Microbiology in Invasive Fungal Sinusitis

Anjali Shetty and Camilla Rodrigues

Introduction

In the past few decades, the incidence of invasive fungal rhinosinusitis cases in tertiary care centers has been steadily increasing with the gradual increase in the number of immunocompromised patients. India contributes to about 40 % of the global burden of zygomycosis [1]. Fungal spores are ubiquitous and are continuously being inhaled, leading to colonization of the sinuses. This colonization may lead to chronic sinusitis and occasionally invasive fungal infection especially in an immunocompromised host.

Fungi are eukaryotes (possess nuclear membrane), depend on an external source for nutrition, and may consist of hyphal segments or unicellular organisms. Fungal infections were a rarity in the past with the predominant problem being allergy, mycotoxicoses from ingested toxins, and mushroom poisoning.

There are four main groups (phyla) of true fungi—*Ascomycota, Basidiomycota, Zygomycota*, and Deuteromycota (Fungi imperfecti). Ascomycota include dermatophytes, *Aspergillus* spp., *Histoplasma capsulatum*, *and Blastomyces dermatitidis*. The most common fungal infections are caused by dermatophytes, fungi that colonize dead keratinized tissue including skin, finger, and toenails. Dermatophytes cause superficial infections such as "ringworm" that are unsightly and difficult to treat, but rarely serious. *Aspergillus fumigatus*, one of the most important of these opportunists, produces small, airborne spores that are frequently inhaled; in some individuals, the fungus starts growing invasively, causing a disease known as aspergillosis, especially in immunocompromised individuals.

A. Shetty, MRCP, FRCPath (✉) • C. Rodrigues, MD
Department of Microbiology, P.D. Hinduja Hospital,
Mahim, Mumbai, Maharashtra 400 016, India
e-mail: anjalishettyuk@yahoo.co.uk;
dr_crodrigues@hindujahospital.com

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Mucormycosis is the common term used to describe infections caused by fungi belonging to the order Mucorales. Zygomycosis, a term which was used earlier to describe these life-threatening infections, has become less accurate based on a recent taxonomic reclassification (based on molecular identification) that abolished Zygomycetes as a class [2, 3].

Mucormycosis and entomophthoramycosis were earlier encompassed by the term zygomycosis. Changes in taxonomy due to molecular phylogenetic analyses have, however, led to the class name Zygomycota being replaced by Glomeromycota. In the current classification, the agents of mucormycosis have been placed under the subphylum Mucormycotina, and the agents of Entomophthoramycosis are now in the subphylum Entomophthoramycotina. Since the phylum Zygomycota does not exist any longer, the disease name zygomycosis has become obsolete.

Mucorales includes *Rhizopus* spp., *Absidia* spp., *Rhizomucor* spp., *and Mucor* spp. These organisms can cause rhinocerebral, pulmonary, gastrointestinal, cutaneous, or disseminated infection in the immunosuppressed host and account for up to 75 % of mucormycosis cases encountered in hematologic malignancy patients [4]. Entomophthoramycosis includes infections due to *Conidiobolus* spp. *and Basidiobolus* spp. They are often seen in tropical environment causing infections of the paranasal sinuses and subcutaneous tissues. These infections are seen in immunocompetent individuals and have a chronic course. *Conidiobolus* spp. affects the head and face. Subcutaneous rhinofacial infection is the most common presentation. Symptoms include nasal discharge, unilateral nasal obstruction, sinus tenderness, and facial swelling (Fig. 1). Basidiobolus spp. involves the subcutaneous tissues of the trunk and arms.

Basidiomycota includes *Cryptococcus neoformans*. This organism is an encapsulated yeast which can cause disease in immunocompetent individuals as well as immunosuppressed patients. Infection occurs following inhalation and meningitis is the most common presentation.

Deuteromycota (Fungi imperfecti) includes *Candida* spp., *Coccidioides immitis*, *and Sporothrix schenckii*. *Candida* species can cause both superficial and invasive infections. It is also part of the normal flora of the gastrointestinal tract.

