al. (2010)
		A. fumigatus and *P. aeruginosa* co-culture lead to a worst pulmonary function.	Unknown	Unknown	−	Amin et al. (2010)
		A. fumigatus convert *P. aeruginosa* metabolites.	*P. aeruginosa* phenazine metabolites were converted by *A. fumigatus* into other chemical entities with alternative properties that include fungal inhibitory activity.	Antagonism	Unknown	Moree et al. (2012)
	P. aeruginosa–B. cenocepacia	The alginate produced by *P. aeruginosa* facilitates *B. cenocepacia* infection by interfering with host innate defense mechanisms.	The presence of alginate impaired *B. cenocepacia* phagocitosis both in vivo and in vitro. Alginate also reduced the proinflammatory responses of CF epithelial cells and alveolar macrophages against *B. cenocepacia* infection.	Synergism	−	Chattoraj et al. (2010)
		B. cenocepacia stimulates *P. aeruginosa* biofilm development; Co-infection in a mouse model by *P. aeruginosa* and *B. cenocepacia* lead to an increased host inflammatory response.	*B. cenocepacia* influenced biofilm formation by *P. aeruginosa*, leading to altered biofilm architecture and increased biomass. Co-infection of by both species increased the production of virulence factors (cytokines IL-1β and chemokines CCL2/JE and CXCL1/KC).	Synergism	−	Bragonzi et al. (2012)
	P. aeruginosa–B. cepacia	*P. aeruginosa* increases the virulence by *B. cepacia*.	*P. aeruginosa* PAO1 exoproducts (autoinducers) increased the production of three virulence factors (siderophore, lipase and protease), by *B. cepacia*.	Synergism	−	Kenney et al. (1995)
	P. aeruginosa–B. cepacia - S. aureus	*P. aeruginosa* dominates over *B. cepacia* and *S. aureus* in mixed culture under a variety of growth conditions.	*P. aeruginosa* dominated by more efficient substrate consumption in regard to obtain high cell concentrations. Conversely, the other bacterial species strongly reduced their viability in mixed culture.	Antagonism (*P. aeruginosa* to *B. cepacia*; (*P. aeruginosa* to *S. aureus*)	Unknown	Rüger et al. (2014)
	P. aeruginosa–C. albicans	*C. albicans* morphology is significantly influenced by the presence of *P. aeruginosa*	3OC12HSL (3-oxo-C12 homoserine lactone), a cell-cell signaling molecule produced by *P. aeruginosa*, was sufficient to inhibit *C. albicans*	Antagonism	+	Hogan et al. (2004)

Table 2 (continued)

Microbes	*Interaction*	*Mechanism*	*Predicted ecological interaction*		*References*
			Within microbes[a]	Effect in host[b]	
		filamentation without affecting fungal growth rates			
	In co-cultures, the presence of farnesol, a sesquiterpene produced by *C. albicans*, decreases the production of PQS (*Pseudomonas* quinolone signal) signaling by *P. aeruginosa*	Farnesol inhibited the production of PQS by inhibition of transcriptions on the pqs operon.	Antagonism	+	Cugini et al. (2007)
	Bacterial supernatant from four *P. aeruginosa* strains strongly reduces the ability of *C. albicans* to form biofilm on silicone.	Up-regulation of YWP1 gene by C. albicans, which encodes a protein known to inhibit biofilm formation, in response to bacterial supernatants of *P. aeruginosa*.	Antagonism	+	Holcombe et al. (2010)
P. aeruginosa–I. limosus; *P. aeruginosa–D. pigrum*	The emergent CF species *I. limosus and D. pigrum* can grow together with *P. aeruginosa*, increasing tolerance of the overall consortia to a wide range of antibiotics.	A possible alteration in the overall biofilm structure and extracellular matrix by both emerging species comparing with *P. aeruginosa* biofilms alone is suggested.	Synergism	−	Lopes et al. (2012)
P. aeruginosa–Oropharyngeal flora (*OF*)	The presence of OF in the lung of a rat model enhances lung damage caused by *P. aeruginosa*.	Auto-inducer-2 (AI-2), a QS mediator used by OF bacteria use to interact with *P. aeruginosa*, modulated *P. aeruginosa* gene expression (up-regulation), increasing its pathogenicity.	Synergism	−	Duan et al. (2003)
P. aeruginosa–Phage (14/1, φKZ, PNM and PT) and Protist (*Tetrahymena termophila* and *Acanthamoebae polyphaga*)	*P. aeruginosa* in the presence of phage and protist decreases its potential for virulence.	Bacteria decreased the protease expression within the host, leading to a reduced virulence potential. The long-term adaptation to the host conditions of the environmental pathogens was associated with reduced defense against natural phages and protists.	Antagonism	+	Friman et al. (2013)
P. aeruginosa–S. aureus	*P. aeruginosa* isolates trigger a wide range of biofilm-stimulatory activities when co-cultured with *S. aureus*.	The ability to stimulate *S. aureus* biofilm formation was strongly associated to the production of HQNO (2-heptyl-4-hydroxy quinoline N-oxide) and PQS (*Pseudomonas* Quinolone Signal) by *P. aeruginosa* isolates.	Unknown	−	Fugère et al. (2014)
	In a murine model of acute lung co-infection, early CF clinical isolate of *P. aeruginosa* could inhibit *S. aureus*. While late CF clinical isolate did not outcompete *S. aureus*	*P. aeruginosa* early CF clinical isolate presented high virulence in an acute infection.	Unknown	-/ Unknown (For late CF clinical isolate)	Baldan et al. (2014)
	Wild type *P. aeruginosa* PAO1 facilitates *S. aureus* microcolony formation.	*P. aeruginosa* type IV pili-mediated interactions between *P. aeruginosa* and *S. aureus* in co-culture biofilms and the level of *P. aeruginosa* piliation has an important impact on microcolony formation.	Synergism	Unknown	Yang et al. (2011b)

