SUPERFICIAL MYCOSES

Dermatophytoses are a category of cutaneous fungal infections caused by more than 30 species dermatophytes. Superficial mycoses are usually confined to the dead outer layers of skin and it appendages (hair, feathers, horn, hooves, claws, nails) and do not invade living viable tissues. Dermatophytes, or more properly, keratinophilic fungi, produce extracellular enzymes (keratinases) which are capable of digesting keratin. Keratin is the primary structural protein of skin, nails, feather, claw, horn hooves and hair. The digestion of keratin manifests as scaling of the skin, loss of hair, and crumbling of the skin. Dermatophyte infections are called ringworm (tinea).

Categorization of fungus as mold or yeast is based upon the microscopic appearance in the tissue or on routine culture media (the asexual stage). If the hyphal structures are observed , the fungi is termed a *mold*; if a single-celled, budding structure is observed, the fungus is termed a *yeast*. On routine culture media, molds will have a ‘’fuzzy’’ or wooly appearance, and a yeast will be bacteria-like in its colonial morphology and consistency. Some pathogenic fungi will produce either hyphal-like structures or yeast like structures, depending upon the conditions in which they are growing. Such fungi are called DIMORPHIC fungi.

The common dermatophytes include Microsporum, Trichophyton, and Epidermophyton.

Morphology

In their nonparasitic state, including culture, dermatophytes produce septate, branching hyphae collectively called mycelium. The asexual reproductive units (conidia) are found in the aerial mycelium. These units may be either macroconidia: pluricellular, podlike structures up to 100 µm long; or microconidia: unicellular spheres or rods less than 10 µm in any dimension. Shape, size, structure, arrangement, and abundance of conidia are diagnostic criteria.

In the parasitic state, only hyphae and arthroconidia (arthrospores), another asexual reproductive unit, are seen.

Growth Characteristics

The traditional medium for propagating dermatophytes (and other pathogenic fungi) is Sabouraud's dextrose agar, a 2% agar containing 1% peptone and 4% glucose. The selectivity is enhanced by the addition cycloheximide (500 µg/ml), which inhibits other fungi, and gentamicin and tetracycline (100 µg/ml of each) or Chloramphenicol (50 µg/ml), which inhibit bacteria. Dermatophytes are aerobes and nonfermenters. Some attack proteins and deaminate amino acids. They grow optimally at 25^{o}C to 30^{o}C and require several days to weeks of incubation.

Some dermatophytes in skin and hair (but not in culture) produce a green fluorescence due to a tryptophan metabolite that is visible under ultraviolet light (λ = 366nm, sometimes referred to as wood's light. Of animal dermatophytes, only *Microsporum canis* produces this reaction.

Ecology

The dermatophytes (which means *skin plants*) causing animal infections may have different natural sources and modes of transmission:

anthropophilic - These are usually associated with humans only; transmission from man to man is by close contact or through contaminated objects.

zoophilic - These are usually associated with animals; transmission to man is by close contact with animals (cats, dogs, cows) or with contaminated products.

geophilic - These are usually found in the soil and are transmitted to man by direct exposure.

Diseases caused by dermatophytes

Group	species	Disease
a. Epidermophyton	E. fluccosum	Infection of skin and nails of fingers and toes
b.Microsporum	M.audouinii	Ringworm of scalp in children
	M. canis	Infection of skin and hair on dogs, cats and other animals. Causes tinea capitis of children
	M. gypseum	A saprophyte in soil and parasite of lower animals
c. Tricophyton	T. mentagrophtyes	Primarily a parasite of hair
	T. rubrum	Causes ringworm and infects hair
	T. verrucosum	Causes a ringworm in cattle
	T. gallinae	Infection in chicken

There are three genera of dermatophytes:

1.Trichophyton species

These infect skin, hair and nails. They rarely cause subcutaneous infections, in immuno-compromised individuals. *Trichophyton* species take 2 to 3 weeks to grow in culture. The conidia are large (macroconidia), smooth, thin-wall, septate (0-10 septa), and pencil-shaped; colonies are a loose aerial mycelium that grow in a variety of colors. Identification requires special biochemical and morphological techniques

2. Microsporum species

These may infect skin and hair, rarely nails. This organism could be easily identified on the scalp because infected hairs fluoresce a bright green color when illuminated with a UV-emitting Wood's light. The loose, cottony mycelia produce macroconidia which are thick-walled, spindle-shaped,

multicellular, and echinulate (spiny). *Microsporum canis* is one of the most common dermatophyte species infecting humans.

3. Epidermophyton floccosum

These infect skin and nails and rarely hair. They form yellow-colored, cottony cultures and are usually readily identified by the thick, bifurcated hyphae with multiple smooth, club-shaped macroconidia

Laboratory diagnosis

Direct Examination

In 50% to 70% of cases, hairs and skin scales infected with *M.canis or M. Audouinii* may emit a bright greenish-yello fluorescence under ultraviolet light e.g woods light.

