Mycology II

OBJECTIVES

• Name the dimorphic fungi and the types of infections they cause.
• Describe the unique structural characteristics of Histoplasma, Blastomyces, Coccidioides, and Sporothrix
• Name the endemic regions where Histoplasma capsulatum, Blastomyces dermatitidis, and Coccidioides immitis are found.
• Name the three genera of dermatophytes and explain how to collect specimens for their recovery in culture.
• Describe features of subcutaneous mycoses including Sporotrichosis and Chromoblastomycosis
SYSTEMIC MYCOSES

Primary Fungal Pathogens

Dimorphic Fungi
- Yeast like cells at 35°C
- Mold form at 25°C
- Yeast forms escape the immune system

Examples:
- *Histoplasma capsulatum*
- *Blastomyces dermatitidis*
- *Coccidioides immitis* (not thermally dimorphic)
- *Sporothrix schenckii*
- *Paracoccidioides brasiliensis*
- *Talaromyces* (Penicillium) *marneffei*

“Yeast in the beast, Mold in the cold”

Histoplasmosis

*Histoplasma capsulatum*

Chicken coop  Bat cave
Characteristics of *H. capsulatum*

- Thermally Dimorphic Fungus
  - Yeast phase (2-4 μm) found in macrophages
  - Mold phase has tuberculate macroconidia
- No capsule
- Found in soil, bird and bat droppings
- Concentrated in Ohio / Mississippi River Valleys

**Histoplasmosis in Americas and US**

Areas Endemic for Histoplasmosis

https://www.cdc.gov/fungal/diseases/histoplasmosis/causes.html

**Route of Infection**

*H. capsulatum*

- Conidia inhaled by host
- Conidia phagocytosed by macrophages and neutrophils
- Convert to yeast intracellularly
- Yeasts multiply and spread to liver, spleen, and bone marrow
- Primary lesion = granuloma in lung
Clinical Manifestations

- Depends on intensity of exposure and immunologic status of the host
- Asymptomatic—most for low intensity exposure
- Pulmonary
  - Infiltrates, mediastinal lymphadenopathy
  - Fever, night sweat, weight loss (like TB)
  - May progress to cavitating lesion—typically with underlying disease
- Disseminated
  - Febrile illness
  - Spread to CNS, skin, adrenals
  - Enlarged liver and spleen
  - Higher incidence in children and immunocompromised adults

Lab Diagnosis of Histoplasmosis

- Direct microscopy
- Culture of bone marrow, biopsy, BAL
  - DNA probe of colony to confirm
- Antigen detection—Serum/Urine Antigen Test
  - Urine more sensitive than blood
  - Helpful for diagnosing disseminated disease

Histoplasmosis

Histology

At 37°C
- Intracellular budding yeast cells are typical
- *Histoplasma capsulatum* predominates at central portion of lesions. Thus, selection of tissue section to be excised for histopathologic and mycologic examination is of particular significance.

Intracellular yeast form in a multinucleate giant cell. H&E, spleen, 1000X
Histoplasmosis Laboratory Diagnosis

At 25°C
- Colonies are slow growing and granular to cottony in appearance.
- Color is white initially and usually becomes buff brown with age; reverse, is yellow or yellowish orange.

Histoplasmosis Laboratory Diagnosis

At 25°C
- Hyphae are septate and hyaline.
- Has both macro- and microconidia.
- Macroconidia are tuberculate, thick-walled, round, unicellular, hyaline, large and often have fingerlike projections on the surface.
Blastomycosis

*Blastomyces dermatitidis*

- “Chicago Disease”

Characteristics of *Blastomyces dermatitidis*

- Dimorphic fungus
  - Large thick wall yeasts with broad based buds
  - Mold phase not unique
- Found in central U.S.
- Associated with soil and wood

Clinical Manifestations

- Infection through inhalation
- Pulmonary
  - May be asymptomatic
  - Fever, chest pain, cough with sputum
  - Hilar lymphadenopathy, nodular lesion
- Cutaneous involvement=classic presentation
  - Skin lesions (necrosis and fibrosis)
  - May be mistaken for squamous cell carcinoma
- Lytic bone lesions
- Disseminated disease
Lab Diagnosis of Blastomycosis

- Direct microscopy
- Culture of sputum, BAL, lung biopsy
  - DNA probe of colony to confirm
- Antigen detection—Serum/Urine Antigen Test
  - Not as sensitive or specific
  - Cross reacts with Histoplasma

Blastomycosis

Histology

- Tissue sections showing large, broad-base, budding yeast-like cells, 8-15mm in diameter.
- Note: tissue sections need to be stained by Gomori’s methenamine silver stain (GMS) or periodic acid-Schiff (PAS) stain to clearly see the yeast-like cells, which are often difficult to observe in H&E stained preps.

