Lecture 2: Laboratory Aspects of Medical Mycology

General Classification Scheme

- Fungal reproductive structures are the basis for the classification and naming of fungi
- Four general groups
  - Chytridiomycota - motile zoospores
  - Zygomycota - thick-walled zygospore
  - Ascomycota - endogenous meiospores
  - Basidiomycota - exogenous meiospores
- Sexually reproductive form of a fungus is termed a teleomorph
- Not all fungi reproduce sexually or infrequently mate
  - Classified within an artificial taxon termed Deuteromycota or Fungi Imperfecti
  - Reproduction is by asexual means via mitotically-derived spores (conidia)
  - Asexual form is termed an anamorph
- Anamorphic fungi are placed into artificial classes primarily based upon their morphology
  - Hyphomycetes - septate hyphae on which conidia are directly produced or are borne on specialized hyphal branches
  - Coelomycetes - septate hyphae, but conidia are formed within spherical, flask-shaped, or spherical structures
  - Blastomycetes - yeasts and pseudohyphae

Fungal Disease Nomenclature

- The International Botanical Code of Nomenclature governs how fungi are given scientific names
- Fungi can be renamed for two reasons
  - Reclassification in the light of new information
  - Discovery of the teleomorph of a previously anamorphic stage
- Fungal disease names are not subject to rules like the Botanical Code
- Past practice was to name disease after the generic name of the causative organism, e.g.,
  - Aspergillosis for *Aspergillus* infections
  - Candidiasis for *Candida* infection
◆ However, when the name of a fungus changes, so does the name of the disease, e.g., previous names of the disease pseudallescheriasis
  ＊ Pseudallescheriasis (*Pseudallescheria*)
  ＊ Monosporiosis (*Monosporium*)
  ＊ Petriellidiosis (*Petriella*)
  ＊ Allescheriasis (*Allescheria*)
  ＊ Scedosporiosis (*Scedosporium*)
◆ Current practice based upon a 1992 recommendation - where possible, name an individual disease in the form of “pathology A due to fungus B”, e.g.,
  ＊ chromoblastomycosis due to *Fonseca pedrosoi*
  ＊ chromoblastomycosis due to *Phialophora verrucosa*
◆ Some commonly used disease names have been retained, but when the specific etiologic is given when known, e.g., aspergillosis due to *Aspergillus fumigatus*

**Laboratory Procedures**
◆ Because the clinical presentation of many fungal infections is non-specific and representative of a number of etiological agents, a definitive diagnosis is based upon laboratory results
◆ Success in laboratory diagnosis is dependent upon appropriate collection of specimens and their handling
◆ Three basic approaches for diagnosing the cause of a fungal infection
  ＊ Direct microscopic detection of etiologic agent in the specimen
  ＊ Isolation and identification of the pathogen in culture
  ＊ Serological evidence
◆ Newer methods are being developed
◆ Direct microscopic examination
  ＊ Observation of fungal material
  ＊ Can be used with histopathological stains
  ＊ Can be, and should be, correlated with results of fungal culture
  ＊ Disadvantages
    ● Less sensitive than culture
    ● Possibility of false-positive and false-negative results
**Histopathology**

- A reliable method to diagnosis fungal infections by observation of
  - Specific types of fungal structures in tissue
  - Visualization of cells using stains to mark cells not normally present in tissue
- Dependent upon
  - Tissue quality
  - Abundance of organism
  - Presence of distinctive structures

**Culture**

- Most fungi grow well on common mycological media
- Growth can be slow and development of diagnostic structures (e.g., spores) can be poor
- Culture techniques (e.g., specific media, incubation temperature, etc.) can be tailored to the fungus based upon suspected diagnosis
- Commonly used media include:
  - Sabouraud’s dextrose (glucose) - supports the growth of most fungi
  - Brain heart infusion - for fastidious fungi like *Histoplasma capsulatum*
  - CHROMagar - chromogenic medium used to distinguish between different *Candida* species
- Other considerations for fungal cultures:
  - Inclusion of antibiotics in media
  - Temperature of incubation
  - Duration of incubation
- Growth of a fungus in culture does not always establish its role in disease
  - Isolation of *Histoplasma* usually is a good indicator that it is the agent of disease since it is not typically found in healthy individuals
  - Isolation of *Aspergillus* or *Candida* is more suspect in the absence of clinical symptoms since these fungi are commonly found in the environment and as normal flora
  - One key to consider - is the specimen from a normally sterile site?
Disadvantages of culture

- Failure to recover an organism (negative culture) does not rule out a diagnosis, particular if symptoms are supportive of a particular diagnosis
- Time needed for culture can be excessive in terms of treatment-dependent diagnosis
- Mixed cultures can present a problem in discerning which fungus is the true etiological agent
- Common fungi can be inadvertently dismissed as contaminants

Fungal identification

- Classically, based upon macroscopic and microscopic observation of morphological characteristics
- Yeasts tend to be less morphologically distinct, therefore many identifications based upon biochemical characteristics much like enteric bacteria
- Other methods of identification:
  - DNA probes
  - Rapid tests such as
    - Germ tube test for Candida
    - Urease test for Cryptococcus
    - Chromogenic assays
  - Molecular methods
    - ITS sequences
    - PCR amplification of diagnostic DNA sequences

Serological testing

- Two types
  - Antibody detection
  - Antigen detection
- Except for specific situations, results are seldom absolute and must be considered with regard to other supportive information
- Antibody testing in immunocompromised individuals is problematic

Antifungal drug susceptibility testing

- In vitro testing can assess if an isolate is susceptible to a particular antifungal drug
  - MIC (minimum inhibitory concentration) levels
  - MFC (minimum fungicidal concentration) levels
- Assumption that testing can predict clinical outcome is not necessarily correct
Studies are ongoing to assess the correlation of MIC/MFC data with treatment outcomes in infections involving yeasts

Factors to be considered in relating MIC data to treatment outcome

- MIC data are not a physical measurement
- Hard to measure host factors affect clinical outcome
- Susceptibility does not uniformly predict success in vivo
- In vitro resistance to antifungal drugs does not always correlate with treatment failure

Types of methods

- Macrodilution tube series
- Microdilution microtiter plate series
- Agar-based techniques
  - Disk diffusion
  - Etest

Routine testing of mold isolates is not recommended due to time/costs involved and absence of definitive interpretation of results with regard to treatment outcome