Fungi isolated in invasive fungal rhinosinusitis while showing geographic variation are often similar in particular forms of fungal disease [5]. For example, *A. fumigatus*, *A. flavus*, *and Rhizopus* sp. are uniformly seen in patients with acute invasive disease worldwide [6–8].

Acute Invasive Fungal Rhinosinusitis

Two distinct patient populations are seen [9]: one group of patients is patients with diabetes, especially with diabetic ketoacidosis and second group with neutropenia. Up to 80 % of invasive fungal infections in the first group are caused by fungi belonging to the order Zygomycetes such *as Rhizopus* sp., *Rhizomucor* sp., *Absidia* sp., *and Mucor* sp. [10]. This disease is more rapidly progressive with high

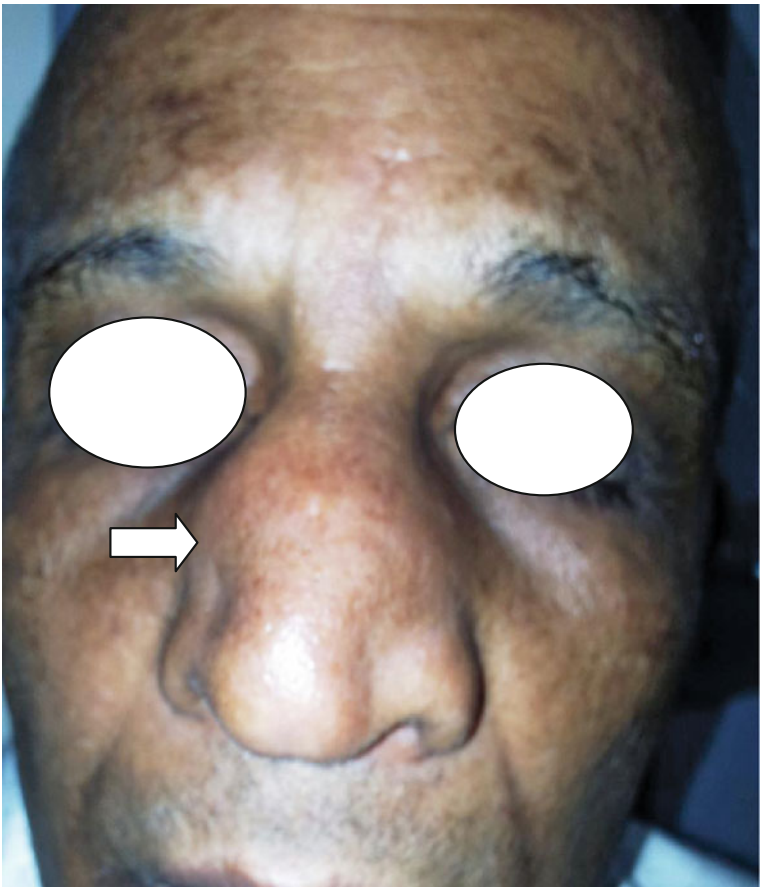

Fig. 1 Subcutaneous (*arrow*) Conidiobolus rhinofacial infection

mortality and morbidity probably due to the high virulence of these fungi as well as due to diagnosis of the disease at a late stage [9].

The other group is immunocompromised patients with severe neutropenia, e.g., patients with hematologic malignancies; patients undergoing chemotherapy or systemic steroid therapy or bone, organ, or stem cell transplantation; or patients with AIDS. Aspergillus species is responsible for up to 80 % of infections in this group [10]. In India, *A. flavus* [11] is the most common (80 %) while in the Middle East, *A. fumigatus* [12] is the most common (50 %) causative agent. Nasal septal ulceration has also been described with Fusarium species and *Pseudallescheria boydii*.

Among the zygomycotic species causing IFS, *Rhizopus arrhizus* is the most frequent agent followed by *Rhizopus microsporus*, *Absidia corymbifera*, *Rhizomucor pusillus*, *and Mucor circinelloides* [13, 14]. Another agent, *Apophysomyces elegans*, is also responsible for zygomycosis in India [1]. Sridhara et al. [15] have reported

an increasing trend of mucormycosis in immunocompetent individuals. Three out of eight immunocompetent cases reported by them were infected by *Apophysomyces elegans* [15].

