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# GUT--MYCOBIOME-QUEST FOR EXCELLENCE, IN THE LUNG DISEASE AND TREATMENT

**Respiratory Medicine**

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## ABSTRACT

The statement "Nobody is fungus-free" is generally true. The fungal microbiome known as the mycobiome is an understudied component of the human microbiome. Fungi produce and secrete a variety of metabolic products. In the gut the fungal community is primarily composed of the genera Aspergillus Saccharomyces spp and Candida. Aspergilloma is the most common clinical manifestation of lung infections caused by Aspergillus species. Allergic Bronchopulmonary Aspergillosis is a result of an immune reaction to the colonization of Aspergillus fumigatus within the airways of patients. "Invasive Pulmonary Aspergillosis-mimicking Tuberculosis". "Chronic fibrosing pulmonary aspergillosis is a cause of 'destroyed lung' syndrome. Gut candida or an overgrowth of the fungus Candida in the digestive system can contribute to lung disease through several mechanisms including the gut-lung axis and the spread of infection. Candida overgrowth can lead to conditions like "leaky gut" which allows byproducts to enter the bloodstream and reach the lungs. Gut fungi regulate host homeostasis pathophysiological and physiological processes and the assembly of the co-residing gut bacterial microbiome. The gut mycobiome is emerging as a key player in precision medicine providing a novel perspective for unravelling complex disease mechanisms and developing highly targeted interventions. Rather than serving solely as biomarkers its distinct fungal signatures actively contribute to shaping diagnostic prognostic and therapeutic advancements across various diseases.

## KEYWORDS

Candida Cladosporium Malassezia Yeasts Bronchopulmonary Aspergillosis Chronic Fibrosing Pulmonary Aspergillosis

## INTRODUCTION

The intestinal microbiota is a complex assemblage of bacteria archaea viruses protists and fungi. Commonly used approaches to inventory and study the microbiota such as sequencing the stretch of DNA that encodes 16S ribosomal RNA (1)

In the gut the fungal community is mostly made up of the genera Aspergillus Candida Debaryomyces Malassezia Penicillium Pichia and Saccharomyces (2)

In immunocompromised individuals the commensal fungi can destabilise the balance of the intestinal microbiota and lead to infections (3).

Individuals undergoing prolonged antifungal therapy show exacerbated colitis and intensified symptoms of allergic airway diseases such as asthma (4).

The gastrointestinal (GI) tract is home to a diverse array of microbial organisms including bacteria fungi viruses and archaea collectively referred to as the microbiome. Bacteria are the dominant members of this community while fungi comprise only around 0.1% of the total gut microbial population (5)

Despite their low abundance recent advances in culture techniques and deep sequencing have revealed the substantial impact of these fungal constituents on intestinal homeostasis and disease (6 7)

This rapidly growing field of research has uncovered a diverse range of fungal species within the human gut including key taxa such as Candida species which reside in the gut as commensals (8)

These discoveries are largely due to advances in deep sequencing technologies and the establishment of several databases dedicated to fungal species such as FungiDB and Mycobank (9 10)

Candida albicans C. tropicalis C. parapsilosis and C. glabrata can all be found as natural asymptomatic components of the human microbiome. Published estimates of C. albicans carriage in healthy individuals range from 30% to 60% (11)

Malassezia has also been reported in significant abundance in fecal samples and may play a role in the gut (12 13)

Saccharomyces cerevisiae – bakers' and brewers' yeast – in the gut presumably originates in food (14)

Colonization of mice with C. albicans can result in dietary antigen leak from the stomach (15)

Aspergillus species are filamentous fungi that are commonly found in soil decaying vegetation and seeds and grains where they thrive as saprophytes. Aspergillus species can be occasionally harmful to humans.(16)

Most Aspergillus species are found in a wide variety of environments and substrates on the Earth throughout the year.(17)

In humans Aspergillus fumigatus is the most common and life-threatening airborne opportunistic fungal pathogen which is particularly important among immunocompromised hosts (18)

In normal host lungs inhaled conidia are cleared by epithelial cells and alveolar macrophages. Conidia escaping these host defenses may germinate into branching filaments called hyphae which is when Aspergillus becomes invasive. Inflammatory mediators released by alveolar macrophages lead to the recruitment of neutrophils which can eliminate the hyphae (19)

