Medical Mycology

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1. Characteristics of fungi important in medical mycology
2. Fungal taxonomy in scope of medical mycology
3. Fungi – pathogenicity factors in general
4. Classification of human fungal diseases
5. Dermatophytes
6. Yeasts
7. Aspergilli
8. Zygomycetes
9. *Pneumocystis jirovecii*
10. Antifungals, mechanism of action, terms fungicidal and fungistatic
11. Laboratory diagnostic methods in medical mycology - intro

Fungi

Heterotrophic metabolism – saprophytic, parasitic

Cell wall built of **chitin**, different polysaccharides (**glucans, galactomannan, mannan)**

Cell membrane contains **ergosterol** (similar to cholesterol to some degree)

Infectious agents affecting humans - 300 – 500 species described, number rises

Sexual (**teleomorph**) and asexual (**anamorph**) forms of different morphology, ecology and pathogenic potential

Changes in taxonomy

2. Taxonomic

Podříše: ROZELLOMYCETA
- Kmen: Rozellomycota – mikrosporidie a kryptomycety, zajímavé absencí chitinové buněčné stěny

Podříše: APHELIDIOMYCETA
- Kmen: Aphelidiomycota (též Aphelida[20]) – afelidie

Podříše: BLASTOCLADIOMYCETA – variabilní, bez buněčné stěny, mají bičíkaté pohyblivé spory (dříve součást chytridiomycet)
- Kmen: Blastocladiomycota (též Allomycota)

Podříše: CHYTRIDIOMYCETA – variabilní, mají bičíkaté pohyblivé spory
- Kmen: Caulochytriomycota (dříve součást kmene Chytridiomycota, třídy Spizellomycetes)
- Kmen: Chytridiomycota (dříve též Archemycota) – chytridiomycety
- Kmen: Monoblepharomycota (dříve součást kmene Chytridiomycota)
- Kmen: Neocallimastigomycota

Podříše: BASIDIOBOLOMYCETA
- Kmen: Basidiobolomycota

Podříše: OLPIDIOMYCETA – variabilní, mají bičíkaté pohyblivé spory (dříve součást chytridiomycet)
- Kmen: Olpidiomycota

Podříše: ZOOPAGOMYCETA – součástí jejich životního cyklu je odolné zygosporangium (dříve součást spájivých hub)
- Kmen: Entomophthoromycota
- Kmen: Kickxellomycota
- Kmen: Zoopagomycota

Podříše: MUCOROMYCETA[pozn. 4] – součástí jejich životního cyklu je odolné zygosporangium (dříve součást spájivých hub)
- Kmen: Calcarisporiellomycota
- Kmen: Glomeromycota – účastní se vnitrobuněčné mykorhizy
- Kmen: Mortierellomycota
- Kmen: Mucoromycota

Podříše: DIKARYA, DIKARYOMYCETA (též NEOMYCOTA)
- Kmen: Ascomycota – houby vřeckovýtrusé[pozn. 5], houby vřeckaté, askomycety[1]
  - Podkmen: Pezizomycotina (dříve též Ascomycotina)[1]
  - Podkmen: Saccharomycotina (dříve též Hemiascomycotina)[1]
  - Podkmen: Taphrinomycotina (dříve též Archiascomycotina)[1]
- Kmen: Basidiomycota – houby stopkovýtrusé[pozn. 6], bazidiomycety[1]
  - Podkmen: Agaricomycotina (obdobné dřívějším Hymenomycetes)
  - Podkmen: Pucciniomycotina (obdobné dřívějším Urediniomycetes)
  - Podkmen: Ustilaginomycotina
  - Podkmen: Wallemiomycotina

Fungi - morphology

Thallus (homogenic tissue structure, no organ differentiation)

Single cell form (blastoconidia) – yeasts

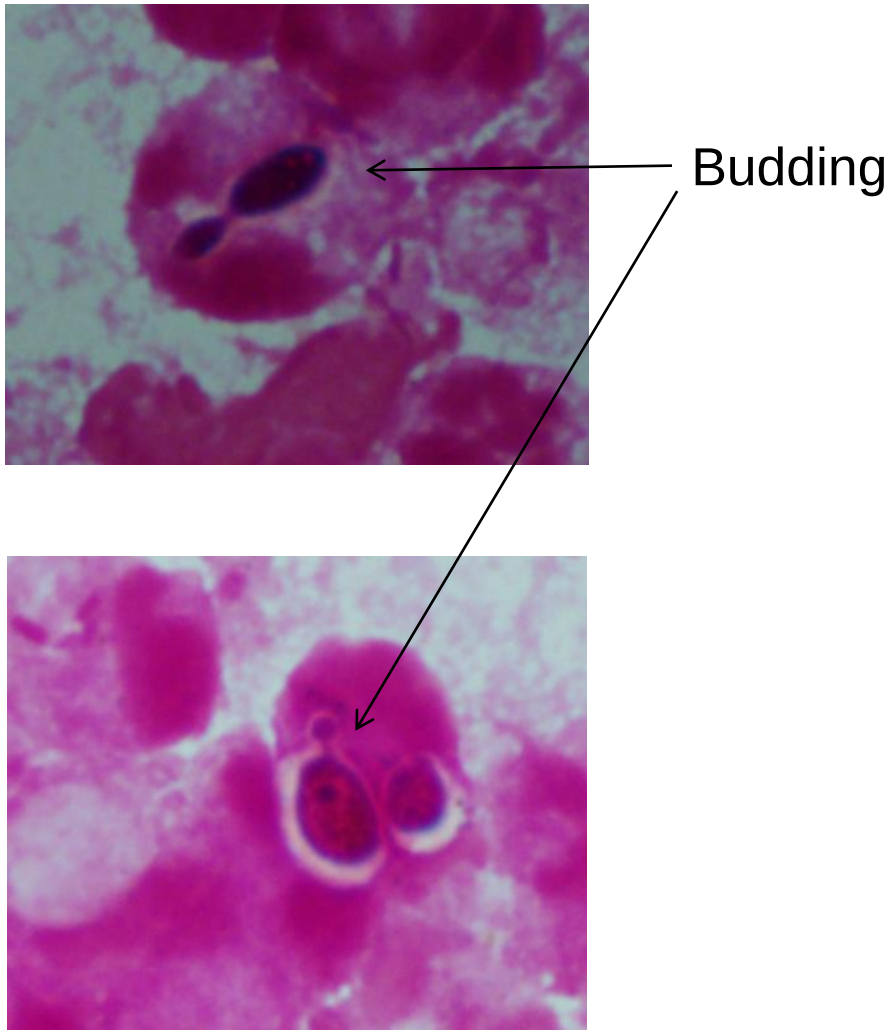
Budding (asexual reproduction), pseudomycelium (pseudohyphae) – blastoconidia elongated

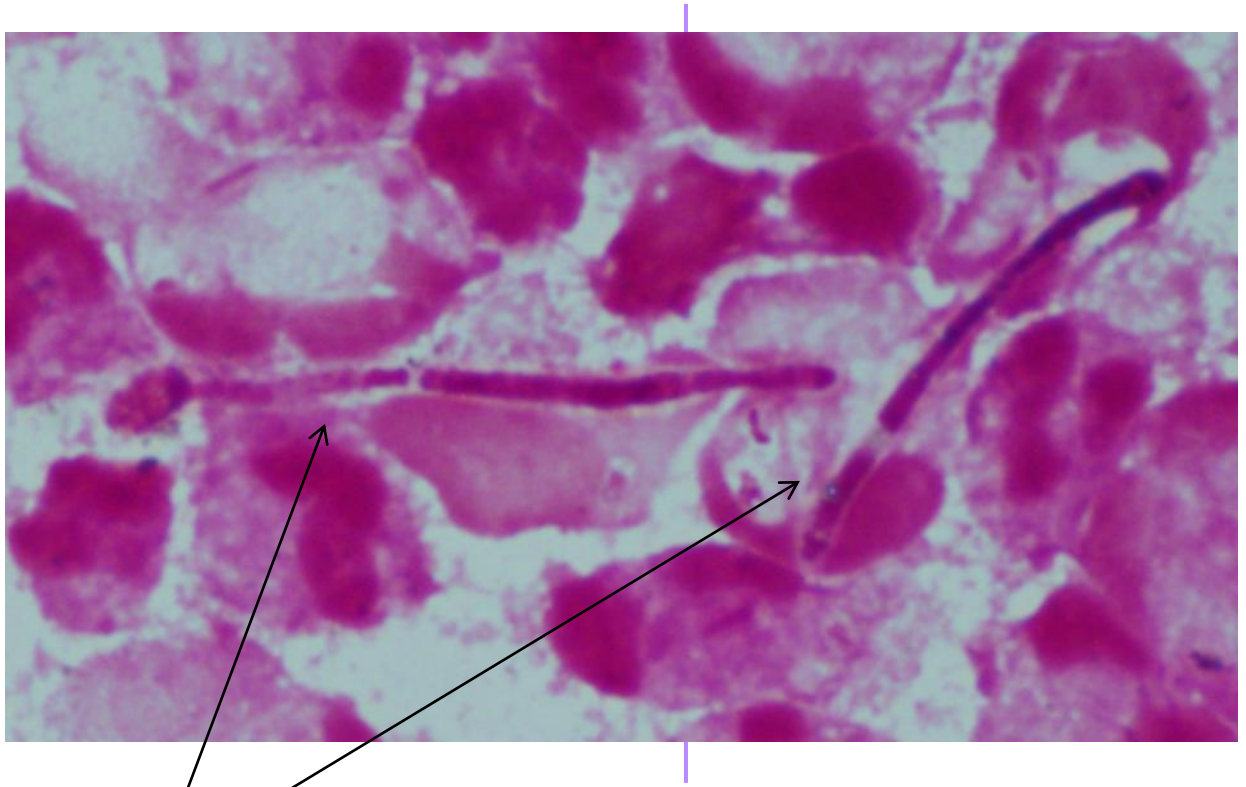
Multicellular form- hyphae (filaments) - moulds

Septate (ascomycetous and basidiomycetous molds
Aseptate (coenocytic - *Zygomycetes*)

Reproduction – asexual (**conidiogenesis, mitosis)**, sexual (sporogenesis, meiosis, fruit body), parasexual (conjugation of haploid hyphae, mitosis)

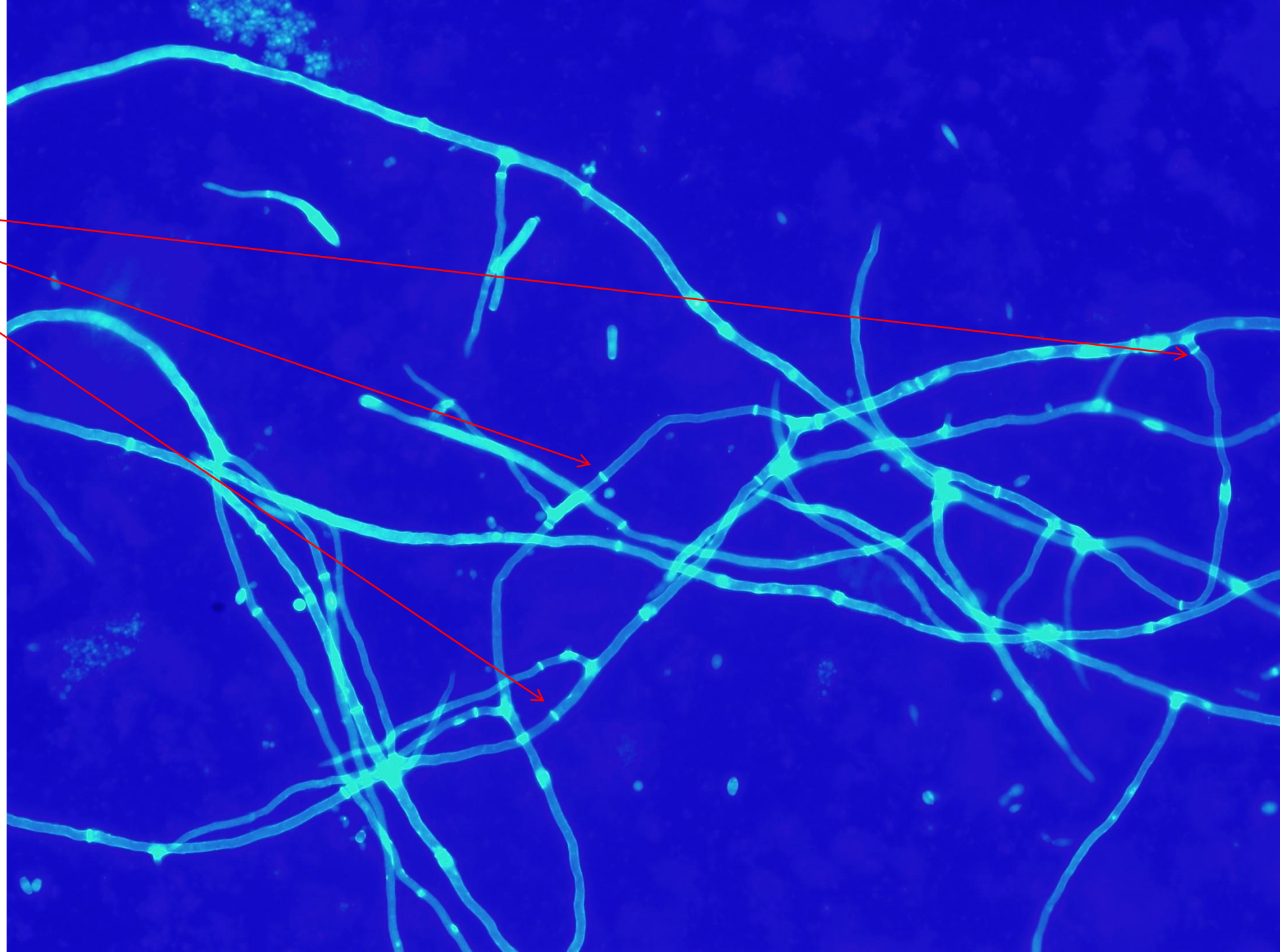
Yeasts – blastoconidia, pseudohyphae – Gram stain