Table 2 (continued)

Microbes		*Interaction*	*Mechanism*	*Predicted ecological interaction*		*References*
				Within microbes[a]	Effect in host[b]	
	P. aeruginosa–S. aureus small colony variants (*SCVs*)	*P. aeruginosa* simultaneously suppresses *S. aureus* respiration and protects it from aminoglycoside antibiotics.	HQNO (2-hydroxy-2-heptyquinoline-N-oxide) produced by *P. aeruginosa* protected *S. aureus* from killing by aminoglycosides, by inhibiting electron transport that is required for aminoglycoside uptake. Furthermore, HQNO had the ability to inhibit *S. aureus* cytochrome activity.	Synergism	−	Hoffman et al. (2006)
	P. aeruginosa–S. maltophilia *P. aeruginosa–B. cenocepacia*	The presence of diffusible signal molecules of DSF family from sputum of patients with CF, produced by *S. maltophilia* and *B. cenocepacia*, led to altered biofilm formation and increased resistance to antibiotics by *P. aeruginosa*.	The sensing of DSF by *P. aeruginosa* leads to alterations in expression of genes encoding a wide range of functions to include biofilm and increased tolerance to polymyxins.	Synergism	−	Twomey et al. (2012)
	P. aeruginosa–S. maltophilia	*S. maltophilia* might confer some selective "fitness advantages to *P. aeruginosa* increasing this virulence. Contrariwise, *P. aeruginosa* might be responsible for the protection of *S. maltophilia* against tobramycin activity.	When grown in mixed biofilm with *S. maltophilia*, *P. aeruginosa* significantly over-expressed *aprA*, and *algD*—codifying for virulence factors protease and alginate, respectively. The induced alginate expression by *P. aeruginosa* might be responsible for the protection of *S. maltophilia* against tobramycin activity we observed in mixed biofilms.	Synergism	−	Pompilio et al. (2015)
Microbial–Host interplay	*B. cenocepacia*–Host	The establishment of a *B. cenocepacia* infection delays the wound repair and also elicited a potent proinflammatory response.	An upregulation of metalloproteases (MMP) genes by 16HBE14o-cells and CFBE41o- cell lines, with increased matrix activity, was observed in response to *B. cenocepacia* infection.	Not determined	−	Pilkington et al. (2011)
		B. cenocepacia infection induces proinflammatory response by the host	*B. cenocepacia* O antigen contributed to macrophage activation due the secretion of proinflammatory cytokine IL-1β.	Not determined	−	Kotrange et al. (2011)
	P. aeruginosa–Host	Early *P. aeruginosa* CF isolates were lethal, while late isolates exhibit reduced or abolished acute virulence in the CF lungs.	The lesions caused by early *P. aeruginosa* strains were due the high leukocytes recruitment and bacterial load in the lungs of mice.	Not determined	(early)/+ (late)	Lorè et al. (2012)
		P. aeruginosa infection causes an excessive stimulated immune inflammatory response.	The expression of IL-8 was up-regulated by translocated nucleoside diphosphate kinase (Ndk) into host cells. The massive influx of neutrophils into *P. aeruginosa*-infected sites was stimulated by an excessive inflammatory response caused by the production and release of IL-8.	Not determined	−	Kim et al. (2014)
		The loss of bacterial motility enable non-motile P. *aeruginosa* to evade to association and ingestion by phagocytes both in vitro and in vivo.	The loss of bacterial motility resulted in reduced inflammatory activation and anti-bacterial IL-1β host response. These mechanisms enabled pathogens to evade the innate immune system.	Not determined	−	Patankar et al. (2013)

