وزارة التعليم العالي والبحث العلمي

الحقيبة التعليمية

القسم العلمي:	تقنيات المختبرات الطبية
اسم المقرر:	Medical Mycology علم الفطريات الطبية
المرحلة / المستوى:	الثالثة
الفصل الدراسي:	الثاني
السنة الدراسية:	2025-2024

Theoretical and Practical Description of

Week	Theory Title	Practical Title
1	Introduction to Mycology. 2. Structure. 3. Classification 4. Reproduction of Fungi	Introduction to Medical Mycology Laboratory – Specimen Collection, Transport and Storage
2	Pathogenesis of Fungal Infection	Direct Microscopic Examination of Fungi
3	Laboratory diagnosis of fungal infections	Culture of Fungi and Identification of Fungal Colonies
4	Superficial Mycoses.	Laboratory Diagnosis of Superficial Mycoses
5	Cutaneous Mycoses.	Laboratory Diagnosis of Cutaneous Mycoses
6	Subcutaneous Mycosis.	Laboratory Diagnosis of Subcutaneous Mycoses

7	Systemic Mycoses.	Laboratory Diagnosis of Systemic Mycoses
8	Paracoccidioidomycosis.	Laboratory Diagnosis of Paracoccidioidomycosis
9	Histoplasmosis.	Practical Laboratory Diagnosis of Histoplasmosis
10	Blastomycosis	Practical Laboratory Diagnosis of Blastomycosis
11	Cryptococcosis	Practical Laboratory Diagnosis of Cryptococcosis
12	Opportunistic Fungal Infection Candidiasis	Laboratory Diagnosis of Candidiasis
13	Aspergillosis	Laboratory Examination and Identification of Aspergillus species
14	Zygomycosis , Penicilliosis	Laboratory Diagnosis of Zygomycosis and Penicillium species
15	Antifungal Drugs	Antifungal Susceptibility Testing Methods

- **المصادر** الاساسية :

References:

1. Textbook of Microbiology and Immunology, 2nd /e Parija ELSEVIER.
2. Review of Medical Microbiology and Immunology. Warren Levinson, 12th May 2012. Mc Graw-Hill (Lange).
3. Jawetz, R., J.L. Melnick, and E.A. Adelberg, Review of Medical Microbiology, Twenty-Eighth Edition, 2019.

1. Introduction to Mycology.

2. Structure.

3. Classification

4. Reproduction of Fungi

First Week

1. Introduction to Mycology

الاسبوع الاول

Mycology is the study of fungi. The name "fungi" is derived from "mykos" meaning mushroom. The fungi are eukaryotic organisms and they differ from the bacteria, which are prokaryotic organisms, in many ways.

TABLE(1-1): Comparison of fungi and bacteria

Feature	Fungi	Bacteria
Diameter	Approximately 4 µm	Approximately 1 µm
Morphology	Yeast and mold	Cocci, bacilli, spirochete, branching filamentous
Staining property	Gram-positive, nonacid fast, stained with PAS and GMS	Gram-positive, Gram-negative, acid fast
Cell wall content	Chitin	Peptidoglycan
Cell membrane	Sterols present	Sterols absent except mycoplasma
Cytoplasm	Mitochondria and endoplasmic reticulum present	Mitochondria and endoplasmic reticulum absent
Nucleus	Eukaryotic	Prokaryotic
Spores	Sexual and asexual spores for reproduction	Endospores for survival, not for reproduction
Thermal dimorphism	Yes (seen in some fungi)	No

PAS, periodic acid-Schiff; GMS, Gomori's methenamine silver.

2. Structure:

The fungi possess rigid cell walls, which possess two characteristic cell structures: chitin and ergosterol.

Chitin: The fungi consist primarily of chitin, unlike peptidoglycan presents in cell wall of bacteria. Hence, fungi are not sensitive to action of penicillin and other antibiotics that inhibit peptidoglycan synthesis. Chitin is a polysaccharide consisting of long chains of N-acetylglucosamine. In addition to chitin, the fungal cell wall also contains mannan and other polysaccharides. Of these, beta-glucan is most important, because it is the target of antifungal drug caspofungin.

Ergosterol: The cell membrane of fungus contains ergosterol, unlike human cell membrane which contains cholesterol. The antifungal agents, such as amphotericin B, fluconazole, and ketoconazole have selective action on the fungi due to this basic difference in membrane sterols.

3. Classification of Fungi:

The fungi can be classified as follows:

1-Taxonomical Classification The fungi are placed in the phylum Thallophyta. There are four classes of fungi: Zygomycetes, Ascomycetes, Basidiomycetes, and Deuteromycetes or Fungi Imperfecti.

2-Morphological Classification The fungi can be classified into the following four main groups based upon the morphology: (a) yeast, (b) yeast-like form, (c) molds, and (d) dimorphic fungi.

a. Yeast: Yeasts are round or oval unicellular fungi that reproduce by asexual budding. On culture medium, such as Sabouraud's dextrose agar (SDA), they produce creamy mucoid colonies. Example: Cryptococcus neoformans.

b. Yeast-like fungi: These are the yeasts with pseudohyphae. Example: Candida albicans.

c. Molds: Molds grow as long filaments called hyphae. They usually measure 2–10 m in width. Some hyphae form transverse walls and hence they are called septate hyphae, whereas others do not produce walls, hence are called nonseptate hyphae. Nonseptate hyphae are multinucleated. The hyphae on their continuous growth form a mat known as mycelium.

The part of the mycelium that projects above the surface in culture medium is called aerial mycelium. Examples include Aspergillus, Penicillium, Rhizopus, etc.

d. Dimorphic fungi: Many of medically important fungi are dimorphic. They exist as hyphal/mycelial forms in the soil and in the cultures at 22–25°C. They occur as yeasts or other structures in human tissue and in the culture at 37°C (Fig 1-1). Examples include Coccidioides immitis, Paracoccidioides brasiliensis, Histoplasma capsulatum, Blastomyces dermatitidis, and Sporothrix schenckii.

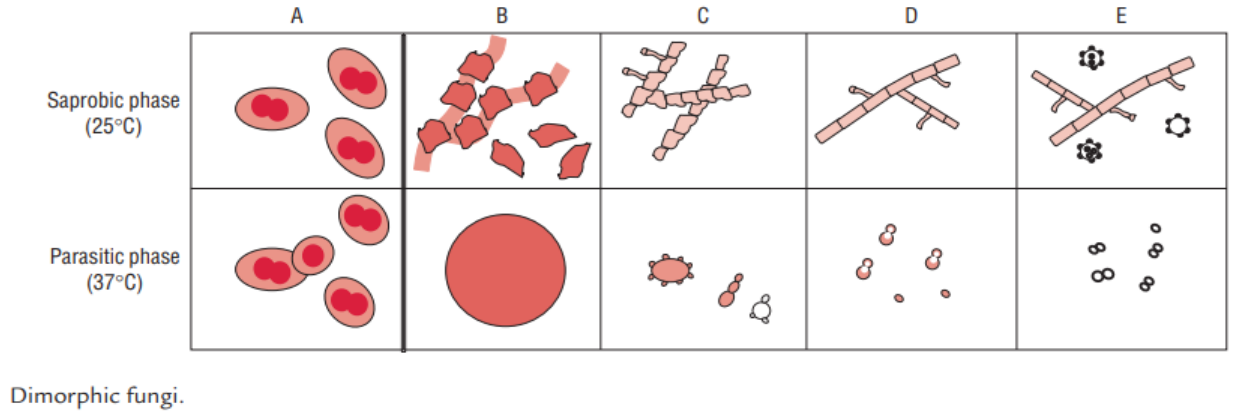
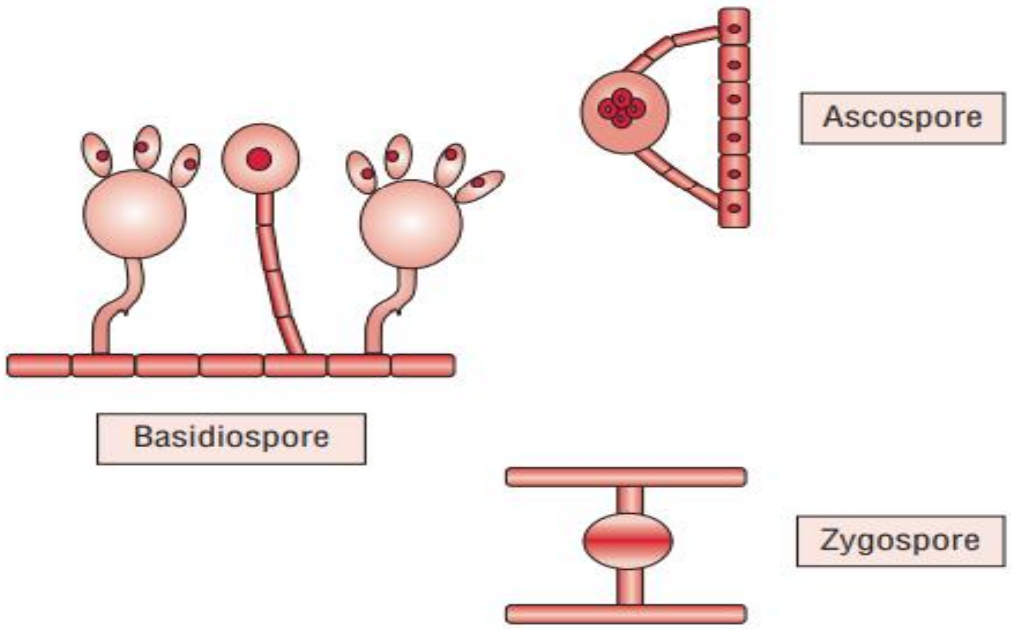


Fig. (1-1)- Dimorphic Fungi

4. Reproduction of Fungi

Fungi can reproduce sexually by forming sexual spores and asexually by forming conidia or asexual spores.