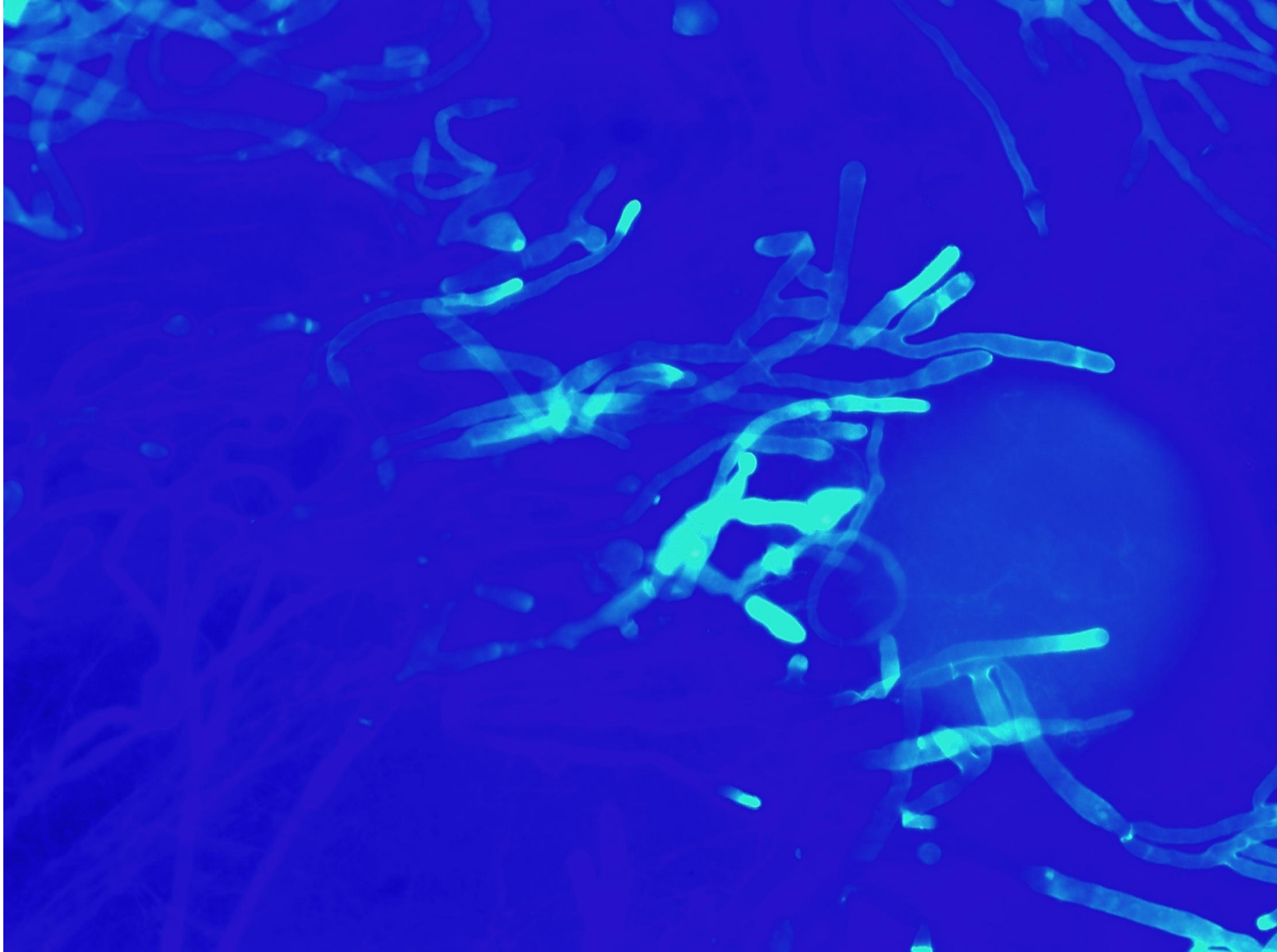
Septate hyphae, Calcofluor White

Septa



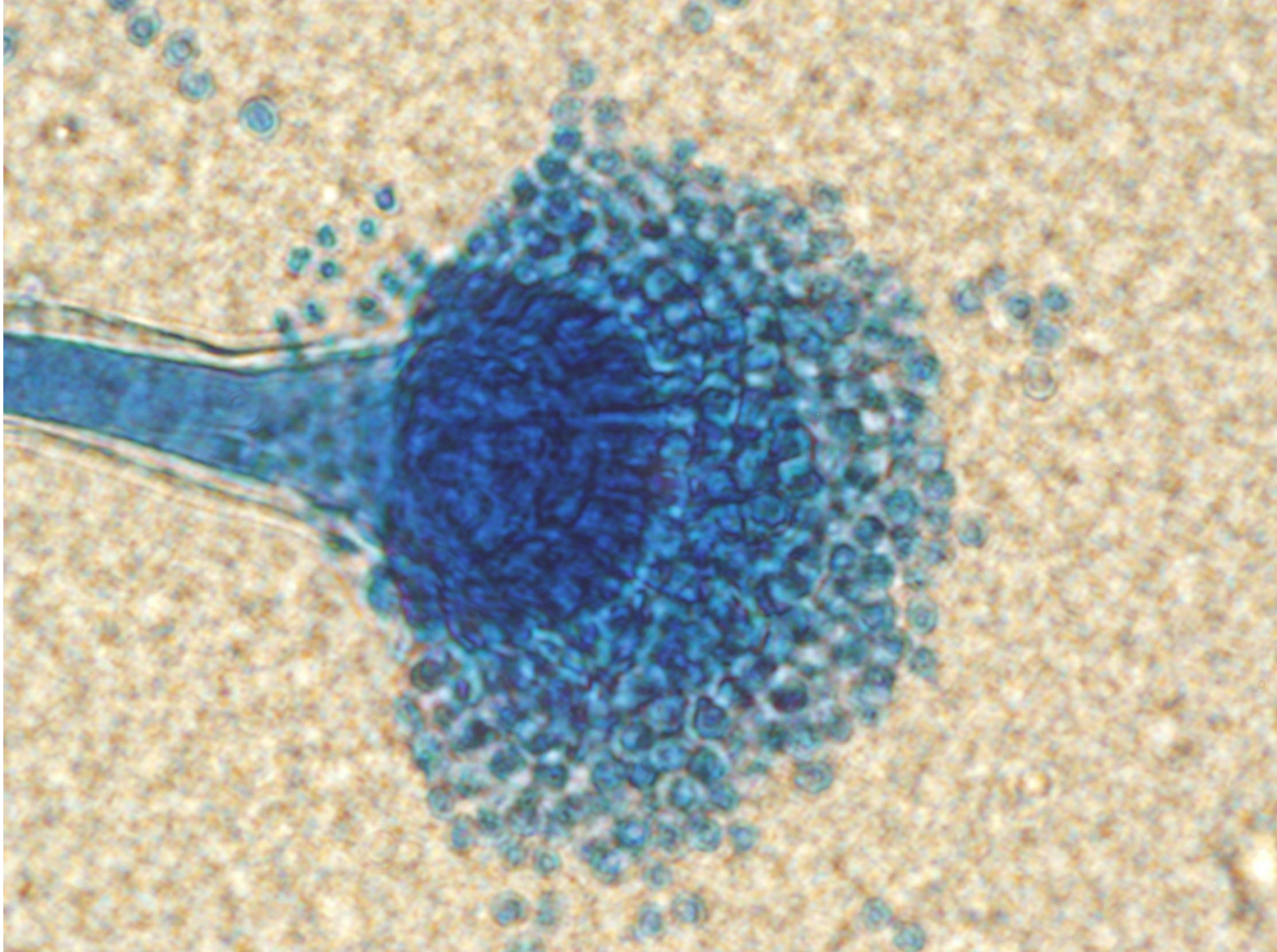
Aseptate hyphae

Calcofluor white



Conidiogenesis

Aspergillus fumigatus



Sporangiospores

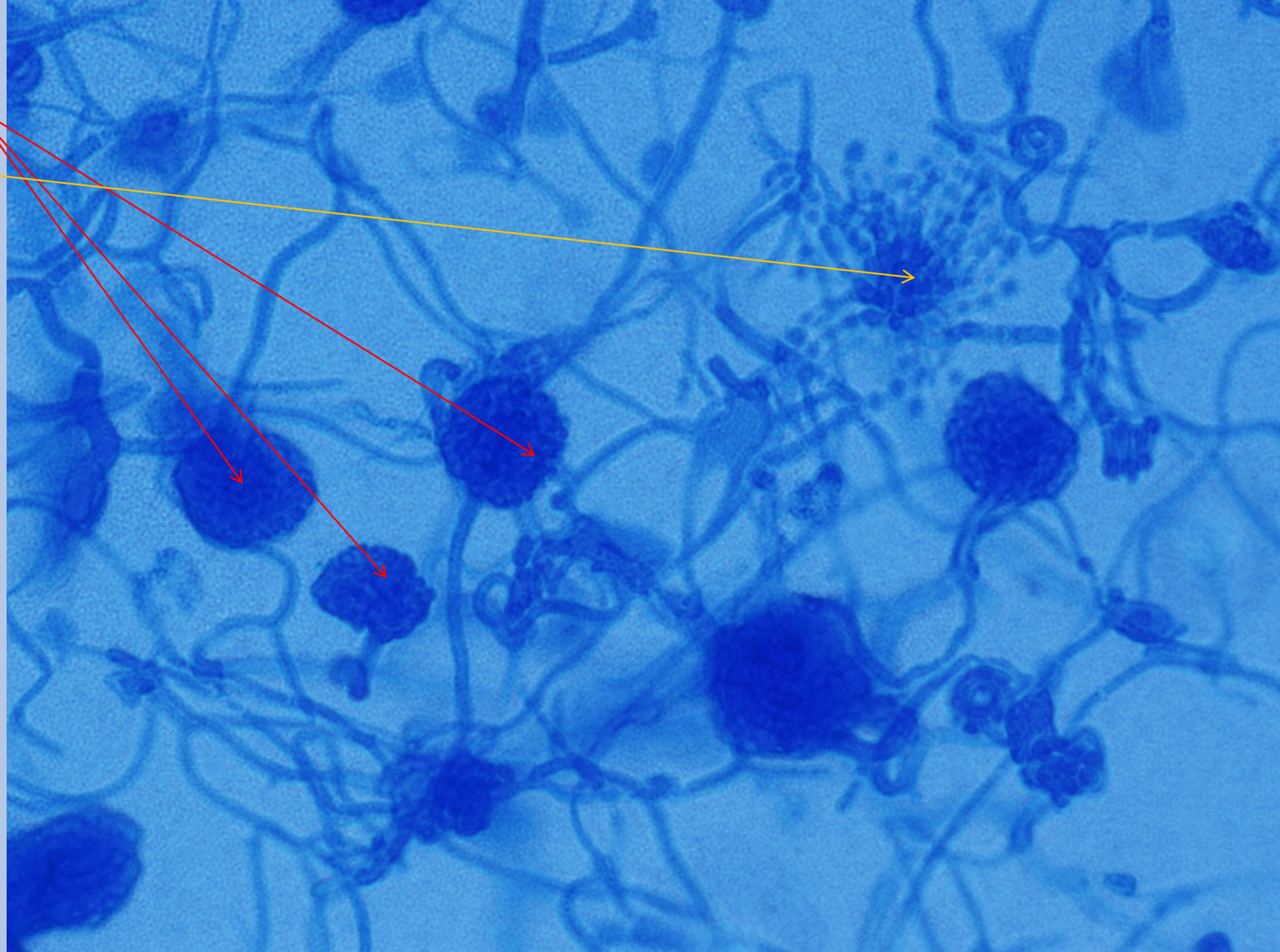
Rhizopus sp.

