Review Article

Mixed bacterial-fungal infections in the CF respiratory tract

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Cystic fibrosis (CF) is a common genetic disease whose major clinical manifestations include repeated episodes of airway infection and inflammation that ultimately result in premature death from respiratory failure. The consequences of infection by individual bacteria have been well studied and the evidence is building that fungal pathogens may be playing an important role in lung disease progression. In contrast, though many CF patients have airway infections characterized by the presence of both bacteria and fungi, our understanding of the impact of such polymicrobial infections on the host is limited. In this review, we discuss what is currently known about incidence of mixed bacterial-fungal infections, and the potential consequences of these mixed infections on the progression of CF lung disease.

Keywords cystic fibrosis, *Pseudomonas aeruginosa, Aspergillus fumigatus*

Abbreviations CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; ENaC, epithelial sodium channels; ABPA, allergic bronchopulmonary aspergillosis; CFF, Cystic Fibrosis Foundation; ERCF, European Epidemiologic Registry of Cystic Fibrosis; PQS, Pseudomonas quinolone signal; 3OC12HSL, 3-oxo-C12-homoserine lactone

Introduction

Cystic fibrosis (CF) is a common genetic disease whose major clinical manifestations include chronic airway infection and inflammation that ultimately results in premature death from respiratory failure (reviewed in [1]). In individuals with CF, the decrease or absence of activity of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, and subsequent over-expression of epithelial sodium channels (ENaC) on airway epithelial cells, leads to a dehydrated airway surface liquid layer, and

impaired cilliary motility and immune cell mobility [1,2]. These factors are likely important contributors to the establishment of chronic infections that cause the progressive obstructive lung disease that results in much of the morbidity and almost all of the mortality in this patient population [1,3]. Most research on the microbiology relating to CF has focused on infections by known pathogens, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, or strains within the *Burkholderia cepacia* complex (Bcc), and many studies show a link between the infection by these pathogens and decreased lung function [4]. Infections by bacteria such as *P. aeruginosa* are very persistent and the same strains are often isolated from a patient over years despite aggressive antibiotic therapy. The incidence and importance of fungi in the CF lung, recently reviewed in [5], is much less well understood in terms of the incidence and duration of infections. A variety of studies indicate that fungi, including *Aspergillus fumigatus*, *Candida albicans*, *Scedosporium apiospermum*, and *Exohiala dermatitidis*,

are commonly found in the CF lung [5–7]. While these fungi have been associated with decreased lung function [6,8–12], a direct causal relationship between fungal infection and compromised lung function has not yet been established. Because bacterial infections are common and persistent within the CF population, fungal infections are often observed in patients whose lungs are simultaneously colonized with bacteria. Furthermore, recent data show that specific bacteria-fungal infections correlate negatively with patient health in comparison to patients with bacterial or fungal infections alone [6,11]. In this review, we summarize the data on the incidence of mixed bacterial-fungal infections in CF and studies on the clinical observations associated with these complex infections. Furthermore, we will discuss the potential consequences of mixed bacterial-fungal infections on the host immune response and the virulence of bacterial and fungal pathogens in the lung. Lastly, we will briefly address issues associated with the management of infections by both bacterial and fungal pathogens, and discuss the challenges associated with obtaining the additional clinical data that are needed to understand the importance of bacterial-fungal infections.