Sexual spores are of three types: zygospores, ascospores, and basidiospores.



(Fig. 1-2) Sexual spores

Ascospores are formed in a sac called ascus, whereas basidiospores are formed outside on the tip of a pedestal called a basidium. Zygospores are single, large spores with thick wall. The fungi that do not produce sexual spores are called imperfect and are classified as Fungi imperfecti. Asexual spores are produced by mitosis. Fungi reproduce asexually by forming conidia. The shape, color, and arrangement of the conidia are helpful for identification of the fungi. Asexual spores can be vegetative or aerial spores as follows:

A. Vegetative spores: These include (a) arthrospores, (b) chlamydospores, and (c) blastospores.

■ **a. Arthrospores** are formed by fragmentations of the ends of hyphae, resulting in rectangular thick-walled spores. The arthrospores are the infective stage of C. immitis

■ **b. Chlamydospores** arise by rounding and thickening of hyphal segments. They are round and thick walled. The terminal chlamydospores help in the identification of C. albicans.

■ **c. Blastospores** are formed by budding process from parent cells, such as yeast. Some yeasts, such as C. albicans can form multiple buds that do not detach from the parent yeast, thus producing elongated structures called pseudohyphae

B. Aerial spores: These include (a) sporangiospores, (b) conidiospores, (c) microconidia, and (d) macroconidia.

■ a. Sporangiospores are spores formed within a sac called sporangium, which develops at the ends of the hyphae called sporangiophores (e.g., Mucor and Rhizopus).

■ b. Conidiospores, or otherwise called conidia, are spores found externally on the sides or tips of hyphae. Conidia can be macroconidia or microconidia.

■ c. Macroconidia are large, aseptate, often multicellular conidia.

■ d. Microconidia are small and single.

الاسبوع الثاني

Pathogenesis of Fungal Infection

Most fungi are obligate aerobes or facultative anaerobes, but none are obligate anaerobes. The natural habitat of most fungi is environment, because all these fungi require a preformed organic source of carbon, hence their constant association with decaying matter.

C. albicans is exception and is an important fungus, which is a part of the normal human flora. Fungi are ubiquitous in nature, i.e., they occur as free-living saprobes, hence determining their role in human infection may sometimes be difficult.

The effects of fungi on humans can be grouped in three major ways as follows:

(a) colonization and disease.

(b) hypersensitive diseases.

(c) diseases caused by mycotoxins or fungal toxins.

a. Colonization and Disease

- Most fungal infections are mild and self-limited.
- Intact skin is an effective host defense against certain fungi. But if the skin is broken, organisms, the fungi enter through that broken skin and initiate the infection.
- Fatty acid content, pH, epithelial turnover, and normal bacterial flora of the skin contribute to host resistance against fungi. For example, the mucous membrane of the nasopharynx traps inhaled fungal spores.

- Immunity:

1. *Cell-mediated immunity* is much important in conferring protection against fungi. Suppression of cell-mediated immunity can lead to reactivation and dissemination of asymptomatic fungal infection and to diseases caused by opportunistic fungi.

2. *The humoral immunity* is mediated by production of IgG and IgM antibody. But their role in protection from fungal disease is uncertain.

- Fungal infection that occurs in the immunocompromised hosts is called as opportunistic mycosis. If such conditions are not rapidly diagnosed and immediately managed, they can prove to be life-threatening.

- Transmission and habitat of some important fungi

Fungi	Habitat	Form transmitted	Portal of entry
Cryptococcus	Soil (pigeon feces)	Yeast	Inhalation into lungs
Aspergillus	Soil and vegetation	Conidia	Inhalation into lungs
Candida	Human body	Yeast	Normal flora of skin, mucosa, gastrointestinal tract, and vagina
Rhinosporidium seeberi	Fresh or stagnant water	Sporangia	Penetration of mucous membrane
Penicilliosis marneffei	Soil	Conidia	Inhalation of conidia

b. Hypersensitivity Diseases

Humans are continually exposed to air-borne fungal spores and other fungal elements present in the environment. These spores can be antigenic stimulants and depending on individual's immunological status may induce a state of hypersensitivity by production of immunoglobulins or sensitized lymphocytes.

Rhinitis, bronchial asthma, alveolitis, and various forms of atopy are the clinical manifestations of hypersensitive pneumonitis.

c. Fungal Toxins & Allergies

• Ingestion of Amanita mushrooms causes liver necrosis due to the presence of two fungal toxins, amanitin and phalloidin. Amanitin inhibits the RNA polymerase that synthesizes cellular mRNA.

• Ingestion of peanuts and grains contaminated with Aspergillus flavus causes liver cancer due to the presence of aflatoxin.

• Inhalation of the spores of Aspergillus fumigatus can cause allergic bronchopulmonary aspergillosis. This is an IgE mediated immediate hypersensitivity response.

Laboratory diagnosis of fungal infections depends on:

(a) direct microscopy.

(b) culture.

(c) serological tests.

(d) nonculture methods.

(e) molecular methods.

A. Direct Microscopy: Direct microscopic examination depends on demonstration of characteristic asexual spores, hyphae, or yeast in various clinical specimens by light microscopy.

- The commonly used clinical specimens are sputum, lung biopsy material, and skin scrapings.
- The specimen is either treated with 10% KOH or stained with special fungal stains. Use of 10% KOH dissolves tissue material, leaving the alkali-resistant fungi intact.
- Calcofluor dye is a fluorescent dye that combines with fungal cell wall and is useful in identification of fungi in tissue specimens.
- Methenamine silver stain is useful for demonstration of fungi in tissues.

- India ink preparation of cerebrospinal fluid (CSF) is a useful method for demonstration of white capsule of C. neoformans in CSF.
- Gram staining is also useful to demonstrate Gram-positive Candida species in the specimen.
- The **disadvantages** of microscopy are that it shows low sensitivity and requires an experienced microscopist for specific identification.

B. Culture Fungal culture is a frequently used method for confirming the diagnosis of fungal infection.

- Sabouraud's dextrose agar (SDA) is the most commonly used medium for fungal culture. Other media include CHROM agar, blood agar, etc.
- The low pH of the medium and addition of chloramphenicol and cycloheximide to the medium inhibit the growth of bacteria in the specimen and thereby facilitate the appearance of slow-growing fungi.
- Fungal colony is identified by rapidity of growth, color, and morphology of the colony at the obverse and pigmentation at the reverse.
- Microscopy of the fungal colony is carried out in lactophenol cotton blue (LPCB) mount to study the morphology of hyphae, spores, and other structures. The appearance of the mycelium and the nature of the asexual spores are very much helpful to identify the fungus.
- Culture, however, is time-consuming in most cases and also the yield is not very good. Culture following lysis of the specimens, such as blood, obviates this problem.

- Blood lysed by addition of certain substances, followed by centrifugation, increases yield of fungi by culture. Yield can be further increased with a shortening of time by combining with BACTEC systems.

C. Serological Tests Demonstration of the antibodies in patient's serum or CSF is useful for diagnosis of fungal infections, especially in systemic fungal infections. A significant rise of antibody titer in a paired sera sample confirms the diagnosis. The complement fixation test was the earliest test used in fungal serology and is still used in the diagnosis of suspected cases of histoplasmosis, blastomycosis, or coccidiomycosis. Recently, newer tests like ELISA (enzyme-linked immunosorbent assay), Western blot, and radioimmunoassays are increasingly used for serodiagnosis of fungal infections.

D. Nonculture Methods These methods include:

(a) detection of fungal antigen.

(b) detection of fungal cell wall markers.

(c) detection of fungal metabolites.

a. <u>Antigen detection:</u> It is useful in immunocompromised hosts where antibody detection is not as sensitive. Detection of fungal antigen in serum, CSF, and urine is increasingly used for diagnosis of many fungal infections. Demonstration of antigen indicates recent or active infection.

- Latex agglutination test is a frequently used test to demonstrate polysaccharide capsular antigen of C. neoformans in CSF for diagnosis of cryptococcal meningitis.

<u>b. Detection of fungal cell wall markers:</u>

- Mannan is a highly immunogenic component of the candidal cell wall. Mannan antigen detection, therefore, is most widely used method in the diagnosis of candidiasis.

- Galactomannan is a heat-stable heteropolysaccharide found in the cell walls of all Aspergillus species. Production of the galactomannan antigen is proportional to fungal load in tissue, hence is being used as the prognostic marker for diagnosis of invasive aspergillosis.

c. Detection of fungal metabolites: Detection of distinctive fungal metabolites is another approach for the diagnosis of fungal infections. Gas liquid chromatography is being used to quantify arabinitol for diagnosis of C. albicans infections.

E. Molecular Diagnosis DNA probes are the recent techniques, which are very useful to identify colonies growing in culture at an earlier stage of growth.