Fruiting bodies

Conidiophore

Aspergillus sp.
lactophenol blue

(environmental specimen)



Fungi – pathogenicity factors

Growth in human body temperature

Dimorphism: ability to transform from yeast to hyphal form, depends on outer conditions (environment or host organism)

Primary fungal pathogens

- *Blastomyces dermatitidis*
- *Coccidioides immitis, Coccidioides posadasii*
- *Histoplasma capsulatum*
- *Paracoccidioides brasiliensis*
- *Talaromyces marneffei*
- *Cryptococcus gattii*

Europe - imported infections
May change with global climatic and environmental changes

Human fungal diseases

Primary pathogens -
Blastomyces, *Coccidioides*, *Histoplasma*, *Paracoccidioides*, *Talaromyces marneffei* - endemic mycoses

Oportunistic pathogens - yeasts, moulds
Individuals with predisposition

Superficial and skin affections
Malassezia furfur – pityriasis versicolor
Dermatophytes - *Trichophyton, Epidermophyton, Microsporum*

Human fungal diseases

Superficial - outmost layers of the skin and hair

Pityriasis versicolor caused by yeast *Malassezia furfur*

Cutaneous and localized subcutaenous mycoses

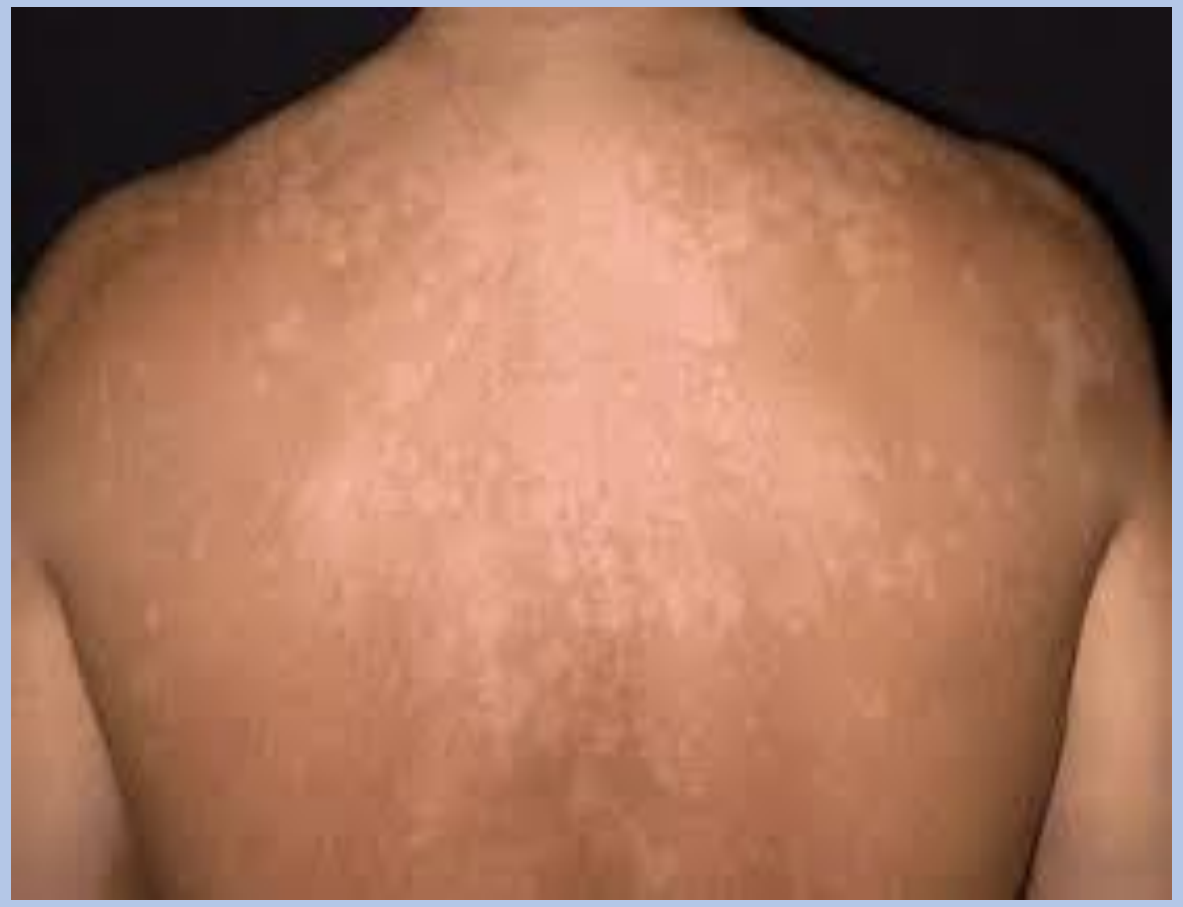
Dermatophytoses, tinea unquium, caused by dermatophytic fungi
Dermatomycoses, caused by nondermatophytic fungi (*Candida*, *Aspergillus*)

Chromoblastomycosis, mycetoma - localized affections in skin, subcutaneous and deeper tissue, melanized fungi, tropical and subtropical lands.

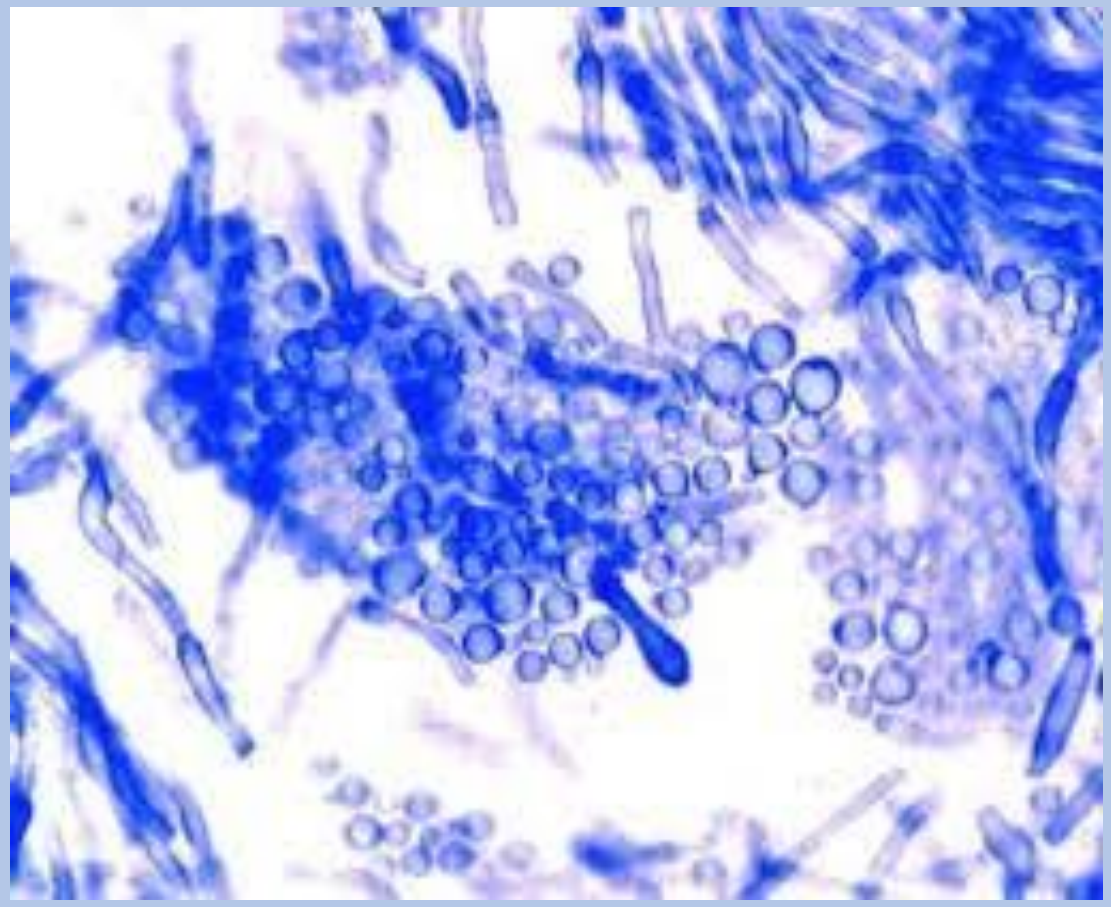
Endemic mycoses
Primary pathogens, endemic in **North and South America**, Africa, Southeast Asia

Opportunistic mycoses
Invasive, life-threatening infections in patients with predisposition
Yeasts including *Cryptococcus* sp., *Malassezia*, sp., aspergilli, mucormycetes, other filamentous fungi (hyalohyphomycosis, phaeohyphomycosis).

Pityriasis versicolor, *Malassezia furfur*



Merckmanuals.com



Link.springer.com

Cutaneous and subcutaneous mycoses

Dermatophytoses caused by dermatophyta
Dermatomycoses caused by nondermatophytic fungi

Dermatophytosis

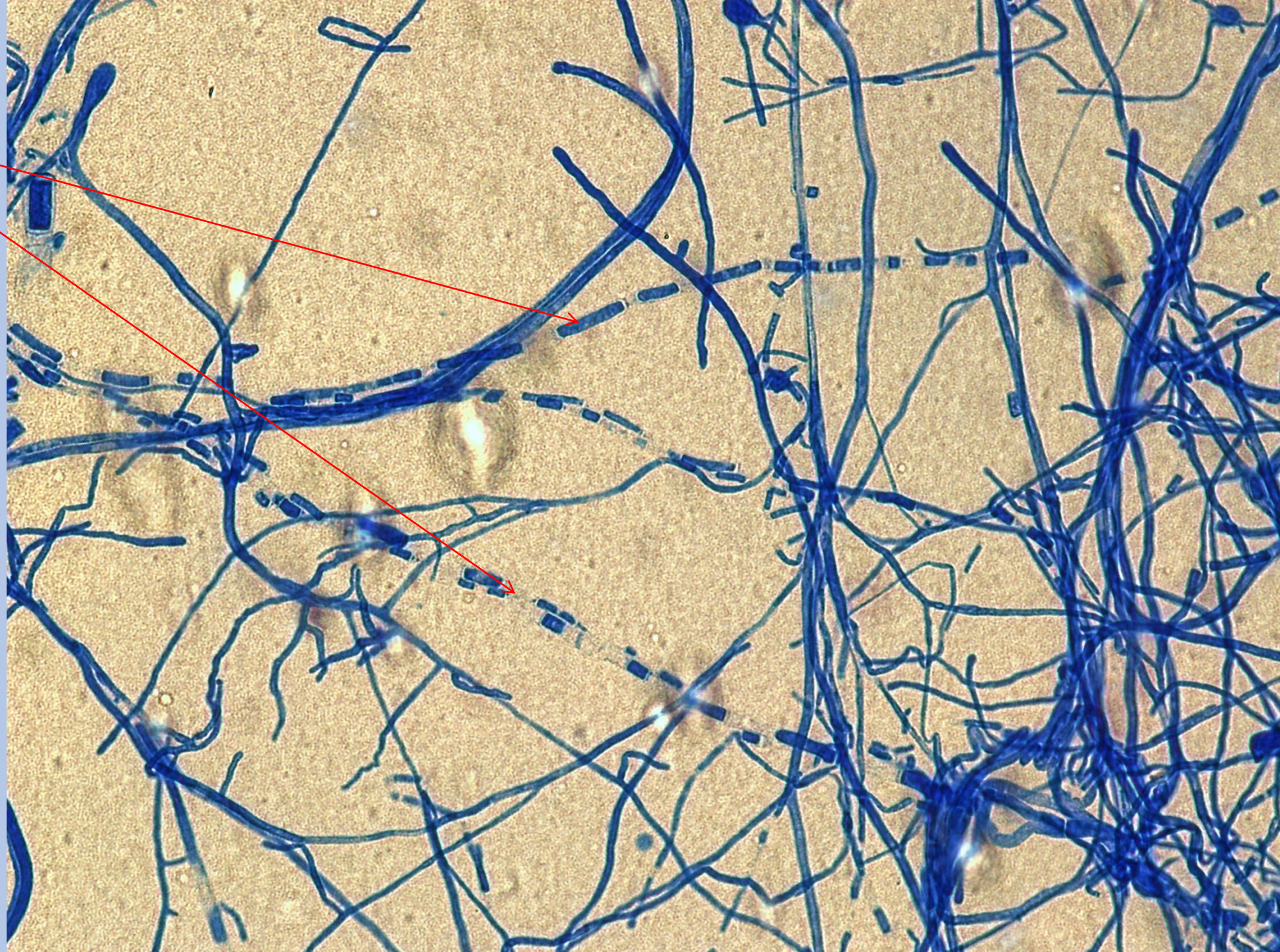
Keratinophilic, keratinolytic agents

Temperature optimum 28 – 30 °C

Invading stratum corneum and keratinized layers of nails and hair
Affections - tinea + anatomical localization