Table 2 (continued)

Microbes	*Interaction*	*Mechanism*	*Predicted ecological interaction*		*References*
			Within microbes[a]	Effect in host[b]	
Rhinovirus–Host and influenza–Host	The presence of *rhinovirus* and *influenza* stimulate inflammatory responses by the host.	Rhinovirus had a pronounced effect on chemokine expression, being associated with greater than two-fold induction of five genes. Influenza induced a more potent response consisting of inflammation, being associated with overexpression of 20 genes, including those encoding the cytokines tumor necrosis factor and IL-12,	Not determined	–	Ramirez et al. (2014)

[a] The terms antagonism refers, in this case, to the result of a negative relationship between the microbes; while the terms synergism is related with a positive or addictive relationship

[b] Predictive ecological effect in host that results from interaction between the microbes. The symbol (+) refers to a positive predictive effect in host; the symbol (−) refers to a negative predictive effect in host

the effects of new therapeutic interventions, dismissing much of the current antibiotic therapy empiricism and increasing its effectiveness.

In addition to microbial–microbial interactions, microbial–host interactions also exist in CF, and the most significant features is the ability of the pathogens to deceive or modulate the multifaceted host response following colonization. The airway epithelium recognizes and responds to pathogens through the interaction between host pathogen recognition receptors and pathogen-associated membrane proteins (Callaghan and McClean 2012). The airway epithelium is one of several sources of chemokine interleukin-8 (IL-8) (Standiford et al. 1990) that acts as the first line of host defense against pathogens. In CF patients, the ΔF508-CFTR mutation results in increased levels of IL-8 and neutrophils, responsible for the development of chronic obstructive and inflammatory lung diseases (Bodas and Vij 2010). Furthermore, neutrophils resulting in DNA release and increased mucous viscosity worsen the problem of bacterial attachment (Callaghan and McClean 2012). Recent studies have demonstrated that the conventional pathogens *P. aeruginosa* and *B. cenocepacia* can trigger an excessive inflammatory response in the host (Kotrange et al. 2011; Pilkington et al. 2011; Lorè et al. 2012; Kim et al. 2014). Deregulation of matrix metalloproteases (MMPs) in CF is another contributor to CF lung disease and to bacterial colonization (Pilkington et al. 2011). So, while many of the modifications and adaptations serve to promote inflammation and to benefit the colonization, other strategies are used to avoid and minimize the host response (Patankar et al. 2013). Additionally, colonization by multiple pathogens may trigger unknown repercussions in the host, although it is suspected that for most cases, adverse effects can occur with greater impact in antibiotic therapy.

The majority of studies about interactions in the polymicrobial CF community focus on the traditional pathogen *P. aeruginosa*, due to its prevalence in CF lung, its ability to form biofilms that protect the organism to the host responses and to numerous antibiotics, and its potential do develop chronic infection. Therefore, more research is needed to provide a better mechanistic insight into the complex interplay between potential pathogenic agents, commensal organisms, and the host response in the polymicrobial infections.

Understanding polymicrobial interactions to better treat CF

The resistance to antimicrobial agents is currently one of the major problems in the healthcare setting worldwide (French 2010). Antimicrobial resistance is potentiated in CF patients due to the extensive use of antimicrobial agents from a young age, both for the prophylaxis and treatment of respiratory infection (Oliver 2010).

When the chronic infection is established, pathogens such as *P. aeruginosa* growing as biofilms in the CF lung can exhibit increased resistance to antibiotics (Hassett et al. 2009; Bjarnsholt et al. 2009; Høiby et al. 2011). In fact, bacteria in the form of biofilms show increased resistance to several antibiotics when compared to planktonic or free living counterparts (Sriramulu et al. 2005). The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of antibiotics to biofilm-growing bacteria may be up to 100–1000-fold higher compared with planktonic bacteria (Anwar et al. 1990; Moskowitz et al. 2004).

