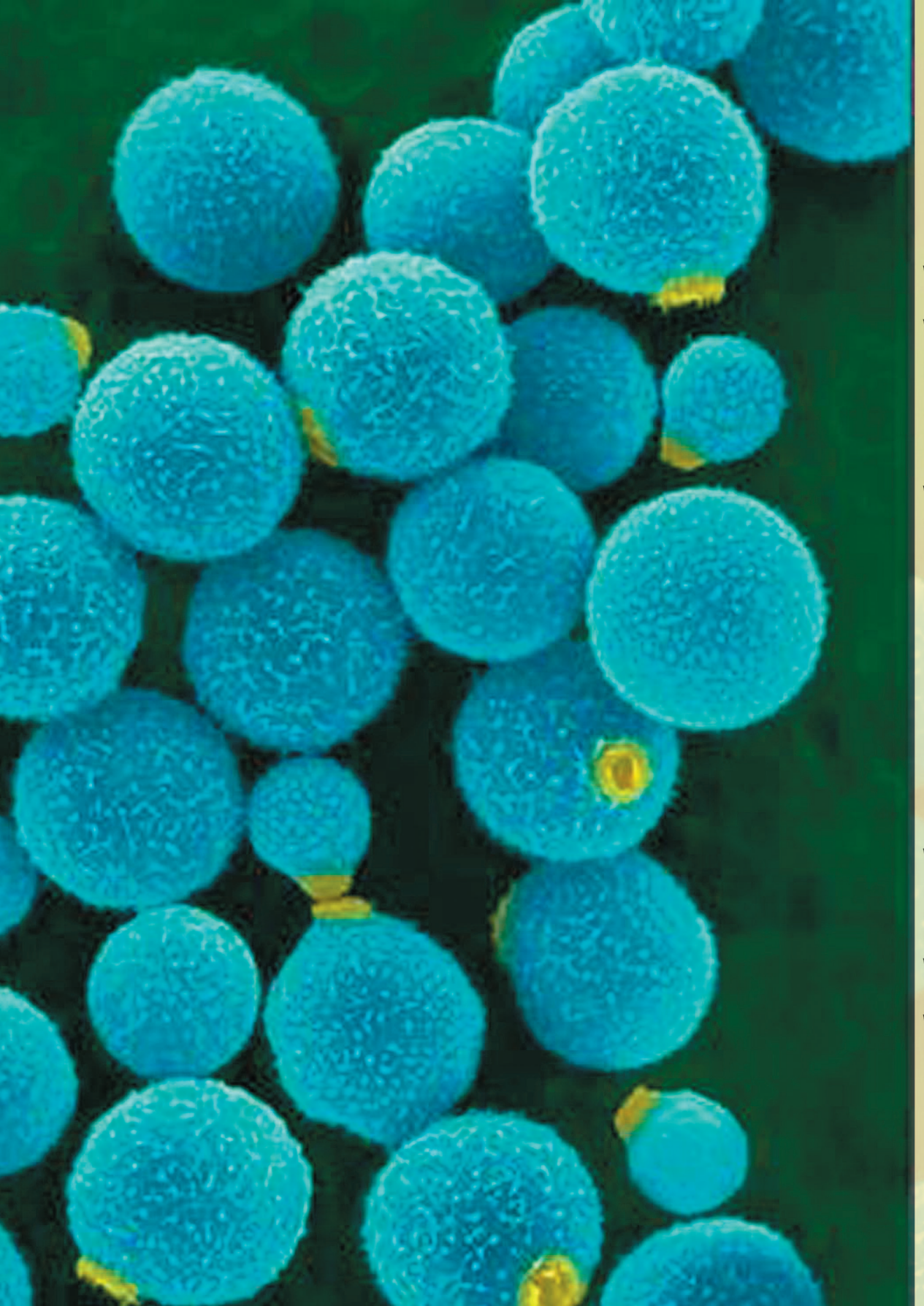
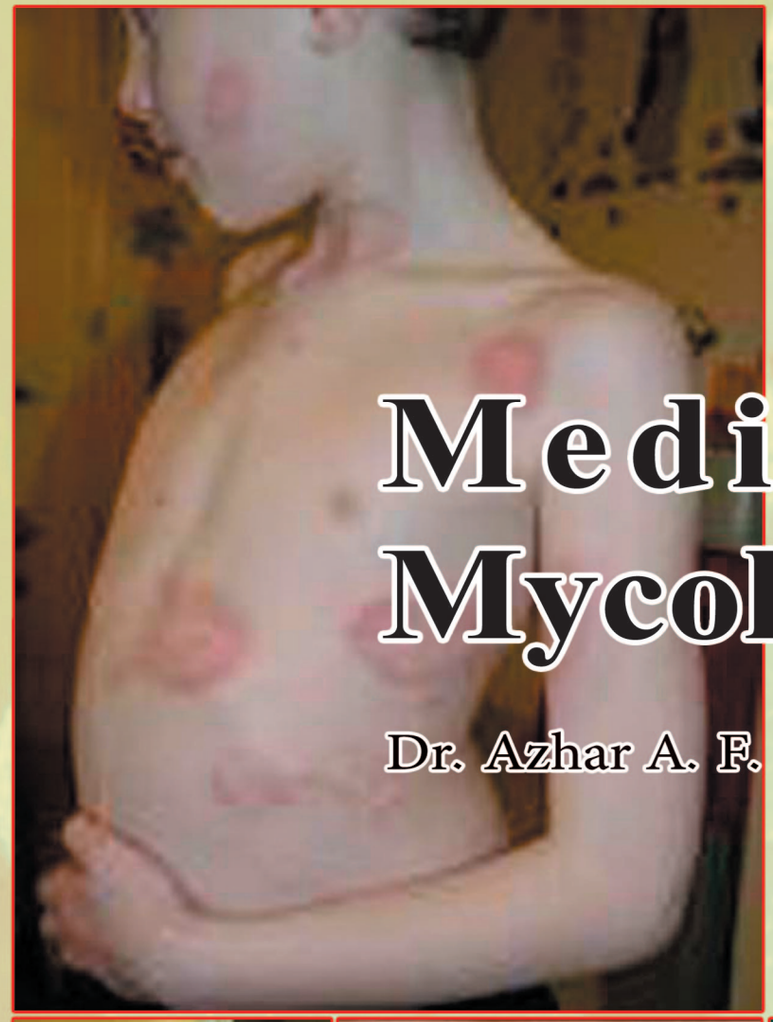