Superficial, Cutaneous, and Subcutaneous Mycoses الاسبوع الرابع

Introduction Fungal infections, depending on the tissues that are initially colonized, can be classified into three major groups as follows:

1. **Superficial mycoses:** These are surface infections of the skin, affecting the outermost layers of skin, hair, and mucosa.
2. **Cutaneous mycoses:** These are infections of the skin involving the epidermis and its integuments, the hair, and nails.
3. **Subcutaneous mycoses:** These are infections of the dermis, subcutaneous tissue, muscle, and fascia.

1. Superficial Mycoses Superficial mycosis caused by different fungi is controlled to the outermost layers of the skin and hair. The condition usually causes cosmetic problem, which can be easily diagnosed and treated. It includes four important conditions:

(a) pityriasis versicolor.

(b) tinea nigra.

(c) black piedra.

(d) white piedra.

Pityriasis Versicolor

Pityriasis versicolor or tinea versicolor is a superficial infection of the skin caused by Malassezia furfur (Pityrosporum orbiculare). M. furfur requires fatty acids for growth, hence is cultured on the Sabouraud's dextrose agar (SDA) overlayed with a layer of olive oil. On incubation at 37°C, the fungus produces creamy colonies within 5–7 days.

The fungus is found in parts of the body rich in sebaceous glands. The lesions of pityriasis versicolor are found most commonly on the upper tissue, arms, and abdomen. They appear as hypopigmented macular lesions often associated with minor scaling or itching. The condition is mostly asymptomatic. It occurs most frequently in hot and humid weather.

Laboratory diagnosis of the condition is usually made by demonstration of both budding yeast cell and hyphae in KOH preparation of skin scrapings. Characteristic "spaghetti and meatballs" appearance of fungus is demonstrated in the microscopy of KOH preparation of the skin. Culture is not carried out routinely for diagnosis.

Tinea Nigra

Tinea nigra is an infection of keratinized layer of skin caused by Exophiala werneckii or Cladosporium werneckii.

C. werneckii is a dimorphic fungus that produces melanin. The fungus on the SDA grows as yeast with many cells in various stages of cell division producing typical two-celled oval structure, on primary isolation from clinical specimen.

A well-demarcated brown-black macular lesion, which appears as brownish spot of the skin, is typical manifestation of the condition. These brownish to black lesions are most commonly seen on palms and soles.

Laboratory diagnosis of tinea nigra is made by microscopy of the KOH preparation of skin scrapings collected from the affected part. Typical darkly pigmented yeast-like cells and hyperfragmented hyphae are demonstrated. Culture of the skin scraping on the SDA confirms the diagnosis.

Black Piedra

Black piedra is a superficial infection of the hair caused by Piedraia hortae, a dematiaceous fungus. The fungus occurs in the perfect state when it colonizes the shaft of hairs. Culture of specimens on SDA shows slow-growing brown to reddish black mycelium, which is considered asexual stage of the fungus.

Infection of shaft of hairs of beard and scalp is the major clinical feature of black piedra.

Laboratory diagnosis of the condition is made by demonstration of nodules containing asci with spindle-shaped ascospores in 10% KOH mount of the hair.

White Piedra

White piedra is an infection of the hair caused by yeast-like organism Trichosporon beigelli. The fungus can be grown on SDA and other media containing cycloheximide. On SDA, it forms green-colored colonies, which subsequently become yellowish gray and wrinkled.

Microscopic examination of the colony shows septate hyphae that break rapidly to form arthroconidia. The latter subsequently become round and develop to blastoconidia.

Laboratory diagnosis of the condition is made by demonstration of fragmented hyphae that develop into arthroconidia or produce blastoconidia in 10% KOH mount of hair. Culture of the fungus from clinical specimen confirms the diagnosis. Features of the organisms causing superficial mycoses are summarized in Table 4-1.

TABLE-Features of the organisms causing superficial mycoses

Disease	Organism	Tissue	Diagnosis
Pityriasis versicolor	*Malassezia furfur*	Skin	Microscopy of skin scraping with 10% KOH shows "spaghetti and meatballs" appearance
Tinea nigra	*Exophiala werneckii*	Skin	Culture on SDA yields black yeast and hyphae
Black piedra	*Piedraia hortae*	Hair	Direct microscopy; culture yields asexual phase
White piedra	*Trichosporon beigelli*	Hair	Direct microscopy; culture on SDA; hyphae, arthroconidia, and blastoconidia

Cutaneous Mycoses الاسبوع الخامس

Dermatophytoses or cutaneous mycoses are diseases of the skin, hair, and nail.

These infections are caused by a homogeneous group of closely related fungi known as dermatophytes.

These dermatophytes infect only superficial keratinized structures, such as skin, hair, and nail, but not deeper tissues.

Dermatophytes The most important dermatophytes that cause infection in humans are classified into the following three genera:

(i) Trichophyton—causes infection of hair, skin, and nail.

(ii) Microsporum—causes infection of hair and skin.

(iii) Epidermophyton—causes infection of skin and nails, but not hair (Fig.)

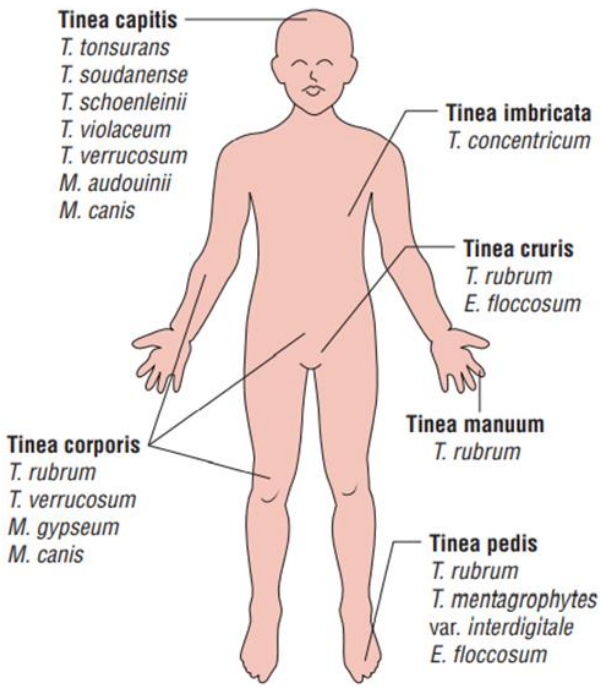


FIG. 5-1. Infections caused by different dermatophytes.

The dermatophytes on the basis of their natural habitat and host preferences can be classified into following groups:

(i) Anthropophilic species: These dermatophytes are typically adapted to live on human host. They are transmitted from human to human through fallen hairs, desquamated epithelium, combs, hair brushes, towels, etc. Examples are Trichophyton rubrum, Microsporum audouinii, and Epidermophyton floccosum.

(ii) Zoophilic species: These are the dermatophytes that live on animals and often cause infection in their animal host. These zoophilic species are transmitted from infected animals to humans by direct and indirect contacts with domestic animals (e.g., cat and dog) and occasionally wild animals. Examples are Trichophyton violaceum and Microsporum canis.

(iii) Geophilic species: These are saprophytic fungi found in soil or in dead organic substances. They occasionally cause infection in humans and animals. Examples are Microsporum gypseum and Trichophyton ajelloi. Dermatophytes usually grow only on keratinized skin and its appendages, and do not penetrate the living tissues. In some infected persons, hypersensitivity to fungus antigen may cause secondary eruptions, such as vesicles on the finger. This reaction is known as dermatophytid (id) reaction. This reaction occurs as a result of hypersensitivity response to circulating fungal antigen, and these lesions do not contain any fungal hyphae.

- **Laboratory diagnosis**

Laboratory diagnosis is based on demonstration of fungal element in clinical specimen by microscopy and confirmation by culture.

The specimens include skin scrapings and nail clippings or hair taken from the areas suspected to be infected by dermatophytes.

These entire specimens are treated with alkali solution to clear epithelial cells and other debris.

Direct microscopy is useful only for diagnosis, while culture is always carried out to identify the specific causative fungal agent.

Direct microscopy

Examination of 10% direct KOH mount may show fungal hyphae. Three types of hair infections can be demonstrated in microscopy of 10% KOH wet mount as follows (Fig. 5-1):

Ectothrix: Ectothrix infection is characterized by presence of a layer of arthrospores on the surface of hair shaft. It is caused by M. audouinii, M. canis, and Trichophyton mentagrophytes.

Endothrix: The clusters of arthrospores are found entirely within the hair shaft in endothrix infection. It is caused by Trichophyton tonsurans, T. violaceum, and Trichophyton schoenleinii.

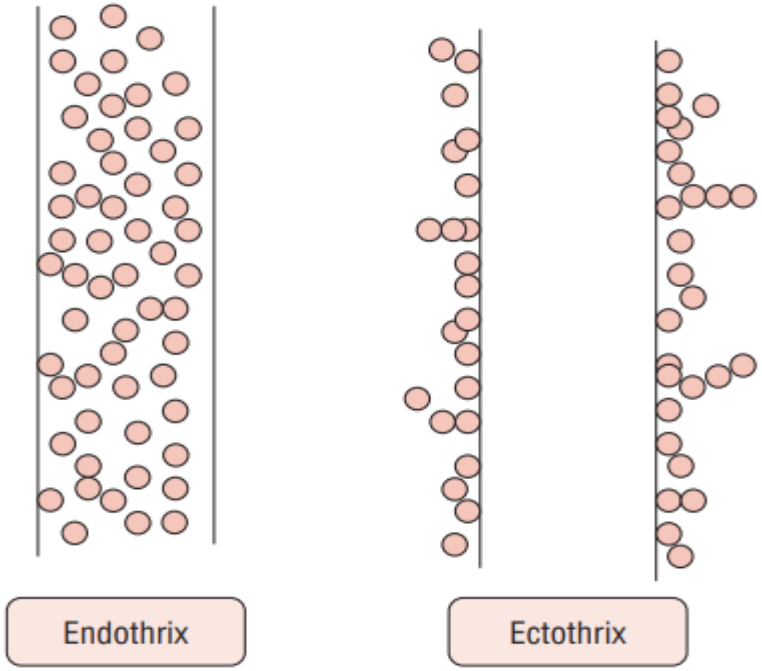


FIG. 5-1. Ectothrix and endothrix infections.

