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Review Article

LABORATORY INVESTIGATIONS FOR FUNGAL INFECTION OF ORAL CAVITY : AN EAGLE EYE VIEW

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ABSTRACT

The incidence of invasive infections caused by fungi has been increasing for the last few decades. This is primarily due to the significant increases in the populations of at-risk patients; this includes those receiving immunosuppressive chemotherapy for various malignancies, solid organ transplant recipients and those receiving prolonged steroid treatment. New diagnostic approaches have been developed based on non-culture-based methods, which may allow early diagnosis and treatment of fungal infections. Laboratory procedures in diagnostic mycology are directed mainly toward the direct demonstration of the pathogenic fungi in clinical specimens by microscopy along with successful isolation of pathogenic fungi by using various culture techniques. They also help in the prediction of possible therapeutic outcome by determining antifungal susceptibility and can also be used in epidemiological studies by tracing the source of infection. The purpose of our review is to provide the reader with comprehensive and up-to-date information on diagnostic methods.

Keywords: Fungi, Spores, Culture, Diagnosis, Candida

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INTRODUCTION

Study of fungi is referred as Mycology (Mykos – mushroom) .Sabouraud is considered as „Father of mycology". Fungi are saprophytic & parasitic eukaryotic organism. Water, soil, & decaying organic debris are their natural habitat. Most of the fungal infections are opportunistic. Fungal diseases are on rise because of immunocompromised states like those receiving immunosuppressive chemotherapy for various malignancies, solid organ transplant recipients and those receiving prolonged steroid treatment. Emergence of fungal diseases from non-pathogenic strains for human Today, Candida species is the fourth most common organism causing disease in HIV/AIDS patients. Many deaths go unnoticed due to lack of knowledge and inadequate diagnostic techniques, even in advanced medical institutions 5% of patients die due to fungal infections.

MORPHOLOGY OF FUNGI

A) Unicellular fungi – Yeast

- Size: generally larger than most bacteria; (1-5) µm; wide and (5-30) µm length
- Shape: oval, elongated or spherical
- Size and shape varies among species
- Lack flagella and other organ of locomotion

B) Multicellular fungi – Moulds

- Morphology enhances their ability to absorb nutrients
- **Hyphae** – branching filaments
- **Mycelium**-tangled mass of hyphae during active growth

C) Structure of fungal cell wall

Fungal cell contains cell wall, cytoplasmic membrane, nucleus which may be single or multiple and various cell organelles similar to other eukaryotes. However, centriole, found in most of the animal eukaryotic cell, is absent in fungi. The fungi could be either septate or aseptate. They possess rigid cell wall containing mannoprotiens, beta glucans & chitin. Cytoplasmic membrane contains sterols. It contains adhesive molecule for attachment and invasion in the host **(Figure 1)**. It acts as protective barrier against antifungal drugs. Detection of these molecules and antibodies directed against them are diagnostic tool. They also attribute to stainability of fungi with differential stains. As the components of the fungal cell wall are not present in humans, this structure is an excellent target for antifungal therapy. Current antifungal drug like amphotericin B and nystatin are based on targeting ergosterol.

In comparison to bacteria, fungal cell possesses complex cytosol. It includes nucleus, nuclear membrane, mitochondria, ribosome, Golgi apparatus, ER, microtubules and micro vesicles. Fungi may be uni-nucleate or multinucleate, and contains DNA. They have true nucleolus rich in RNA.

C) Reproduction:

Fungi can reproduce in multiple ways depending upon the type of fungus and the environmental conditions - Budding, Fragmentation, Production of spores asexually, Production of spores sexually. Budding occurs in yeasts, which are only made up of one cell. Budding is somewhat similar to binary fission in bacteria, in that the single cell divides into two separate cells. Fragmentation is a mode of reproduction used by those fungi that form hyphae. During fragmentation, some of the hyphae break off and simply start growing as new individuals. Spores are tiny single cells that are produced by fungi that have hyphae. They can be produced asexually by a process in which the tips of the hyphae form specially encased cells – the spores. Some fungi also produce spores sexually. Two types of special cells called "gametes" are produced. One of each type unites to produce a new individual spore. Spores are tiny single cells that are usually very resistant to environmental changes. They can remain dormant for long periods of time until the conditions are right for them to develop into mature individuals.