Dr. Azhar A. F. Ibrahim

Medical Mycology



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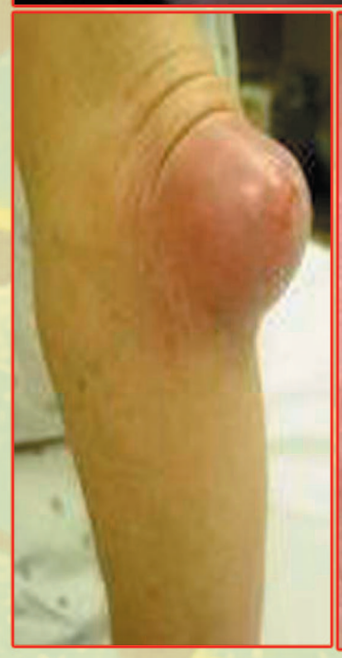

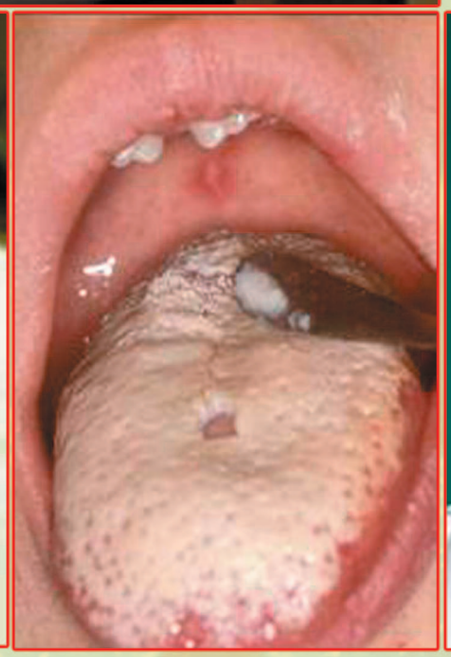

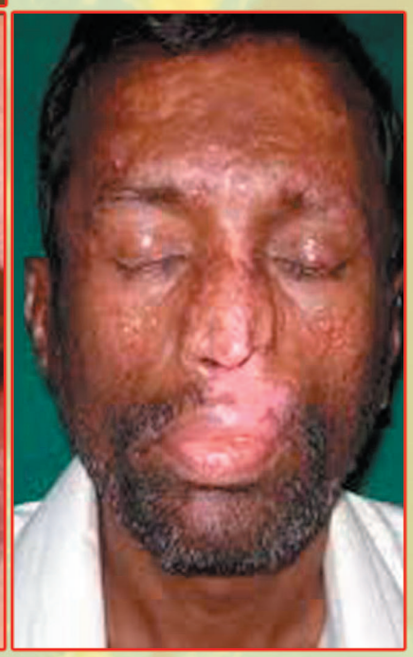

Medical Mycology

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Dedication

To my beloved husband who designed this book

To my beloved son, Mustafa

To my candle of life, beloved mother

To the memory of my darling father

To my dear sisters and brother

With my commitment

Azhar

Contents:

Cutaneous mycosis

Subcutaneous mycosis

Deep Mycosis (Systemic Mycosis)

Fungal opportunistic infections in HIV/AIDS patients

Common opportunistic fungal infections in HIV/AIDS

Mycotoxicosis

Antimycotic drugs

Classes

Preface

A mycosis (plural: Mycoses) is a fungal infection of animals, including humans. Mycoses are common, and a variety of environmental and physiological conditions can contribute to the development of fungal diseases. Inhalation of fungal spores or localized colonization of the skin may initiate persistent infections; therefore, mycoses often start in the lungs or on the skin. People are at risk of fungal infections when they are taking strong antibiotics for a long period of time because antibiotics kill not only damaging bacteria, but healthy bacteria as well. This alters the balance of microorganisms in the mouth, vagina, intestines and other places in the body, and results in an overgrowth of fungus.

Individuals with weakened immune systems are also at risk of developing fungal infections. This is the case of people with HIV/AIDS, people under steroid treatments, and people taking chemotherapy. People with diabetes also tend to develop fungal infections. Very young and very old people, also, are groups at risk.

Superficial mycoses are limited to the outermost layers of the skin and hair. An example of a fungal infection is Tinea versicolor, a fungus infection that commonly affects the skin of young people, especially the chest, back, and upper arms and legs. Tinea versicolor is caused by a fungus that lives in the skin of some adults. It doesn't usually affect the face. This fungus produces spots that are either lighter than the skin or a reddish-brown. This fungus exists in two forms, one of them

causing visible spots. Factors that can cause the fungus to become more visible include high humidity, as well as immune or hormone abnormalities. However, almost all people with this very common condition are healthy.

Cutaneous mycoses extend deeper into the epidermis, and also include invasive hair and nail diseases. These diseases are restricted to the keratinized layers of the skin, hair, and nails. Unlike the superficial mycoses, host immune responses may be evoked, resulting in pathologic changes expressed in the deeper layers of the skin. The organisms that cause these diseases are called dermatophytes. The resulting diseases are often called ringworm (even though there is no worm involved) or tinea. Cutaneous mycoses are caused by Microsporum, Trichophyton, and Epidermophyton fungi, which together comprise 41 species. One common disease is the athlete's foot which most commonly affects men and children before puberty. It is divided in three categories: chronic interdigital athlete's foot, chronic scaly athlete's foot, and acute vesicular athlete's foot.

Subcutaneous mycoses involve the dermis, subcutaneous tissues, muscle, and fascia. These infections are chronic and can be initiated by piercing trauma to the skin, which allows the fungi to enter. These infections are difficult to treat and may require surgical interventions such as debridement.

Systemic mycoses due to primary pathogens originate primarily in the lungs and may spread to many organ systems. Organisms that cause systemic mycoses are

inherently virulent. Generally, primary pathogens that cause systemic mycoses are dimorphic. Systemic mycoses due to opportunistic pathogens are infections of patients with immune deficiencies who would otherwise not be infected. Examples of immunocompromised conditions include AIDS, alteration of normal flora by antibiotics, immunosuppressive therapy, and metastatic cancer. Examples of opportunistic mycoses include Candidiasis, Cryptococcosis and Aspergillosis.

Mycotoxicosis is the disease caused by ingestion of mycotoxins, which are toxins produced by fungi living on or in a plant at harvest time.

Mycotoxins are those secondary metabolites of fungi that have the capacity to impair animal health and productivity. The diverse effects precipitated by these compounds are conventionally considered under the generic term

"mycotoxicosis", and include distinct syndromes as well as non-specific conditions.

This disease is very serious because of the possible severe reactions (including death in some cases). Thankfully, it is also a fairly rare disease.

Some of these toxins are fatal within hours of ingestion, such as ergotamine, produced from ergots from the fungi *Claviceps* spp. This fungi has been found contaminating fresh pasture in Europe.

Antifungal medicines work by either:

- **killing the fungal cells – for example, by affecting a substance in the cell wall, causing the contents of the cell to leak out and the cell to die.**
- **preventing the fungal cells from growing and reproducing.**

Antifungal medicines are used in several ways, depending on specific fungal infection. The main types of antifungal medicines include:

topical antifungals, applied to the skin, hair or nails

oral antifungals, swallowed in capsule, pill or liquid form

intravenous antifungals, injected into bloodstream.

