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Minority Report: The Intestinal Mycobiota in Systemic Infections

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Abstract

Compared to bacteria, fungi often exhibit a lower abundance and a higher temporal volatility in the intestinal microbiota. Analysis of fungi in the microbiota (mycobiota) faces technical limitations with tools that were originally developed for analyzing bacteria. Dysbiotic states of the intestinal mycobiota, often associated with disruption of the healthy bacterial microbiota, are characterized by overgrowth (domination) of specific fungal taxa and loss of diversity. Intestinal domination by *Candida* species has been shown to be a major source of *Candida* bloodstream infections. Fungal dysbiosis is also linked to the development and treatment response in non-fungal infections, for example *Clostridioides difficile* colitis and HIV. Further research is needed to define the contribution of intestinal mycobiota to human fungal and non-fungal infections.

Introduction

The human intestinal microbiota is a complex reservoir of viruses, bacteria, archaea, fungi, and protozoa. The impact of the bacterial microbiota on systemic infections, especially in allogeneic hematopoietic cell transplant (allo-HCT) patients, has been extensively investigated [1–5]. In contrast, investigations on the impact of the fungal compartment of the intestinal microbial flora (i.e. mycobiota) have only started recently, in part because fungi generally represent a minor microbial component in the intestinal niche. In this review, we discuss features of the human intestinal mycobiota and technical challenges in its analysis. We focus on summarizing recent research developments that link the mycobiota to various human infectious diseases.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Demographics of the human mycobiota

Current analytical tools for microbiota largely rely on next-generation sequencing techniques and were initially developed for analyzing bacteria. These tools have been adapted to explore the demographics of the mycobiota. Internal transcribed spacer 1 and 2 (ITS1 and ITS2) regions of fungal ribosomal DNA are both common targets in amplicon-based sequencing method and have provided similar observations [6–8]: In the healthy human gastrointestinal tract, the most common fungal taxa are yeasts such as *Candida*, *Saccharomyces*, and *Malassezia* and filamentous fungi such as *Aspergillus*, *Cladosporium*, and *Penicillium*. Collectively, these fungal constituents represent core mycobiota species.

Shotgun metagenomics sequencing is another commonly used methodology for microbiota analysis and provides reads of the total microbiota without a potential bias from targeted PCR amplification. Shotgun sequencing of healthy donor samples within the Human Microbiota Project confirmed the fungal composition obtained by ITS sequencing [7]. Although the presence of many species detected in sequencing data can be verified by cultivation methods [6], a recent study showed that a considerable portion of recovered fungal DNA from healthy human fecal samples were in fact derived from food or other transient contents [9**]. This study correlated a reduction in fecal *Candida albicans* levels with an increase in the frequency of oral hygiene. These observations suggest that core mycobiota species have a higher volatility than the core bacterial microbiota in the healthy human gut.

In contrast, in patients with a high risk of fungal infection, such as allogeneic hematopoietic cell transplant (allo-HCT) recipients, longitudinal sampling of fecal specimens demonstrated that certain *Candida* species could rapidly expand and dominate the intestinal mycobiota, which precedes systemic fungal infections [10**]. Therefore, the intestinal mycobiota is characterized by distinct patterns and constituents under different pathological conditions.

Current limitations of mycobiota analysis

Metagenomics data in analyzing bacterial microbiota provide unbiased reads number of different taxa and a functional characterization of the community, for example the frequency of drug resistance genes or of genes implicated in metabolic pathways. However, this method is impractical for detailed characterization of the mycobiota. Multiple studies have shown that less than 0.1% of shotgun reads from healthy human fecal samples aligned to fungal genomes [7,11], ranging from a few hundred to several thousand reads (Figure 1). Even in a fecal sample with a drastic expansion of *Candida parapsilosis*, in which nearly half of the shotgun reads mapped to one dominant fungal species, the fungal sequences (8.4×10^4 reads, 8.4 Mb sequence size) did not amount to one full coverage of the *C. parapsilosis* genome (13Mb). In general, shotgun sequencing fails to provide sufficient fungal reads for robust compositional or functional mycobiota analysis. An alternative approach for functional analysis would be to isolate the fungus from fecal samples and to perform whole genome sequencing on the isolates. This approach would bypass the issue of sequencing depth with fungal genomes but is only applicable to viable fungal cultures from fecal samples.