Infectious particles (propagules) – **arthroconidia, hyphae**, transmitted via **fomites** (keratinized layers desquamation)

Formation of arthroconidia



Dermatophytes – ecology, epidemiology

- **Anthropophilic – interhuman transmission, perfect adaptation to human host**

Chronic course, mild inflammatory reaction, long-term and difficult treatment
Infectious particles (propagules) – **arthroconidia, hyphae**, transmitted via **fomites** (keratinized layers desquamation)

Trichophyton rubrum*, Epidermophyton floccosum, Microsporum audouini*

- **Zoophilic – low adaptation to human host**

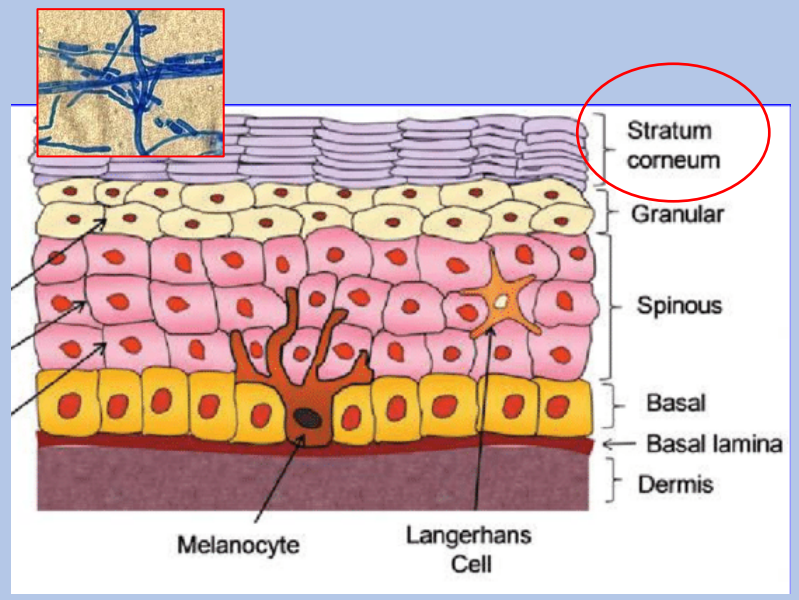
Acute course, severe inflammatory reaction , good and rapid response to treatment

***Trichophyton mentagrophytes* complex**, *Microsporum canis*

- **Geophilic – low adaptation to human host**

Microsporum gypseum, course similar to zoophlic, accidental infection – rather rare occurence

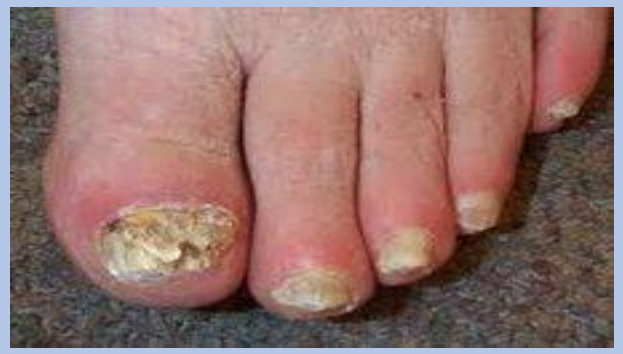
Anthropophilic dermatophytes - transmission



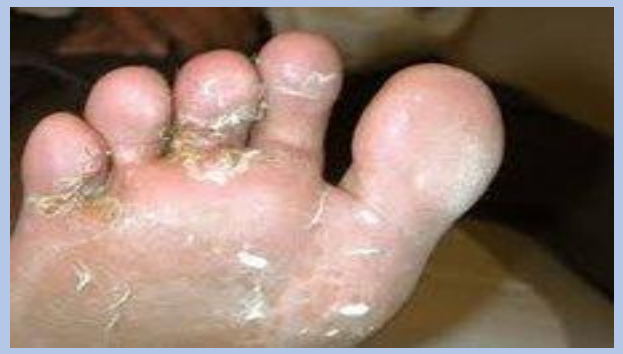
Zoophilic dermatophytes - transmission

Dermatophytosis

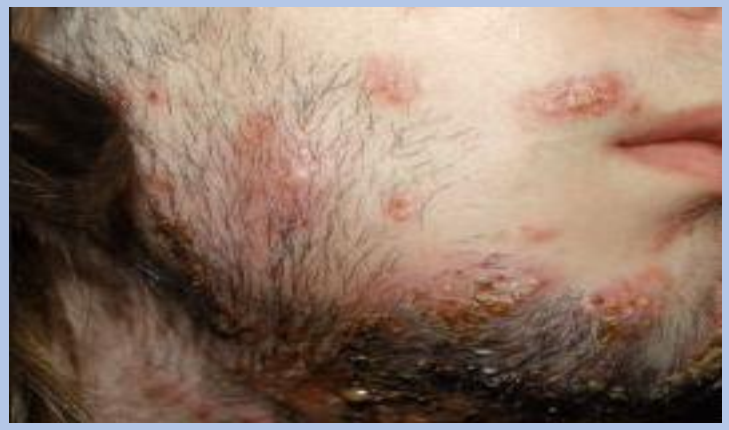
Tinea unguium
dermnentz.org



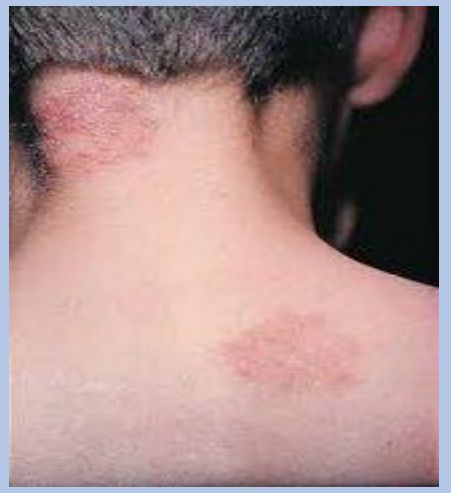
Tinea pedis
natural-health-news.com



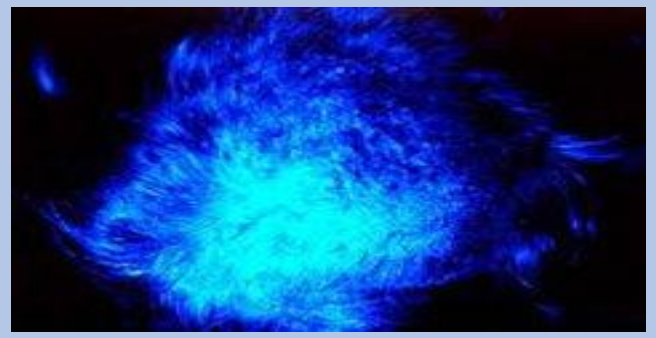
Tinea barbae
By Maddyportelli - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=48807927



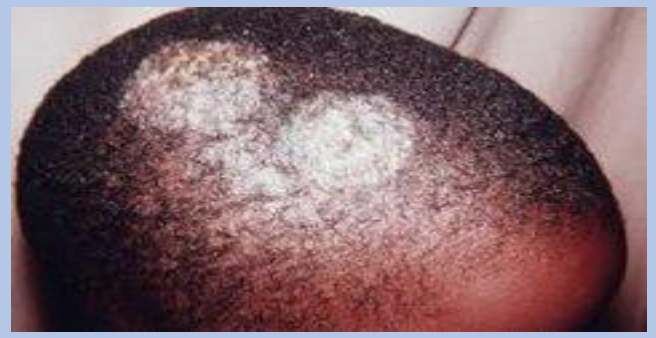
Tinea corporis
sciencedirect.com



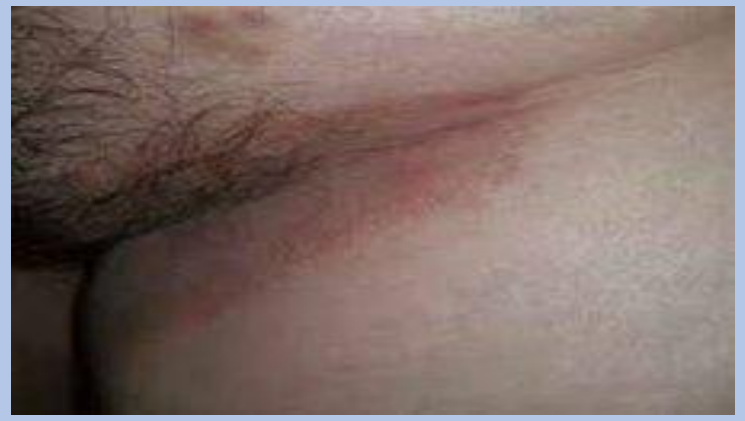
Tinea capitis
Wood lamp
medscape



Tinea capitis
medicinenet.com



Tinea cruris
mitchmedical.us



Dermatophytosis - treatment

Localized lesions, hair and nails not involved- local treatment (imidazoles, terbinafin)

More severe cases - systemic treatment - terbinafin, itraconazole, (griseofulvin)

Dermatomycosis – other fungal organisms

Candida, *Aspergillus*, other hyphomycetes

Opportunistic mycoses

Patients with predisposition – different in different fungal pathogens

Generally – cellular immunity mechanisms impairment, skin and mucosal barriers impairment

Causative agents

- *Candida*
- *Aspergillus*
- *Cryptococcus neoformans,*
- *Pneumocystis jirovecii,*
- zygomycetes

Fungal infections – immune response

Major surgery, burn wounds, major skin and soft tissue wounds, ATB

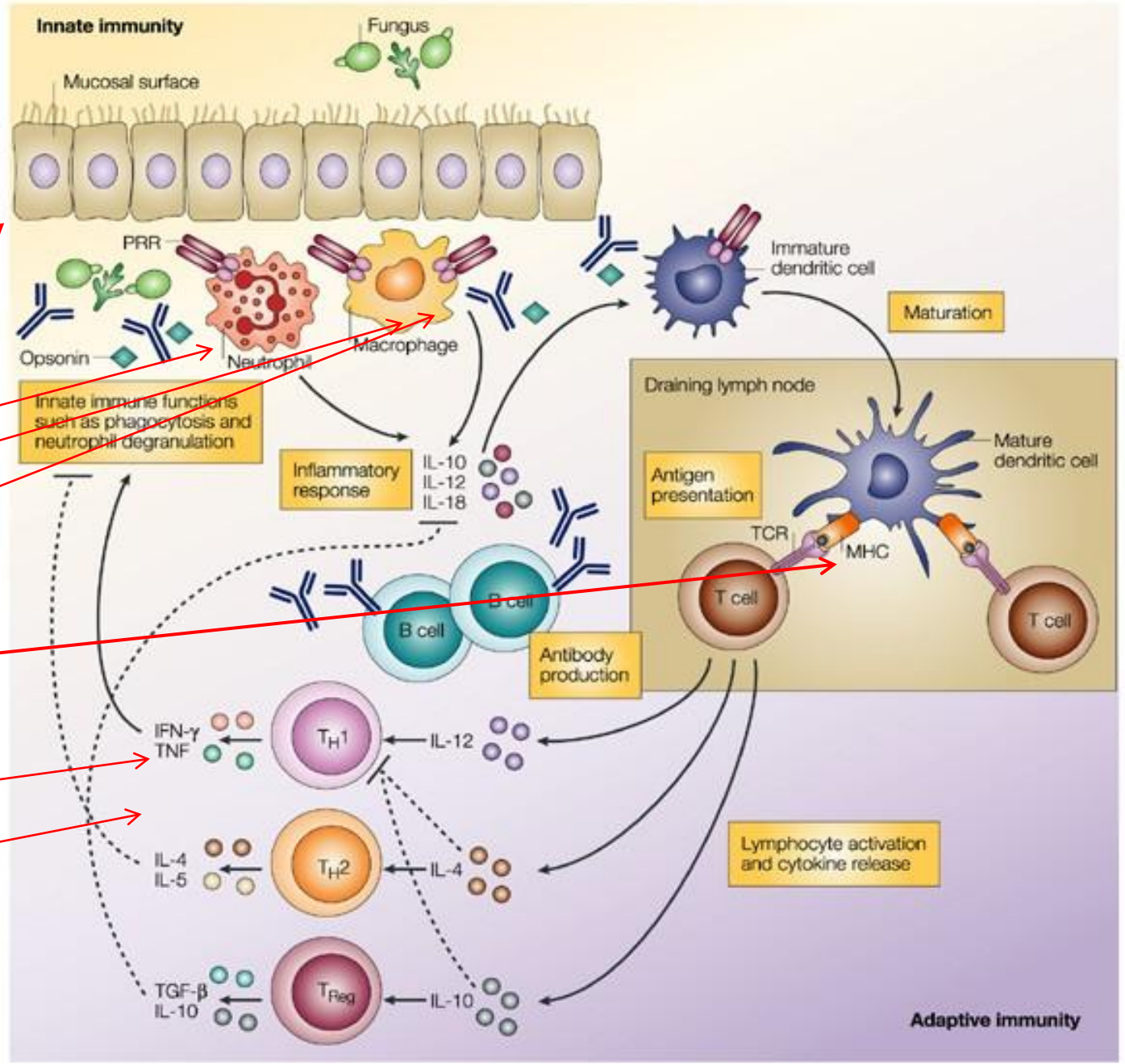
Intravenous catheters

Diabetes mellitus: decreased functions (chemotaxis, phagocytosis, killing) of diabetic polymorphonuclear cells and diabetic monocytes/macrophages compared to cells of controls