Clinical data regarding mixed bacterial-fungal infections in CF

The consequences of co-infections by bacteria and fungi are not well known, but two recent studies indicate that this category of infections correlates with decreased lung function when compared to infections by either bacteria or fungi alone [6,11]. In a retrospective study that reviewed the records of 230 patients under the age of nineteen, Amin *et al*. [11] examined the effects of persistent *A. fumigatus* infections, defined as two or more sequential *A. fumigatus*-positive sputum or bronchoalveolar lavage cultures in a year, on a variety of factors relating to patient health. *P. aeruginosa* was detected in 54.1% of the patient group with persistent infection by *A. fumigatus*, relative to 44.1% in the *A. fumigatus* negative control group ($P = 0.01$). More importantly, when *P. aeruginosa* was present along with *A. fumigatus*, there was a statistically-significant negative association with lung function relative to what was observed with *P. aeruginosa* or *A. fumigatus* alone [11]. Similar studies in older CF patients and at other sites are needed to support these very interesting findings. Chotirmall *et al*. [6] suggest a link between *C. albicans* and forced expiratory volume (FEV1) decline and frequency of exacerbations through a prospective examination of 89 CF patients (and 3916 sputum samples) over eleven years. Out of 89 patients, 18 patients experienced chronic *C. albicans* colonization, determined by the presence of this fungus in more than 50% of sputum samples from those patients, and 26 were intermittently colonized. Because *C. albicans* can also be part of the oral microflora, care was taken to avoid contamination of sputum samples by *C. albicans* from the mouth. In analyses where the chronically and intermittently colonized groups were combined, 86.4% of *C. albicans*-colonized patients were co-colonized with *P. aeruginosa*, whereas *P. aeruginosa* was only present in 31.1% of the control group who did not have a history of *C. albicans* colonization. When patients colonized with *C. albicans* were compared to those infected with both *C. albicans* and *P. aeruginosa*, there was a significant increase in rate the decline of BMI, but not FEV1. However, when the same patients were compared pre-and post acquisition of *C. albicans*, significant declines in both BMI ($P < 0.0001$) and percent predicted FEV1 ($P < 0.001$) were reported. The decrease in pulmonary function associated with infection by both *P. aeruginosa* and *C. albicans* was −4.86% of the predicted FEV1. As is the case for fungal infections, it is not yet known if concomitant bacterial-fungal infections are worsening a patient's respiratory status or if the detection of fungi is indicative of other differences that dictate the clinical course. In addition, one must not only consider the presence or absence of fungi, but also fungal burden as an important contributor to lung disease severity. Quantitative analysis of 46 sputa with *C. albicans* found that more than half contained relatively high levels of the fungus with more than 10^6 colony-forming units (CFUs) per gram of sputum; the range of fungal concentrations was 10^3 to 10^8 CFU per gram [13]. Future work is needed to determine if there is a link between fungal load within a patient and the potential for clinical impact.

Studies that examine the benefits of antifungal therapy are likely to be key in addressing this question of whether eradication of fungi from mixed bacterial-fungal infections (and fungal infections alone) will improve patient status. A recent case series of six patients colonized with *A. fumigatus* showed clinical improvement following treatment with an antifungal therapy [12]. Five of the six patients had simultaneous bacterial infections (*P. aeruginosa*, four patients; *S. maltophilia*, one patient; Bcc, one patient; and non-tuberculosis Mycobacterium, two patients) but did not respond to antibacterial therapy alone [12]. This study supports the potential importance of fungi in CF lung infections, and again brings up the notion that microbes within the CF airway may act synergistically in ways that negatively impact the course of disease. Additional studies that examine the benefits of antifungal therapy, such as an ongoing clinical trial [ClinicalTrails.gov, NCT00528190] that examines the benefit of oral itraconazole for patients chronically colonized with *A. fumigatus*, will likely be a key part of answering this question.

The prevalence of simultaneous colonization by bacteria and fungi in the CF lung

Additional clinical studies provide some insight into the frequency of mixed bacterial-fungal infections in association with CF. Because *P. aeruginosa* is so common in the CF population, many of the studies find that sputa contain both *P. aeruginosa* and fungi. In Bakare *et al*. [14], a study of 94 patients, with a median age of 28 years, found *A. fumigatus* and *C. albicans* present in 45.7% and 75% of patients, respectively. *P. aeruginosa*, predominantly in the alginate-overproducing mucoid form, was present in 76.6% of the 94 patients studied, and *A. fumigatus* and *P. aeruginosa* were detected together in 64.2% of *A. fumigatus*-positive samples indicating a high frequency of mixed bacterial and fungal infections. While the rate at which *C. albicans* and *P. aeruginosa* were detected together was not reported, the prevalence of both species in this population suggests that they are also commonly found in the same sputum samples. The co-incidence of *C. albicans* or *A. fumigatus* and bacterial pathogens such as *Hemophilus influenzae*, Bcc strains, and *Stenotrophomonas maltophilia*, was not reported as these species were found in less than 10% of patients. Similarly, no mention was made of sputum containing fungi and *S. aureus*, a Gram-positive bacterium detected in 19.1% of patients. Other studies also report associations between *P. aeruginosa* and fungi such as *C. albicans* or *A. fumigatus*. In Storey *et al*. [15], 20 out of 84 sputum samples contained *C. albicans*, and 18 of those also had *P. aeruginosa* present as the predominant organism. In a study with 102 patients who provided sputum samples over 22 months, Bauernfeind *et al*. [13] found *P. aeruginosa* in 83.4% of patients, *C. albicans* in 29.4% of patients (9.6% of samples) and *A. fumigatus* in 5.9% of patients (1.9% of samples). Among these 472 sputum samples, *P. aeruginosa* and *C. albicans* were found together in 6.9% of samples; *C. albicans* co-colonization with other bacteria was less frequent.