Favus: In favus, there is sparse hyphal growth and formation of air spaces within hair shaft. It is caused by T. violaceum, T. schoenleinii, and M. gypseum.

Culture

The clinical specimens are cultured by inoculation on SDA containing antibiotics like cycloheximide. The media after inoculation are incubated at 25–30°C for 3 weeks.

At 25°C most of the pathogenic fungi grow well, while saprophytic fungi and bacteria are inhibited.

The cultures are examined at regular intervals, and dermatophytes are identified based on (a) colony morphology, (b) pigment production, and (c) presence of microconidia and macroconidia.

The LPCB preparation of the colonies shows microconidia, macroconidia, or both.

*Only macroconidia are present in the Epidermophyton infection.

*Few macroconidia and more microconidia are present in Trichophyton infection.

*Macroconidia are predominantly present in Microsporum infection.

General characteristics of macroconidia and microconidia of dermatophytes

	Microsporum	*Epidermophyton*	*Trichophyton*
Macroconidia	Thick-walled, rough, numerous	Smooth-walled, numerous	Thin-walled, smooth, rare
Microconidia	Rare	Absent	Abundant

Other tests

- **Hair perforation test:**

This test is performed to differentiate T. rubrum from T. mentagrophytes. The test is also used to differentiate M. canis from Microsporum equinus.

- **Urease test:** Urease test is carried out to differentiate T. mentagrophytes from T. rubrum.
- **Growth on rice grains:** This test is useful to differentiate M. canis from M. audouinii.

Subcutaneous Mycosis الاسبوع السادس

Subcutaneous mycosis is defined as fungal infection associated with development of characteristic lesion in subcutaneous tissue and overlying skin with or without extension to bone and muscle. This is caused by a heterogeneous group of fungal infection of low pathogenic potential introduced in the body percutaneously from a trivial trauma. Table 6-1 shows the classification of subcutaneous mycoses.

Table 6-1 Causative agents of subcutaneous mycoses

Condition	Causative agents
Mycetoma	Bacteria, fungi
Chromoblastomycosis	*Fonsecaea pedrosoi* *Fonsecaea compactum* *Phialophora verrucosa* *Cladophialophora carrionii* *Rhinocladiella aquaspersa*
Phaeohyphomycosis	*Exophiala jeanselmei* *Bipolaris spicifera* *Wangiella dermatitidis*
Sporotrichosis	*Sporothrix schenckii*
Rhinosporidiosis	*Rhinosporidium seeberi*

Mycetoma

Mycetoma is a slowly progressive, chronic granulomatous infection of skin and subcutaneous tissues with occasional involvement of underlying fascia and bone usually affecting extremities.

The condition is characterized by a triad of (a) tumefaction, (b) draining sinuses, and (c) grains or granules.

Mycetoma is caused by a number of actinomycetes and filamentous fungi that enter through penetrating injuries resulting from thorn pricks, splinters, etc (Table 6-2).

Table 6-2 Important causative agents of mycetoma

Grain	Causative agents
Black grain	*Madurella mycetomatis*
	Madurella grisea
	Exophiala jeanselmei
	Curvularia geniculata
White grain	*Pseudoallescheria boydii*
	Acremonium falciforme
	Actinomadura madurae
	Nocardia brasiliensis
Red grain	*Actinomadura pelletieri*

Lower extremities are most commonly involved.

Laboratory diagnosis

depends on demonstration of the fungi and fungal filaments in granules, pus, and biopsy tissue by microscopy.

■ The granules on naked eye examination may be hard or soft, round or lobulated, and vary in size from 0.5 to 3 mm. These may be black, white to cream, or red.

■ Microscopy of the granules may reveal very thin fungal filaments, measuring less than 1 m in diameters in cases of actinomycotic mycetomas. However, the fungal filaments may be broader and often show septate hyphae and chlamydospores in mycotic mycetoma .

Sporotrichosis

Sporotrichosis is a chronic pyogenic granulomatous lesion of the skin and subcutaneous tissue caused by Sporothrix schenckii.

- S. schenckii is a dimorphic fungus found all over the world.
- The fungus is found in soil, decaying woods, thorns, and on infected animals including rats, cats, dogs, and horses.
- Spore is the infective stage of the fungus.
- It causes infection primarily on the hand or the forearm through direct contact of the skin by spores. Typically, infection is introduced in skin through a penetration of thorn.

S. schenckii occurs in two phases.

Yeast phase occurs in tissue and in culture at 37°C, while mycelium phase occurs in culture at 22–25°C. In infected tissue, the yeast appears as round, oval, or cigarshaped cells with irregular borders. Periodic acid-Schiff (PAS) or Gomori's methenamine silver (GMS) stain is useful to demonstrate these structures in the stained smears.

The fungus on SDA at 25°C produces black and shiny colonies, which become wrinkled and foggy during course of time. The mold contains hyphae bearing flower-like structures of small conidia on delicate sterigmata.

Laboratory diagnosis of sporotrichosis is made by demonstration of asteroid bodies in pus of the abscesses.

(***Asteroid bodies*** consist of a central basophilic budding yeast cell with eosinophilic material, which radiates from the center).

Systemic Mycoses الاسبوع السابع

Introduction

Systemic mycoses are caused by fungi of soil, which are characteristically virulent and cause disease in healthy humans.

The systemic mycoses include:

1. Coccidioidomycosis
2. Paracoccidioidomycosis
3. Histoplasmosis
4. Blastomycosis
5. Cryptococcosis

1. Coccidioidomycosis

- Coccidioidomycosis caused by Coccidioides immitis was first recognized as a distinct disease entity in 1892.

- C. immitis is a dimorphic fungus, which occurs as a mold in soil and in culture at 25°C and as a spherule in tissue and in culture at 37°C.

- The spherule is oval with a thick, double refractile wall that is filled with endospores.

- C. immitis grows in mycelial form in the soil of endemic areas. Subsequently, the hyphal cells either develop into barrelshaped structures or shrink and die, producing the characteristic arthroconidia.

- The arthroconidia are the infective stage of the fungus.

When the soil is disrupted, the arthroconidia become air-borne and if inhaled by a susceptible host, initiate the infection.

- The arthrospore inside the pulmonary acinus gives off its outer layer, swells, and develops to a spherical structure called the spherule. The spherule is the parasitic stage of the organism, which reproduces by a process known as endosporulation.
- Rupture of the spherule leads to release of endospores, each of which matures into spherules and the cycle is repeated.
- If the organism is cultured, it re-enters the mycelial phase with hyphae formation. The spherule is the characteristic tissue form of the organism.
- In symptomatic cases, C. immitis causes a primary pulmonary disease and disseminated disease. Pulmonary infection is the most frequent presentation in symptomatic patients. In disseminated disease, virtually every tissue of the body including central nervous system (CNS), skin, and bones is involved.

Laboratory diagnosis of coccidioidomycosis is made by

1- demonstration of spherules containing endospores in

(a) sputum, or smears from the lesion stained by calcofluor white and (b) in biopsy material stained by hematoxylin and eosin, silver, or periodic acid-Schiff stains.

2- **Culture** is the most definitive method for diagnosis. The fungus grows well on Sabouraud's dextrose agar (SDA) and other media producing white and cottony colony within 5 days. The demonstration of typical arthroconidia is useful to identify the organism. However, arthroconidia are infectious, hence pose a significant risk to laboratory personnel.

3- Serodiagnosis of coccidioidomycosis is based on the demonstration of antibodies to coccidioidal antigens in patient's serum by:

*Tube-precipitating antigen

The complement fixation antigen are the two major antigens used to detect antibodies.

*Enzyme immunoassay (EIA) is the most frequently used test to detect serum tube-precipitating antibodies that are IgM antibodies to mycelial phase antigens.

4- DNA probe is a recent method used for accurate identification of the fungus.

2. Paracoccidioidomycosis الاسبوع الثامن

- Paracoccidioidomycosis, also known as South American blastomycosis and Lutz–Splendore–Almeida disease, is a chronic progressive systemic mycosis caused by Paracoccidioides brasiliensis.

- P. brasiliensis is a thermally dimorphic fungus found in the soil.

It occurs as a mold in the soil and as a yeast in tissue.

- These yeasts are unique in that one large mother cell produces multiple blastoconidia (daughter cells) that arise from multiple sites, resembling a "Mickey Mouse head" or a "pilot wheel." This characteristic appearance helps to differentiate this yeast from Blastomyces dermatitidis and all other yeasts.
- Infection occurs by inhalation of conidia or mycelial fragments.
- The lungs are the primary site of infection. From this site, the fungus then disseminates to other organs through the venous and lymphatic systems.
- Cell-mediated immunity (CMI) is the most important defense mechanism in an immunocompetent host.
- However, most initial infections are subclinical. In adults, the course of disease is long-term and the outcome is better with appropriate therapy. Pulmonary infection is the most common manifestation. The disease in younger patients is subacute and carries a worse prognosis.
- Bats and saguis may serve as reservoirs. The infection can also be transmitted orally by ingestion.