Glucocorticoids

Immunosupressants administered after bone marrow and solid organ transplantation or autoimmune diseases

AIDS



Candidiasis

Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida krusei

Natural inhabitants of mucosal and skin surfaces - oral cavity, vagina, GIT, skin - most cases **endogenous**

Superficial, mucosal- thrush, vaginal candidiasis, esophagitis

Risk factors
Antibiotic treatment, diabetes mellitus, AIDS, radiotherapy (head, neck), pregnancy, prosthetic teeth

Local treatment except esophagitis (disinfectants, azoles, polyenes)

Invasive candidiasis

Risk factors

- Immunodeficiency, particularly in cellular imunity, phagocytosis (neutropenia following oncological treatment, bone marrow and solid organ transplantation, corticosteroid administration
- diabetes mellitus
- Major abdominal surgery
- Premature birth
- Intravascular catheters (also exogenous origin of candidiasis)
- Broad-spectrum antibiotic treatment

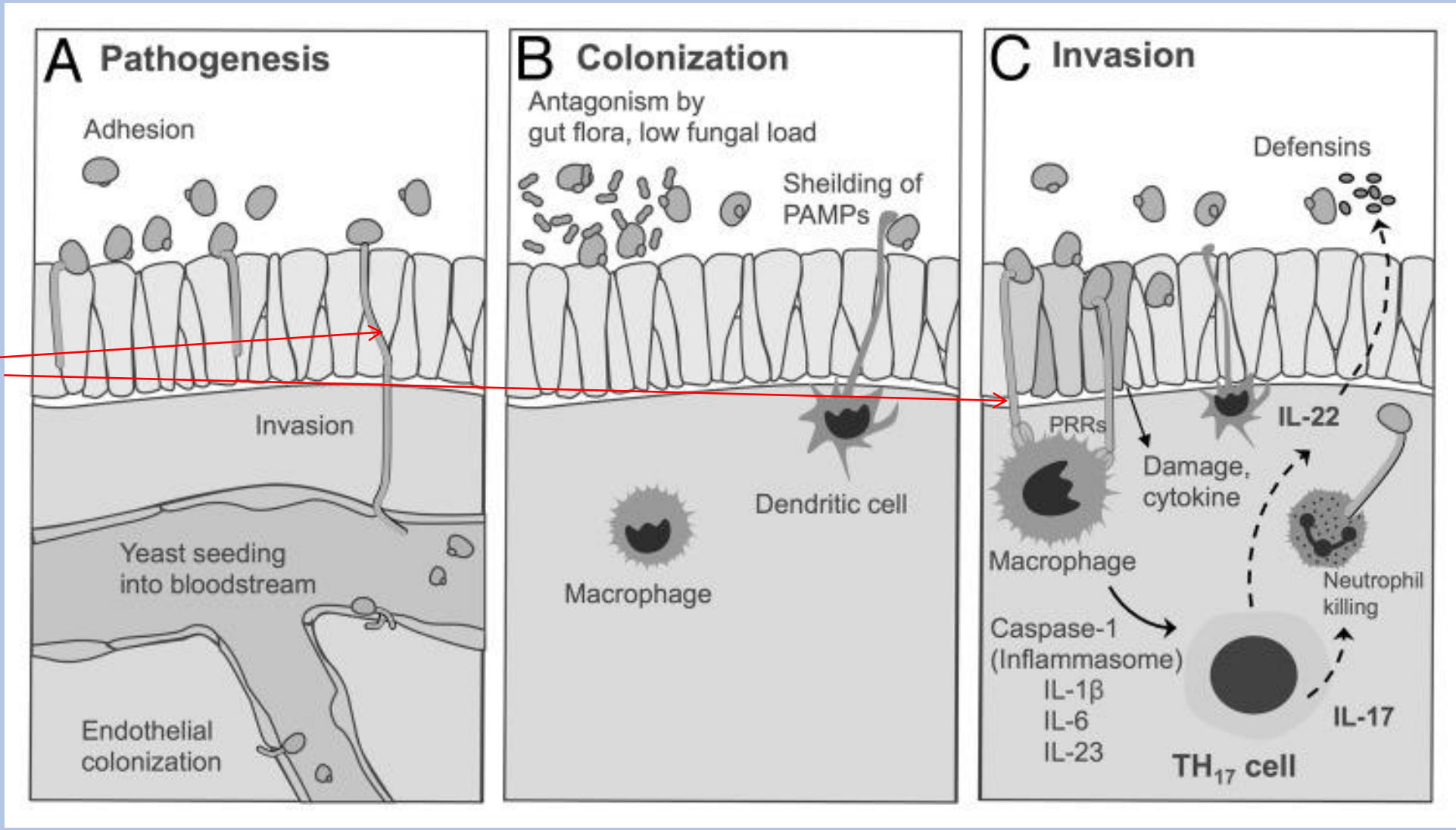
Candida sp. – pathogenicity factors

- Survival and growth in body temperature 37 °C
- Adherence
- Pseudohyphae
- Hydrophobic cell surface
- Mannan in cell wall
- Enzymes - proteases, phospholipases

Pathogenesis – overgrowth on mucosal surfaces, translocation (especially GIT), dissemination (through blood to organs)

Invasive candidiasis - pathogenesis

pseudohyphae



Lewis RE et al. The potential impact of antifungal drug resistance mechanisms on the host immune response to Candida. Virulence 3(4):368-76 · July 2012

Invasive candidasis

Clinical manifestation

Bloodstream infections, peritonitis, urinary tract infections, organ dissemination - liver, spleen, eye, brain, heart (endocarditis, pericarditis), kidneys

Treatment - systemic, **echinocandins – drugs of choice**, amphotericin B+- flucytosine, fluconazole in CNS involvement

Every case of candidemia – examination of heart valves (and eye) indicated, control blood sample collection after treatment is initiated

Reconstitution of hematopoiesis – reduction of immunosupression, growth factor therapy
Source control (surgery, implanted devices removal)

Prophylaxis: fluconazole

Cryptococcus neoformans

Infection source – contaminated soil, dust (bird droppings), **inhalation**

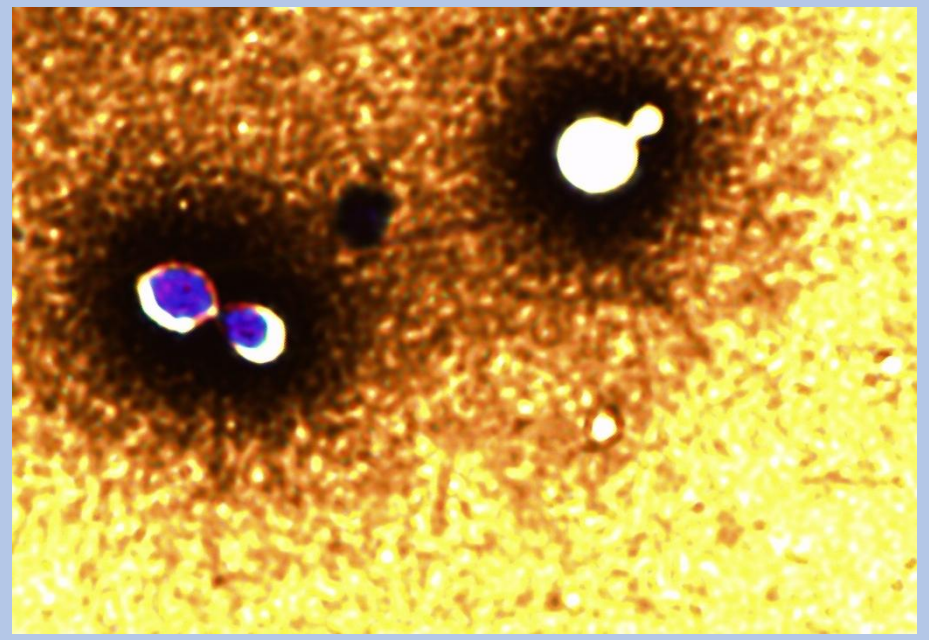
Risk factors
- Cellular immunodeficiency, AIDS

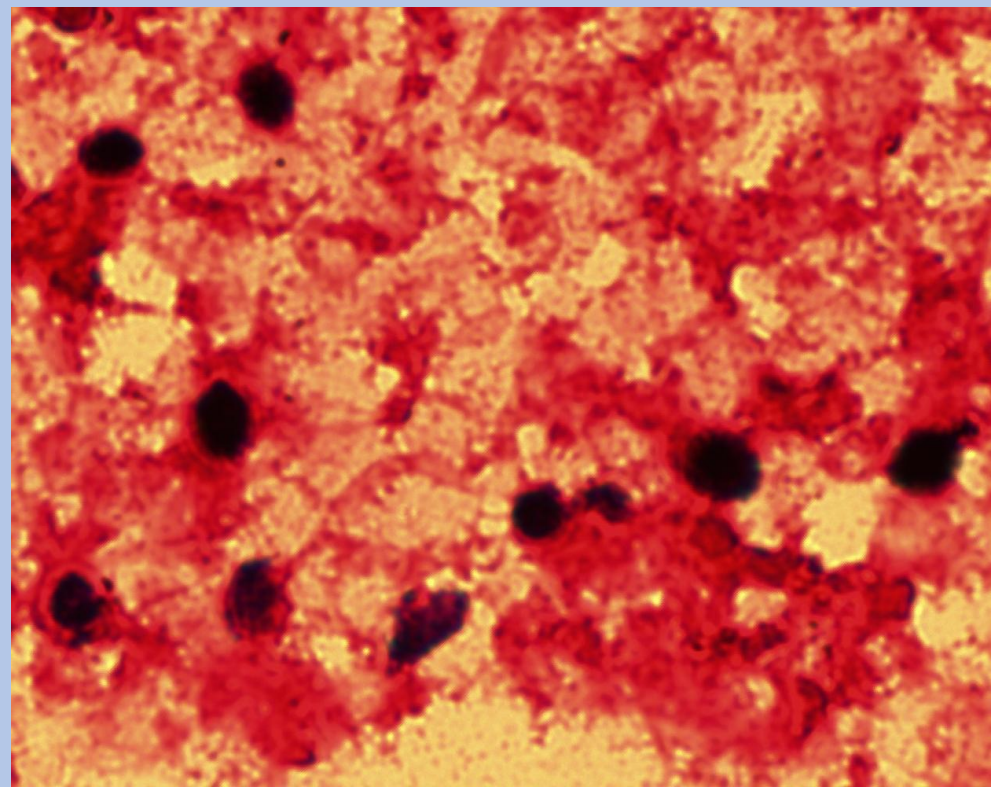
Pathogenicity factors
- Polysaccharide capsule
- Melanin
- Body temperature 37 °C survival and growth

Pathogenesis - after inhalation blastoconidia are engulfed by alveolar macrophages, but they survive, dissemination in macrophages via blood and lymph, predilected localization: CNS

Clinical manifestation: meningoencephalitis, disseminated disease in most cases, subacute/chronic course