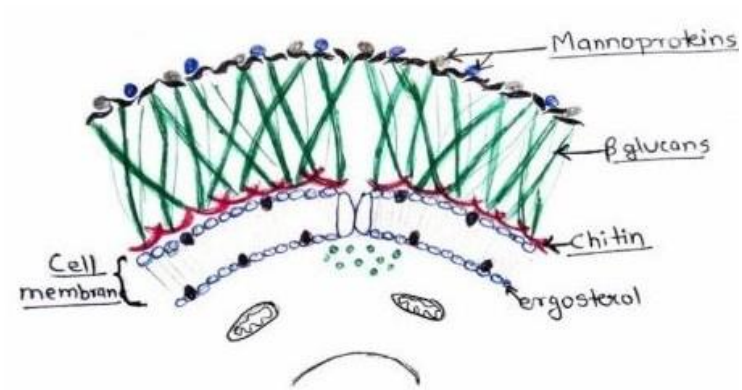
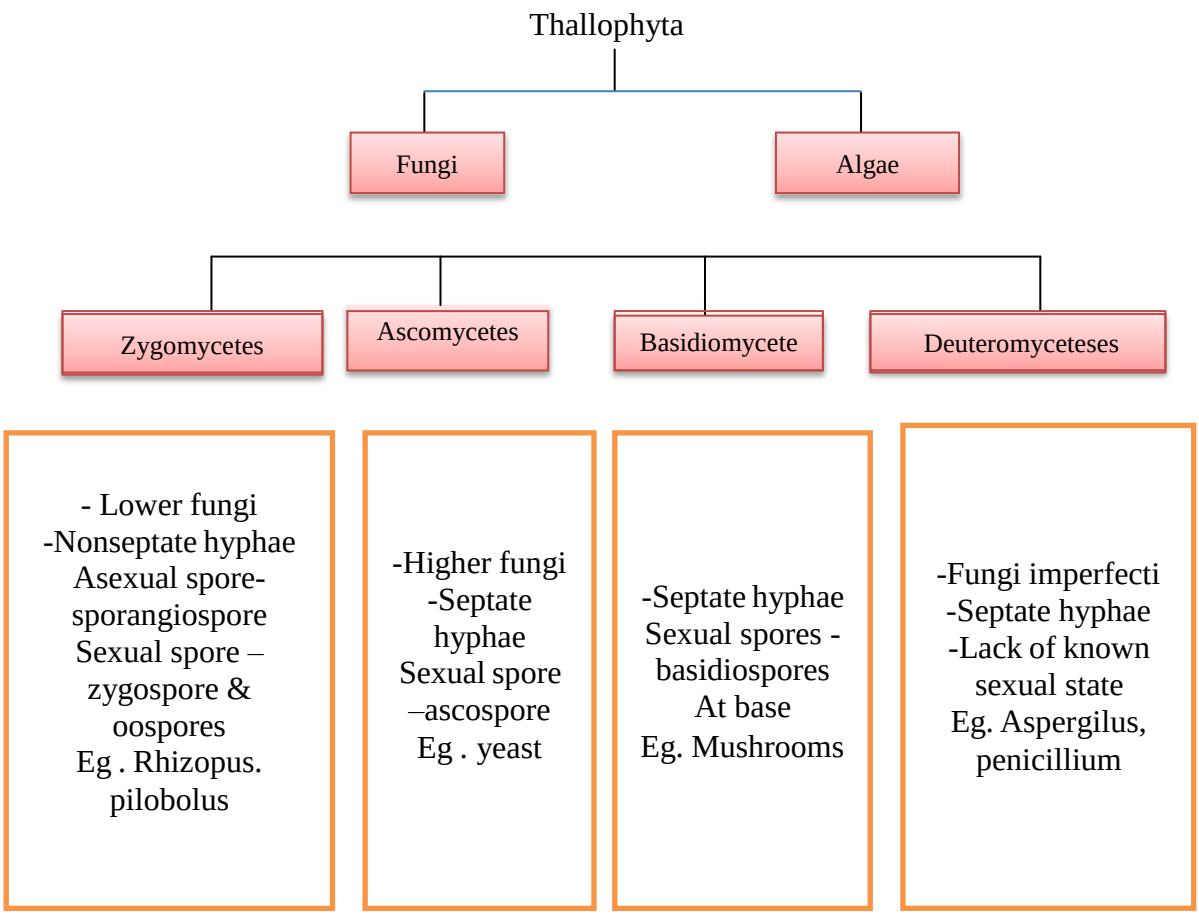