Wueppenhorst et al. [16] have reported a case of fulminant invasive fungal sinusitis caused by *Conidiobolus incongruus* in Germany. They concluded that diagnostics relying exclusively on histopathological findings could misdiagnose entomophthoramycosis as mucormycosis, and therefore, species identification is indispensable for collection of data for the adequate treatment of the condition.

Chronic Invasive Fungal Rhinosinusitis

Aspergillus species, dematiaceous molds such as *Bipolaris*, *Curvularia*, and *Pseudallescheria boydii* are the fungi implicated in this disease. *Aspergillus fumigatus* is the most commonly isolated fungus [17] although *Mucor* sp. is also known to be a causative agent especially in diabetics.

Chronic Granulomatous Rhinosinusitis

Aspergillus flavus is the fungus most often implicated in this disease. Paranasal granuloma is a peculiar syndrome associated with proptosis that has also been called indolent fungal sinusitis in immune competent persons. The fungus *A. flavus* shows exuberant growth with regional tissue invasion, non-caseating granulomas, giant cells, and plasma cells. This condition is known to occur in Saudi Arabia, Sudan, India, and Pakistan [11, 18]. This is rarely seen in the USA [19].

Diagnosis

Specimen – Tissue samples or aspirates are recommended as opposed to swabs as the material obtained in tissue and aspirate is much more than in swabs, thus increasing the yield.

Microscopy

KOH Wet Mount

Potassium hydroxide digests proteinaceous material and debris and allows visualization of the fungal hyphae under a light microscope (Fig. 2).