Recent studies have demonstrated that the bacterial GI community

reassembly after broad-spectrum antibiotics can be altered if the fungal community blooms in the GI tract and this can impact the development of allergic airway disease (20)

**"Invasive Pulmonary Aspergillosis-mimicking Tuberculosis"**

Aspergilloma a fungal ball grows within and is usually restricted to an existing lung cavity and mimics old tuberculosis or bronchiectasis. In this type of colonizing Aspergillosis surgical removal becomes necessary as the disease commonly causes massive hemoptysis. In invasive Aspergillosis the fungus first causes pneumonia and later disseminates to involve other organs. E.g. the brain kidney or heart. Patients who develop this type of disease which may be fatal are usually immunocompromised or debilitated due to prolonged treatment with antibiotics steroids and cytotoxic drugs. Obstructive bronchial aspergillosis usually occurs in the setting of underlying pulmonary disease such as cystic fibrosis chronic bronchitis or bronchiectasis. The colonization of old fibrocavitary disease by Aspergillus is the most common cause of a pulmonary fungus ball. Aspergillus fungus balls show heterogeneous staining intensity on H&E stain. giving the impression of alternating zones of growth. The wall of the fungus ball frequently shows an increased number of tissue eosinophils. The walls of the cavity are lined with granulation tissue granulomatous inflammation or metaplastic squamous epithelium depending on the activity of the disease (21)

The rapid expansion of a cavity is typically caused by vascular thrombosis induced by various Aspergillus species most commonly Aspergillus niger. "Chronic fibrosing pulmonary aspergillosis: a cause of 'destroyed lung' syndrome." This condition is masked by the formation of bronchial casts or plugs composed of hyphal elements and mucinous material.(22)

Occasionally in debilitated subject's oral thrush extends into the respiratory tract to involve the bronchi or lungs. Candida infection may be a secondary invader of the lungs where pre-existing disease is present (eg TB or cancer). Candida pneumonia is a rare infection of the lungs with the majority of cases occurring secondary to hematological dissemination of Candida organisms from a distant site usually the gastrointestinal tract or skin (23).

Candida auris enters the bloodstream and causes serious invasive infections. This yeast is often resistant to antifungal drugs and difficult to treat. Ambulatory post-COVID-19 patients with corticosteroid therapy central venous catheter or other lines or tubes entering their body or who have previously received antibiotics or antifungal medications appear to be at the highest risk of infection with this yeast

**Fungal Influence on Immunity**

Studies have demonstrated that gut fungi can modulate the immune system influencing inflammation and the development of immune responses.

Life-threatening fungal infections have risen sharply in recent years owing to the advances and intensity of medical care that may blunt immunity in patients. This emerging crisis has created the growing need to clarify immune defence mechanisms against fungi with the ultimate goal of therapeutic intervention.(24)

Mice's inhalation of Aspergillus fumigatus leads to a rapid increase in phagocytic numbers in the spleen and blood but also in the lung (25)

This increase is IL-3 dependent.IL-3 is important for the recruitment of basophils into mediastinal lymph nodes following Nippostrongylus basiliensis infection.(26)

Life-threatening fungal infections have risen sharply in recent years owing to advances and intensity of medical care that may blunt immunity in patients. This emerging crisis has created the growing need to clarify immune defense mechanisms against fungi with the ultimate goal of therapeutic intervention. Dendritic cells and subsets are mobilized against fungi in various anatomical components. Fungal molecular patterns and their corresponding receptors that signal responses and shape the differentiation of T-cell subsets and B-cell subsets ultimately. The effector and regulatory mechanisms eliminate these invaders while constraining collateral damage to vital tissues. These insights create a foundation for the development of new immune-based strategies for prevention or enhanced clearance of fungal diseases.(27)

A recent surge in the discovery of newly described inborn errors of immune function-related genes that result in susceptibility to fungal disease has significantly enhanced the understanding of the cellular and molecular basis of antifungal immune responses. Characterization of single gene defects that predispose to various combinations of superficial and deep-seated infections caused by yeast mold and dimorphic fungi has unmasked the critical role of novel molecules and signaling pathways in mucosal and systemic antifungal host defense. These experiments of nature offer a unique opportunity for developing new knowledge in immunological research and for devising immune-based therapeutic approaches for patients infected with fungal pathogens (28).