- **Laboratory diagnosis** depends on demonstration of large, multiple budding yeasts (blastoconidia) in 30% KOH wet mount preparation of sputum. Gomori's methenamine silver (GMS) staining of biopsy specimens shows yeast cells measuring 2–30 m in diameter. The organism is isolated by culture on SDA at 37°C after 20–30 days.

3. Histoplasmosis الاسبوع التاسع

- Histoplasmosis is primarily a disease of reticuloendothelial system caused by an intracellular fungus Histoplasma capsulatum.
- H. capsulatum is a dimorphic fungus, which occurs in two stages: as a mold in soil and as yeast at body temperature in mammals. On SDA medium at 37°C, this fungus produces cottony mycelial growth.
- The colony is characterized by thin, branching, septate hyphae that produce tuberculate macroconidia and microconidia.
- H. capsulatum infection is acquired by inhalation of conidia and mycelial fragments from contaminated soil.
- Once inhaled, it is deposited in alveoli of the lung and transformation from the mycelial to the pathogenic yeast form occurs intracellularly inside the macrophages. The yeast inside the macrophages multiplies in approximately 15–18 hours. Multiplication continues within the phagosomes despite fusion with lysosomes.
- This is possibly due to production of certain proteins by yeasts that inhibit the activity of lysosomal proteases.
- CMI is key component of host defense against the fungus.

- T lymphocytes are crucial in limiting the extent of infection. In persons with impaired CMI, Histoplasma species which remained latent in healed granulomas may be reactivated, resulting in histoplasmosis.
- Most infected individuals are asymptomatic.
- But, H. capsulatum causes acute pulmonary histoplasmosis, chronic pulmonary histoplasmosis, and progressive disseminated histoplasmosis. Majority of patients with acute pulmonary histoplasmosis are asymptomatic.
- Incubation period varies from 3 to 14 days. Fever, headache, malaise, myalgia, abdominal pain, and chills are common symptoms. Cough, hemoptysis, dyspnea, and/or chest pain may be present.
- Chronic pulmonary histoplasmosis is seen in patients with underlying pulmonary disease. Cough, weight loss, fevers, and malaise are symptoms.
- Progressive disseminated histoplasmosis is seen in patients who are immunocompromised, such as patients with AIDS.
- Bats play an important role in transmission of the disease. They can become infected, and they transmit the fungus through their droppings.
- The soil contaminated with birds' excretions and droppings of bats remains highly infectious for years.
- **Laboratory diagnosis** depends on demonstration of oval yeast cells within macrophages in bone marrow aspirates and in tissue biopsy specimens.
- Biopsy of oropharyngeal ulcers is usually diagnostic (refer the box Biopsy).

- Culture of sputum and blood on SDA at 25°C shows hyphae with tuberculoid macroconidia and at 37°C shows yeasts.
- Complement-fixation test (CFT) and immunodiffusion (ID) are useful serological tests for demonstration of specific antibodies in serum.
- The ID test detects antibodies to two glycoproteins, H and M, of H. capsulatum. Anti-H antibody is more specific for active histoplasmosis, is positive in 50–80% of patients, and remains elevated for years.

4. Blastomycosis الاسبوع العاشر

- Blastomycosis is a granulomatous fungal infection caused by B. dermatitidis.

- B. dermatitidis is a dimorphic fungus, which occurs in two stages: as mold in soil and as yeast in tissue. On culture at 37°C and in tissue, the yeast is a round structure with a double refractile wall and a single broad-based bud. This appearance helps to differentiate it from the Cryptococcus neoformans yeast, which has a narrow-based bud. On culture at 25°C, the fungus produces a mycelial growth showing typical pyriform microconidia, which measure 2–4 m in diameter.

B. dermatitidis infection occurs by inhalation of microconidia found in soil, its natural habitat.

- Inside the lungs, the conidia are transformed to the yeast phase at body temperature. The thick cell wall of the yeasts offers resistance to phagocytosis. Then, the yeasts replicate and may disseminate through the blood and lymphatics to other organs to cause the disease.

- Pulmonary infection is the major clinical manifestation.

- Symptoms:

1. A flu-like illness with fever, chills, myalgia, headache, and a nonproductive cough may occur, which resolves within days.

2. In some cases, it may develop to a progressive pulmonary disease or may result in extrapulmonary dissemination.

3. Extrapulmonary dissemination to other organs, more often, occurs in patients with chronic pulmonary illness and in those who are immunocompromised.

* The skin is the most common site of extrapulmonary blastomycosis.

5.Cryptococcosis الاسبوع الحادي عشر

- Cryptococcosis, also called **European blastomycosis**, is an acute to chronic disease caused by an encapsulated yeast, C. neoformans.
- Cryptococcosis is the most common life-threatening fungal disease in patients with AIDS.

Morphology

■ C. neoformans is a true yeast.

■ It is an oval and budding cyst and measures 3–6 m in diameter. The yeast may be single or may have a single budding daughter cell.

■ Within the host and in certain culture media, the yeast is surrounded by a wide polysaccharide capsule.

The polysaccharide capsule is composed of mannose, xylose, and glucuronic acid.

■ C. neoformans on SDA medium forms smooth, convex, cream-colored colonies at 20–37°C.

Lactophenol cotton blue (LPCB) wet mount of the colony shows budding yeast cells.

*C. neoformans has two varieties: C. neoformans var neoformans and C. neoformans var gattii.

Pathogenesis and Immunity The immune status of the host is the crucial factor in pathogenesis of cryptococcosis.

C. neoformans usually causes most serious infections in patients with impaired CMI. These include:

■ patients with AIDS

■ patients undergoing corticosteroid treatment

■ patients undergoing organ transplantation

■ patients with reticuloendothelial malignancy

■ patients with sarcoidosis.

C. neoformans is primarily transmitted by inhalation (Fig. 7-1). Following inhalation, the yeasts are deposited into the pulmonary alveoli, in which they survive before they are phagocytosed by alveolar macrophages. Glucosylceramide synthase has been identified as an essential factor in the survival of C. neoformans in pulmonary alveoli.

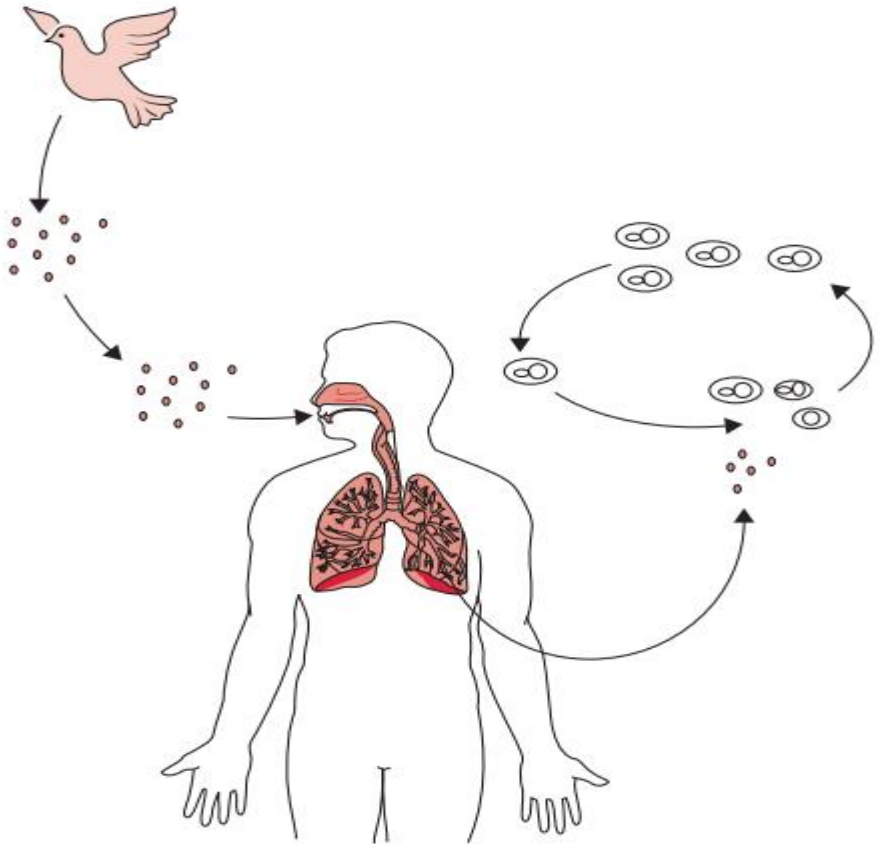


FIG. 7-1. Transmission of Cryptococcus neoformans

Cryptococcal polysaccharide capsule has antiphagocytic properties. Hence, unencapsulated yeast are readily phagocytosed and destroyed than the encapsulated organisms, which are more resistant to phagocytosis. The antiphagocytic properties of

the capsule prevent recognition of the yeast by phagocytes and inhibit leukocyte migration into the area of fungal replication.