This simple introduction represent general informations about medical mycology. I hope that every interested person will enjoy with.

Assistant professor

Dr. Azhar A. F. Ibrahim

Chapter 1

Cutaneous mycosis

Dermatophytosis

Dermatophytosis or ringworm is a clinical condition caused by fungal infection of the skin in humans, pets such as cats, and domesticated animals such as sheep and cattle. The term "ringworm" is a misnomer, since the condition is caused by fungi of several different species and not by parasitic worms. The fungi that cause parasitic infection (dermatophytes) feed on keratin, the material found in the outer layer of skin, hair, and nails. These fungi thrive on skin that is warm and moist, but may also survive directly on the outsides of hair shafts or in their interiors. In pets, the fungus responsible for the disease survives in skin and on the outer surface of hairs.

It has been estimated that currently up to twenty percent of the population may be infected by ringworm or one of the other dermatophytoses. It is especially common among people who play sports, wrestling in particular. Wrestlers with ringworm may be withheld from competition until their skin condition is deemed non-infectious by the proper authorities.(1)

Misdiagnosis and treatment of ringworm with a topical steroid, a standard treatment of the superficially similar pityriasis rosea, can result in tinea incognito, a condition where ringworm fungus will grow without typical features like a distinctive raised border.

A number of different species of fungi are involved. Dermatophytes of the genera *Trichophyton* and *Microsporum* are the most common causative agents. These fungi attack

various parts of the body and lead to the following conditions. Note that the Latin names are for the conditions (disease patterns), not the agents that cause them. The disease patterns below identify the type of fungus that causes them only in the cases listed:

- **Tinea pedis (athlete's foot) affects the feet**
- **Tinea unguium affects the fingernails and toenails**
- **Tinea corporis affects the arms, legs, and trunk**
- **Tinea cruris (jock itch) affects the groin area**
- **Tinea manuum affects the hands and palm area**
- **Tinea capitis affects the scalp**
- **Tinea barbae affects facial hair**
- **Tinea faciei (face fungus) affects the face.**

I. Tinea pedis:

Ring worm of the feet, particularly of the inter-digital webs and soles, is the most common site among people who wearing shoes.

In the toe webs, scaling, fissuring, maceration and erythema may be associated with an itching or burning sensation.

If bacterial superinfection supervenes, erythema increase and may be associated with edema and pain.

The sole appears hyperkeratotic and often covered with fine scales, fissuring may occur. Causative agent is *Epidermophyton floccusum*. (2). Figure(1,2)

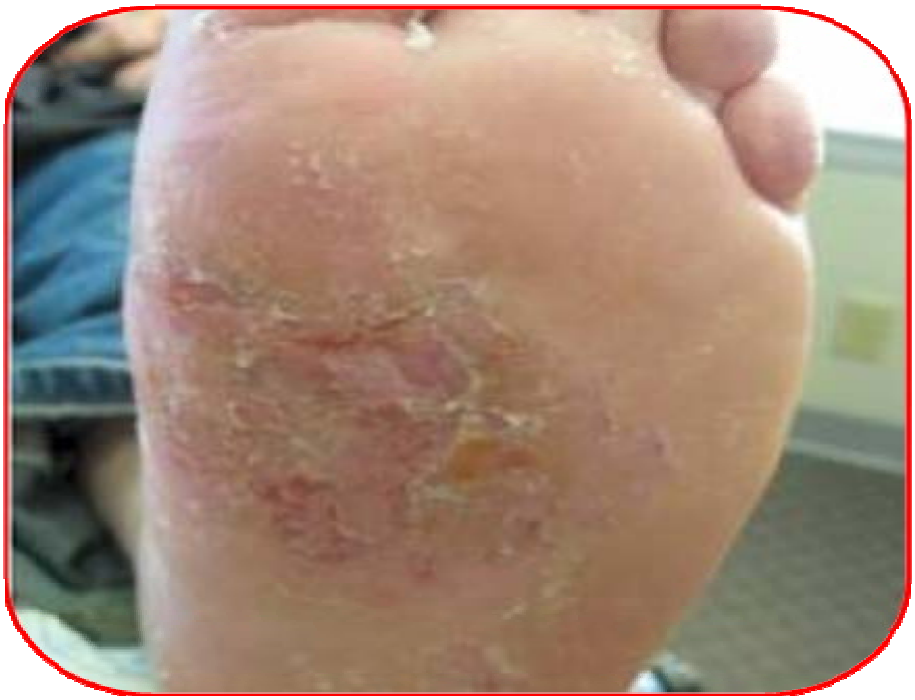


Figure 1: Tinera pedis(myfootshop.com)

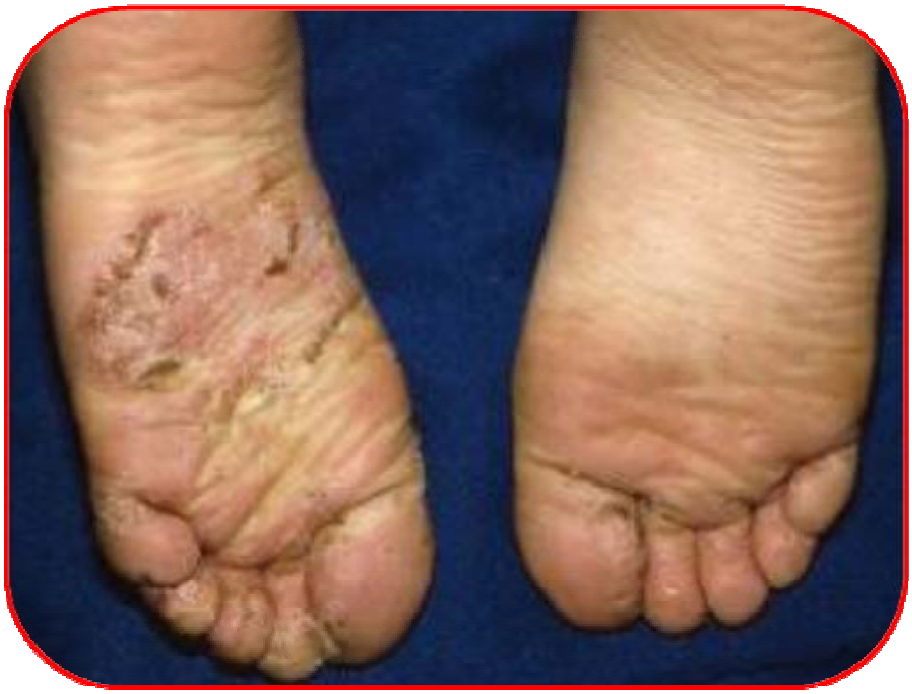


Figure 2: Tinea pedis(fixinfect.co.uk)

II. Tinea capitis:

Infection of the hair shafts distinguishes scalp infection from that on glabrous skin.