Given the limitations of shotgun metagenomics, current characterization of the mycobiota relies on ITS amplicon-based methods. Although the strategy of fungal ITS sequencing is analogous to the bacterial 16S sequencing, fungal sequence analyses encounter technical issues unprecedented in bacterial sequencing analyses. While the tandem repeats of rDNA operons vary between 1–15 copies in different bacterial species [12], this variation ranges between 14 – 1442 copies in fungal species [13]. An ideal mycobiota pipeline would normalize the relative abundance of each species according to the rDNA copy numbers. However, this normalization is problematic given the lack of a reference copy number for all fungal species. Meanwhile, unlike the largely uniform length of 16S amplicons in bacteria, the ITS1 amplicons from different fungal species ranges from less than 200 bp to over 500 bp in length [14]. Since the widely used Illumina Miseq platform preferentially sequences smaller DNA fragments [15], fungal species with long ITS regions (e.g. *Saccharomyces cerevisiae* [16]) can be underrepresented in the final data output. The effect of this underrepresentation will be lower in samples characterized by exponential overgrowth of specific fungal species than in samples that contain a diverse mycobiota.

Although new sequencing strategies, for example full-length rDNA sequencing [17] may bypass the size limitations of the Illumina platform, at the present time amplicon-based sequencing is still the best approach to characterize the intestinal mycobiota. Due to the limitations of the ITS sequencing pipeline and the inherently higher variability of the intestinal mycobiota, a single sampling timepoint may not reflect the dynamic nature of intestinal mycobiota and yield spurious findings in clinical studies. Instead, researchers should focus on longitudinal collection of patient samples to provide a dynamic view of intestinal mycobiota and to reduce sampling noise in the analysis.

Simultaneous analysis of intestinal fungal and bacterial flora

As discussed above, mycobiota represents a minority of the total intestinal flora. In most instances, bacteria, especially anaerobes, are the major constituents in the intestinal niche. Researchers have associated the presence of anaerobes with intestinal colonization resistance to fungi, particularly *Candida* species. Consistent with this idea, prior exposure to broad-spectrum anti-bacterial antibiotics increased the risk of developing candidemia [18]. In a murine model, administration of antibiotics was required for intestinal *Candida albicans* colonization and dissemination [19]. A follow-up study showed that the anaerobic bacteria, *Blautia producta* (Family: *Lachnospiraceae*) and *Bacteroides thetaiotamicron* (Phylum: *Bacteroidetes*), conferred colonization resistance against *Candida albicans* [20]. Clinical datasets agreed with these laboratory studies and demonstrated that human intestinal samples with *Candida* species expansion exhibited a significant reduction in these bacterial taxa [10**,21,22]. These findings point to cross-kingdom interactions between bacteria and fungi in the intestinal microbiota. Therefore, it is crucial to analyze fungal and bacterial dynamics in parallel to assess complementary and antagonistic functions of members of both kingdoms in the same ecological niche.

The mycobiota and fungal bloodstream infections

Candida species cause over 20,000 bloodstream infection cases per year in the United States alone [23]. Even with contemporary antifungal drugs, fungus-attributable mortality is higher than 25% [23,24]. Patients in intensive care units and immunosuppressed patients, especially those with prolonged neutropenia, are at an increased risk of invasive candidiasis [25]. Early identification of high-risk patients and preemptive treatment may reduce *Candida*-related morbidity and mortality [25].

The observation that ingestion of a high dose of live *Candida albicans* leads to candidemia in a healthy volunteer raised the hypothesis of an intestinal source for *Candida* bloodstream infections [26]. Further studies showed that *Candida* bloodstream infections are associated with a high absolute *Candida* intestinal burden, and that *Candida* can be detected in stool samples prior to the development of a bloodstream infection in neutropenic patients and in neonates (systematically reviewed in [27]).

The intestinal *Candida* reservoir was confirmed to be an important source for *Candida* bloodstream infections by ITS1 sequencing of longitudinally collected fecal samples in a case-control studies of allo-HCT patients [10**]. All but one of patient in the *Candida* bloodstream infection group experienced a marked intestinal expansion, both in terms of relative and absolute abundance, of corresponding *Candida* species (*Candida parapsilosis* complex species and *Candida albicans*) prior to bloodstream infection. Similar levels of fungal dysbiosis were not observed in control patients during the study period. Whole genome sequencing of recovered *Candida* isolates confirmed the similarity of strains found in paired stool and blood samples from individual patients. Therefore, the intestinal *Candida* expansion could serve as a biomarker to help identify patients at high risk for systemic candidiasis.

This unusual expansion of specific *Candida* species during allo-HCT was influenced by multiple factors. First, the expansion of *Candida* was associated with the loss of the intestinal bacterial density and diversity, especially of the anaerobes. The dysbiosis of the anaerobic community was linked to the prolonged administration of broad-spectrum antibiotics (Figure 2). Second, *C. parapsilosis* complex species were frequently observed as the driver of intestinal domination and causative agent of bloodstream infections in this cohort. This predominance may be associated with to the use of standard antifungal prophylaxis with micafungin during the first week of allo-HCT, since *C. parapsilosis* complex species are more resistant to micafungin than other *Candida* species [28].