- **Host immunity**

  - The host immunity in cryptococcal infection is mediated by both cellular and humoral responses.
  - CMI is mediated by natural killer cells and T lymphocytes can inhibit or kill cryptococci.
  - An increase in helper T-cell activity, skin test conversion, and a reduction in the number of viable organisms in the tissues indicates a successful host response against the fungus.
  - Humoral immunity is mediated by anticryptococcal antibodies and soluble anticryptococcal factors.
  - Both anticryptococcal antibodies and the complement play a crucial role in facilitating the macrophage- and lymphocyte-mediated immune response to the organism.

Clinical Syndromes

C. neoformans causes (a) pulmonary cryptococcosis in immunocompetent hosts and in immunocompromised hosts, (b) CNS cryptococcosis, and (c) disseminated nonpulmonary non-CNS cryptococcosis.

Epidemiology C. neoformans is distributed worldwide. The incidence of cryptococcosis is increasing and now it represents a major lifethreatening fungal infection in patients with AIDS.

Laboratory Diagnosis

- Laboratory diagnosis of cryptococcal infection is made by demonstration of the yeast in CSF, sputum, pus, and brain biopsy tissue by smear and culture.
- Methenamine silver or periodic acid Schiff stains are used to stain the tissue specimens for demonstration of the capsule of C. neoformans.
- Fixed tissue may also be stained with mucicarmine, which preferentially stains C. neoformans.
- India ink preparation is commonly used to detect budding yeast cells in the CSF (Fig. 7-2).
- The capsule appears as a clear halo around the yeast cells. By this method, cryptococci can be demonstrated in 25–50% of patients with cryptococcal meningitis.
- Gram-stained smear of the CSF shows Gram-positive yeast cells. The culture of centrifuged CSF specimens confirms diagnosis of the condition. This fungus is identified based on its microscopic appearance, biochemical test results, and ability to grow at 37°C.
- Latex agglutination test (LAT) is a frequently used serological test to detect cryptococcal polysaccharide antigen in the serum or CSF for diagnosis of meningitis. LAT is an extremely important adjunct to the diagnosis.

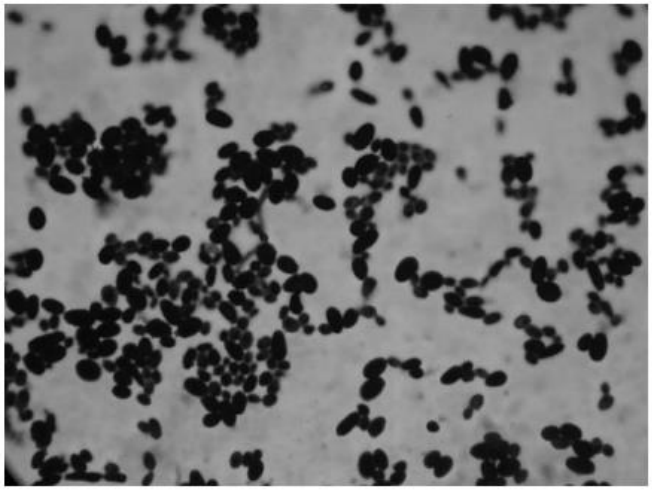


FIG. 7-2. India ink preparation showing capsule of Cryptococcus neoformans (x400).

Opportunistic Fungal Infection الاسبوع الثانى عشر

Introduction

The opportunistic fungi usually cause infections in persons with impaired host defense, but do not cause disease in most of the immunocompetent hosts. Since these fungi become pathogens in individuals with impaired immunity by taking advantage of the host's debilitated conditions, they are called opportunistic fungi. In recent times, there is an increasing list of exotic and rare fungi, which have been associated to cause opportunistic infections. But most opportunistic infections are caused by Candida albicans, Aspergillus spp., Penicillium marneffei, and various Zygomycetes (Table 74-1).

Disease	Causative fungus
Candidiasis	*Candida albicans*, *Candida tropicalis*, and other species
Aspergillosis	*Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus niger*, and other species
Zygomycosis	*Mucor*, *Rhizopus*, and *Absidia* species
Pneumocystis carinii pneumonia (PCP)	*Pneumocystis jiroveci*
Penicilliosis	*Penicillium marneffei*
Pseudoallescheria boydii infection	*Pseudoallescheria boydii*
Fusarium solani infection	*Fusarium solani*
Meningitis	*Cryptococcus neoformans*

Table8-1 Common opportunistic infections

Candidiasis Candida species are the most common fungal pathogens that affect humans.

- These species are true opportunistic pathogens that take advantage of the host's debilitated condition and gain access to the circulation and deep tissues.

- The genus Candida includes more than 100 species, of which only few cause diseases in humans.
- C. albicans and occasionally other species cause candidiasis, a major infection in immunocompromised hosts.

Candida albicans

- C. albicans is the most common Candida species, which causes opportunistic infections in immunocompromised hosts.
- It forms the part of the normal flora of the mucous membrane of the gastrointestinal, genitourinary, and respiratory tract.

Properties

■ C. albicans is ovoid or spherical yeast with a single bud.

■ It forms the part of the normal flora of the mucous membrane of the gastrointestinal, genitourinary, and respiratory tract.

- It produces pseudohyphae in the cultures and in tissues.

- Pseudohyphae are elongated yeast that may resemble hyphae morphologically, but are really not true hyphae.

- Candida grows readily on Sabouraud's dextrose agar and on bacteriological culture media. C. albicans produces creamy white, smooth colonies with a yeasty odor.

■ It can be differentiated from other Candida species by carbohydrate fermentation reaction and by characteristic growth properties.

■ Only C. albicans produces chlamydospores on cornmeal agar culture at 25°C.

Pathogenesis and Immunity Candida spp. are usually present as part of normal flora on healthy mucosal surface of the oral cavity, gastrointestinal tract, and vagina.

- Candida shows colonization at these sites in more than 80% of healthy people.
- The organism, however, is rarely present on the surface of normal human skin, except occasionally from certain intertriginous area, such as the groin.

◗ **Pathogenesis of Candida infection**

- Under certain conditions, Candida gains access to systemic circulation from the oropharynx of the gastrointestinal tract.
- Colonization of the mucocutaneous surface is the first stage in the pathogenesis of Candidal infection.
- The fungus causes invasion in human tissue through different routes. Disruption of the skin or mucosa allows the organism access to the blood stream.
- Massive colonization with large numbers of Candida also permits the organism to pass directly into the blood stream, causing the infection.
- In immunocompromised hosts, Candida may disseminate to many organs, such as lung, spleen, liver, heart, and brain.
- Candida may induce inflammation of the eye, causing endophthalmitis and also may involve skin in 10–30% of patients with disseminated infection.

- Deficiency in host defence mechanisms plays a significant role in development of Candida infection.

- **Host immunity** Both cell-mediated and humoral antibodies confer protection against Candida in healthy adults. Cell-mediated immunity (CMI) is, however, most important. Alteration in CMI may cause extensive superficial candidiasis, despite having normal or elevated humoral antibodies. The humoral antibodies appear to play minimal role in protection against the disease. Humoral antibodies confer protection against Candida in healthy adults.

Clinical Syndromes Candida causes a wide spectrum of clinical illnesses as follows:

Cutaneous candidiasis: Candida species in immunocompetent host can cause infection of any warm and moist part of the body exposed to environment. It causes infection of the nail, rectum, and other skin folds. **Mucocutaneous candidiasis:** Mucocutaneous candidiasis (thrush, perianal disease, etc.) is the most common manifestation of candidiasis, but usually does not cause any mortality. In patients with advanced immunodeficiency due to HIV infection, Candida species can cause severe oropharyngeal and esophageal candidiasis that result in poor intake of food, leading to malnutrition, wasting, and early death. These patients are also usually resistant to treatment with antifungal therapy.

Chronic mucocutaneous candidiasis: This is a heterogeneous group of clinical syndromes. This syndrome is characterized by chronic, treatment-resistant, superficial Candida infection of the skin, nails, and oropharynx. However, these patients do not show any evidence of disseminated candidiasis.

Systemic candidiasis: These include endocarditis, gastrointestinal tract candidiasis, respiratory tract candidiasis, genitourinary candidiasis, and hepatosplenic candidiasis. Systemic candidiasis may be candidemia and disseminated

candidiasis. In patients with AIDS, oral thrush and Candida esophagitis are more common but not candidemia and disseminated candidiasis. Candida endophthalmitis and central nervous infection (CNS) infection due to Candida species are other complications of Candida infection.

Disseminated candidiasis: This is increasingly becoming a problem in patients with serious hematologic malignancies that are treated with immunosuppressive drugs for over a long period of time. Severe neutropenia in these patients is the most important predisposing condition for life-threatening infection caused by Candida.