Cryptococcus neoformans and C. gattii are facultative intracellular yeasts that possess several virulence factors contributing to their pathogenicity. One of their most notable features is the production of a thick polysaccharide capsule which plays a crucial role in immune evasion. This capsule suppresses cytokine production by immune cells sequesters complement components to interfere with immune signaling and reduces the antigen-presenting capacity of monocytes effectively shielding the fungus from detection. By masking itself from phagocytes Cryptococcus species can avoid being engulfed and destroyed. In addition to capsule formation other significant virulence traits include melanin production which provides protection against oxidative stress and enhances survival in hostile environments the ability to thrive at human body temperature (37 °C) and the secretion of extracellular enzymes that facilitate tissue invasion and nutrient acquisition (29)

**Development of the Gut Mycobiome in Humans**

Fungal species assemble in the gut before birth as detected by both amplicon sequencing and culture-based techniques; however this is still a matter of debate as there are immense controversies over in-utero colonisation of microbes versus studies reporting gut fungal assembly after birth (30)

Studies have found that the gut mycobiome of infants is initially dominated by Saccharomycetales and Malasseziales spp at the first month followed by a gradual decline of Malasseziales to an undetectable amount at 5 months of age (31)

At 1–2 years of age—a crucial window of infant dietary shift from breastmilk to solid food—Saccharomyces cerevisiae becomes the most abundant species concomitant with the appearance of Cystofilobasidium spp Ascomycota spp and Monographella spp indicating a prominent role for diet in shaping the gut mycobiome.(32)

After that the gut mycobiome undergoes further changes and matures to an adulthood-like fungal community that features substantially increased gut mycobiome diversity and compositional dominance by the phyla Ascomycota Basidiomycota and Zygomycota (33)

**A Study Says Antibiotics Can Lead to Life-threatening Fungal Infections Because of Disruption to the Gut Microbiome**

Candida is a fungus that is a common cause of fungal infections in humans. Thrush a yeast infection is caused by Candida. But it can also cause a life-threatening bloodstream infection called invasive candidiasis. Fungal infections kill around the same number of people each year as tuberculosis. They mostly take hold in people who are vulnerable because they have a defective immune system caused by an underlying disease such as cancer or a viral infection such as HIV or COVID. New study shows that antibiotics can cause immune system defects that increase the risk of dangerous fungal infections. Candida is a fungus that is a common cause of fungal infections in humans.

**Delhi Hospital Reports 'first-ever' White Fungus Case Causing Severe Damage to the Entire Intestine**

Delhi's Sir Ganga Ram Hospital (SGRH) has come across a first-of-its-kind case of white fungus causing multiple perforations "throughout the intestine" in a COVID-19 patient.

**Black Fungus in the Intestine: Rarest of Rare Cases Found Treated at Delhi's Sir Ganga Ram Hospital**

Mucormycosis or Black Fungus of the small intestine was detected and treated in two recovered Covid-19 patients at the Sir Ganga Ram Hospital in New Delhi.

**Biotechnology For Molecular Diagnosis of Aspergillus--(Pulmonary Fungi)**

A 10% KOH preparation of sputum bronchoalveolar lavage transbronchial biopsy and other biopsies reveals non-pigmented septate hyphae 3-5 µm in diameter with characteristic dichotomous branching at 45-degree angles. The hyphae tend to branch repeatedly. In a majority of pulmonary and disseminated lesions only hyphal forms are seen. In allergic aspergillosis there is usually abundant fungus in the sputum and mycelial plugs may be present. In aspergilloma the fungus may be difficult to find in the sputum. In invasive aspergillosis the sputum smear is often negative. The most reliable method for the diagnosis of acute invasive aspergillosis is the examination of stained tissue sections.

The morphology of Aspergillus is fairly characteristic usually demonstrating nonpigmented narrow septate hyphae with acute-angle branching. The histological section can be stained with hematoxylin and eosin(H&E) and Gomori methenamine silver(GMS) and examined for characteristic hyphae. In chronic lesions short distorted hyphae may be as wide as 12 microm. Conidial heads may occasionally form in areas exposed to air eg in pulmonary cavities and ear or skin infections.