Figure 1 showing the diagrammatic representation of fungal cell wall

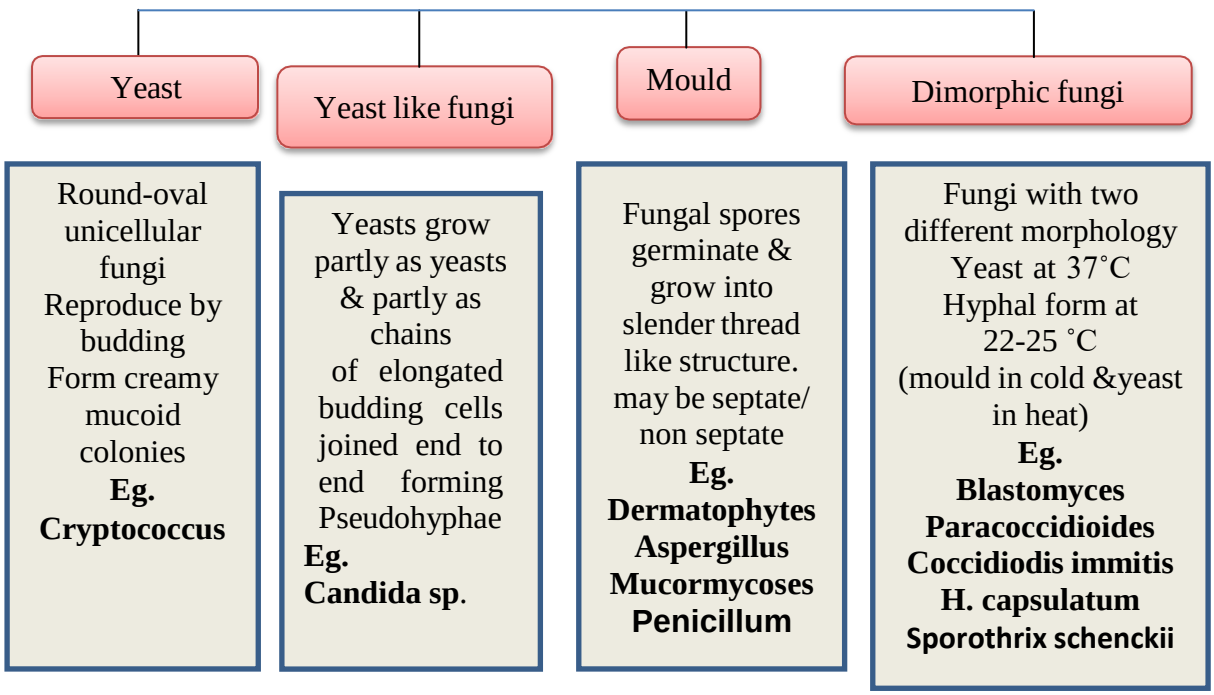
CLASSIFICATION

The following table shows the classification of the fungi based on Taxonomy & Morphology.

A) Taxonomical classificat



B) Morphological classification



LABORATORY INVESTIGATIONS

Diagnosis of fungal lesions is always a comprehensive diagnosis: Combination of Clinical observation and Laboratory investigations. Direct examination includes wet mount, staining, histopathology and wood lamp examination. Non culture methods include Germ tube test & Hair perforation test. Serological methods include antigen & antibody detection. Tests for detection of metabolites & CMI (cell mediated immunity) are also included. Recent advances include Molecular methods, MALDI-TOF MS & Commercial yeast identification system.