Microscopic Examination

Skin scrapings and hair are examined microscopically for the presence of hyphae and arthroconidia. The scraping should include materials from the margins of any lesion. The sample is placed on a slide , flooded with 10% to 20% potassium hydroxide, covered with a cover slip, and heated gently. Microscopic examination should begin under low power (100X) and subdued light. Infected hairs are encased in an irregular sheath of arthrospores that may double their normal thickness. At higher magnification (400X) of such hairs, individual, spherical arthroconidia are recognizable.

Stains and penetrating and wetting agents (permanent ink, lactophenol cotton blue, dimethylsulfoxide) improve visualization. Calcoflour white reagent imparts fluorescence to fungal structures and facilitates diagnosis where a fluorescent microscope is available.

Culture

Scrapings are planted onto and into the surface of selective media (Sabouraud's agar with chloramphenicol and cycloheximide, Dermatophyte Test Medium [DTM],Rapid Sporulation Medium [RPM], which are incubated at 25^{o}C (room temperature) for up to 3-weeks. An alkaline reaction suggests presence of dermatophyte.

THERAPY

Skin infections can be treated (more or less successfully) with a variety of drugs, such as:

Tolfnatate (Tinactin) available over the counter - Topical

Ketoconazole seems to be most effective for tinea versicolor and other dermatophytes.

Itraconazole - oral

Terbinifine (Lamisil) - oral, topical.

Echinocandins (caspofungin)

For infections involving the scalp and particularly the nails, griseofulvin is commonly used. This antimycotic must be incorporated into the newly produced keratin layer to form a barrier against further invasion by the fungus. This is a very slow process requiring oral administration of the drug for long periods - up to 6 to 9 months for fingernail infections and 12 to18 months for toenail infections.

Itraconazole and terbinafine are the drugs of choice for onychomycoses.

DIMORPHIC FUNGI

A.BLASTOMYCOSIS (*Blastomyces dermatitidis*)

Most of the systemic fungi have a specific niche in nature where they are commonly found. *Blastomyces dermatitidis* survives in soil that contains organic debris (rotting wood, animal droppings, plant material) and infects people collecting firewood, tearing down old buildings or engaged in other outdoor activities which disrupt the soil. In addition to an ecological niche, most fungi that cause systemic infections have a limited geographic distribution where they occur most frequently. Blastomycosis occurs in eastern North America and Africa.

HISTOPLASMOSIS (*Histoplasma capsulatum*) Histoplasmosis is a systemic disease, mostly o the reticuloendothelial system, manifesting itself in the bone marrow, lungs , liver, and the spleen In fact, hepatosplenomegaly is the primary sign in the young, while in adults, histoplasmosis mor commonly appears as pulmonary disease. This is one of the most common fungal infections, occurring frequently in South Carolina, particularly the northwestern portion of the state. The ecological niche of *H. capsulatum* is in blackbird roosts, chicken houses and bat guano. Typically patient will have spread chicken manure around his garden and 3 weeks later will develop pulmonary infection. There have been several outbreaks in South Carolina where workers have cleared canebrakes which served as blackbird roosts with bulldozers. All who were exposed, workers and bystanders, contracted histoplasmosis. Histoplasmosis is a significant occupational disease in bat caves in Mexico when workers harvest the guano for fertilizer. In the endemic area the majority of patients who develop histoplasmosis (95%) are asymptomatic. The diagnosis is made from their history, serologic testing or skin test. In the patients who are clinically ill, histoplasmosis generally occurs in one of three forms: acute pulmonary, chronic pulmonary or disseminated. There is generally complete recovery from the acute pulmonary form (another "flu-like" illness). However, if untreated, the disseminated form of disease is usually fatal. Patients wi first notice shortness of breath and a cough which becomes productive. The sputum may be purul or bloody. Patients will become anorexic and lose weight. They have night sweats. This again sounds like tuberculosis, and the lung x- ray also looks like tuberculosis, but today radiologists ca distinguish between these diseases on the chest film (histoplasmosis usually appears as bilateral interstitial infiltrates). Histoplasmosis is prevalent primarily in the eastern U.S. In S.C., a histoplasmin skin test survey of lifetime, one county residents, white males, 17 to 21 years old, w performed on Navy recruits. The greatest number of positive skin tests appeared in the northwest

part of the state. A similar study of medical students conducted at Medical University of South Carolina, about 25 years ago, bore the same distribution.The skin test is NOT used for diagnostic purposes, because it interferes with serological tests. Skin tests are used for epidemiological surveys.

Clinical specimens sent to the lab depend on the presentation of the disease: Sputum or Bronchial alveolar lavage, if it is pulmonary disease, or biopsy material from the diseased organ. Bone marrow is an excellent source of the fungus, which tends to grow in the reticulo-endothelial syste Peripheral blood is also a source of visualizing the organism histologically. The yeast is usually found in monocytes or in PMN's. Many times an astute medical technologist performing a white blood cell count will be the first one to make the diagnosis of histoplasmosis. In peripheral blood, *H. capsulatum* appears as a small yeast about 5-6 microns in diameter. (Blastomyces is 12 to 15 microns). Gastric washings are also a source of *H. capsulatum* as people with pulmonary disease produce sputum and frequently swallow their sputum.