Associations between fungi and bacteria other than *P. aeruginosa* have also been found. Bakare *et al*. [14] found that all of the patients with *B. cepacia* complex strains or *S. maltophilia* also had *A. fumigatus* in their sputum at least once during the study prompting the suggestion that there may be a correlation. Marchac *et al*. [16] also noted a positive correlation between the presence of *A. fumigatus* and *Stenotrophomonas maltophilia*. In this 63-patient study, *A. fumigatus* was found in 51% of patients with *S. maltophilia* versus in 9% of controls (*P* < 0.001), and the increased incidence of *A. fumigatus* in these patients could not be explained by corticosteroid use or allergic bronchopulmonary aspergillosis (ABPA).

In studies where the presence of bacterial and fungal pathogens was reported, but specific co-occurrence rates for bacteria and fungi were not noted, the high frequencies with which bacterial and fungal pathogens are detected does imply the frequent occurrence of mixed infections. Data from four such studies are summarized below. A cross-sectional analysis of data from over 7,000 Europeans with CF found *Candida* spp. in 34% of patients and *Aspergillus* spp. in 17% within the 180-day study period [10]; *P. aeruginosa* was found in 68% of patients and Bcc was found in 5.6%. If one were to make the assumption that there is no bias towards or against the co-infection by *P. aeruginosa* and commonly detected fungal pathogens, one would expect that 22% and 11% of patients to have been colonized by both *P. aeruginosa* and either *Candida* spp. or *Aspergillus* spp., respectively. In fact, data described above from [6,11] suggests that there is an increased likelihood of finding fungi in patients colonized with *P. aeruginosa* compared patients without *P. aeruginosa* for reasons that are not yet understood [6,11]. The increased use of antibiotics in patients with *P. aeruginosa* may play a role in the increased prevalence of fungi in sputum samples. Smaller studies also show similar relative levels of infection by *P. aeruginosa*, *C. albicans* and *A. fumigatus*. In Haase *et al*. [17], analysis of 121 patients, 70% of whom were infected with *P. aeruginosa*, found *C. albicans* in 70% of samples, *A. fumigatus* in 30% of samples and *E. dermatitidis* in 9%. Other recent studies have found similarly high levels of *A. fumigatus* and *C. albicans*, and documented persistent colonization over weeks and months by these fungi in some patients [7].

Host response to simultaneous exposure to bacteria and fungi

Because bacterial-fungal co-infections are both common and potentially detrimental to lung function, it is critical that we understand potential synergism associated with simultaneous infection by these two groups of pathogens. One way in which bacterial-fungal infections could influence the host is through the immune system. Fungi and bacteria stimulate differential immune responses [18,19], and little is known about the effects on the immune response when bacterial and fungal microbial products are simultaneously present within the lung. In the case of *P. aeruginosa*, initial infection results in rapid neutrophil recruitment followed by an adaptive immune response characterized by IFNγ and IL-17 production [20,21]. Fungi, such as *C. albicans* or *A. fumigatus*, elicit neutrophilia early, with a subsequent adaptive response characterized by IL-4 production and eosinophilia [22,23]. A recent study from our lab shows that while exposure to *P. aeruginosa* antigens *in vivo* results in a robust Th1-type immune response and *C. albicans* a robust Th2 response, combined exposure

to both *P. aeruginosa* and *C. albicans* immunodeviates the response to *C. albicans* from Th2 to Th1 and this effect is independent of LPS [24]. These studies have been performed in mice with functioning CFTR and thus need to be performed in models with mutated alleles of *CFTR* because there is evidence for immune system differences associated with decreased CTFR activity [25–30]. Furthermore, similar studies with live organisms are essential to determine if the immunodeviation that occurs with bacterial and fungal coexposure results in important clinical consequences for the host such as prolonged colonization with either bacteria or fungi. Preliminary studies from our group suggest that if *C. albicans* and *P. aeruginosa* are coadministered to mouse lung, significant increases in the persistence of both species *C. albicans* and *P. aeruginosa* infection was observed compared to monospecies infections [unpublished data]. While additional studies are required to understand this phenomenon, these findings potentially support the notion that, when together, *P. aeruginosa* and *C. albicans*, or possibly other fungi, can impact the ability of the host immune response to clear infection.

