Mycology

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Fungi are eukaryotic cells

• each fungal cell has:
  – nucleus and nuclear membrane
  – endoplasmic reticulum
  – mitochondria

• most fungi are obligate or facultative aerobes
• most pathogenic fungi are exogenous
  – their natural habitats being water, soil, and organic debris

• the mycoses with the highest incidence (candidiasis and dermatophytosis) are caused by fungi that are part of the normal microbial flora or highly adapted to survival on the human host
Fungi grows in 2 basic forms:

• **Yeast:**
  - unicellular growth pattern
  - can be spherical or ellipsoidal
  - they reproduce by budding
  - when the buds are not able to separate → pseudohyphae

• **Mould (Mycelia):**
  - produces multicellular filamentous colonies which contains the following:
    • **Hyphae** - cylindrical tubules composed of fungal cells attached end to end
    • **Mycelium** - the collective term for a mass of hyphae
    • **Vegetative hyphae** – penetrates the supporting medium and absorb nutrients
    • **Aerial hyphae** – project above the surface of the mycelium and bear the reproductive structures of the mould
• Dimorphic:
  – can grow either as yeast or a mould
  – depending on the environmental conditions and temperature
  – at body temperature they grow as yeast, at 25°C they grow as moulds
Summary of important cell features of fungal cells

Cell wall
- multilayered
- composed of carbohydrates, polysaccharides and some proteins (chitin, glucans, mannans, glucomannans, galactomannans)
- some are capsulated (Cryptococcus neophormans – stained by Indian ink)

Cell membrane
- bilayer
- composed of glycoproteins, lipids, and ergosterol (sterol)
- differs from mammalian cell membranes, which contain cholesterol
Nucleus
• Membrane-bound (eukaryotic)
• Multichromosomal and can be haploid or diploid

Cytosol complex
• contains several organelles
• nucleus, mitochondria, Golgi apparatus, ribosomes, a well-defined endoplasmic reticulum and other inclusions

Shape and size
Yeast - are oval to round; 2-10 \( \mu m \) in diameter;
Moulds - are filamentous; 2-10 \( \mu m \) in diameter, several hundred \( \mu ms \) in length
Metabolism

- exclusively heterotrophic
- produces various metabolites - primary (e.g. citric acid, ethanol) and secondary (e.g. alpha amanitin, aflatoxin)
- doubling time is long (hours) compared with most bacteria (minutes)

Staining properties

- false Gram-positive
- can be stained by
  - methenamine-silver
  - periodic acid Schiff-stain (PAS)
Reproduction of the human pathogenic fungi

- Fungi can produce spores to enhance their survival
- Spores can dispersed, they are more resistant and can germinate
- The spores can be used for both asexual and sexual reproduction

Asexual reproduction
Asexual spores are mitosis progeny and genetically identical
Asexual spores are:
Conidia
- non-sexual bud produces from transformation of vegetative yeast or hyphal cell
Blastospore
- conidial formation through a budding process (yeast)
Arthrospore
- type of segmented spore. Very light → carried easily by air
Chlamydospor e
- large thick wall produced from terminal or intracalary hyphal cells
Sporangiospore
- spore formed within a sac
Sexual reproduction

Sexual spores are products of meiosis

Sexual spores:

- **Ascospore**
  - a spore formed within an ascus (a sac like)

- **Basidiospore**
  - a spore formed on basidia

- **Zygospore**
Specimens:

- scrapings from the skin and the nails
- hairs plucked from involved areas
- *Microsporum*-infected hairs fluoresce under Wood's light (365 nm) in a darkened room
Culture:

• the identification of dermatophyte species requires cultures

• specimens are inoculated onto inhibitory mould agar or Sabouraud's agar plates containing cycloheximidine and chloramphenicol

• species are identified on the basis:
  – colonial morphology (growth rate, surface texture, and any pigmentation)
  – microscopic morphology (macroconidia, microconidia)
  – nutritional requirements
Sabouraud's agar