Treatment amphotericin B + 5-FC, high-dose fluconazole, long-term



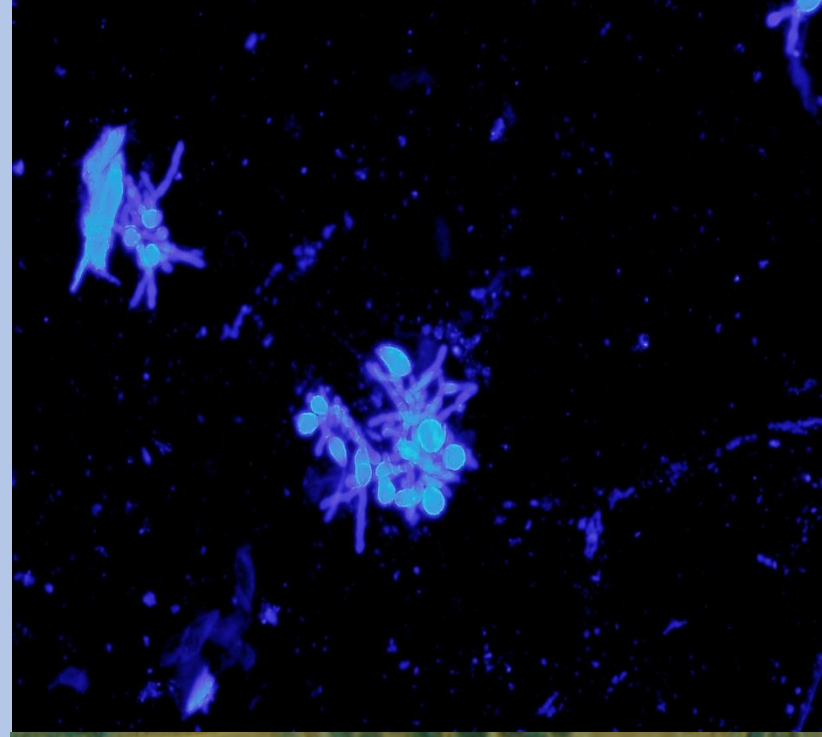


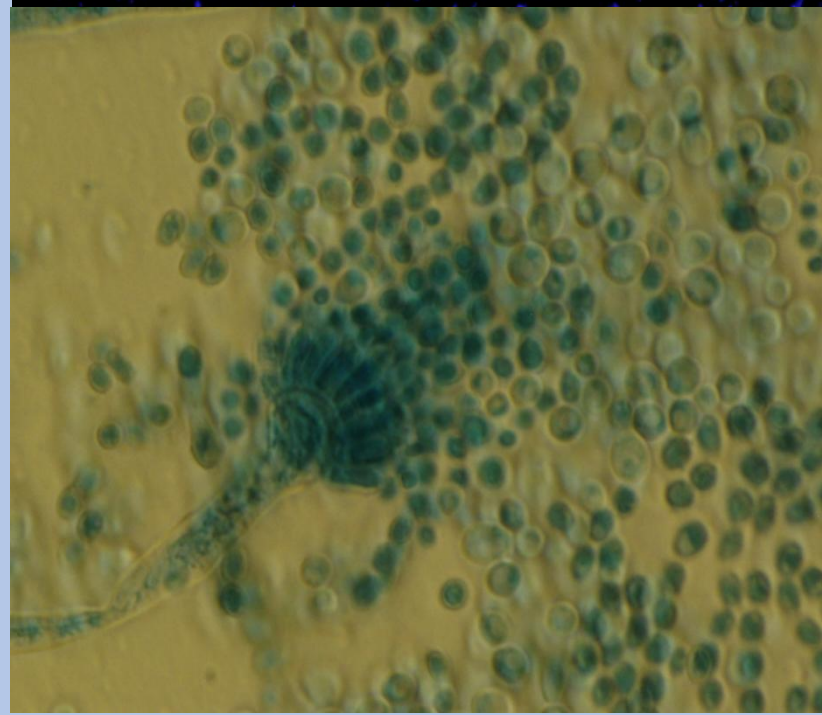
Aspergillosis

A. fumigatus, *A. niger, A. flavus*
Source: environment - soil, pot flowers, food, household (conidia, inhalation)

Risk factors
- Neutropenia (neutrophiles able to destruct conidia)
- T lymphocytes/macrophage impairment (leading to restricted ability to destruct hyphae)
- ICU treatment, mechanical ventilation, lung tissue destruction (viruses – influenza, SARS-CoV2)
- Chronic pulmonary disease

Pathogenicity factors
- Adherence – fibronectin, laminin (conidia)
- Gliotoxin (inhibits macrophage activity and and T- cell proliferation)
- Enzymes - catalase, phospholipase, elastase, proteases





Aspergillosis – clinical forms (depending on host´s condition)

- **Allergic broncopulmonary aspergillosis** - paranasal sinuses or bronchial mucosa colonization followed by allergic reaction

- **Aspergiloma -** previously formed cavity (paranasal sinuses, lungs (tumor necrosis, bronchiectasis, evacuated abscess…), adhesion of inhaled conidia, hyphae formation, finally cavity filled with fungal thallus, no invasion to surrounding tissue

- **Chronic pulmonary aspergillosis –** chronic pulmonary disease, tissue invasion

- **Invasive aspergillosis** – conidia inhalation and adhesion, growing hyphae invade surrounding tissue, angioinvasion, tissue necrosis and destruction, hematogenous dissemination (CNS, heart, GIT, kidneys, liver).

Aspergillosis – clinical manifestation and treatment

Complex diagnostic procedure (history, immunology, imaging, microbiology, histopathology)

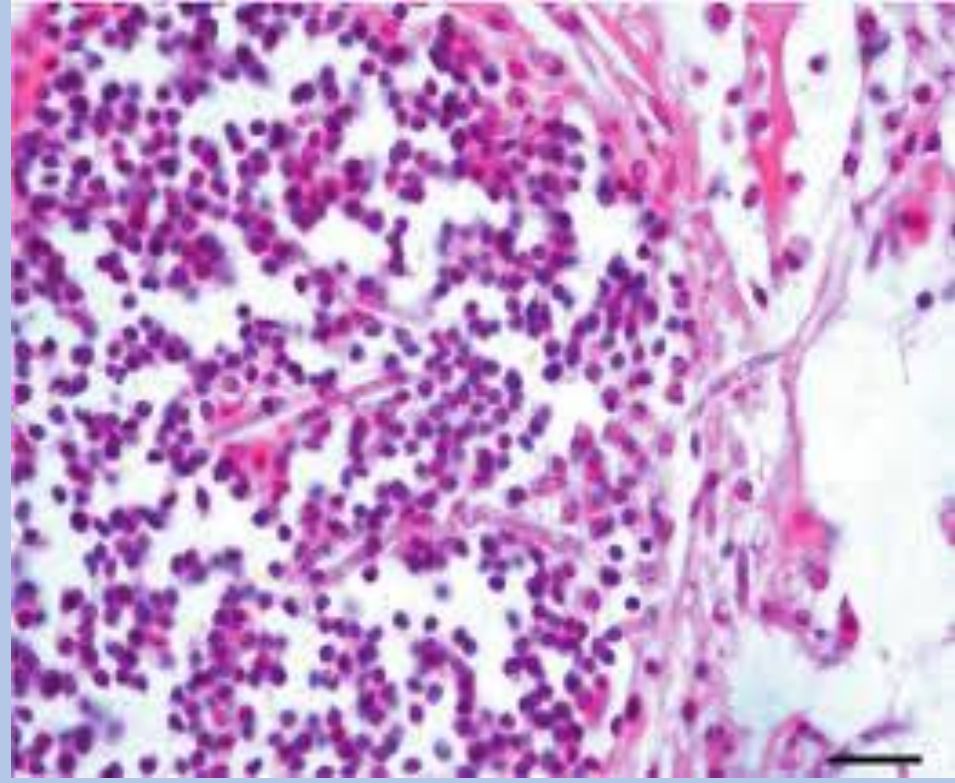
Colonization, bronchial obstruction, allergic bronchopulmonary aspergillosis - cough, asthma

Antiallergic and symptomatic therapy in most cases

Aspergilloma if bleeding is a threat, surgery and antifungal therapy is indicated

Invasive aspergilosis – fever refractory to antibiotic treatment, pulmonary infiltrates, hemoptysis, pleuritis.

Systemic antifungal therapy - **voriconazole, isavuconazole**



Aspergillus - angioinvasion, hematoxylin-eosin, journals.sagepub. com

Mucormycosis (zygomycosis)

Rhizopus, Mucor, Lichtheimia, Rhizomucor – environment (soil), sporangiospores

Risk factors

- Cellular immunity impairment – mainly phagocytosis
- Diabetes mellitus, ketoacidosis
- Renal impairment, hemodialysis, iron chelators administration
- Corticosteroid therapy

Pathogenicity factors

Angioinvasivity – adhaerence to endothelial surfaces (receptor-ligand, up-regulated by glucosis and iron abundance)
Immunomodulation

Mucormycosis - pathogenesis

Sporangiospores are inhaled or contaminate bandages or any wound coverage
Rapid growth and tissue destruction, necrosis and haemorrhage due to angioinvasion

