

# Epidemiology and Laboratory Diagnosis of Fungal Diseases

## Epidemiology of Mycoses

- Mycosis (pl., mycoses) - an infection caused by a fungus
- Two broad categories of mycoses
  - Nosocomial (hospital acquired)
  - Community acquired
- Nosocomial infections are considered opportunistic in origin
- Community-acquired infections can be opportunistic, but also include endemic mycoses
- Over the past 20 years, both nosocomial and community-acquired mycoses have increased dramatically
- Excluding HIV/AIDS patients, mycoses are the 7th most common cause of infectious disease
- Contributing factors to increased mycoses:
  - Growing population of immune compromised individuals
  - Mobile population/immigration
  - More older adults with chronic medical conditions
  - Aggressive medical therapies
    - Surgery
    - Antibiotics
    - Chemotherapies/Organ transplants
  - Environmental changes

## Nosocomial Mycoses

- From 1980 to 1990, a marked increase was noted in nosocomial mycotic infections regardless of institution type
  - Teaching hospitals
    - Large (>500 beds): 2.4% to 6.6%
    - Small (<500 beds): 2.1% to 3.5%

- Non-teaching hospitals
  - Large (>200 beds): 1.2% to 2.5%
  - Small (<200 beds): 0.9% to 2.4%
- These increases occurred regardless of:
  - Body site (4 studied)
  - Major specialty service provided
  - Subspecialty service provided
- Deaths due to fungal infections increased nearly three-fold from 1980 (680 deaths) to 1990 (2300 deaths)
- Fungemia patients were more likely to die than those bloodstream infections by other types of microbes
- Independent risk factors for fungemia (i.e., those determined to enhance infection alone)
  - Number and duration of antimicrobial agents
  - Chemotherapy
  - Previous colonization
  - Indwelling catheter
  - Neutropenia
  - Hemodialysis
- Organ transplant patients experience the highest risk of fungal infection
  - Rates of fungal infection by type of transplant
    - Renal, <5%
    - Bone marrow, 2-30%
    - Heart, 10-35%
    - Liver, 28-42%
  - Risk factors include large corticosteroid doses, tissue rejection, poor tissue function, hyperglycemia, leukopenia, and age

- Molecular epidemiology
  - Increased emphasis on characterizing pathogens at the subspecies level to:
    - Better define infectious process
    - Modes of transmission
  - Classically, epidemiology was based upon physiological factors
  - Today, epidemiological studies tend to be DNA-based using a variety of molecular methods
  - Molecular methods include
    - RFLP (restriction fragment length polymorphisms) analysis
    - Electrophoretic karyotyping
    - Polymerase chain reaction-based methods of DNA fingerprinting
  - Protein-based methods include
    - Immunoblot fingerprinting
    - Polyacrylamide gel electrophoresis
    - Multilocus enzyme electrophoresis
  - Key question asked by epidemiology - are two or more isolates associated with an outbreak “the same” or “different”?
    - If different, probably reflect different sources or modes of infection
    - If same, infection is assumed to be cross-contamination from one patient to another, or from a common source
  - Typing methods can also help distinguish between a relapse of infection or the acquisition of a new one
  - Epidemiology data can also be used to help develop strategies of prevention and control which must consider if the origin of infection is
    - Endogenous - mainly yeast infections, particular *Candida* and *Candida*-like species
    - Exogenous - mainly mold species, but also yeasts from contaminated sources

## Community-Acquired Mycoses

- Etiological agents of community-acquired fungal infections include
  - Endemic dimorphic fungi
    - *Blastomyces dermatitidis*
    - *Coccidioides immitis* and *C. posadasii*
    - *Histoplasma capsulatum* var. *capsulatum* and *H. capsulatum* var. *dubosii*
    - *Paracoccidioides brasiliensis*
    - *Penicillium marneffeii*
  - Opportunistic pathogens
    - *Candida* species and other yeast and yeast-like fungi
    - Non-pigmented (in vivo) molds causing hyalophycomycosis
    - Pigmented (in vivo) molds causing phaeohycomycosis
    - Subcutaneous pathogens causing sporotrichosis, chromoblastomycosis, and mycetoma
    - Zygomycetes
- Endemic, dimorphic fungi
  - Acquired in specific geographic regions
  - Afflict both immune competent and immunocompromised individuals, but more common in HIV-infected persons and organ transplant recipients
  - Endemic regions
    - *Histoplasma capsulatum*
      - var. *capsulatum* - Ohio River valley of the U.S. and Latin America
      - var. *dubosii* - portions of Africa
    - *Coccidioides* spp. - desert southwestern U.S., northern Mexico, and Central America
    - *Paracoccidioides brasiliensis*. - Central and South America
    - *Blastomyces dermatitidis* - Ohio River and Mississippi valley areas of U.S. and portions of Africa
    - *Penicillium marneffeii* - Southeast Asia
- Opportunistic fungal pathogens
  - Includes virtually any fungus present in the environment
  - Incidence is unknown because these types of infections are not required to be reported

- Among the most notable is *Cryptococcus neoformans*
  - Rare infection prior to HIV pandemic
  - Two varieties
    - var. *neoformans* - worldwide distribution
    - var. *gattii* - tropical and subtropical regions

### Laboratory Diagnosis

- Diagnosis of a fungal infection is complicated and requires diverse expertise
- Most common methods are traditional, i.e., isolation, culture, and observation
- “Gold standard” is the recovery of the etiological agent from the clinical specimen
- Current methods include a mixture of the traditional, commercially-available kits, and new molecular approaches
- A proper means to diagnosis includes the following:
  - Specimen selection
    - Proper anatomical site
    - Use of suitable container for transport
  - Specimen collection
    - Choose an active lesion
    - Use aseptic technique
    - Obtain an appropriate quantity
    - Use sterile, appropriately labeled containers
  - Specimen transport and storage
    - Examine microscopically as soon as possible
    - Transport immediately using pertinent transport media if required
    - Do not freeze specimens
    - Do not refrigerate if not likely to contain contaminating microbes
    - Do not desiccate
    - Plate onto appropriate media
  - Culture: most infectious agents identified growth from clinical specimens
    - Media - various media have been developed, some quite traditional, and have been very successful in helping identify etiological agents

- Incubation conditions
  - Optimal temperature: 30°C (range 25-30°C)
  - Need a humid environment
  - Incubation period of 4-6 weeks often used
  - To assess form transitions, dimorphic fungi cultured at 35-37°C
- Direct microscopic examination
  - Requires recovery and identification of fungus from cultures or examination of infected tissue
  - Trained individual can often make diagnosis based upon this examination
  - Types of observations
    - Wet mounts (unstained)
    - Stained preparations
      - » Fungal cells
      - » Tissue samples
- Methods of identification and diagnosis
  - Morphology - some fungi exhibit characteristic cellular features both in vitro
  - Histopathology - some fungi exhibit characteristic cellular features both in vitro
  - Nucleic acid probes
  - Serology
  - Radiological surveys