Roux *et al.* [31] have shown that rats inoculated with *P. aeruginosa* are at higher risk of developing pneumonia if they were previously exposed to live, but not heat killed *C. albicans*. They further demonstrate that this finding may be secondary to decreased macrophage reactive oxidant species generation following *Candida* exposure [31]. These studies raise the possibility that simultaneous exposure to *P. aeruginosa* and *C. albicans* can have important consequences on host immunity and potentially provide a rationale for treatment of seemingly 'benign' fungi such as *C. albicans*.

Mechanisms by which bacteria and fungi may directly affect one another

Interactions between bacteria and fungi may directly lead to changes in a microbe's ability to colonize a host, the amount of virulence factors produced, or the behavior of one of the interacting species. In the oral cavity, the physical interactions between bacteria and *C. albicans* are thought to contribute to increased fungal colonization of denture material, which can ultimately lead to denture stomatitis [32,33]. In studies of mechanically-ventilated patients with respiratory failure, Azoulay *et al.* [34] found that 'colonization' of airway secretions with *C. albicans*, a common phenomenon for patients supported for longer than two days, was a risk factor for the development of subsequent *P. aeruginosa* ventilator-associated pneumonia. Interestingly, the presence of *C. albicans* did not correlate with an increased risk of *S. aureus* pneumonia which may be indicative of some level of specificity in the interactions

between *P. aeruginosa* and *C. albicans* [34]. In another study, *P. aeruginosa* and *C. albicans* were commonly co-isolated from endotracheal tube biofilms in patients with ventilator-associated pneumonia suggesting that they preferentially favor each other in the setting of mixed infections [35]. While there are few studies that examine the relationship between other CF-relevant fungi and subsequent colonization by pathogens such as *P. aeruginosa*, there is evidence that initial colonization by fungi can promote subsequent acquisition of *P. aeruginosa* in onychomycosis [36].

Direct physical interactions between *P. aeruginosa* and *C. albicans* have been observed in *in vitro* studies [37]. *P. aeruginosa* cells can attach to the surface of *C. albicans* hyphae, and form a dense population of cells surrounded by an extracellular matrix, or a biofilm, on the surface of *C. albicans* hyphae over time [37]. *Burkholderia cenocepacia* can also form mixed species biofilms in association with *C. albicans* hyphae [38]. Additional studies are clearly needed to determine if the presence of *C. albicans* or other fungi is a predisposing factor for the acquisition of bacterial pathogens such as *P. aeruginosa* or *Burkholderia cencocepacia* and, if so, whether coaggregation between bacteria and fungi plays a role. If such a correlation is found, new strategies for delaying *P. aeruginosa* colonization in the CF lung may be developed based on the prevention of or treatment of fungal colonization. Therapies to delay *P. aeruginosa* infection are often implemented based on epidemiological data that indicate that *P. aerguinosa* presages a more rapid decline in lung function in CF [39–41]. The observation that biofilm formation develops in *P. aeruginosa*-*C. albicans* cocultures is of particular interest with respect to CF as the infecting microbes are thought to be in a biofilm-like state when in the lung [42,43]. Single species biofilms develop high levels of antibiotic resistance and are recalcitrant to host clearance [44,45], and these properties may be further altered when multiple species are present. For example, antibiotic resistance of *S. aureus* and *S. epidermidis* [46,47] increases in biofilms that also contain *C. albicans* [46]. One potential mechanism for increased resistance to antibacterials in mixed species biofilms with *C. albicans* is relates to the ability for the β-glucan rich matrix to bind antibiotic compounds [48]. Extracytoplasmic glucans have been previously shown to protect *P. aeruginosa* from several antibiotics relevant to CF [49].