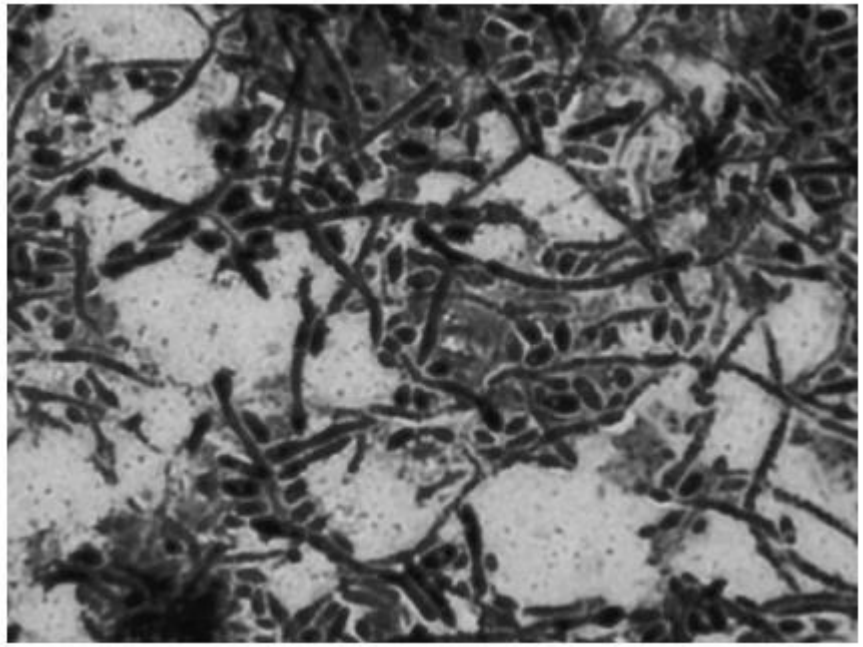
Epidemiology

- Candida species is distributed worldwide.
- In recent times, Candida species have replaced Cryptococcus species as the most common fungi affecting the CNS of immunocompromised patients worldwide.
- C. albicans and Candida glabrata are responsible for causing infection in 70–80% of patients with invasive candidiasis.
- Since Candida is present as a part of normal flora already in the skin and mucous membrane of the host, it causes infection in the infected host; it is therefore not transmitted.

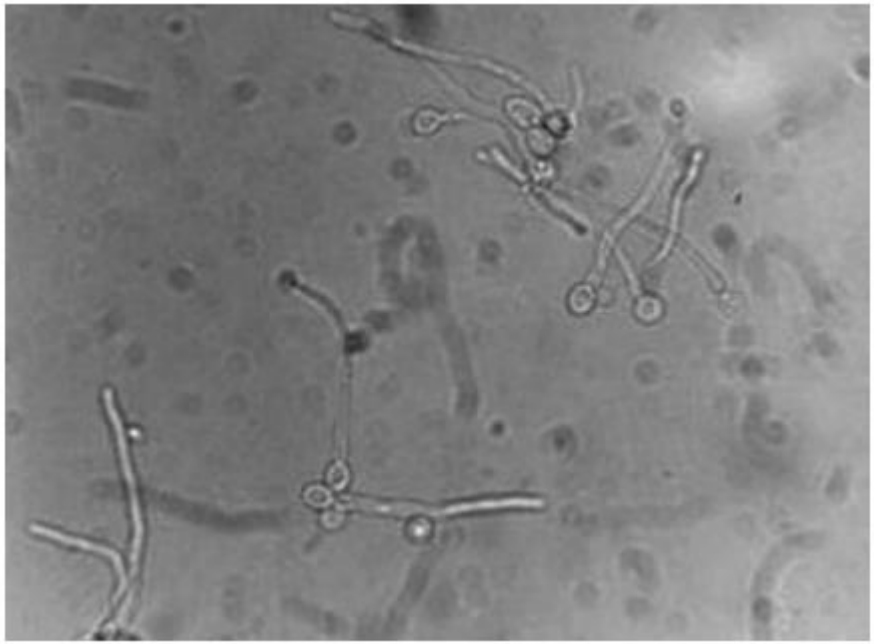
Laboratory Diagnosis

- **Specimens** These include exudates or tissues for microscopy obtained from skin or nails examined by microscope for demonstration of pseudohyphae or budding yeast cells of Candida.

- **Microscopy**
  - Gram-stained smear of the exudates or tissue shows Gram positive, oval, budding yeast and pseudohyphae (Fig. 74-1).
  - Since Candida is found as a part of normal flora on normal skin or mucosa, only the presence of large numbers of Candida is of significance. Demonstration of pseudohyphae indicates infection, and tissue invasion is of more diagnostic value.
- **Culture** Culture on Sabouraud's dextrose agar (SDA) produces typical creamy white, smooth colonies.



Gram-stained smear showing Gram-positive, oval, budding yeast and pseudohyphae (x1000).



Candida albicans showing formation of the germ tube (x400).

- Different Candida species are identified by their growth characteristics, sugar fermentation, and assimilation tests.
- Germ tube is a rapid method for identification of C. albicans and Candida dubliniensis.
- This test depends on the ability of C. albicans to produce germ tube within 2 hours when incubated in human serum at 37°C.
- This phenomenon is called Reynold–Braude phenomenon (Fig. 74-2, Color Photo 66).

- Chlamydospores are typically produced by C. albicans on cornmeal agar at 25°C, but not by other Candida species. Moreover, CHROM agar allows for the presumptive identification of several Candida species by using color reaction in specialized media, thereby showing different colors of the colonies depending on the Candida species.

- **Nonculture Candida detection tests** These include (a) Candida mannan assay, (b) Candida heat-labile-antigen assay, (c) D-arabinitol assay, (d) D-inositol assay, and (e) 1,3-beta-D-glucan assay.

- **Immunological tests**

  - Serological tests are not that useful in diagnosis of patients with candidiasis because antibodies against Candida appear in sera of patients as well as in that of normal persons.
  - Skin test with Candida antigen is a delayed hypersensitivity skin test, which is used as an indicator of functions of the CMI.
  - The skin test is uniformly positive in immunocompetent adults and indicates that the person has intact CMI.
  - The skin test is negative in individuals who have deficient CMI. Such a person is anergic and is negative to other skin tests, such as purified protein derivative (PPD) skin test for tuberculosis.

Molecular Diagnosis DNA probe and polymerase chain reaction (PCR) are still under evaluation, but appear to be promising.

Treatment Antifungal therapy forms the mainstay of treatment of the infections caused by Candida. These agents include azoles (fluconazole, triazole, ketoconazole), nystatin, and amphotericin B. C. glabrata is becoming increasingly important worldwide and is intrinsically less susceptible to amphotericin B and other azoles (ketoconazole, fluconazole, etc).

Prevention and Control Antifungal prophylaxis is indicated for patients with invasive candidiasis who are at high risk of developing invasive candiasis. There is no vaccine available against candidiasis.

Aspergillosis الاسبوع الثالث عشر

A broad spectrum of diseases in humans ranging from direct invasion to hypersensitive reactions are caused by Aspergillus species. Although more than 100 species have been described, the majority of human diseases are caused by Aspergillus fumigatus and Aspergillus niger, and less frequently by Aspergillus flavus and Aspergillus clavatus.

Aspergillus Species Properties

■ Aspergillus species are molds.

■ They have septate hyphae that form V-shaped dichotomous branches.

The Aspergillus species are identified by (a) their morphological features, (b) the pattern of conidiophore development, and (c) the color of the conidia.

■ The fungus grows rapidly on SDA and other culture media at 25°C. Aspergillus produces colonies within 1–2 days and shows a velvety surface.

Pathogenesis and Immunity Aspergillus species rarely cause infections in immunocompetent individuals. They cause invasive infections mostly in the patients who are immunocompromised either due to (a) use of immunosuppressive drugs, (b) underlying lung diseases, or (c) immunodeficiency diseases, such as HIV. In immunocompromised host, Aspergillus species cause invasion of the blood, thereby causing infarction, hemorrhage, and necrosis of lung tissues. Aspergillus spp. also produces toxic metabolites that inhibit macrophage and neutrophil phagocytosis, facilitating dissemination of the infection. Aspergillus species unlike Candida species do not form the part of normal flora of humans. They are ubiquitous in the environment; hence transmission of infection is mostly exogenous.

Clinical Syndromes

In immunocompetent hosts, Aspergillus species may primarily affect the lungs, causing four main syndromes including (a) allergic bronchopulmonary aspergillosis, (b) chronic necrotizing aspergillus pneumonia, (c) aspergilloma, and (d) invasive aspergillosis.

Epidemiology Various clinical manifestations of Aspergillus infection have been documented worldwide.

Laboratory Diagnosis Laboratory diagnosis of invasive aspergillosis or chronic necrotizing aspergillus pneumonia depends on demonstration of Aspergillus in tissue by direct microscopy and culture.

Direct KOH preparation of the specimen shows nonpigmented, septate hyphae with characteristic dichotomous branching at an angle of 45°.

Biopsy specimens show septate, branching hyphae invading the tissues.

■ The specimens are inoculated on SDA without cycloheximide and are incubated at 25°C for 1–2 days.

■ Estimation of serum galactomannan is useful for diagnosis of invasive aspergillosis. Demonstration of elevated galactomannan in bronchoalveolar lavage is also useful for diagnosis of pulmonary aspergillosis.

■ The diagnosis of allergic bronchopulmonary aspergillosis is established by a positive skin test for A. fumigatus. Highly increased level of serum IgE (1000 IU/cc) and a positive serology for Aspergillus precipitation or Aspergillus-specific IgG or IgE antibodies are suggestive of the condition.

■ Serum IgG levels are usually positive in aspergilloma.