***M. audouinii, M. canis, M. equinum and Trichophyton.* spp: *T. rubrum, T. schoeleinii, T. mentagrophytes, T. violacium*, invade hair shafts in a distinctive manner. Infected hair appear dull gray.**

Some hairs are broken off a few millimeters above the follicle orifice, creating the appearance of partial alopecia.

Endothrix means infection of hyphea forming arthrospores within the hair shaft, it caused by *Trichophyton* spp.

Ectothrix means no invasion of the hair shaft by *Microsporum* spp. Figure (3, 4).

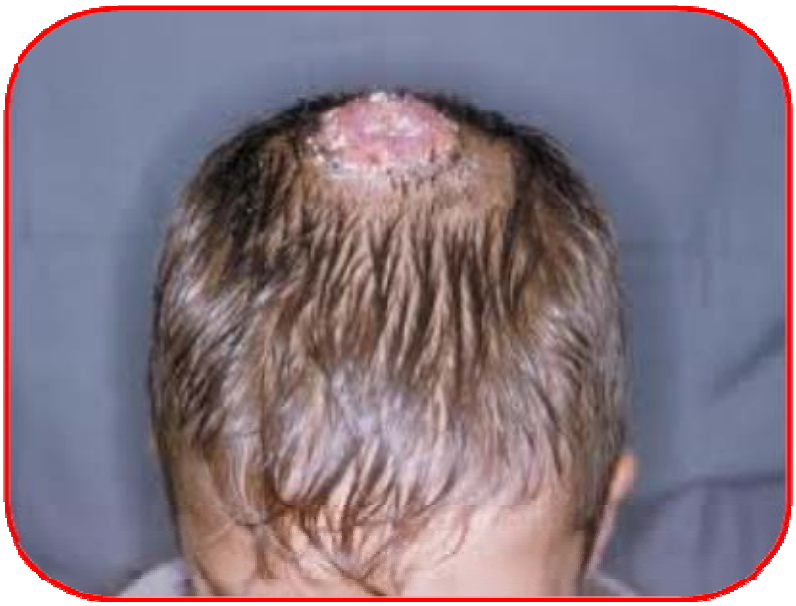


Figure 3: Tinea capitis
(turks-doctor.blogspot)

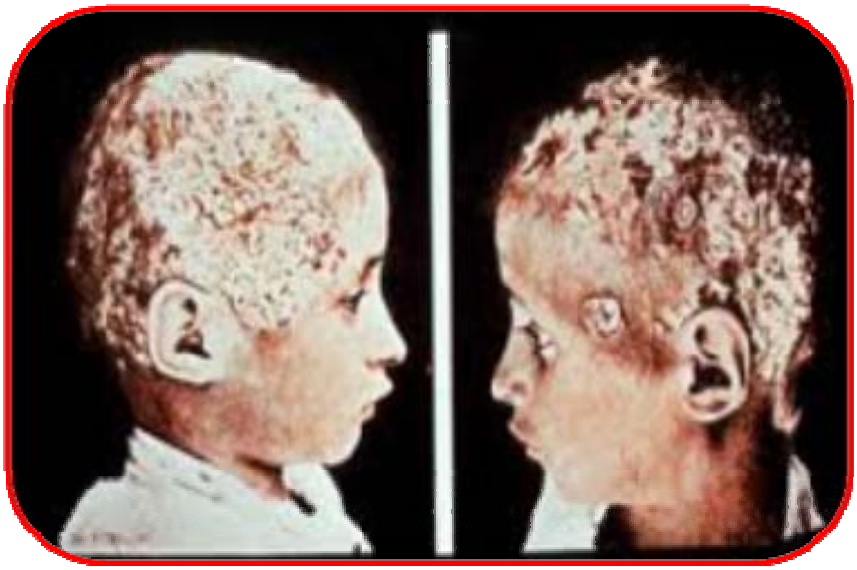


Figure 4: Tinea favusa
(doctorfungus.org)

III. Tinea barbae:

Ring worm of the bearded area of men, is caused by species of *Microsporum*, or *Trichophyton*. Erythematous patches of the face and neck show scaling, fragile, lusterless hairs and tendency to folliculitis. Figure(5)

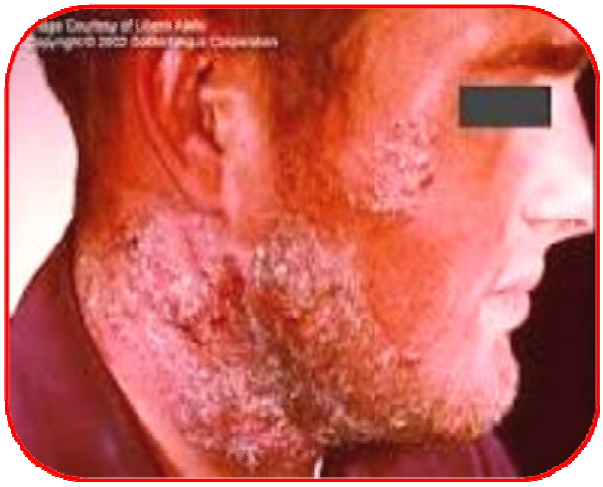


Figure 5: Tinea barbae(healthmad.com)

IV. Tinea corporis:

Infection of the body may results from extension of infection in the scalp, groin, or beard, but the term tinea corporis is used mainly for lesions of glabrous skin. Lesions are annular, sharply marginated, have a raised border, and may a single, multiple or confluent.

Infection of hair follicles can lead to a deep dermal inflammatory reaction lead to a pustular well-circumscribed, elevated crusted lesions referred to as Majocchi's granuloma. Figure(6).

