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 marneffei*
  - Opportunistic pathogens
    - *Candida* species and other yeast and yeast-like fungi
    - Non-pigmented (in vivo) molds causing hyalophyphomycosis
    - Pigmented (in vivo) molds causing phaeohyphomycosis
    - Subcutaneous pathogens causing sporotrichosis, chromoblastomycosis, and mycetoma
    - Zygometes

- 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 marneffei* - 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