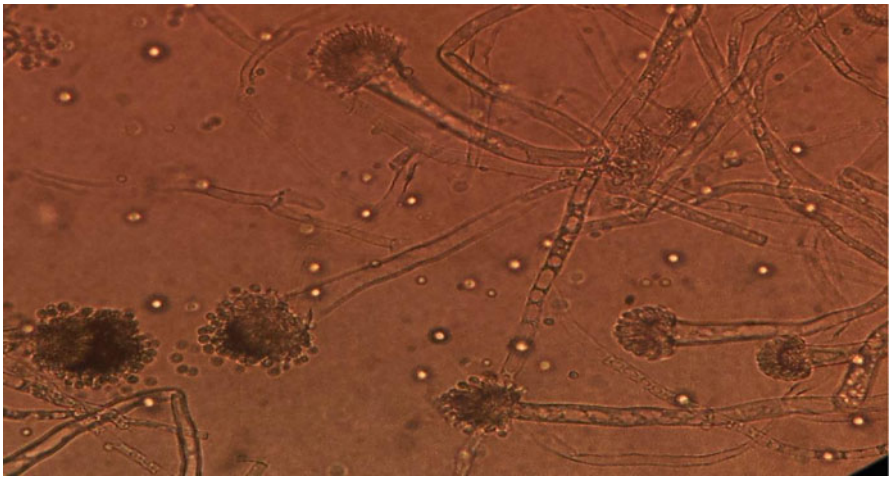

Fig. 2 KOH mount of *Aspergillus*

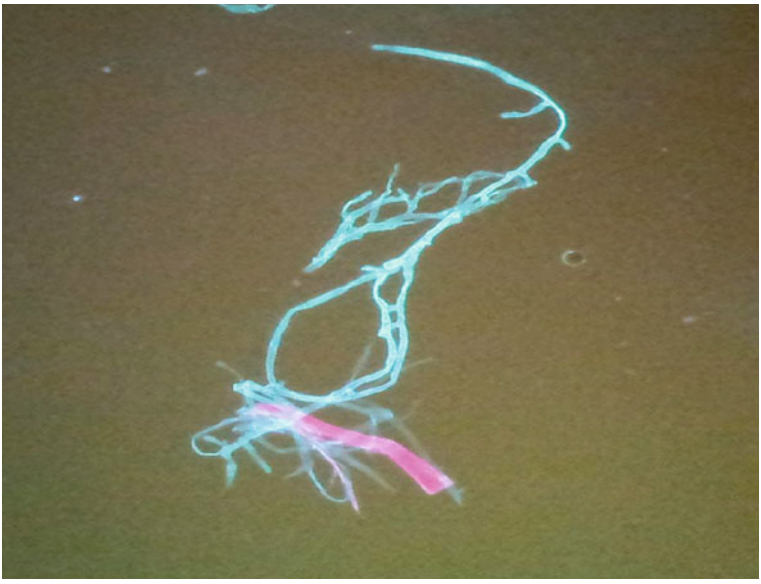

Fig. 3 Calcofluor preparation of aseptate fungal filaments

Calcofluor Staining

It is difficult to stain fungi with routine stains, but this stain binds to the chitin and cellulose in the fungal cell wall and demonstrates bright green to blue fluorescence under a fluorescent microscope making it easier to demonstrate the fungi (Figs. 3 and 4). Sensitivity increased by 15 % in demonstrating fungal hyphae when

Fig. 4 Calcofluor preparation of septate fungi

calcofluor white was added to KOH wet mounts in a Chinese study on fungal keratitis [22]. For rapid diagnosis, clinicians should request for calcofluor/KOH mount.

Lactophenol cotton blue is a widely used method of staining and observing fungi (Figs. 5a, b and 6). On microscopy one can comment on the presence or absence of septae. Aseptate fungi are Mucorales (Fig. 3). Septate fungi are *Aspergillus* sp., *Fusarium* spp., *Scedosporium* spp., etc. (Fig. 4). *Aspergillus* spp. demonstrates acute angle branching and Mucorales demonstrates right angle branching. Practically, it may be difficult to comment on the branching pattern.

Culture

Fungal culture is difficult and often no growth is achieved. Fungal culture specimens should be obtained preferably before starting antifungal therapy. Initiation of antifungals prior to culture reduces the chances of growing fungus on culture. While processing the sample for culture, it is important to remember that grinding and freeze-thawing of the specimen will lead to a decreased yield of Mucorales.

The samples are cultured on agar such as Sabouraud dextrose agar (Figs. 7, 8, 9, 10 and 11), brain-heart infusion agar, etc. with antibiotics. The agar is incubated at both room temperature and 37 °C. Macroscopic and microscopic examination of cultures aids in the diagnosis of fungus. Fungal cultures should be examined biweekly for a period of 4 weeks before they are declared as negative.

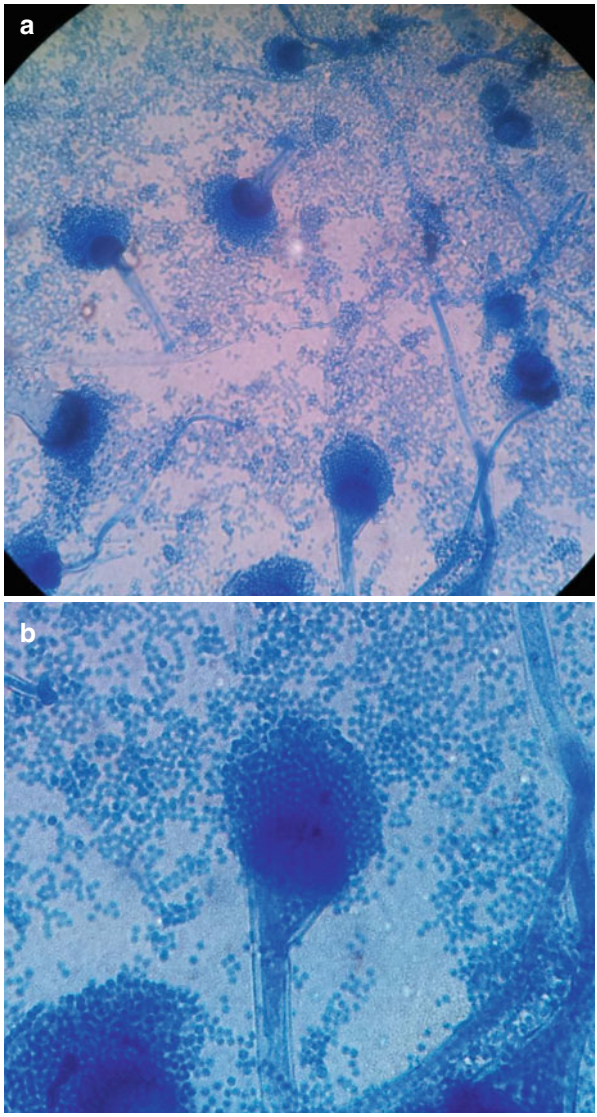

Fig. 5 (**a**) Lactophenol cotton *blue* preparation of *Aspergillus fumigatus*. (**b**) LCB preparation of *Aspergillus fumigatus* head