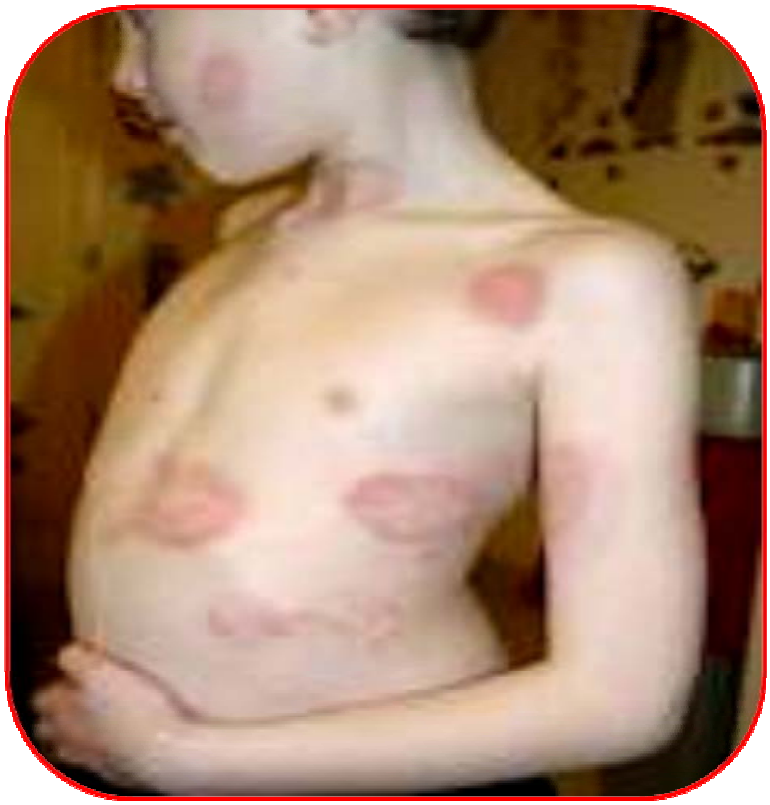


Figure 6: Tinea coprporis. Multiple lesions covered different areas of a child body (hxbenefit.com)

V. Tinea cruris:

Infection of the groin is more common in men, involved the perineum, scrotum, and perianal area. Other sites are under pendulous breasts, in the axilla, and around the ambilicus of obese pts.

Erythema of the entire area is common, some times with vesicles, with itching and burning. Chronic lesions are less erythematous, ans hyperpigmented. Figure(7)

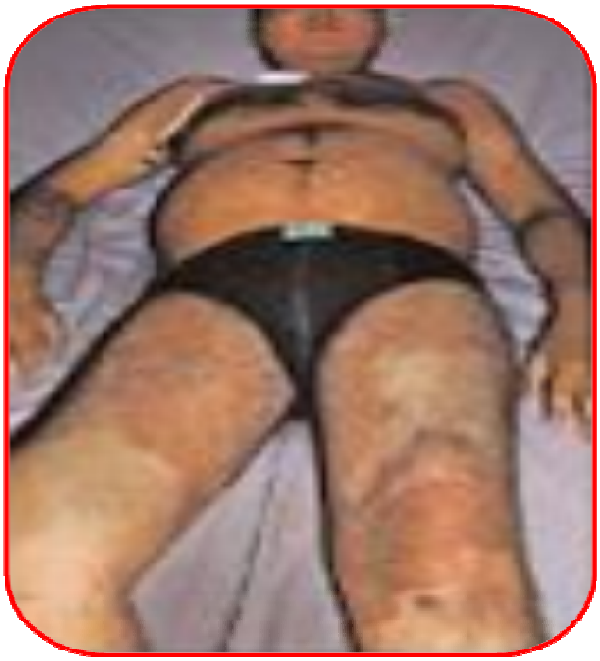


Figure 7
Tinea cruris, note extension to the thigh (medkaav.com)

VI. Tinea unguium:

Ring worm is the most common cause of onychomycosis. Infection begins under the leading edge of the nail or a long the lateral borders and may continue until the entire nail and its bed is infected.

Infected nails is usually opaque, chalky or yellow and thick, with loosing of distal ends. Figure(8)

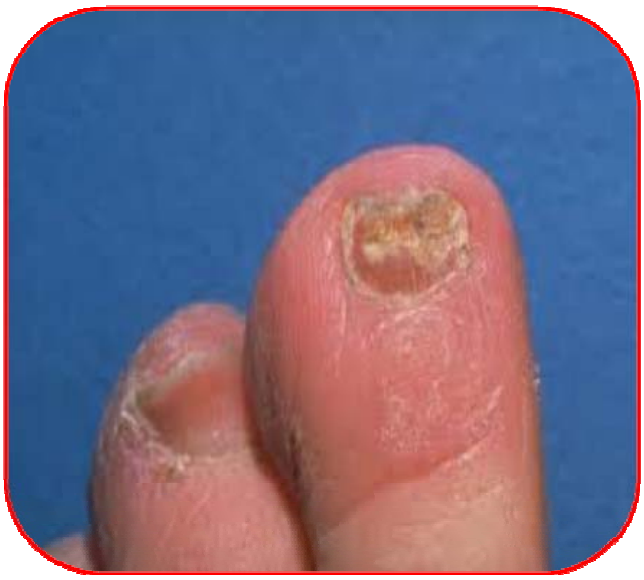


Figure 8
Tinea unguium (familymedicine-mehelp.com)

VII. Tinea imbricate:

Is an unusual form of tinea corporis, caused by *Trichophyton concentricum*. Concentric rings of scaling spread out peripherally over many years, creating a distinctive appearance.

Prevention:

Advice often given includes:

***Avoid sharing clothing, sports equipment, towels, or sheets.**

***Washing clothes in hot water with fungicidal soap after suspected exposure to ringworm.**

***Avoid walking barefoot, instead wear appropriate protective shoes in locker rooms and sandals at the beach. (3,4, 5)**

***After being exposed to places where the potential of being infected is great, one should wash with an antibacterial and anti-fungal soap or one that contains tea tree oil, which contains terpinen-4-ol. (6,7)**

***Avoid touching pets with bald spots as they are often carriers of the fungus.**

Treatment:

Antifungal treatments include topical agents such as miconazole, terbinafine, clotrimazole, ketoconazole, or tolnaftate applied twice daily until symptoms resolve — usually within one or two weeks. (8) Topical treatments should then be continued for a further 7 days after resolution of visible symptoms to prevent recurrence. (8,9) The total duration of treatment is therefore generally two weeks. (10,11) but may be as long as three.

In more severe cases or where there is scalp ringworm, systemic treatment with oral medications may be given.

To prevent spreading the infection, lesions should not be touched, and good hygiene maintained with washing of hands and the body.

Laboratory diagnosis:

Direct examination:

Diagnosis of dermatophytes can be riadly made with KOH preparations of the infected specimens such as hair stumbs, skin, and nail scrapings.

In tinea capitis caused by *Microsporum* spp., a gray, dusty lesions is often seen because the infected hair breaks 1-2 mm above the scalp surface and the hair stub is covered with a mass of arthrospores.

Wood's lamp (U.V) may be used to detect the invaded hairs: e.g. *M. audouinii*, *M. canis* show a green fluorescence.

For microscopic examination a few hair stubs are pulled out with fine foreceps, placed on a slide with a drop of KOH (10%) and covered with a cover slip.

In order to observe the true position of the fungus (ectothrix or endothrix), the outer walls of the hair should not be damaged during this preparation. The slide can be gently heated before examination.

In tinea pedis, the white macerated skin from the interdigital space should be cleaned by rubbing it with a gause spong with 70% alcohol before stripping the epidermal scales from the edge of the lesion.

The epidermal scales from tinea corporis can be processed similarly, except it is not necessary to discard the superficial material before obtaining the scales.