Apart from the conventional resistance mechanisms presented by bacteria (e.g., chromosomal beta-lactamase, upregulated efflux pumps, and mutations in antibiotic target molecules), biofilms also present an extracellular polymeric matrix. The reduced diffusion of antibiotics through the exopolysaccharide matrix (alginate, in the case of *P. aeruginosa* biofilm) retards the movement of antimicrobial agents (Costerton 2001; Bagge et al. 2004; Chan et al. 2005; Anderson and O'Toole 2008; Vettoretti et al. 2009) and contributes for the resistance and/or tolerance of biofilms to the antimicrobial agents (Høiby et al. 2010a; Sriramulu 2013).

When CF was first described in 1938 (Andersen 1938). the predicted survival age of patients was only 6 months (Cohen-Cymberknoh et al. 2011). For patients born in the 1990s, median survival is now predicted to exceed 40 years, due to the introduction of multiple therapies that treat the symptoms of CF (Wilschanski 2013).

Antibiotic therapy for CF patients is directed at preventing, eradicating, or controlling respiratory infections. The therapy generally starts with oral and inhaled therapies in an outpatient setting and the use of intravenous route for patients with severe exacerbations (McCaughey et al. 2012; Sriramulu 2013).

The fluoroquinolones (e.g., ciprofloxacin) are the most commonly used oral agents to treat acute exacerbations caused by *P. aeruginosa* infection (Sriramulu 2013). Other agents that have long been used by inhalation in CF patient for the treatment of *P. aeruginosa* lung infection are tobramycin, aztreonam, or colistin (Sriramulu 2013). Current standard care guidelines for antibiotic recommend in CF patients for most commonly bacterial species are described in Table 3.

Recently, new antibiotic combinations have been developed (MacLeod et al. 2009; McCaughey et al. 2012; McCaughey et al. 2013; Anderson et al. 2013). One example is the combination of fosfomycin/tobramycin (FTI), an inhaled antibiotic with broad-spectrum antibacterial activity for treatment of bacterial respiratory infections. FTI consists of fosfomycin (F) and tobramycin (T) in a 4:1 weight-to-weight ratio (*w*/*w*); this combination has promising activity against MRSA and *P. aeruginosa* with greater activity under aerobic and physiologically relevant anaerobic conditions, compared to F or T alone (MacLeod et al. 2009; McCaughey et al. 2012; McCaughey et al. 2013; Anderson et al. 2013). Lam and colleagues (2013) reported that tobramycin inhalation powder (TIP) represents the first dry powder inhaled antibiotic available for use in CF. TIP was approved in the USA in 2013 (Fiel 2014). Inhaled antibiotics have been probably the safest and most effective therapy for *P. aeruginosa* chronic lung infection in CF patients (Máiz et al. 2013). The use of inhaled antibiotics allows it to be delivered directly to the target area, with a lower dose than more conventional oral or intravenous delivery methods, with reduced

Table 3 Antibiotic therapy used for bacterial species most commonly associated with CF airway disease (Döring et al. 2012)

Species	*Infection phase*	*Antibiotic therapy*
P. aeruginosa	First isolated from patients	Oral ciprofloxacin or Inhaled colistin or tobramycin or aztreonam
	Chronic infection	Two inhaled antibiotics among the following: colistin, tobramycin, aztreonam
H. influenzae	–	Oral or intravenous amoxicillin + clavulanic acid depending on the severity.
S. aureus	First isolated from patients	Oral flucloxacillin or Oral flucloxacillin + oral or intravenous rifampicin or fusidic acid
	Chronic infection	Oral flucloxacillin
MRSA: *Methicillin-resistant Staphylococcus aureus*	First isolated from patients	Oral rifampicin + fusidic acid.
	Chronic infection	Intravenous vancomycin or teicoplanin or linezolid
B. cepacia	–	At least two intravenous antibiotics: Intravenous ticarcillin + clavulanic acid or piperacillin + tazobactam

systemic absorption and consequently reduced risk of toxic effects (Traini and Young 2009; Hoppentocht et al. 2014).

With the increased antibiotic resistance in CF patients, the need for new strategies in the lifelong treatment of pulmonary infection has to be validated (van Westreenen and Tiddens 2010). In a cross-sectional study, the detection of 2,3-butanedione in the breath gases of CF patients indicated the presence of *Streptococcus spp*. (Whiteson et al. 2014). Linking together products that are unique to microbial metabolism with the genes detected by metagenomic sequences of microbial communities in sputum may enable development of biomarkers for early detection of exacerbations.

Lim and colleagues (2014) combined the use of metagenomic sequencing and clinical microbiology for monitoring polymicrobial infections in individual patients. Their findings highlighted that information on the predicted resistance of the whole microbial community is perhaps one of the most useful pieces of information extracted from metagenome sequencing, which will be important to understand how quickly antibiotic resistance might change in the microbial community. The implementation of metagenomic analysis as a clinical diagnostic tool can give rise to vital information for clinicians to prescribe the appropriate antibiotic therapy.