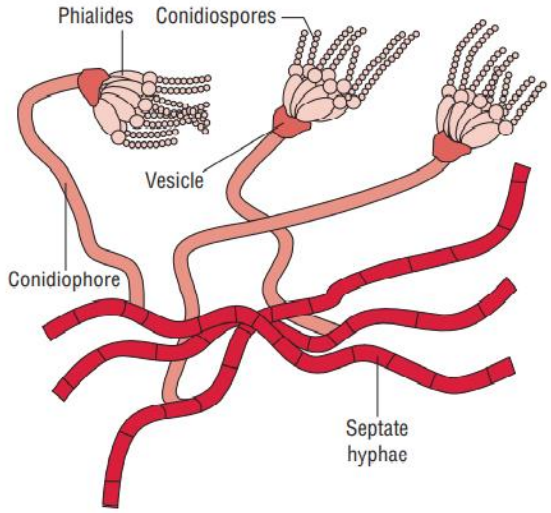
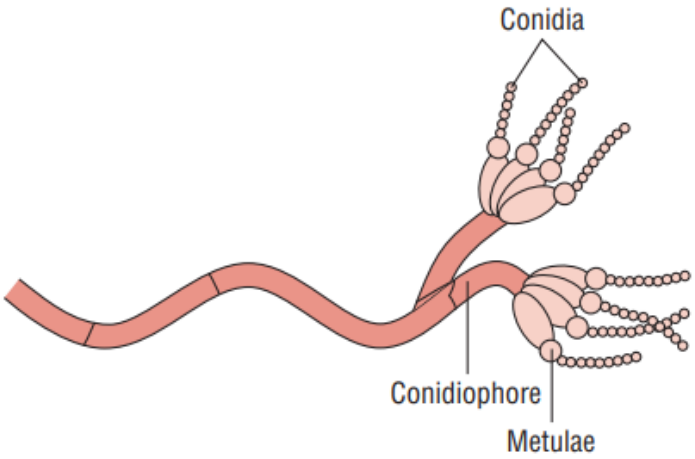


FIG. 74-3. *Aspergillus* species with septate hyphae that form V-shaped dichotomous branches.

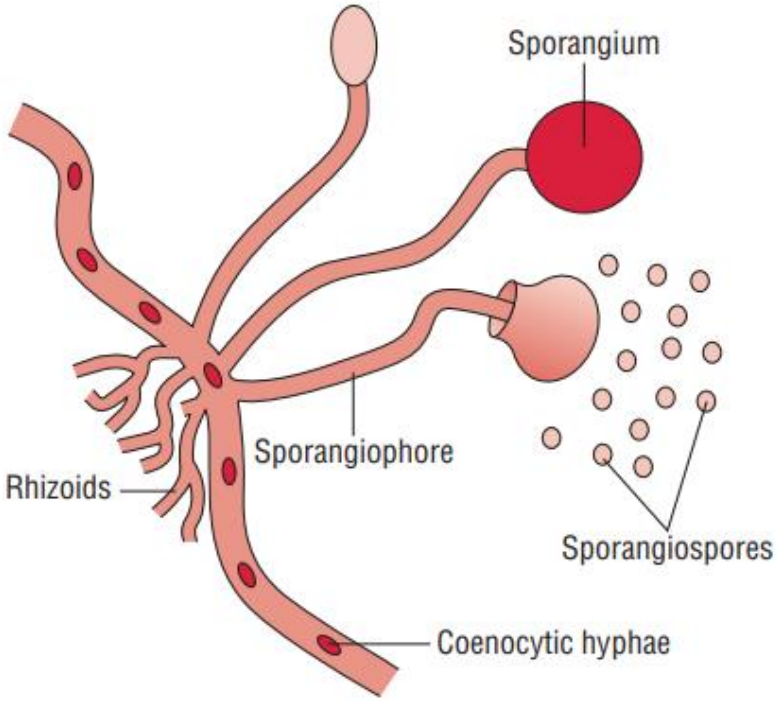
Zygomycosis الاسبوع الرابع عشر

Zygomycosis, also known as mucormycosis or phycomycosis, is an infection caused by saprophytic molds, such as Mucor (Fig. 74-4), Rhizopus (Fig. 74-5), and Absidia. These fungi are ubiquitous in the environment and generally saprophytic. They rarely cause disease in immunocompetent hosts, but they are the third most frequent cause of invasive fungal infection in immunocompromised patients. Rhizopus species are the most common causative agents of zygomycosis in humans. Of many Rhizopus species, Rhizopus arrhizus is the most common agent of zygomycosis. The fungalagents of zygomycosis

have a high degree of predilection to invade major blood vessels, leading to ischemia, necrosis, and infarction of adjacent tissues. They are also known to affect patients with acidosis secondary to renal insufficiency, diarrhea, and aspirin intake. Most of the infections produced by Zygomycetes are acute and are usually fatal despite early diagnosis and treatment. The agents of zygomycosis are transmitted by air, through their asexual spores. In humans, they invade tissues of patients with reduced host defense. From there, they enter the blood vessels and proliferate in the walls of blood vessels particularly paranasal sinuses, lungs, or intestines. This results in infarction and necrosis of tissues distal to blocked vessels. Zygomycetes cause a spectrum of diseases in humans depending on the immune status of the host and the portal of entry. These cause rhinocerebral zygomycosis, pulmonary zygomycosis, and gastrointestinal zygomycosis. Rhinocerebral zygomycosis is the most common manifestation of disease seen in patients with diabetic acidosis. Fever, unilateral facial pain or headache, nasal congestion, epistaxis, visual disturbance, and lethargy are the common manifestations. Clinical diagnosis of the zygomycosis is frequently difficult. It requires a high degree of suspicion and a host with appropriate risk factors. Laboratory diagnosis is by microscopy, culture, and histopathology:



Mucor species.



Rhizopus species.

Penicillium species rarely cause opportunistic infections in humans. The Penicillium species are identified by their typical morphology (Fig. 74-6), culture characteristics on the SDA medium, and microscopy. P. marneffei is the only dimorphic fungus in the genus Penicillium known to cause opportunistic infection.

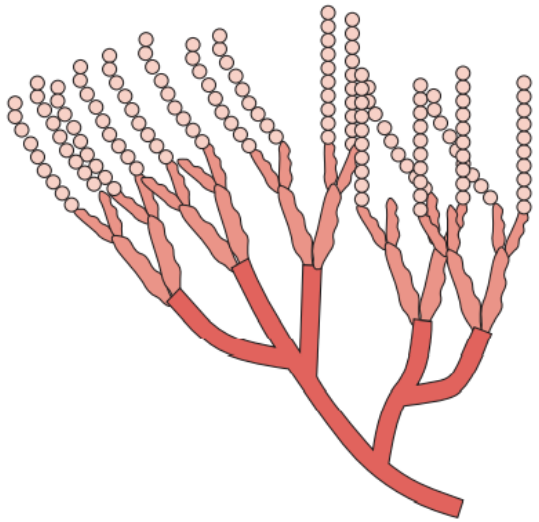
Penicillium marneffei

■ Demonstration of P. marneffei in the skin and mucosal scrapings, sputum, stool, blood, urine, lymph node, bone marrow, lung and liver biopsy specimens. Staining of skin, lymph node, and bone marrow aspirate by hematoxylin and eosin, PAS (periodic acid-Schiff), Wright's, and calcofluor white stain demonstrates yeast cells with transverse septa.

■ Immunohistochemical assay using monoclonal antibody against an external wall epitope is used to identify P. marneffei in tissues. Demonstration of fungal antigen in affected tissue by using direct immunofluorescence antibody also helps in diagnosis of the condition. Peripheral blood smear shows P. marneffei in patients with AIDS.

■ Culture is the gold standard. P. marneffei is a highly infectious fungus, hence culture should be done in a laboratory with a biohazard safety level-2 precautions. The fungus on SDA without cycloheximide and at 25°C produces mycelial grayish white colonies, green center, white periphery, and bright rose pigmented reverse (Color Photo 71). Microscopy of the colony shows septate hyaline hyphae, branched conidiophores, and three to five medullae which produce phialides that bear conidia in chains. The fungus on SDA at 37°C produces yeast-like colonies and cream-colored mucoid with brown red pigment. Microscopy of the colony shows pleomorphic ellipsoidal to rectangular yeast cells with transverse septum.

■ Indirect immunofluorescent antibody test, immunoblot assay, and immunodiffusion method using mycelial phase culture antigen are used to demonstrate serum IgG antibodies. Immunodiffusion and latex agglutination tests are used to detect P. marneffei antigen in serum and urine.



Penicillium species.

Antifungal Drugs الاسبوع الخامس عشر

A limited but increasing number of antibiotics can be used to treat mycotic infections. Most have one or more limitations, such as;

1. profound side effects.

2. a narrow antifungal spectrum.

3. poor penetration of certain tissues.

4. the selection of resistant fungi.

Finding suitable fungal targets is difficult because fungi, like humans, are eukaryotes. Many of the cellular and molecular processes are similar, and there is often extensive homology among the genes and proteins.

Table 3-1 summarizes the common antifungal agents and their primary sites of activity.

(Table 3-1) Antifungal agents and primary sites of activity

Group of compounds	Antifungal agent	Mechanism of action
Polyenes	Amphotericin B Nystatin	Bind to ergosterol
Azole derivatives	Miconazole Ketoconazole Fluconazole Itraconazole	Inhibit cytochrome P-450 dependent enzymes
Nucleoside analogs	5-fluoro-cytosine	Inhibits DNA and RNA synthesis
Grisans	Griseofulvin	Inhibits microtubular function
Allylamines	Naftifine Terbinafine	Squalene epoxidase inhibitors
Thiocarbamates	Tolnaftate Tolciclate	Squalene epoxidase inhibitors
Morpholines	Amorolfine	Inhibits ergosterol biosynthesis
Echinocandins	Caspofungin, Anidulafungin	B-1, 3 glucan synthetase inhibitors