Blastomycosis

Laboratory Diagnosis

- Broad-based budding yeast on KOH Prep
Blastomycosis
Laboratory Diagnosis

At 25°C

- The growth rate is slow to moderately rapid. The colony diameter is 0.5 to 3 cm following incubation for 7 days on potato glucose agar. The texture is membranous and downy to woolly. The surface color is white to beige and reverse is pale to brownish.

- Septate hyaline hyphae and unbranched short conidiophores are observed.
- Conidiophores arise at right angles to the hyphae. Conidia are hyaline and unicellular. They are solitary and pyriform to globose in shape (Lollipop).

Blastomycosis
Laboratory Diagnosis

At 37°C

- After incubation at 37°C on an enriched medium or in infected tissue sections, the fungus appears as budding yeast cells.
- The yeast cells (8-12 µm in diameter) typically have double-contoured refractile walls and a broad base attaching the bud to the parent cell.
Broad Based Budding = Blastomycosis

Coccidioidomycosis

*Coccidioides immitis/posadasii*

- “Valley Fever”

Areas Endemic for Coccidioidomycosis

https://www.cdc.gov/fungal/diseases/coccidioidomycosis/causes.html
Characteristics of Coccidioides

- Dimorphic fungus
  - In tissue: spherule filled with endospores
  - In culture: mold with barrel shaped arthroconidia
- *Coccidioides immitis* (typically in California) and *Coccidioides posadasii* (typically outside of California)
- Thought to be geographically restricted to desert climate (Southwest U.S.)
- Not transmitted person-to-person

Coccidiomycosis Clinical Manifestations

- Primary infection characterized by fever, cough, chest pain, malaise and occ. hypersensitivity reactions. CXR shows infiltrate, hilar adenopathy, or pleural effusion. Mild eosinophilia may be seen
- Most cases resolve spontaneously with complete recovery
- Dissemination to bone, skin, squamous and mucosal tissues, meninges, joints and other sites can follow primary pulmonary infection
- Dissemination occurs more frequently in African Americans, Filipinos, Native American, Mexican-Americans, Pregnant women, and IC patients

Lab Diagnosis of Coccidiomycosis

- Microscopic exam to detect spherules
- Culture
  - Demonstrate alternating arthroconidia
  - DNA probe of colony
- Serology useful unless anergic
  - Antibody decrease as disease resolves
Coccidiomycosis
Histology

- Spherules containing endospores are typical structures formed in infected tissues.

Coccidiomycosis
Laboratory Diagnosis

- Colonies grow rapidly. Macroscopic morphology may be very variable.
- At 25 or 37°C and on Sabouraud dextrose agar, the colonies are moist, glabrous, membranous, and grayish initially, later producing white and cottony aerial mycelium. With age, colonies become tan to brown in color.

- At 25°C Arthroconidia are thick-walled, barrel-shaped, 2-4 x 3-6µm in size.
- Typically, these arthroconidia alternate with empty disjunctor cells.
Coccidiomycosis
Infection
• Inhalation of arthroconidia bypasses upper airway into alveoli
• Monocytes ingest arthroconidia
• Arthroconidia converts to spherule

SUBCUTANEOUS MYCOSES
• Involves infection of skin and underlying tissues (without dissemination)
• Humans often accidental hosts (trauma with saprophytic fungi)

Dermatophytosis (Ringworm, Tinea)
• Fungal infection of keratinized tissues (skin, hair, nails) caused by a group of specialized fungi, the dermatophytes
• Onychomycosis—nail infections caused by any fungus
• Invasion of subcutaneous or deep tissues is very rare
• Slow growing in culture (25°C)
• Transmission requires close personal contact
Dermatophytes-Classification

- Classified into 3 genera
  - *Epidermophyton* – 2 species
  - *Microsporum* – 16 species
  - *Trichophyton* – 24 species