- glucose, pepton, acidic pH
- morphologic characteristic of fungi are used for identification
- antibiotic (gentamycin, chloramphenicol) \(\rightarrow\) inhibition of the bacteria
- long time incubation (for negative result) about 14 days

Microscopy

- wet mount
- simple staining (eg. methylene blue)
- before native examination 30% KOH \(\rightarrow\) keratolysis
Biochemical reactions

• sugar fermentation
• sugar assimilation test
• nitrate assimilation test

Serology

Molecular biology
Therapy of fungal infection

Polyenes
- Integration into cell membrane

5-Fluorocytosine
- Interruption of DNA & RNA synthesis

Azoles
- Interruption of sterol biosynthesis (cell and mitochondrial membranes)

Cell wall

Plasma membrane

Mitochondrion
Azoles (e.g., fluconazole)
- act by inhibiting ergosterol biosynthesis
- the more recent triazole derivatives, such as fluconazole, itraconazole, and voriconazol, owe their antifungal activity to inhibition of cytochrome P450-dependent 14α-sterol demethylase
- the earlier imidazole derivatives, such as miconazole, econazole and ketokonazole, have a complex mode of action, inhibiting several membrane-bound enzymes as well as membrane lipid biosynthesis

Allylamines/Thiocarbamates (e.g., naftifine, terbinafine/tolnaftate)
- act by inhibiting early steps of ergosterol biosynthesis
- target: squalene epoxidase

Morpholines (e.g., amorolfine) - Inhibit ergosterol biosynthesis (inhibition of Δ14-reductase and Δ7-Δ8-isomerase)
Polyenes (e.g., amphotericin B, nystatine)
- the polyene antifungal compounds bind to ergosterol
- resulting in the production of aqueous pores that lead to altered permeability leakage of vital cytoplasmic components, and death of the organism
  Ambisome® = liposomal amphotericin B

Nucleotide (e.g., 5-fluorocytosin) - Inhibits DNA and RNA synthesis

Grisans (e.g., griseofulvin) –
- interacts specifically with tubulin and acts as a mitotic poison
- inhibits microtubular function

Chitin synthase inhibitors
- Inhibit cell wall synthesis

Glucan synthesis inhibitors (Caspofungin)
- Inhibit cell wall synthesis
Classification of fungal disease

Fungal allergies

- “sick building syndrome”, generally occur in individuals with other allergies

Mycotoxicosis

- may result from ingestion of fungal-contaminated foods (generally an animal problem) or mushrooms
Superficial mycoses
• infections limited to the outermost layers of the skin and hair

Cutaneous mycoses
• infections that extend deeper into the epidermis, as well as invasive hair and nail diseases
Subcutaneous mycoses
• infections involving the dermis, subcutaneous tissues, muscle and fascia

Systemic mycoses
• infections that originate primarily in the lung but may spread to many organ

Opportunistic mycoses
• infections in immunocompromised patients
Clinical manifestations suggestive of fungal infection

- **flu-type infection:** that has lasted longer than a viral flu
- **chronic respiratory problem:** with weight loss and night sweats
- **fever of unknown origin:** that does not respond to antibacterial agents
• any infection with negative bacterial cultures: that does not respond to antibiotics and that does not appear viral
• signs of meningitis
• exposure to dust with bird or bat guano
Predisposal factors of fungal infections

Diseases

• diseases causing immunosuppression: tumours, AIDS, steroid therapy, diabetes mellitus
• trauma: burnt wounds - moulds, stabbed injuries - subcutaneous mycosis
• iatrogenic mycosis: during the immunosuppression therapy: irradiation, corticosteroid therapy, administration of broad spectrum antibiotics, cytostatic therapy
• nosocomial infections: fungi associated to plastic surfaces (catheters)
• „voluntarily risks”: alcoholism, drug abusers, microbiologists, mycologists
Superficial mycoses
- affect the outermost layer of skin and hair
- generally do not induce a cellular response to the infection