Invasive aspergillus pneumonia Galactomannan ELISA may be positive in the blood very early before clinical suspicion of invasive fungal infection. Serological testing for antibodies to Aspergillus antigens is helpful to diagnose aspergilloma. or allergic broncho-pulmonary aspergillosis in immunocompetent individuals but unfortunately serology plays a little role in the diagnosis in the immunocompromised patient because of Aspergillus growth does not correlate with an increase in anti-Aspergillus antibody titer (34)

Galactomannan(GM) is a major cell wall component of Aspergillus and it is known that the highest concentrations of GM are released in the terminal phases of the disease. An ELISA technique was introduced using a rat anti-GM monoclonal antibody EB-A2 which recognizes 1---5 Beta-D-Galactio furanoside side chains of the GM molecule (35)

This sandwich ELISA technique is used in the current commercially available GM assay for the diagnosis of IA. In a meta-analysis conducted to characterize the clinical utility of the GM assay 27 studies were identified and overall the GM assay had a sensitivity of 71 % and a specificity of 89% for proven cases of IA as defined by the specific clinical criteria (36)

Unfortunately the GM assay has decreased sensitivity in the setting of a patient receiving Aspergillus antifungals although specificity for detection does not change

The sensitivity increases markedly when BAL fluid is tested instead of the patient's serum. False-positive results may be obtained in patients receiving piperacillin-tazobactam antibiotics or Plasmalyte intravenous fluids. Beta-glucan testing is also helpful in diagnosing invasive aspergillosis is found to be comparable or more sensitive than galactomannan assays. False-positive results with beta-glucan assays have been observed in patients who were receiving fungal-derived antibiotics. Sometimes false positives may be noted with Pseudomonas aeruginosa infections. Perimonitoring of the galactomannan polymerase chain reaction is also useful. These assays are more sensitive than Enzyme immunoassays and latex agglutination techniques.(37)

Radiological manifestations include nodular shadows patchy infiltrates and cavitating lesions are the common findings on the chest radiograph. Computed tomography (CT) scan is useful in the evaluation of non-specific infiltrates in immunocompromised patients. It is imperative to achieve an early diagnosis to avoid the development of long-standing complications and to reduce the mortality rates as much as possible. The ' halo sign' on CT is now regarded as an early indicator of invasive pulmonary Aspergillosis. (38)

**Treatment**

Possible interactions with other drugs must be considered before Azoles are prescribed. In addition plasma azole concentrations vary substantially from one patient to another and many authorities recommend monitoring levels to ensure that drug concentrations are adequate but not excessive especially withitraconazole and voriconazole.(39)

Initial IV administration is preferred for acute invasive aspergillosis and oral administration for all other diseases that require anti fungal therapy.(40)

Treatment of chronic necrotizing pulmonary aspergillosis (CNPA) and invasive aspergillosis differs significantly from treatment of allergic bronchopulmonary aspergillosis (ABPA) and aspergilloma Allergic bronchopulmonary aspergillosis(ABPA) is a hypersensitivity reaction that requires treatment with oral corticosteroids. Inhaled steroids are not effective (41)

Recent guidelines recommend voriconazole and/or isavuconazole for the primary treatment of IA with liposomal amphotericin B being the first alternative and posaconazole as well as echinocandins primarily recommended for salvage treatment. Few studies have evaluated treatment options for chronic pulmonary aspergillosis (CPA) where long-term oral itraconazole or voriconazole remain the treatment of choice. (42)

**The Need for New Antifungal Agents**

Potential pharmacological strategies include the use of (i) new formulations of antifungals such as liposomal amphotericin B amphotericin B lipid complex amphotericin B colloidal dispersion amphotericin B into a lipid nanosphere formulation itraconazole and β-cyclodextrin itraconazole or (ii) combination therapies of one or more antifungal compounds for example amphotericin B + flucytosine fluconazole + flucytosine amphotericin B + fluconazole caspofungin + liposomal amphotericin B and caspofungin + fluconazole

**Significant Gap in Research**

The study of gut fungi is an emerging field in understanding its complex interactions with the gut microbiome and its impact on human health. Future research should focus on standardized methods exploring the role of the mycobiome in various diseases and developing targeted therapeutics.