Mixed infections may also alter the levels or spectrum of virulence factors produced by bacteria or fungi. For example, phenazine toxins produced by *P. aeruginosa* are thought to contribute to the damage of the host lung [50,51]. *P. aeruginosa* phenazines also kill *C. albicans* [37,52,53], and, *in vitro*, *P. aeruginosa* produces higher levels of phenazines in the presence of *C. albicans* [52].

Furthermore, we found that the *P. aeruginosa* LasR loss-of-function mutants, which commonly arise in the CF lung [54], produce very high amounts of phenazines in the presence of either *C. albicans* or farnesol, a molecule secreted by *C. albicans* [Cugini and Hogan, unpublished]. The finding that *C. albicans* can stimulate virulence factor production might suggest a mechanism by which a fungal coinfection or superinfection could lead to an exacerbation in a *P. aeruginosa*-infected patient. Farnesol also alters *P. aeruginosa* motility which may translate into an increased ability to form biofilms [55]. In addition to the effects of *C. albicans* on the levels of *P. aeruginosa*-produced phenazines and other similarly-regulated virulence factors, *C. albicans* may alter the spectrum of virulence factors produced. Gibson *et al.* reported that a *P. aeruginosa* phenazine that is not normally released at high concentrations in single species cultures is secreted when *C. albicans* is present [52]. Thus, these studies highlight the possibility that although *C. albicans* itself is an infrequent cause of invasive infections in the lung, it may have alternative effects that put the patient at risk. Very little is known about the effects of other fungi on *P. aeruginosa* virulence factor production; studies on *P. aeruginosa-A. fumigatus* interactions will be of particular interest.

While *C. albicans* may promote *P. aeruginosa* survival in the host by increasing virulence factor production, *P. aeruginosa* can secrete factors that promote *C. albicans* survival. *P. aeruginosa* produces an extracellular signaling molecule, 3-oxo-C12-homoserine lactone (3OC12HSL), which positively regulates bacterial virulence factor expression [56,57]. 3OC12HSL, which is frequently at detectable levels in sputum from CF patients infected with *P. aeruginosa* [43,58,59], inhibits *C. albicans* hyphal growth [60]. Subsequent analysis confirmed that 3OC12HSL-producing *P. aeruginosa* CF isolates suppress *C. albicans* filamentation in the laboratory [55]. Interestingly, a CF isolate of *Burkholderia cenocepia* produces a molecule with a similar ability to repress *C. albicans* hyphal growth [38]. The *C. albicans* response to 3OC12HSL and related compounds is likely mediated through the Ras1-cAMP-controlled signaling pathway in the fungus [61]. This response may aid in *C. albicans* survival as other molecules that similarly inhibit the Ras1-cAMP pathway induce *C. albicans* oxidative stress resistance and thus may increase its resistance to the host phagocytic cells [62].

A further level of complexity to consider in the study of bacterial-fungal interactions in the CF lung is the evolution of strains over time. *P. aeruginosa* mutants that lose the ability to produce high amounts of 3OC12HSL, due to mutation of the LasR transcriptional regulator, frequently arise in chronic CF infections [54]; these strains no longer alter fungal morphology [55]. Interestingly, LasR-defective CF laboratory strains and clinical isolates strains produced even higher levels of virulence factors in the presence of *C. albicans* than their LasR-positive counterparts [Cugini C, Morales DK, Hogan DA. Candida albicans-produced farnesol stimulates Pseudomonas quinolone signal production in LasR-defective Pseudomonas aeruginosa strains. *Microbiology* 2010; **156**: 3096–3107.] Consequently, the appearance of LasR mutants in the CF lung may also bring about changes in bacterial-fungal and fungal-host interactions. Studies on the interactions between mucoid *P. aeruginosa* strains and fungi, and on the relationships between CF-related bacteria and fungi other than *C. albicans*, particularly *A. fumigatus*, are needed.