In the case of tinea unguium, the upper portion of the infected nail is scraped away before obtaining thin specimens from deeper layers of nails.

Direct culture:

Cleanse dermal and nail lesions with 70% alcohol, remove the specimens, and place them directly on an agar medium(Sabouraud's agar) containing cyclohexamide and chloramphenicol.

The use of medium containing gentamycin is recommended for specimens heavily contaminated with bacteria.

The dermatophytes test medium (DTM) is widely used in the lab. when the growth turn the medium red by producing alkaline PH. Incubation of culture is at 25-30^{o}C for at least 4 weeks.

Keratomycosis (Mycotic keratitis)

A fungal keratitis is an 'inflammation of the eye's cornea' (called keratitis) that results from infection by a fungal organism. Keratomycosis is the Greek terminology equivalent of fungal keratitis - it is the fungal infection of the cornea, the anterior part of the eye which covers the pupil. Those experiencing these symptoms are typically advised to immediately visit the appropriate eye care professional.

Causes:

Filamentous fungi:

- ***Aspergillus fumigatus***
- ***Fusarium* spp.**

Yeasts:

- ***Candida* spp. (12,13)**

Clinical manifestations:

A foreign-body sensation in the eye, with burning, stinging discomfort. Over several days, pains become more intense, along with photophobia.

The eye becomes red and vision is blurred. Examination at this time reveals a break in the corneal epithelium and in Bowman's mem., usually grossly obvious as a corneal ulcer.

Untreated, the disease progresses to corneal perforation and loss of the eye. The periocular tissues are not invaded, but the blind eye requires enucleation because of the pain. Figure(9).

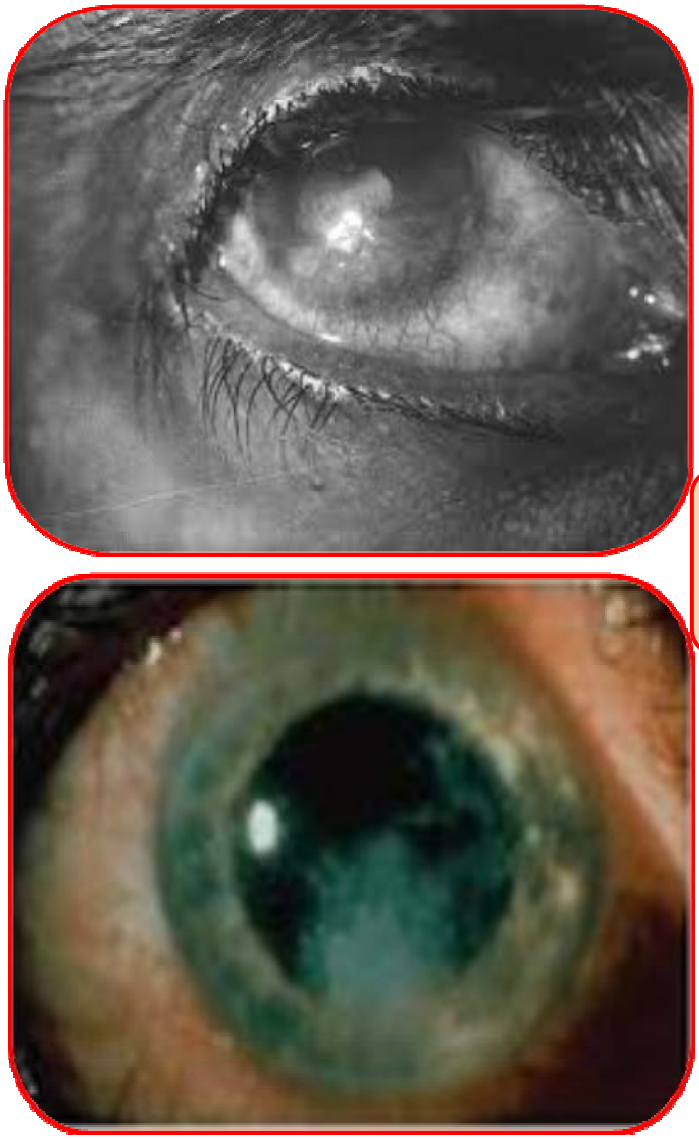


Figure 9
Fungal keratitis
(health.reply.com)

Treatment and management:

A presumptive diagnosis of fungal keratitis requires immediate empirical therapy. Natamycin ophthalmic suspension is the drug of choice for filamentous fungal infection. Fluconazole ophthalmic solution is recommended for *Candida* infection of the cornea. Amphotericin B eye drops may be required for non-responding cases, but can be quite toxic and requires expert pharmacist for preparation. Other medications have also been tried with moderate success. consult your eye care professional in any case as they will have the best treatment.

Prevention:

Prevention of trauma with vegetable / organic matter, particularly in agricultural workers while harvesting can reduce the incidence of fungal keratitis. Wearing of broad protective glasses with side shields is recommended for people at risk for such injuries.

Tinea versicolor (Pityriasis versicolor)

Tinea versicolor (also known as Dermatomycosis furfuracea. (14) Pityriasis versicolor, and Tinea flava) is a condition characterized by a rash on the trunk and proximal extremities. Recent research has shown that the majority of Tinea versicolor is caused by the *Malassezia globosa* fungus, although *Malassezia furfur* is responsible for a small number of cases. (15,16) These yeasts are normally found on the human skin and only become troublesome under certain

circumstances, such as a warm and humid environment, although the exact conditions that cause initiation of the disease process are poorly understood. (17) The condition pityriasis versicolor was first identified in 1846. (18). Figure(10).