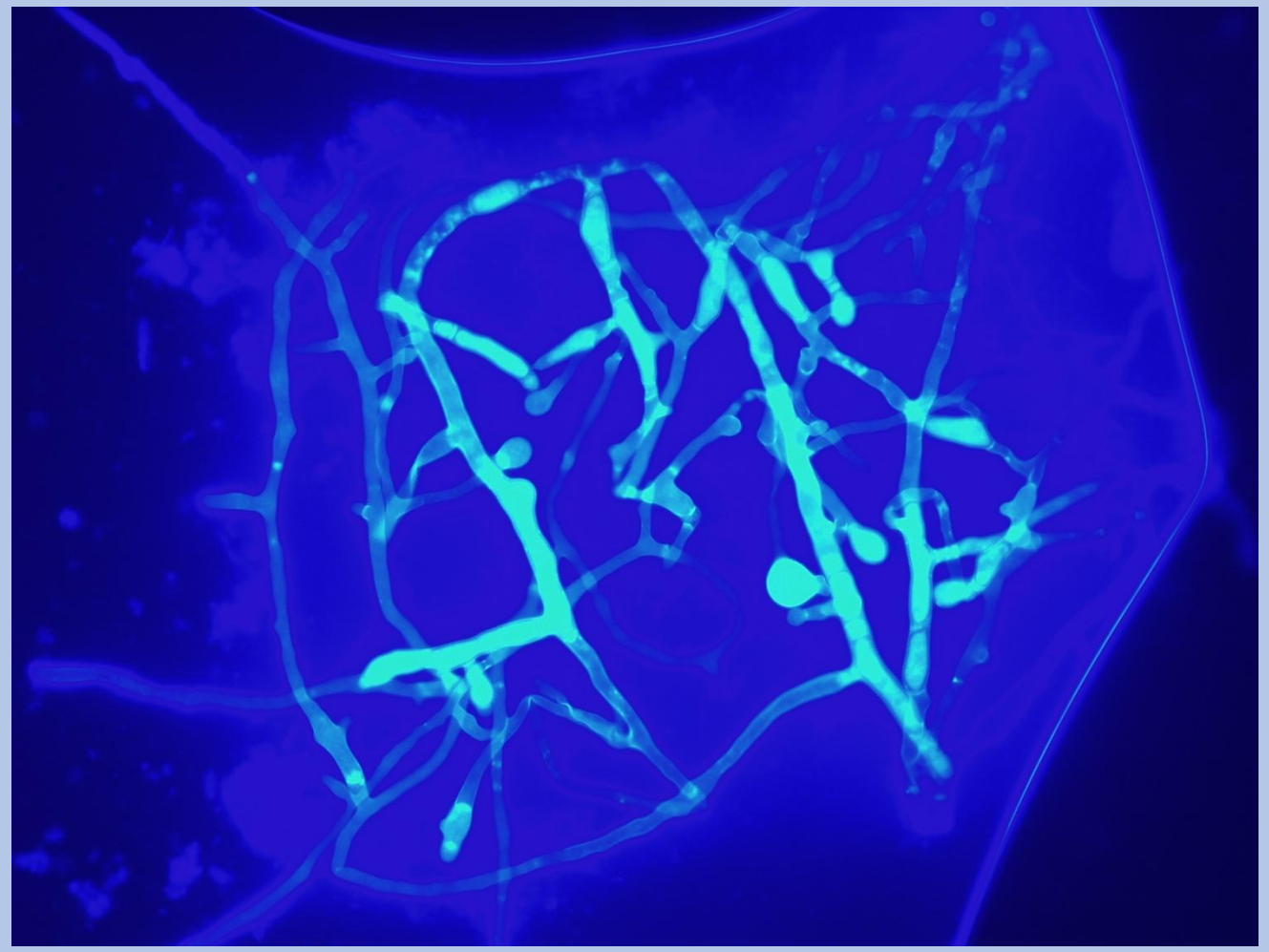
Clinical forms

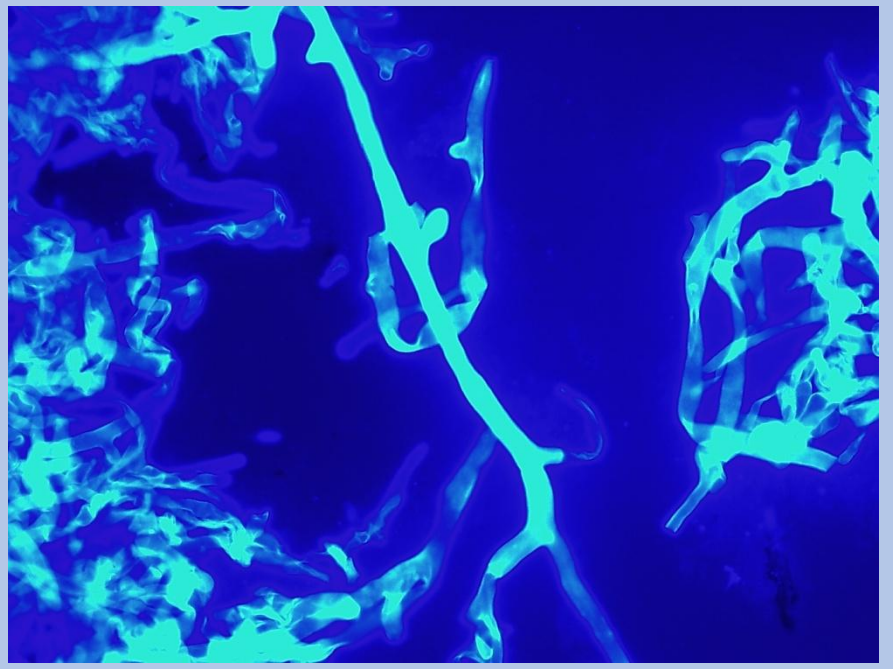
Rhinocerbral – RF diabetic ketoacidosis
Pulmonary – rapidly progressing haemorrhagic-necrotizing pneumonia
Disseminated - angioinvazsion, rapid dissemination, fatal bleeding
Skin (always suspect dissemination!) or posttraumatic wound infection (burn wounds, hurricane and tsunami associated trauma)

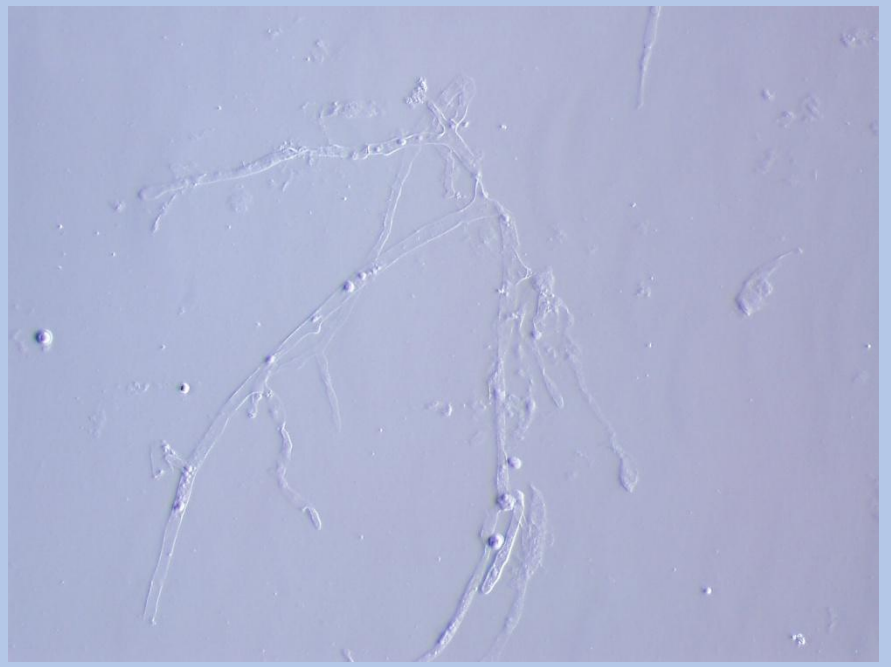
Therapy – surgery whenever possible

Amphotericin B lipid complex, isavuconazole

Mucormycosis, direct microscopy – wet mount, calcofluor white







Pneumocystis jirovecii

Ascomycetous fungus previously classified as protozoon

Both sexual and asexual life cycle occurs in **alveolar tissue**

Risk factors
AIDS
Immaturity, premature birth
Cellular immunity impairment

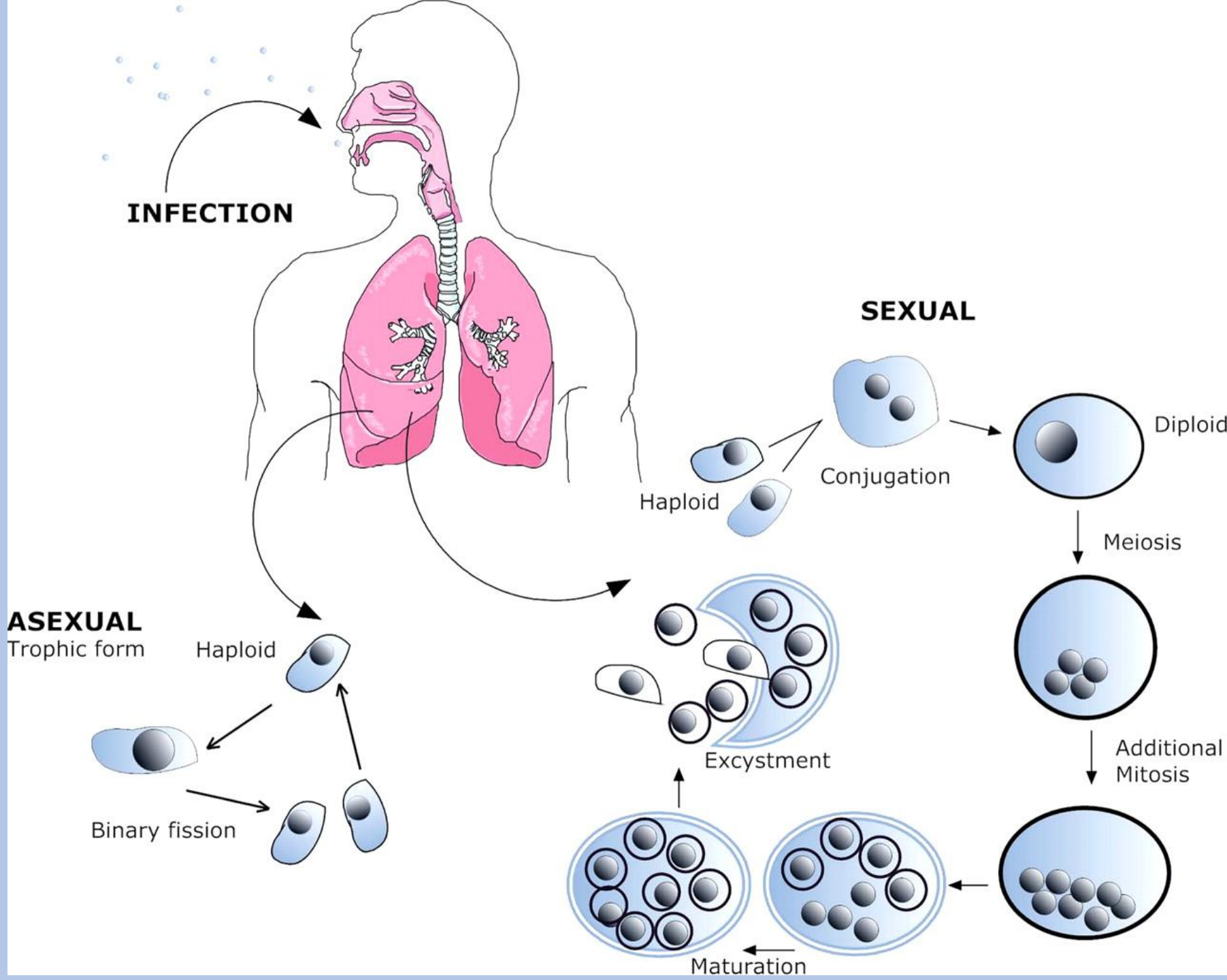
Clinical manifestation - interstitial pneumonia, granulomatous inflammatory process diffuse alveolar damage
Respiratory distress, low oxygen saturation, dyspnea, long-term course with gradual deterioration of lung functions

Co-trimoxazole both fot treatment (high-dose) and prophylaxis (low-dose)

P. jirovecii

Life cycle

Asm.org



Antifungal agents

Local therapy

- Polyenes - nystatin, amfotericin B
- Imidazoles (clotrimazole, econazole, miconazole)
- Allylamines - naftifin, terbinafin
- Griseofulvin

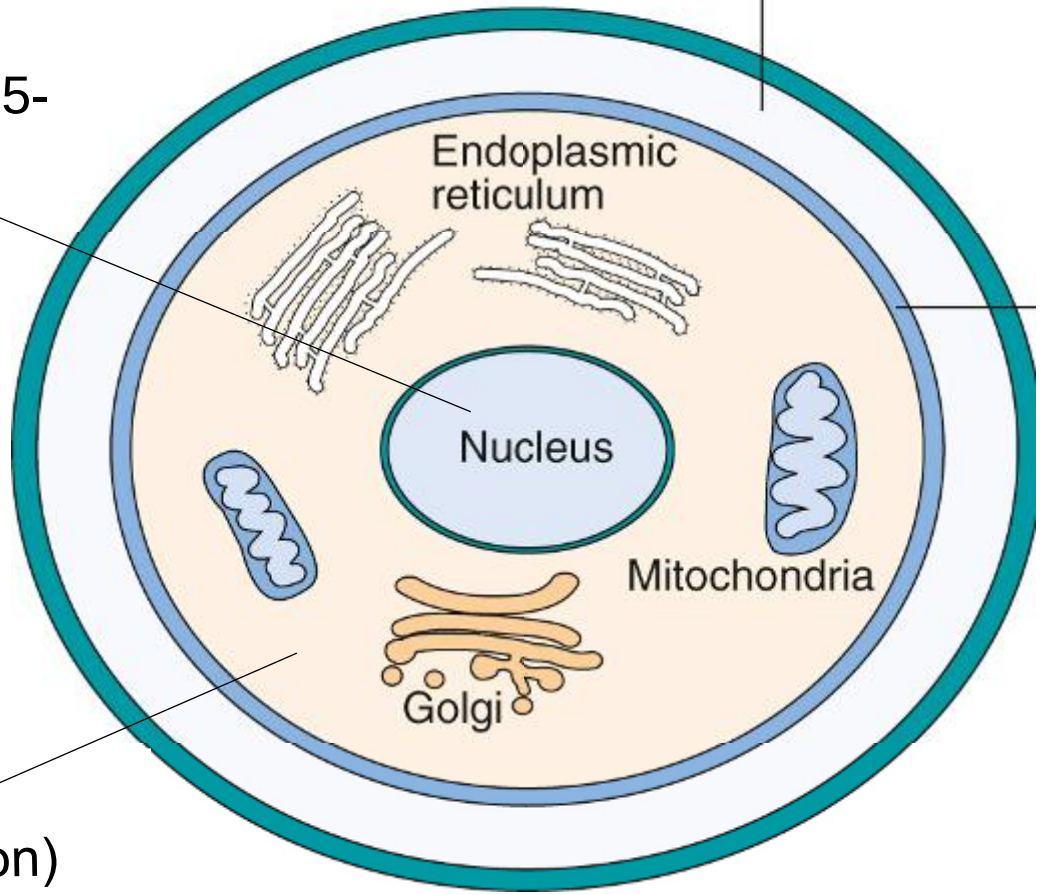
Systemic

- 5-fluorocytosin
- amotericin B
- Griseofulvin
- triazoles
- echinocandins

Antifungal agents mechanism of action

Cell wall – Glucan synthesis inhibition
Echinocandins

DNA and RNA
synthesis inhibition - 5-
fluorocytosin



Cell membrane
Ergosterol synthesis inhibition
azoles, allylamines
Membrane disruption - polyenes

Mitosis inhibition
(microtubule disruption)
Griseofulvin

Antifungals - systemic

Polyenes - Amphotericin B

Mechanism of action

- **Binding to ergosterol**, ion channel formation, osmotic gradient impairment, cell death
- Oxidation cascade, direct membrane destruction

Similarity of cholesterol and ergosterol molecule – polyenes bind also to animal cell membranes – mechanism of **nephrotoxicity**