Effects of antibiotic therapy on patients on dynamics of bacterial-fungal infections

While the benefits of antibiotic therapy are indisputable, treatment with antibiotics may increase lung fungal load. Increases in fungi such as *C. albicans* and *Aspergillus* spp. [63] or fungi-associated diseases [64] in patients with frequent exposure to antibacterial therapy have been documented. In a 520-patient study, Burns *et al.* [63] found an increase in the number of patients with detectable levels of *C. albicans* and *A. fumigatus* after six months of intermittent inhaled tobramycin therapy (28-days on drug followed by 28-days off drug) relative to the placebo control group. The 'treatment emergent' *C. albicans* was isolated in 22% of the treatment group relative to 16% in the control group; 'treatment emergent' *A. fumigatus* was found in 18% of the treatment group relative to 9% of the control group. It is important to note that in this study, there were no changes in patient status that necessitated antifungal therapy over the course of this study. It is not yet known if antibacterial therapy leads to the acquisition of fungal species in the lung or if fungal strains already present increase in number after treatment. In another study, quantitative analysis of CF sputum found *P. aeruginosa* concentrations to be between 10^7 and 10^9 CFUs/g sputum and *C. albicans* at 10^4 to 10^7 CFUs per gram suggesting a significant fungal burden albeit less than bacteria [13]. A separate study by Hoppe *et al.* [65] found similar relative levels of *P. aeruginosa* (10^7 CFUs recovered) and *C. albicans* (10^4 CFUs recovered) by throat swab after coughing in CF patients. While Kerr [53] reported the clinical observation that *C. albicans* levels increased as bacterial loads declined, we are unaware of quantitative studies that look at how fungal loads change in direct response to the administration of antibacterials. Potential suppression of *C. albicans* growth in CF sputum may involve some of the same factors, such as phenazines or phospholipase C, that allow *P. aeruginosa* to suppress *C. albicans* growth on laboratory media [37,53,66]. In some patients, there may be a role for antifungal therapy concomitant with aggressive use of antibacterials.

Challenges associated with assessing mixed bacterial-fungal infections

In order to truly assess the frequency of mixed bacterial-fungal infections and their clinical consequences, we must address the challenges in detecting fungi in sputum. The diagnosis of less frequently encountered fungi often requires bronchoalveolar lavage to accurately identify potential pathogens. The lack of standardized methods and/or reporting, as well as difficulties associated with culturing some fungi [67,68], may present challenges in both study design and retrospective analyses. Furthermore, especially for studies focused on *C. albicans*, studies must make efforts to exclude contamination of sputum by fungi that reside in the oral cavity.

The use of nucleic acid-based detection methods for the characterization of the CF sputum microflora will greatly increase our understanding of this complex community. Studies focused on the molecular characterization of the bacterial microflora found in CF sputum have identified new species common in CF sputum that may play a role in this polymicrobial disease [69]. Molecular analyses of fungi within CF sputum have also been reported [7,70,71]. To date, however, a concomitant analysis of bacteria and fungi within the same samples has not been performed. Analysis of bacterial populations, often using the tools that detect 16S rDNA common to bacteria, and fungi, generally using regions associated with their 18S rDNA sequences, in the same samples will require the use of two methods in parallel or the development of new markers for the profiling microbial populations.

Conclusions

It is not common practice to treat fungal pathogens identified by routine screening in CF sputum. Rather, antifungal therapies are frequently reserved for those instances when there are concerns about acute infection. If, however, results continue to show that fungal colonization, alone or in the presence of bacteria, correlates with decreased lung function or patient status, the use of antifungal therapy would need to be considered in a new context. Even outside of the CF lung, our understanding of complex polymicrobial infections is very limited. It may be the case that certain combinations of organisms, or even certain strains of organism, are detrimental while other bacterial-fungal combinations are not. For example, the effects of fungal-*P. aeruginosa* interactions may depend on the specific *P. aeruginosa* strains involved (i.e., mucoid versus nonmucoid [72] or LasR+ versus LasR− [54]). The importance of bacterial-fungal interactions may also differ depending on the nature of the interaction. For example, particular organisms may promote subsequent pathogen acquisition, the virulence or antibiotic resistance of particular species during chronic infection. Furthermore, the simultaneous presence of bacteria and fungi may have important effects on the host innate or adaptive immune responses. Many additional clinical studies, animal model experiments, and *in vitro* analyses will be required in order to understand the importance and nature of the interactions between bacteria and fungi that likely occur in the CF lung. Standardized methods for the reporting of fungi along with bacterial pathogens will also greatly aid future studies aimed at understanding of the consequences of mixed bacterial-fungal interactions and bacterial-fungal interactions.

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