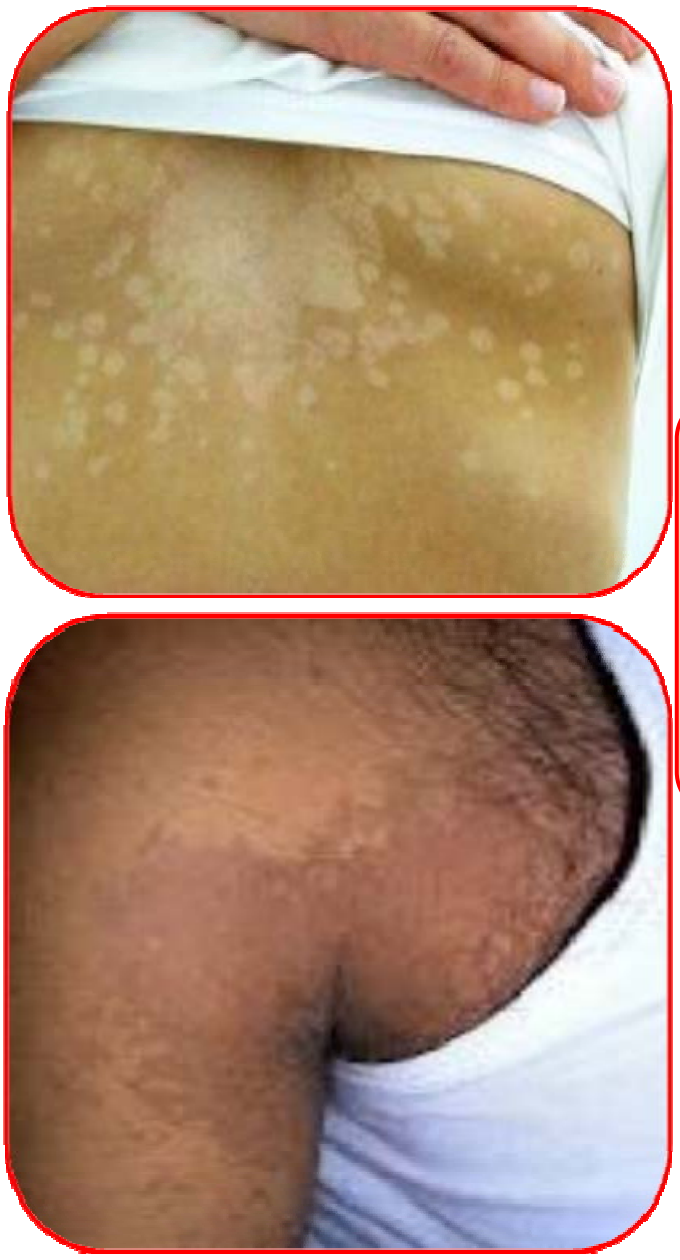


Figure 10
Hypo and hyperpigmentation of tinea versicolor above picture (reversingibs.com) below picture (dermaamin.com)

This agent appear as spaghetti and ball of meat under the microscope. Figure(11).

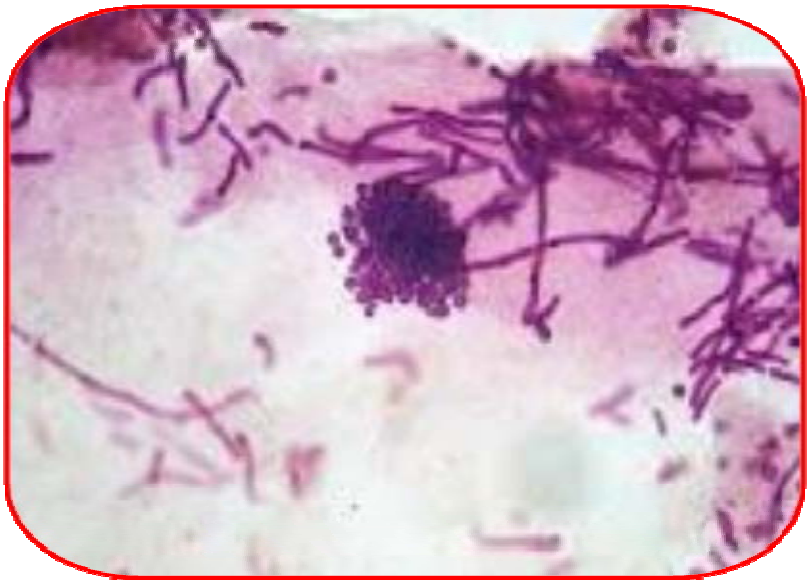


Figure 11: Malassizia furfur
(balls of meat and spaghetti)(huidziekten.nl)

Clinical manifestations:

Pityriasis versicolor usually presents as asymptomatic patches of hypo- or hyper-pigmented macules, varying in size, shape and color.

The most common sites are the chest, upper back, shoulders, upper arms and abdomen. Extension to the thigh, neck, and forearms may occur, but lesions of the scalp, palms and feet are rare. Hair shaft and nail are not infected. An uncommon variant is folliculitis, with erythematous papules or rarely, papulopustular lesions that resemble acne, it is occurred in patients who often receiving antibiotics or steroids.

M. furfur **cause catheter-acquired sepsis in neonates and adults with prolonged IV lipids. Clinical signs may be absent or the patient may be febrile (in such cases). Neonates often have had substantial thrombocytopenia and leukocytosis. Pneumonia may develop in such patients.**

Treatment:

Treatments for tinea versicolor include:

Tinaderm (Tolnaftate topical solution) is a very effective lotion. Its effect is moderately long lasting. This is a water soluble lotion and is externally applied on the affected parts of skin, preferably during night hours. Its composition is 10 mg of Tolnaftate USP in Polyethylene and Glycol 400 USP NF base q.s.

Topical antifungal medications containing 2.5% selenium sulfide (Selsun Extra Strength shampoo) are often recommended. Selsun Blue works for some people, but not all, because it only contains 1% selenium sulfide. Products containing more than 1% selenium sulfide are considered prescription strength. Other products that contain 1% selenium sulfide include (ZunSpot) medicated cream. Ketoconazole (Nizoral ointment and shampoo) is another treatment. It is normally applied to dry skin and washed off after 10 minutes, repeated daily for 2 weeks. Ciclopirox (Ciclopirox olamine) is an alternative treatment to ketoconazole as it suppresses growth of the yeast *Malassezia furfur*. Initial results show similar efficacy to ketoconazole

with a relative increase in subjective symptom relief due to its inherent anti-inflammatory properties. (19) Other topical antifungal agents such as clotrimazole, miconazole or terbinafine are less widely recommended. Additionally, hydrogen peroxide has been known to lessen symptoms, and on certain occasions, remove the problem, although permanent scarring occurs with this treatment. Clotrimazole (1%) is also used combined with selenium sulfide (2.5%).

Oral antifungal prescription-only medications include 400 mg of ketoconazole or fluconazole in a single dose, or ketoconazole 200 mg daily for 7 days, or itraconazole. (20,21), 400 mg daily for 3–7 days. The single-dose regimens, or pulse therapy regimes, can be made more effective by having the patient exercise 1–2 hours after the dose, to induce sweating. The sweat is allowed to evaporate, and showering is delayed for a day, leaving a film of the medication on the skin.

Recurrence is common and may be reduced by intermittent application of topical anti-fungal agents like tea tree oil or selenium sulfide.

Piedra (Tinea nodosa)

Piedra is an infection limited to hair shaft and is characterized by firm, irregular nodules composed of fungal elements.

Black piedra caused by *Piedra hortae*, and white piedra which caused by *Trichosporon beigelii* are the only two recognized varieties of piedra.