The diagnosis of fungal infections is dependent entirely on the selection and collection of an appropriate clinical specimen for culture. Many fungal infections are similar clinically to mycobacterial infections, and often the same clinical specimen is cultured for both fungi and mycobacteria. It is common for many years, such as Candida species to be recovered in routine bacteriology media and fungal culture media. The diagnosis of the fungal infections is dependent on the selection and collection of an appropriate clinical specimen. Samples should be collected before the start of any treatment. It can be collected as imprint, swab, oral rinse, expectoration, paper points & smear. The specimen to be collected from advancing edge of lesion & it should be placed in sterile saline and not in formalin. Specimen should be collected from active lesion. Old burn out lesions don‟t contain viable organisms. Specimen should be collected under aseptic conditions. Specimens transported to the lab. without any delay to prevent bacterial overgrowth. Antibiotics to be added in case of delay (Penicillin 20 u/ml, streptomycin 100000ug/ml & chloramphenicol 0.2mg/ml). Refrigeration of the specimen at 4°C for not longer than 24 hrs (blood & CSF to be kept at room temp.)

Wet preparations include Potassium hydroxide mount, India ink, Nigrosin, Calcofluor white, Lactophenol cotton blue, Neutral red. Differential stains include Periodoc acid-schiff stain, Grocott-Gomori‟s methenamine silver stain, Hematoxylin & eosin, May Grunwald fiemsa stain, Gram‟s stain, Nigrosin, Kinyoun‟s stain, Gridley‟s fungal stain, Mayer‟s mucicarmine stain. Although cultural method is considered as Gold stranded in diagnosis of fungal disease, few recent methods have evolved for rapid and accurate diagnosis of fungal infection. They are based on the detection of circulating antigen, fungal constitutive macromolecules, fungus specific metabolites, fungus specific nucleic acid sequences and antibodies. Serological tests also used to predict the prognosis of fungal disease and response to antifungal therapy and can give clue about active disease by measuring the antibody or antigen titre. It is done either to demonstration of antigen or antibody in the serum or body fluids of suspected fungal infections.

COLONY MORPHOLOGY

Each fungi forms unique colonies on the medium which helps in identifying the causative organism. It forms various morphologies such as cotton, glabrous, granular & velvety (Table 1)

Colony characteristics	**Fungal speciessuspected**
Creamy white, smooth,convex colonies	Candida species
Moist, mucoid, slightly raised,shiny colonies	Cryptococcus neoformans
Moist, wrinkled, yeast like colonies with waxy surface &cream, tan or pink in color	Histoplasmacapsulatum
Creamy white powdery colonies that emulsify whentouched with sterile loop	Geotrichumspecies
Extremely fast growth, gray to black in color and of fluffy or wooly appearance covering the entire surface of the culture medium.	Mucor
Fluffy to granular, blue-green to gray-green, confluentgrowth	Aspergillusfumigatus
Extremely slow growth, heaped, wrinkled, moist, yeast like colonies that become darker with time and gets covered with a short aerial mycelium	Paracoccidioides
Delicate, cobweb like growth, fluffy white colonies	Coccidioides immitis

Table 1 showing the colony characteristics of various fungal organisms

FUNGAL INFECTIONS IN HUMAN

Candidiasis is referred to the infection caused by any of the > 160 spp. Of genus *candida*. They are normal commensal of mucosal surfaces and causes infection when immunity goes down. *Candida spp*. are the fourth most common cause of the nosocomial BSI and been associated with significant mortality. *Candida* is the leading cause of invasive fungal infection in neonates and critically ill patient in ICUs. *Candida* is 2nd cause of invasive fungal infection in severely ill immunocompromised (IC) patient. Fungal infections in humans can be Superficial involving surface & cutaneous or deep which involves subcutaneous & systemic organs.

CONCLUSION

Direct microscopy is important for rapid diagnosis of mycoses. Fungal culture is the gold standard but slow. Immunodiagnostics may be used but have lesser sensitivity and specificity. Nucleic acid amplification techniques are rapid diagnostic tests with better sensitivity and specificity than microscopy but exponentially more expensive.

FUNDING INFORMATION
Nil

CONFLICT OF INTEREST
Nil

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