Mycology

When it is grown on Sabouraud dextrose agar at 25 degrees C, it appears as a white, cotto-nnn ny mycelium after 2 to 3 weeks. As the colony ages, it becomes tan. In the mold form, *Histoplasma* l very distinct spore called a tuberculate macroconidium . The tubercles are diagnostic, however th are some non-pathogens which appear similar. A medical mycologist will be able to distinguish tl Grown at 37 degrees, C the yeast form appears. It is a white to tan colony. The yeast cell is 5-6 microns in diameter and slightly oval in shape. This is not diagnostic. To confirm the diagnosis, o must convert the organism from yeast to mycelium or vice-versa or use the DNA probe.

Serology

Serology for histoplasmosis is a little more complicated than for other mycoses, but it provides

more information than blastomycosis serology.

There are 4 tests:

- Complement Fixation
- Immunodiffusion
- EIA (antibody)
- EIA (antigen)

Each of these serological tests has different characteristics that make them useful.

The complement fixation test is like the one for blastomycosis, except there are 2 antigens, one to the yeast form of the organism and the other to the mycelial form. Some patients react to one form and not the other, while some individuals react to both. The reason for the different responses is n clear. One disadvantage is that complement fixing antibody develops late in the disease, about 2 t 3 months after onset. A second disadvantage is that it cross reacts with other mycotic infections. A advantage of the C-F test is that it is quantitative, so the physician can follow the course of the disease by observing the titer of several samples. The interpretation of the immunodiffusion test i little more complicated than with blastomycosis because there are two bands which may appear. A H band indicates active disease and will appear in 2 to 3 weeks. An M band can indicate past or present disease, or result from a skin test. This is one reason why skin tests are not used for diagnosis because they can interfere with other tests. Skin tests will also affect the complement fixation test.

Recently, a radioimmunoassay for histoplasma polysaccharide antigen has been developed. This i proprietary test so the evaluation of the results have been questioned. The drug of choice (DOC) i amphotericin B, with all its side effects. Itraconazole is now also being used for mild cases.

C.COCCIDIOIDOMYCOSIS (*Coccidioides immitis*)

Coccidioidomycosis is primarily a pulmonary disease. About 60 % of the infections in the endemic area are asymptomatic. About 25 % suffer a "flu-like" illness and recover without therapy. This disease exhibits the typical symptoms of a pulmonary fungal disease: anorexia, weight loss, cough, hemoptysis, and resembles TB. CNS infection with *C. immitis* is more common while it is less frequent with the other fungal diseases. The ecological niche of *C. immitis* is the Sonoran desert, which includes the deserts of the Southwest (California, Arizona, New Mexico, Nevada, Utah and Texas) and northern Mexico. It is also found in small foci in Central and South America.

Desert soil, pottery, archaeological middens, cotton, and rodent burrows all harbor *C. immitis. C. immitis* is a dimorphic fungus with 2 life cycles. The organism follows the SAPROPHYTIC cycle in the soil and the PARASITIC cycle in man or animals. The saprophytic cycle starts in the soil with spores (arthroconidia) that develop into mycelium. The mycelium then matures and forms alternating spores within itself. The arthroconidia are then released, and germinate back into mycelia. The parasitic cycle involves the inhalation of the arthroconidia by animals which then form spherules filled with endospores. The ambient temperature and availability of oxygen appear to govern the pathway The organism can be carried by the wind and therefore spread hundreds of miles in storms so the distribution is quite wide. In 1978, cases were seen in Sacramento 500 miles north of the endemic area, from a dust storm in Southern California. The spores of the organism are readily airborne. The cases that occur in South Carolina are usually in patients who have visited an endemic area and brought back pottery, or blankets purchase from a dusty roadside stand, or in Navy and Air Force personnel who were exposed when they were stationed in the endemic area. The disease manifests itself after they are transferred to a base in South Carolina. A few interesting cases occurred in cotton mills in Burlington and Charlotte, N.C. The cotton, grown in the desert of the

Southwest, was contaminated with the fungus spores and the mill workers inhaled the spores while handling the raw cotton and developed coccidioidomycosis.

Histopathology

The inflammatory reaction is both purulent and granulomatous. Recently released endospores incite a polymorphonuclear response. As the endospores mature into spherules, the acute reaction is replaced by lymphocytes, plasma cells, epithelioid cells and giant cells.

Serology

There are four tests for diagnosis:

- Complement-Fixation
- Slide agglutination
- Immunodiffusion
- EIA C-F antibody is slow to rise and develop in about 1 month. This test is excellent for coccidioidomycosis because it is quantitative. However, these antibodies cross-react wit some other fungi (Blastomyces and Histoplasma). The C-F test is also a PROGNOSTIC test. If the titer keeps rising, then the patient is responding poorly and the course may be fatal. If the C-F titer is dropping then the prognosis for that patient is favorable. A titer o greater than 1:128 usually indicates extensive dissemination. Life-long immunity usuall follows infection with *C. immitis.* There is a much greater mortality rate in dark-skinned people (Mexicans, Filipinos, and Blacks). They are 25 times more likely to develop progressive disease and death. The reason for this is obscure.

Amphotericin B, fluconazole and itraconazole are the drugs of choice.