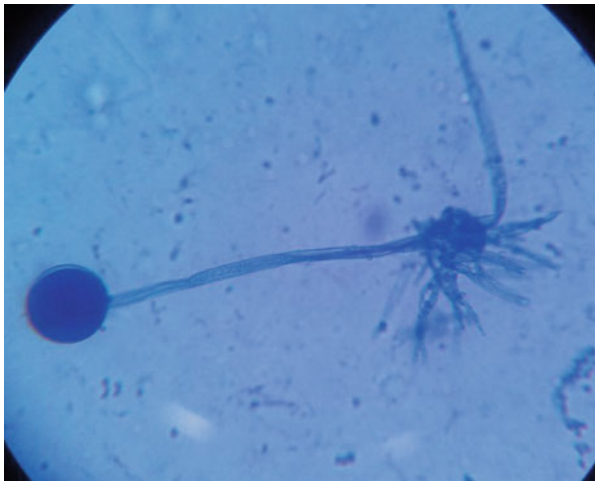

Fig. 6 Lactophenol cotton *blue* preparation of *Rhizopus* spp

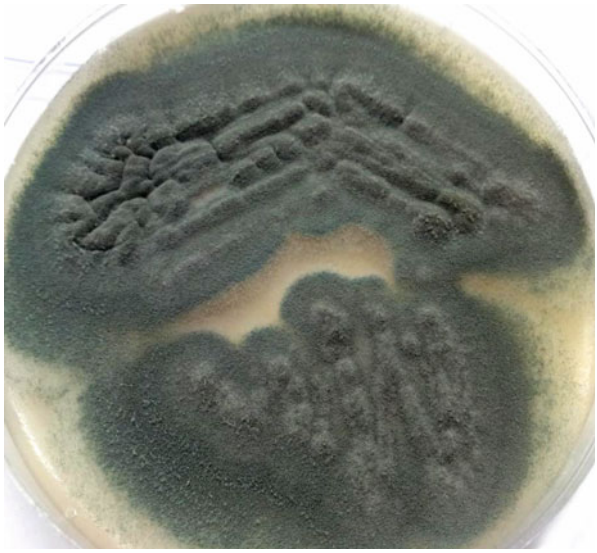

Fig. 7 *Aspergillus fumigatus* on Sabouraud dextrose agar (SDA)

Aspergillus Galactomannan

This is a noninvasive test to diagnose invasive aspergillosis. It detects galactomannan, which is a polysaccharide cell wall component that is released by *Aspergillus* spp. during hyphal growth. The latex test had poor sensitivity and has been largely replaced by a double sandwich ELISA. FDA cutoff for this test is 0.5 ng/ml, though