Dermatophytes Clinical Manifestations

- Inflammation often greatest at the advancing margin leaving a central area with some clearing
- The name “ringworm” follows from the irregular inflammatory border of the skin lesion
- Naming of clinical disease is done by appending a Latin term designating body site to the word, tinea

Dermatophytes Clinical Manifestations – Tinea pedis

- Tinea pedis (Athlete’s foot) – most common dermatophyte infection
- Begins as weeping, peeling lesion between 4th and 5th toes. May extend to other toes
- In toe webs, scaling, fissuring, maceration, and erythema may be associated with an itching or burning sensation
- Most common species: *T. rubrum, T. mentagrophytes, E. floccosum*
**Dermatophytes**

**Clinical Manifestations – Tinea capitis**

- Dermatophytosis of scalp due to invasion of hair
- Hair becomes dull, lusterless and tends to break off 1-2 mm above hair follicle orifice
- Infection may be patchy or extensive and with time may involve entire scalp
- Predominant cause of tinea capitis in USA is *T. tonsurans*

**Dermatophytes**

**Clinical Manifestations – Tinea corporis**

- Dermatophytosis of glabrous skin. Most commonly occurs in children and may be seen on face, shoulder, arms, or other surfaces
- Lesions variable in size, annular, sharply margined, exhibit raised, red, serpiginous border ("ringworm")
- Agents of tinea corporis include: *M. canis, M. gypseum,* *T. mentagrophytes, T. rubrum*

**Dermatophytes**

**Clinical Manifestations – Tinea cruris**

- Infection of groin, perineum, scrotum, perianal area. More common in men
- Erythema, pustule formation, hyperpigmentation of affected areas associated with chronic infection
- Itching or burning common
- Common agents are *T. rubrum and E. floccosum*
Pathogenesis of Dermatophytes

- Inoculation via minor trauma
- Penetrates stratum corneum and proliferates
- Infection spreads laterally, not deeper
- Spread to other keratinized structures
- Cell mediated immunity for resolution
- Chronic infection associated with decreased T cell function

Lab Diagnosis of Dermatophytosis

- Direct exam of leading edge of skin lesion by KOH or calcofluor
- Examine hair under Wood’s light
  - Damaged hair fluoresces
  - Remove infected hair at shaft for culture
- Culture to rule out other pathogens
- ID by color, growth, conidia shape

KOH Preparation/Mount

- 20% potassium hydroxide
- Rapid detection of fungal elements from a clinical specimen
- Sample is placed on a slide with KOH solution
- Solution slowly dissolves the skin cells but not the fungus. The fungus can then be seen with a microscope.
Diagnosis of Dermatophytes

Scrape leading edge of skin lesion

KOH Prep of Hair

Dermatophytes
Laboratory Diagnosis – Culture

SUBCUTANEOUS MYCOSES

• Sporotrichosis
• Chromoblastomycosis
Sporotrichosis

*Sporothrix schenckii*

• “Rose gardeners disease”

Characteristics of *Sporothrix schenckii*

• Thermally Dimorphic Fungus
  – Cigar shaped yeast at 37°C
  – Mold with daisy like conidiophore at 25°C
• Found in soil
• Associated with rose thorns and moss

Clinical Manifestations

• Papular skin lesion enlarges and ulcerates
• Appearance of subcutaneous firm nodules that progress along dermal and lymphatic vessels.
  – It is also called nodular lymphangiitis
• Multiple ulcers
• Disseminates to bones, lungs, eyes, CNS
Sporotrichosis Skin Lesions

Sporotrichosis Infection

- Inhalation of conidia or traumatic inoculation of skin
- Organism multiplies at local site
- Pyogenic and granulomatous response
- Infection spreads via the lymphatics
- Pulmonary sporotrichosis is rare

Lab Diagnosis of Sporotrichosis

- Sporothrix in culture and microscopic mold findings
**Sporothrix Yeast Phase**

**Chromoblastomycosis**

- Posttraumatic chronic infection of subcutaneous tissue
- Papules → verrucous cauliflower-like lesions on lower extremities
- Systemic invasion is very rare

**Chromoblastomycosis**

- Etiologic Agents
  - *Phialophora verrucosa*
  - *Fonsecaea pedrosoi*
  - *Fonsecaea compacta*
  - *Cladophialophora carrionii*
  - *Rhinocladiella aquaspersa*
Chromoblastomycosis

Histology

- Histologic examination reveal the presence of dark-walled, or dematiaceous (melanized) septate hyphae
- Copper pennies