Malassezia furfur - Pityriasis versicolor
- yeast, required lipid in the medium of growth
- rarely may cause an opportunistic fungemia, folliculitis
- is a chronic mild superficial disease of the stratum corneum epidermidis
- hyper- or hypopigmented maculae occur on the skin
  • Diagnosis: direct microscopic examination of scrapings of infected skin treated with 10-20% KOH
  • Treatment: selenium sulfide, azoles
Subcutaneous mycosis

- the fungi that cause subcutaneous mycoses normally reside in soil or on vegetation
- they enter the skin or subcutaneous tissue by traumatic inoculation with contaminated material
- extension via the lymphatics draining the lesion is slow except in sporotrichosis.
- in rare cases they become systemic and produce life-threatening disease

Sporothrix schenckii

- is a thermally dimorphic fungus that lives on vegetation: grasses, trees, sphagnum moss, rose bushes, and other horticultural plants
- initial lesions is usually on the extremities
- initial lesion develops as a granulomatosus nodule that may progress to form necrotic lesion
- treatment: itraconazole, azoles, amphotericin B
Cutaneous mycoses

- infect only the superficial keratinized tissue (skin, hair, nails)
- most are unable to grow at 37°C or in the presence of serum
- 25°C on Sabourod’s dextrose agar for 2 weeks
- are classified as: geophilic, zoophilic, anthropophilic depending on their usual habitat
• infections begin in the skin after trauma and contact

• **risk factors**: moisture, warmth, specific skin chemistry, composition of sebum, prespiration, youth heavy exposure, genetic predisposition

• trichophytin: is a crude antigen preparation that can be used to detect immediate or delayed type hypersensitivity to dermatophytytic antigens
Tinea Pedis (Athlete's Foot): Trichophyton rubrum, Epidermophyton floccosum

- Tinea pedis is the most prevalent of all dermatophytoses
- A chronic infection of the toe webs
- Other varieties are: the vesicular, ulcerative, and moccasin types, with hyperkeratosis of the sole.
- There is itching between the toes and the development of small vesicles that rupture and discharge a thin fluid
- The skin of the toe webs becomes macerated and peels
- Chronic form: peeling and cracking of the skin are the principal manifestations, accompanied by pain and pruritus
Tinea unguium (Onychomycosis): Trichophyton rubrum, Epidermophyton floccosum

- nail infection may follow prolonged tinea pedis
- the nails become yellow, brittle, thickened, and crumbly
- treatment: nail infections are the most difficult to treat
- requiring months of oral itraconazole or terbinafine as well as surgical removal of the nail
- relapses are common
Tinea corporis (Microsporum canis) – ringworm
Tinea cruris and Tinea manus (Trichophyton rubrum, Epidermophyton floccosum)

- dermatophytosis of the glabrous skin commonly gives rise to the annular lesions with a clearing, scaly centre surrounded by a red advancing border that may be dry or vesicular
- the dermatophyte grows only within dead, keratinized tissue
- fungal metabolites, enzymes, and antigens diffuse through the viable layers of the epidermis to cause erythema, vesicle formation, and pruritus
- the lesions expand centrifugally, and active hyphal growth is at the periphery, which is the most likely region from which to obtain material for diagnosis
• treatment: itraconazole and terbinafine
• topical preparations may be used: miconazole nitrate, tolnaftate, and clotrimazole
• applied for at least 2-4 weeks, the cures rates are usually 70-100%
• treatment should be continued for 1-2 weeks after clearing of the lesions
Tinea capitis (Microsporum sp.) and Tinea barbae (Trichophyton rubrum, T. mentagrophytes)

- tinea capitis is dermatophytosis of the scalp and hair
- infection of the hair takes place just above the hair root
- the infection produces dull gray, circular patches of alopecia, scaling, and itching
- in prepubescent children, epidemic tinea capitis is usually self limiting
• **favus**: an acute inflammatory infection of the hair follicle caused by *T. schoenleinii*

• **tinea barbae**: involves the bearded region - cattle

• **treatment**: scalp infections are treated with griseofulvin for 4 - 6 weeks

• shampoos and miconazole cream or other topical antifungal agents may be effective if used for weeks