Because CF infection is no longer viewed as being caused by a single pathogen, antibiotics used to target a small group of species recognized as key CF pathogens are generally ineffective when other atypical species are present (Lopes et al. 2012, 2014b) or fail in many cases (Leekha et al. 2011). This problem is compounded by the huge polymicrobial CF community and the bacterial interactions occurring in lung. Due to the complex interactions that result between traditional and emergent CF pathogens—for instance, a study by Lopes and colleagues (2012) demonstrated that the association among atypical and conventional CF bacteria could result in the impact of the antibiotic resistance—a new approach where antibiotic therapy is personalized to each patient, based on comprehensive microbiological analyses, could be development for treating lung infections (Short et al. 2014). There is an increasing appreciation of the polymicrobial nature of many bacterial infections such as those associated with CF and of the potentially important role for interspecies interactions in influencing both bacterial virulence and response to therapy, as previously discussed in the earlier section. Twomey and colleagues (2012) demonstrated that antibiotic resistance of *P. aeruginosa* biofilms was enhanced in the presence of diffusible signal molecules, produced by *Stenotrophomas maltophilia* and *B. cenocepacia*. On the other hand, a recent study showed that *P. aeruginosa* might be responsible for the protection of *S. maltophilia* against tobramycin (Pompilio et al. 2015).

Antivirulence drugs are a new type of therapeutic drug that target virulence factors, without killing or inhibiting bacterial growth. Many antivirulence strategies are being explored, including inhibiting bacterial adhesion to the host cell (inhibiting biofilm formation), inhibiting cell-to-cell signaling (known as quorum quenchers by inhibiting QS systems), and interfering with gene regulation of virulence traits (Rasko and Sperandio 2010; Rutherford and Bassler 2012; Allen et al. 2014). Other innovative therapeutic approach is the development of CFTR-modulating drug as potential treatment for cystic fibrosis. Ivacaftor is the first licensed CFTR modulator drug and, although only targets ~5 % of CF patients, may indeed be one of many therapeutic agents that point to the emergence of a new era of personalized medicine (Ramsey et al. 2011). These drugs will allow treatment of the basic defect in CF disease and open the door for therapy according to gene sequencing—true personalized medicine (Wilschanski 2013). Moreover, every person with CF is unique and requires personalized diagnosis and therapy.

In addition to recognizing the polymicrobial nature of CF community, understanding the molecular mechanisms and biological effects from the microbe–microbe and host–microbe interactions is also crucial to improve therapy regimens and also define new antimicrobial agents, new targets and strategies for CF disease control. We are facing a postantibiotic era with limited capability to combat polymicrobial infections.

Conclusions

In the past decades, technological advances in diagnostic tools have led to the recognition that many infections, including CF, are far more complex than originally believed. It has become apparent that, although therapies are focused on the treatment of the dominant microbial species of an infection, other microbes may have a profound significance on both the response to antibiotic therapy and virulence. While microbes–microbes and microbes–host interactions are not fully understood, it is suspected that the consequences of such interplay carry out synergistic and antagonistic effects either for CF treatment or for antibiotic resistance, ultimately contributing to disease progress and clinical outcome.

Accordingly, the challenge is now to explore multispecies biofilms in further detail, by examining their physiology, function, and underlying mechanisms but specifically enhancing the focus for microbial–microbial and/or microbial–host interactions in these communities. Understanding the physical and chemical interactions between microorganisms in these polymicrobial communities will help to define potential new targets for disrupting biofilm-community development and, in cystic fibrosis, affect the ecology of biofilms in the airways of patients. Since several pathogens employ QS regulation to express a specific broad range of virulence factors, this has spurred interest in QS inhibitors as antivirulence drugs.

The medical community is now starting to recognize the significance of polymicrobial diseases and the major types of

microbial community interactions associated with human health and disease. Many traditional therapies are just starting to take into account the polymicrobial cause of diseases and the repercussions of treatment and prevention. By taking into account the complexity of infecting organisms and their interplay, it is intended to develop a new approach where therapy is personalized to each patient or to a group of patients. If successful, the approach may pave the way for more effective therapeutic regimens and ultimately contribute to personalized treatment for these diseases, based on unique microbial profile of a given patient, and further extrapolate for analogous polymicrobial infections.

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Compliance with ethical standards

Declaration of interest The authors report no declarations of interest.

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