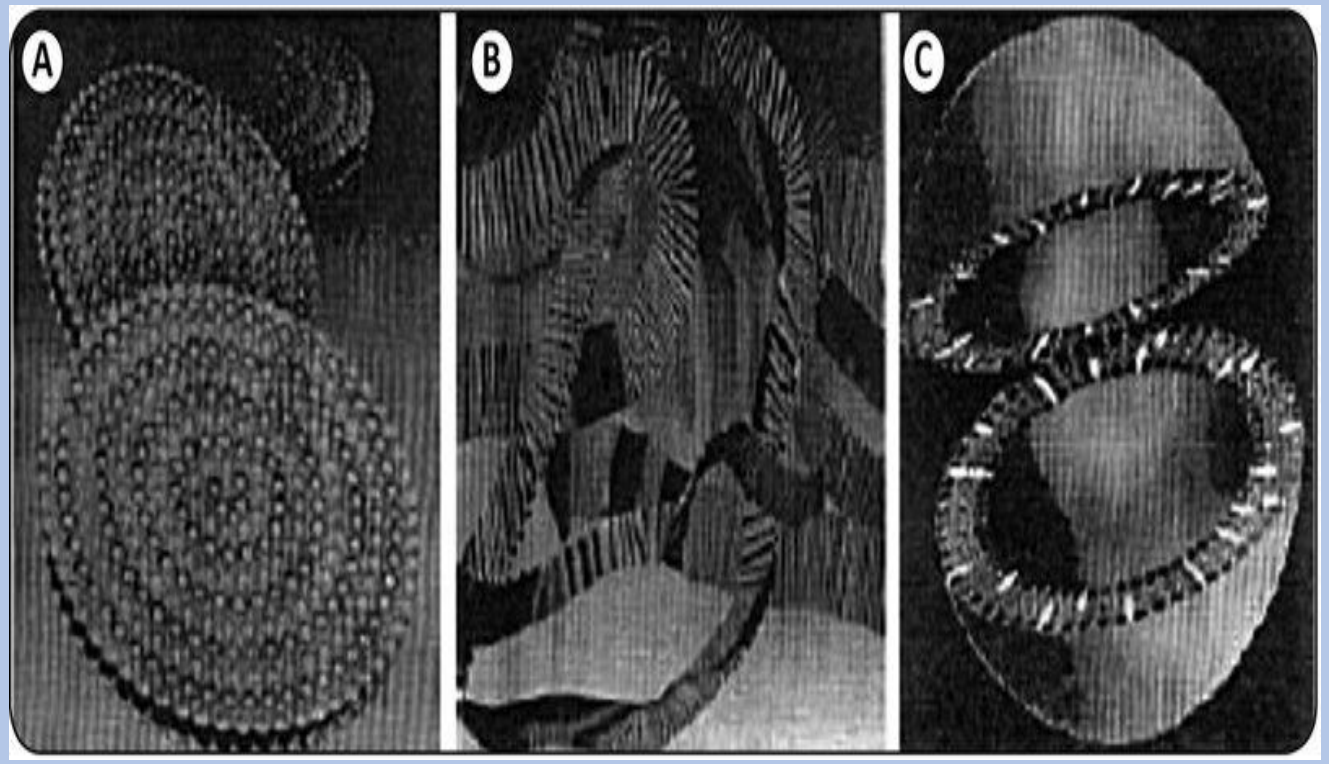
Fungicidal in most fungi aktivita, broad spectrum (yeasts, molds including zygomycetes)

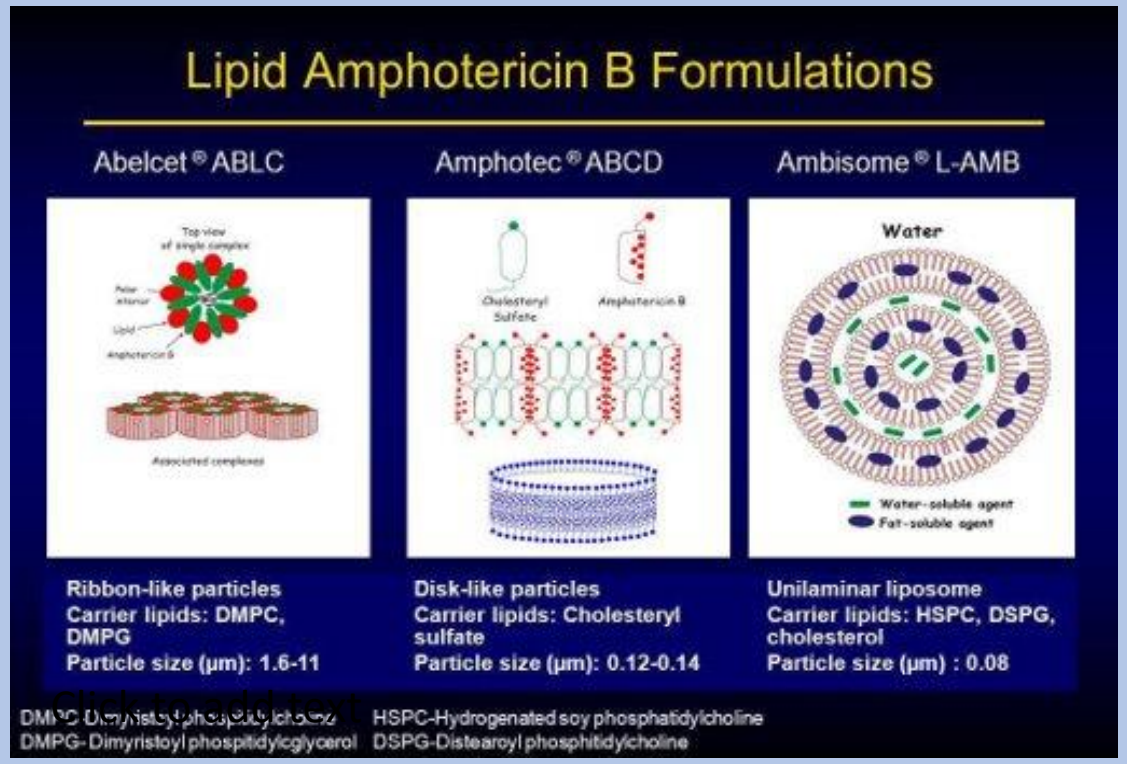
Some *Aspergillus* species and most melanized fungi resistant

Lipid formulations: Liposomal amphotericin B, amphotericin B lipid complex - less severe adverse effects

Acquired resistance rare, target site alteration (membrane composition)

Amphotericin B lipid formulations





SciElo

Antifungals – systemic: Azoles

Imidazoles - ketoconazole only has systemic effect (lipophilic, severe adverse effects- GIT, hepatotoxicity, endocrine system)

Triazoles - lower toxicity, systemic effect, different antifungal spectrum - fluconazole, itraconazole, voriconazole, posaconazole, isavuconazole

Mechanimu of action: **lanosterole 14-alpha-demethylase inhibition,** lanosterole to ergosterole transformation

Fungistatic/fungicidal effect depends on targeted fungal organism

Acquired resistance - target structure (enzyme) mutations, gene expression regulation – overexpression and enzyme overproduction, efflux

Described in *Candida* sp., *Aspergillus fumigatus* (environmental, fungicides – agriculture)

Fluconazole - oral and i.v., good bioavailability and tissue penetration including CNS
Yeasts except *C. krusei, C. glabrata* limited susceptibility, most hyphomycetes resistant
Invasive candidiasis prophylaxis

Itraconazole - oral, lipophilic
Yeasts, aspergilli, zygomycetes resistant
Skin and mucosal candidiasis treatment, dermatophytoses systemic treatment

Voriconazole - oral, i. v., good tissue penetration including CNS
Yeasts, aspergilli, zygomycetes resistant
Invasive aspergillosis – drug of choice

Posaconazole– oral., i. v.,
Yeasts, aspergilli, zygomycetes
Opportunistic mycoses prophylaxis in high-risk patients

Isavuconazole- oral, i. v.
Invasive mucormycosis and aspergillosis

Antifungals systemic - echinocandins

Mechanism of action: **1,3-beta-D-glucan synthesis inhibition** - low toxicity
Yeasts – fungicidal, moulds - fungistatic
1st choice in invasive candidiasis

caspofungin, micafungin, anidulafungin, **rezafungin**, all i. v.
Acquired resistance – target enzyme cascade modification

Antimetabolites: 5-fluorocytosin

Mechanism of action : **DNA and RNA synthesis inhibition**, toxic (hepatotoxicity, inhibits hematopoiesis)
Oral, penetrates to CNS, yeasts including cryptococci, only in combination

Resistance - restricted permeability, restricted penetrance into fungal cell, loss of activity in enzyme transformation of a precursor to active agent

Griseofulvin

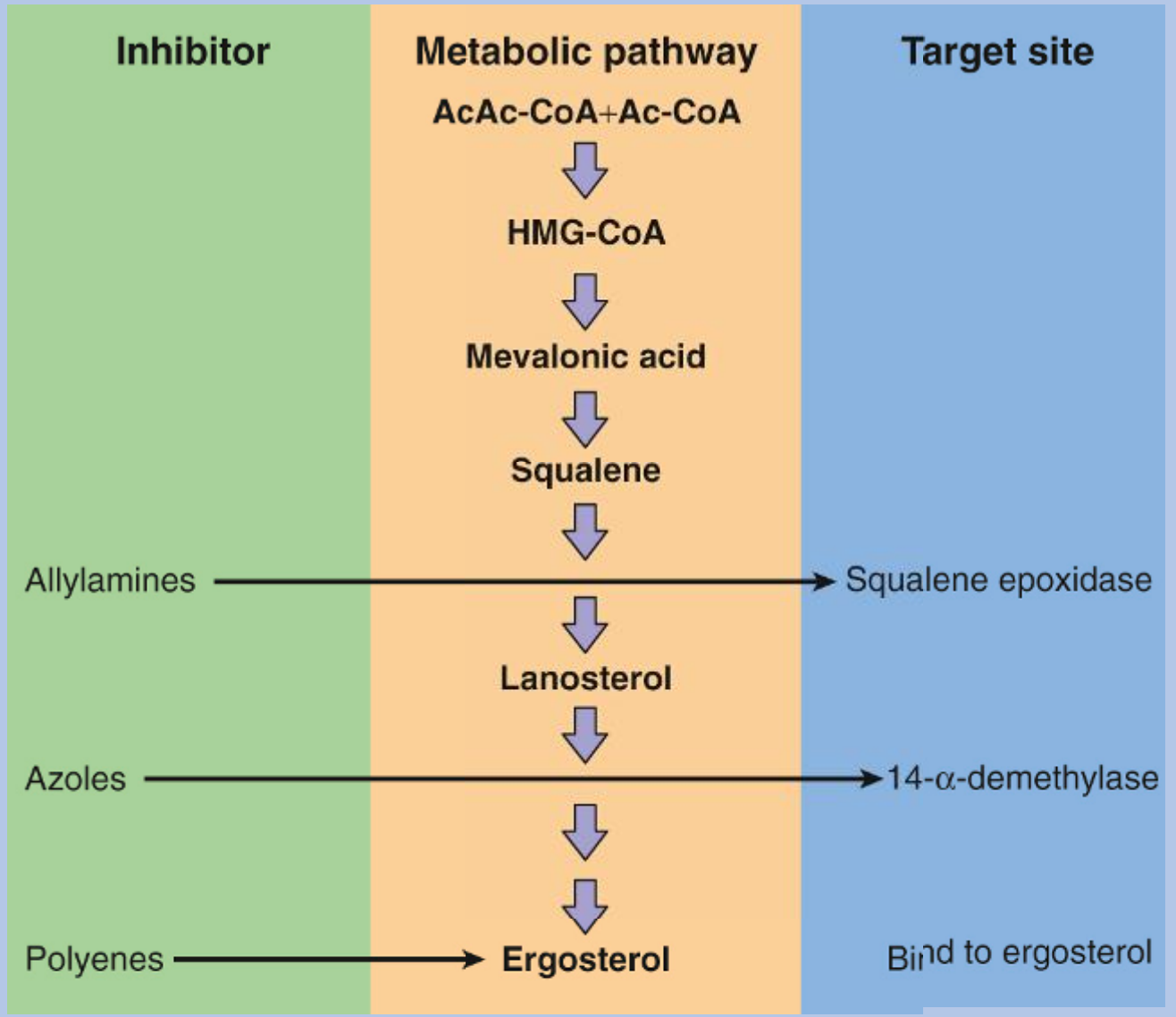
Mechanism of action: Mitosis inhibition via interaction with microtubules
Dermatophytoses – systemic treatment

Allylamines

Mechanism of action

Squalenepoxidase inhibition (step in ergosterole synthesis)

Terbinafine – oral
Lipofphilic, high concentrations in skin, subcutaneous tissue and hair and nails
Dermatophytoses – systemic therapy



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