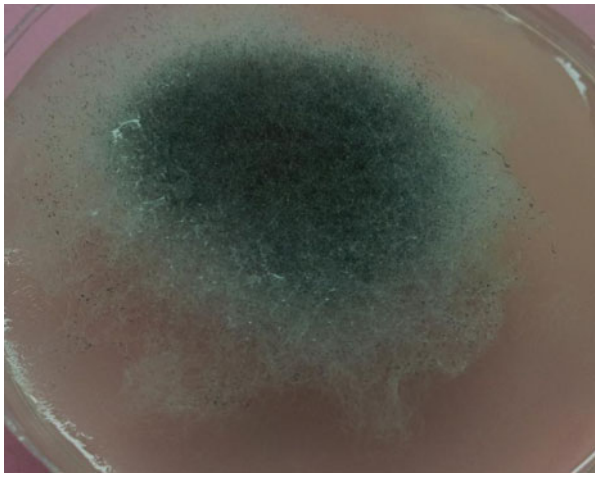

Fig. 8 Mucor on Sabouraud dextrose agar

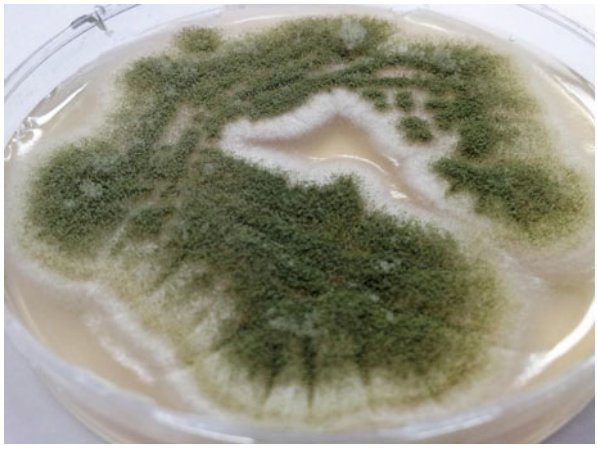

Fig. 9 *Aspergillus flavus* on Sabourad dextrose agar

studies use different cutoffs of positivity. Together with host factors and clinical criteria, a positive serum galactomannan test would suggest probable invasive aspergillosis [1]. This test has shown variable sensitivity and specificity and is impacted by prior antifungal therapy. False positivity is known to occur due to the use of piperacillin-tazobactam and also in children. Cross reaction occurs with other fungi such as *Paecilomyces* spp., *Alternaria* spp., *Penicillium* spp., etc. A study in patients with hematological malignancy in Taiwan [21] had 16 patients with invasive fungal sinusitis who had serial follow-up of Aspergillus galactomannan. Sensitivity was 64 % and specificity was 60 % for the diagnosis of invasive aspergillus sinusitis when compared to the EORTC criteria [22].

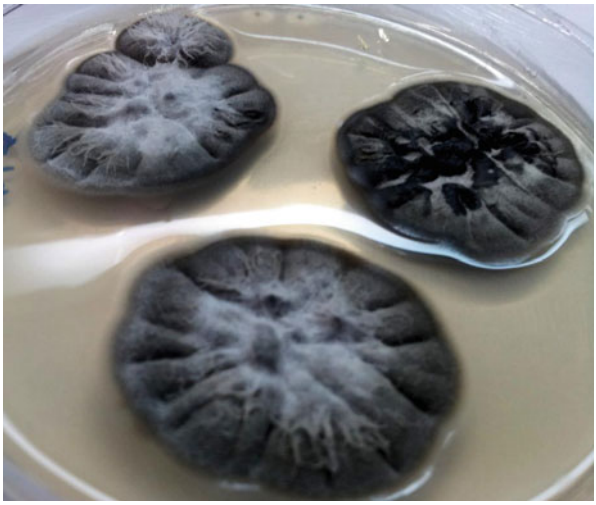

Fig. 10 *Scedosporium* on SDA

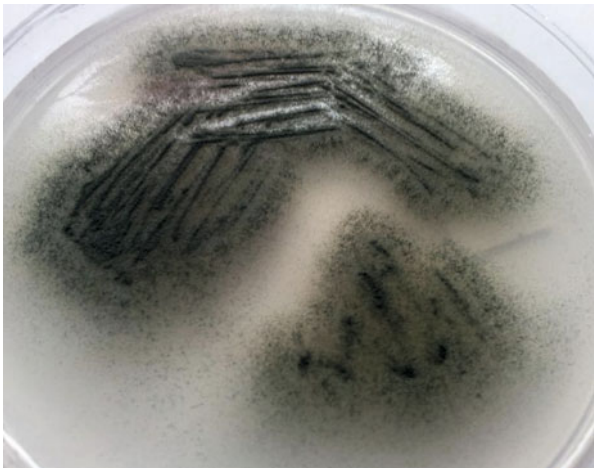

Fig. 11 *A. fumigatus* on CZA (Czapek's agar)

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