**Literature Gaps:**
**Potential Significance**

Even though fungus has potential significance it is hard to notice the differentiation from bacteria

**Lack of Organized Methods**

There's a need demand and urgency for organized methods to estimate the gut fungus.

**Restricted Comprehension of Interrelationships**

Greater research is needed to understand the intricate interrelationships between fungi bacteria and the host immune system.

**Future Research Directions:**
**Systemic Procedure**

Advanced sequencing technologies and computational biology methods will help in studying fungi

**Disease Associations:**

Scrutinize the role of fungal dysbiosis in diseases like IBD colorectal cancer and neurological disorders.

**Targeted Therapies:**

Exploring potential therapeutic interventions targeting the gut mycobiome such as probiotics or antifungal agents.

**Precision Medicine:**

Using the gut mycobiome for biomarker discovery disease diagnostics and targeted therapeutics.

**Information on Accelerating Scientific Innovation**

Sequencing technologies have significantly expanded our understanding of the gut mycobiome revealing that fungi play a crucial role in gut health immunity and disease. Research has shown that fungal communities in the gut particularly species like Candida albicans can influence host immunity interact with bacteria and contribute to both health and disease.

**Key Advances and Discoveries:**
**Next-generation Sequencing (NGS):**

NGS has enabled researchers to identify a wider range of gut fungi and study their interactions with the host and other gut microbes.

**Fungal Dysbiosis in Disease:**

Disruptions in the gut mycobiome or fungal dysbiosis have been linked to various diseases including inflammatory bowel disease (IBD) metabolic disorders and even cancer.

**Fungal Interactions With Bacteria:**
Research has revealed complex interactions between fungi and bacteria in the gut where fungi can either promote or inhibit bacterial growth and colonization.

**Fungal Probiotics:**
The potential for using fungi as probiotics to treat gut-related disorders is an emerging area of research.

**Fungal Metabolites:**
Fungi produce a wide range of metabolites that can impact gut health and other aspects of host biology.

**Gut-brain Axis:**
Research suggests that gut fungi can influence the gut-brain axis impacting behavior and mental health.

**Fungal Role in Metabolic Diseases:**
Studies have identified correlations between gut fungi and metabolic diseases like obesity type 2 diabetes and non-alcoholic fatty liver disease (NAFLD).

**Fungal Role in Biofuel Production:**
Some anaerobic gut fungi have unique cellulolytic enzymes that could be harnessed for biofuel production.

**Fungal Interactions with the Host Immune System:**
Gut fungi can interact with host immune cells and receptors influencing immune responses and contributing to inflammation.

**Fungal Diversity:**
The gut mycobiome is diverse and understanding the role of various fungal species is crucial for a comprehensive understanding of gut health and disease.

**Ongoing Research:**
**Elucidating the Precise Mechanisms of Fungal Interactions with Bacteria and the Host Immune System:**
Understanding these interactions will be crucial for developing targeted therapies.
**Identifying Specific Fungal Species Associated with Different Diseases:**
This knowledge can help in the development of diagnostic tools and targeted interventions.
**Exploring the Potential of Fungal Probiotics For Treating Gut-related Disorders:**
Research is ongoing to determine the safety and efficacy of using fungi as probiotics.
**Developing New Antifungal Therapies:**
New approaches to treat fungal infections and dysbiosis are being explored.
**Investigating the Impact of Diet and Other Lifestyle Factors on the Gut Mycobiome:**
Understanding these factors can help in optimizing gut health and preventing diseases.

**WHERE WILL THE RESEARCH GO NEXT?**

**CONCLUSION**
The human gastrointestinal tract harbors a vast array of microorganisms which play essential roles in maintaining metabolic balance and immune function. Compared to bacterial communities the human gut mycobiome is low in diversity and dominated by yeast including Saccharomyces Malassezia and Candida. In spite of low quantity fungi may significantly influence both health and disease. Advances in next-generation sequencing metagenomics metatranscriptomics metaproteomics metabolomics and computational biology have provided novel opportunities to study the gut mycobiome shedding light on its composition functional genes and metabolite interactions. Emerging evidence links fungal dysbiosis to various diseases including Aspergilloma Chronic fibrosing pulmonary aspergillosis A rare condition of candida pneumonia. inflammatory bowel disease colorectal cancer metabolic disorders and neurological conditions.

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