Beyond external factors such as administration of antibiotics, a recent study proposed a pathogenic synergy between bacterial and fungal pathogens at the onset of systemic candidiasis [29*]. In a murine model, *C. albicans* gavage and colonization induced a reduction in oral and intestinal bacterial diversity, specifically with an overgrowth of *Enterococcus spp.* in both sites. This observation suggested that a high burden of *Enterococci* diminished oral mucosal integrity and facilitated *C. albicans* invasion. This hypothesis requires further exploration in relevant clinical studies.

The mycobiota and *Clostridioides difficile* infection

Clostridioides difficile is the main cause of nosocomially acquired diarrhea (*C. difficile* infection, CDI) [30,31]. Broad-spectrum antibiotic use and the ensuing disturbances in the bacterial microbiota predispose patients to CDI [32,33]. Three studies have shown differential enrichment of multiple fungal taxa in patients with CDI by association analyses [34–36]. A limitation of these studies is their low sample size (10 – 18 patients with CDI) and the possibility of an inflated type I error due to multiple testing. Additionally, the clinical relevance of the reported taxa enrichment (such as *Helotiales* and *Byssoschlamys*) is unclear, as they are not generally considered as part of the core mycobiota [37].

A randomized controlled trial of fecal microbiota transfer (FMT) for the treatment of CDI showed that CDI patients had a higher baseline (prior to FMT) intestinal fungal load with lower fungal alpha-diversity than healthy controls, mainly due to an enrichment in *Candida albicans* [38*]. FMT non-responders had a lower fungal diversity with a higher abundance of *C. albicans* compared to responders (post FMT). This finding was possibly linked to a reduction in bacterial taxa that conferred *Candida* colonization resistance (specifically *Lachnospiraceae* and *Bacteroidetes* [20]) in the non-responders. Similarly, in a study comparing fidaxomicin to vancomycin for CDI, *Candida* intestinal colonization was more frequently observed in the vancomycin arm [39], since fidaxomicin and not vancomycin was known to spare *Bacteroides* [40]. Collectively, these findings were consistent with idea that *C. albicans* is not directly associated with CDI but rather represents a marker for bacterial dysbiosis observed in CDI.

On the other hand, administration of *C. albicans* prior or concomitant with FMT reduced FMT efficacy in a murine model of CDI [38*]. This result suggests that intestinal colonized *C. albicans* may directly influence the efficacy of FMT. In another study, pre-colonization with *C. albicans* prolonged mouse survival after induction of CDI, without affecting *C. difficile* growth or toxin titers. The improved survival was linked to an enhanced protective IL-17 response in *Candida*-colonized mice [41]. These mouse data suggest differential effects of *C. albicans* on CDI outcomes, based on the timing of *Candida* administration and study setting and will require validation in human cohorts.

The mycobiota and HIV infection

Advanced HIV infection with low CD4 T-cell counts is a known risk factor for opportunistic fungal infections. Several studies suggest that patients infected with HIV have increased serum levels of beta-D-glucan (BDG, a fungal cell wall component) compared to non-infected controls [42*,43]. Elevated BDG levels are associated with immune activation and high circulating inflammatory markers in these patients [44,45]. This observation led to the hypothesis that fungal translocation from the gut or other mucosal surfaces could lead to immune activation and disease progression in HIV patients, similarly to what has been postulated for bacteria [46,47]. This hypothesis will need further validation due to a lack of data on the intestinal mycobiota in HIV patients [48].

Future outlook

For many gastrointestinal infections, such as spontaneous peritonitis [49], cholecystitis [50], and superinfected pancreatic pseudocysts [51], fungi are well-known causative pathogens. The gastrointestinal mycobiota appears to be a likely source for many of these infections. Longitudinal sampling of intestinal or fecal specimens from these patients may facilitate monitoring mycobiota dynamics and may help provide more definitive answers about a causal link. In the future, it will be imperative to analyze the intestinal mycobiota in parallel with bacterial flora to reveal comprehensive cross-kingdom microbial interactions in this complex niche and to define their impact on human disease development.

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Highlights

- The human mycobiota contains clinically relevant fungal species;
- Longitudinal sampling provides robust insights into the human mycobiota;
- Simultaneous analysis of intestinal fungi and bacteria is critical in clinical studies;
- The intestinal mycobiota is an important source of fungal bloodstream infections;
- The mycobiota may play a role in bacterial or viral systemic infections.

Figure 1.

The fungal reads and non-fungal reads in metagenomic sequencing data of human fecal samples (A) without *Candida* domination, and (B) with *Candida* domination. Sample B was collected from a patient with active candidemia. The fungal reads account for <0.01% of total reads in sample A, and close to 50% of total reads in sample B.

Figure 2.

The dynamics of intestinal fungal and bacterial flora in the context of fungal bloodstream infection. This abstractive figure was plotted by re-analyzing data from a previously published study [10**].