• ketoconazole, itraconazole and terbinafine
Candida infections
Opportunistic mycoses

Candida albicans (yeast)
- member of the normal flora of the: oral cavity, gastrointestinal, vagina
- non albicans: C. krusei, C. lusitaniae

Candida infection:
- in patients with normal immunity:
  - oral - soor
  - vaginal
  - interdigital
- in patients with immunodeficiency:
  - disseminated oral candidiasis
  - oesophagitis
  - pneumonia
  - nephritis
  - endocarditis and sepsis
Cryptococcosis (Cryptococcus neoformans)

- is a yeast
- may be isolated from fruit, milk, vegetation and soil
- meningitis - found in very large numbers in dry pigeon faeces
Aspergilloses (Aspergillus fumigatus)

- is a mould
- found in decaying vegetation
  - allergic bronchopulmonary aspergillosis
  - fungus ball → the fungus grows in a preexisting cavity (tuberculoid cavity) → surgical resection
- invasive form → spreading into the lung → necrotising pneumonia → dissemination to other organs (meningitis)
Mucormycosis (Mucor mucedo)

- is a mould
- localisation in the wall of the vessels → prone to thrombosis → possible ischemic necrosis throughout the body
- forms: rhinocerebral, pulmonary, cutaneous, gastrointestinal, central nervous system
Systemic mycosis

- affect internal organs and may disseminate to multiple sites of the body
- are named as: deep mycoses
- are caused by pathogenic fungi, which can invade and cause disease in healthy or compromised hosts

Histoplasmosis (Histoplasma capsulatum)
- is a dimorphic fungus
- does not have a capsule
- **source**: guano
- **can be classified as**: local, disseminated, acute, chronic, fulminant
- inhalation of the spores → pneumonia → chronic. inflammation → heals with calcification
Coccioidiosis (Coccidioides immitis)
- is a dimorphic fungus
- inhalation of the spores → 2/3 asymptomatic
  - influenza like disease
  - erythema nodosum
  - arthritis
- dissemination: meningitis, osteomyelitis
Blastomycosis (Blastomyces dermatitidis)

- dimorphic fungus
- is a pulmonary, disseminated and cutaneous fungal disease
- inhalation of the spores → dissemination → pulmonary manifestation → skin lesions (rarely asymptomatic infection)
Paracoccidioides brasiliensis

- dimorphic fungus
- mother cells may produce 10 to 12 uniform or variably sized buds
- cause systemic granulomatous disease
- involves mainly the lungs, phagocytic mononuclear system, mucous membranes, skin, and adrenals
- is the most common endemic human mycosis in Latin America
- has been isolated from:
  - soil, animals such as armadillos and bats, dog food, and penguin faeces
  - has also been isolated from the intestinal contents of bats
- the respiratory route is the main portal of entry and the lung is the primary site of infection
- the fungus spreads to other parts of the lung through peribronchial lymphatic vessels and drains into regional lymph nodes
**Pneumocystis jiroveci** previously known as **Pneumocystis carinii**

Geographic Distribution: Worldwide, in humans and animals

- *Pneumocystis* pneumonia occurs in immunosuppressed individuals and in premature, malnourished infants.

Clinical presentation:

- These fungi are found in the lungs of mammals where they reside without causing overt infection until the host's immune system becomes debilitating. Then, an often lethal pneumonia (PCP= *Pneumocystis pneumonia*) can result.

**Infantile pneumonia** - epidemic, in premature and malnourished infants

**Pneumonia of immunocompromised host** - sporadic, may affect any host with congenital or acquired immunodeficiency

**Extrapulmonary infection** - result of dissemination to other organs, including lymph nodes, spleen, bone marrow, liver, kidney, heart, brain, pancreas, skin and other organs

Laboratory identification

- Gomori methenamine silver stain method

The walls of the cysts are stained black and often appear crescent shaped or like crushed ping-pong balls

- treatment: Trimethoprim-sulfamethoxazole, pentamidine, atovaquone, echinocandins (micafungin), sordarins, azasordarins, terbinafine