Clinical manifestations:

1. Black piedra:

Is usually found only on scalp hair. Although the infected hair appears normal in this case, the infected hair is rough, sandy, or granular to the touch. Infection with this fungus is charactarized by asymptomatic nodules vary in size from microscopic to a few millimeters in diameter. The thickness is usually greater one end, tapering at the opposite one. *P. hortae* not penetrate the cortex of the hair shaft. Figure (12, 13)

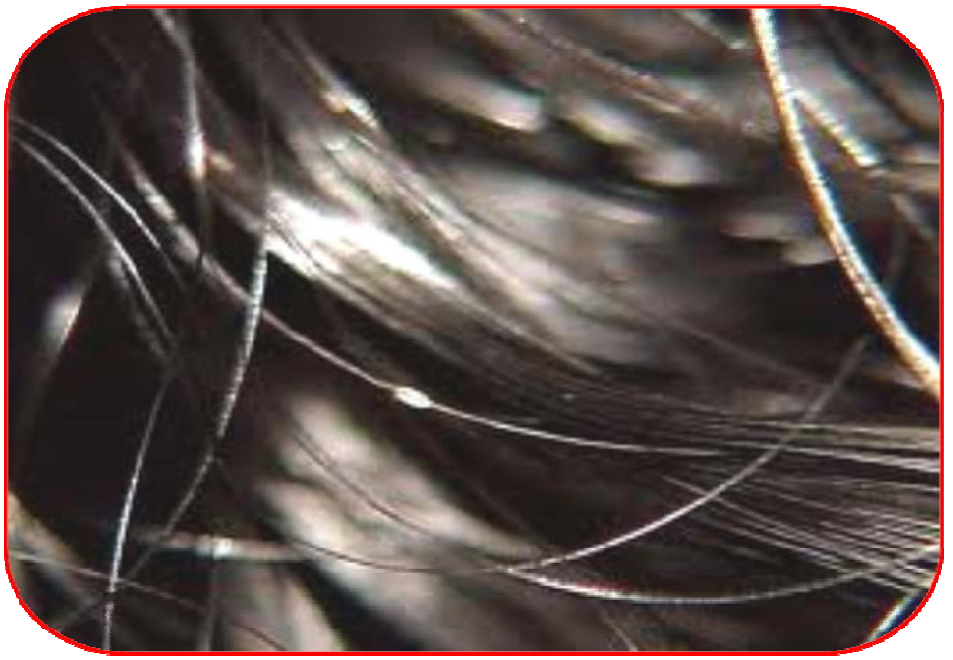


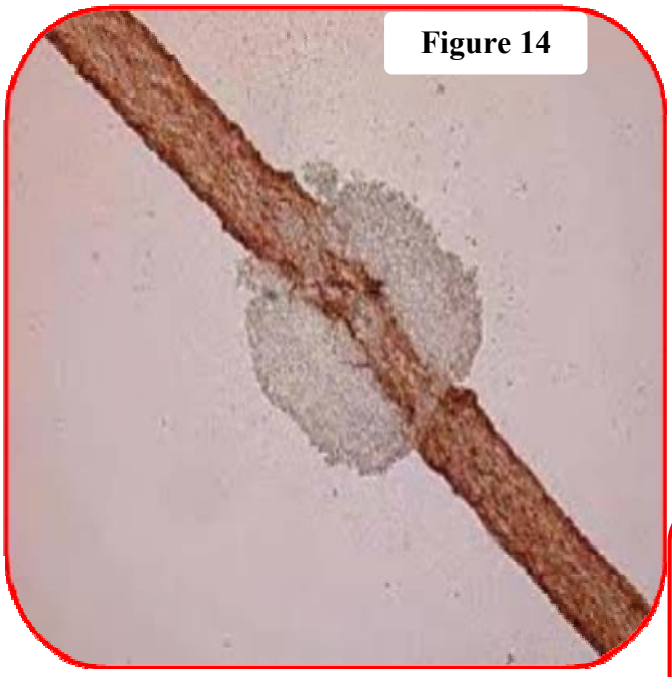
Figure 12: Black piedra(dermaamin.com)

Figure 13: Hair shaft with black Piedra (doctorfungus.com)

2. White piedra:

White piedra is characterized by soft, mucilaginous, white greenish-yellow to light brown nodules seen more often on hairs of the genital area, beard, and mustache than on scalp, eyebrows or eyelashes.

The nodules are usually thickest at the center but may show variation in shape and size. Unlike the nodules of black piedra the fungal mass of *T. beigelii* can easily be pulled off the hair shaft. The clinical growth of *T. beigelii* start beneath the cuticle of the hair shaft. (22) Figure(14,15)



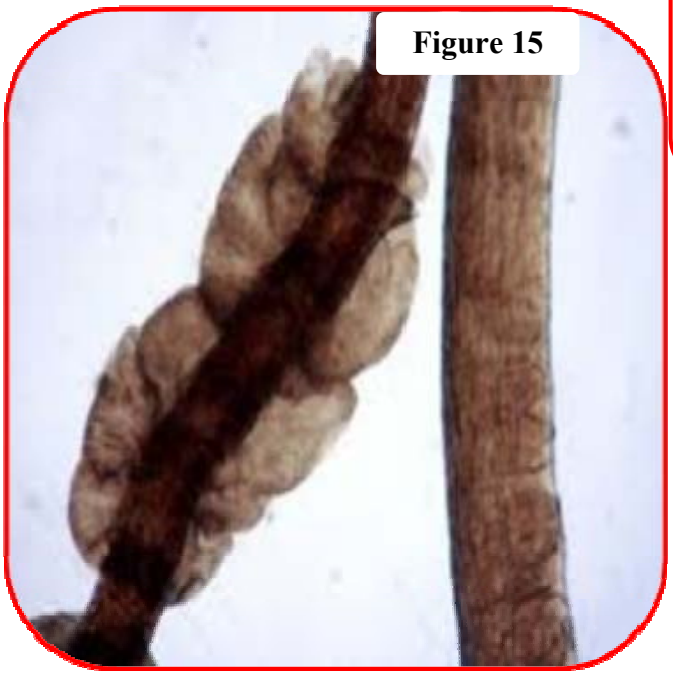


Figure 14and15: Hair shaft under the microscope reveal white piedra, 14 (cueflash.com), 15 (ebongskin.com)

Treatment:

Treatment of tinea nodosa consists in frequent shaving or clipping and the application of a mild parasiticide. (23)

Tinea nigra (Nigricans palmaris)

Tinea Nigra (also known as "superficial phaeohyphomycosis,"(and "Tinea nigra palmaris et plantaris" (24)) is a superficial fungal infection that causes dark brown to black painless patches on the palms of the hands and the soles of the feet. (25)

The lesions are most commonly found on the palms of the hands and less frequently on the sole of the foot or other area of the skin. Causative agent is *Exophiala werenckii*, but more recently classified as *Hortaea werneckii* . (26)

Clinical manifestations:

Tinea nigra usually presents as an asymptomatic dark patch of the skin on the palm of one hand and rarely on the both.

Other locations include the sole, interdigital web of the hand, palmer aspects of the fingers and rarely on wrist, forearm, trunk, or neck. The lesions usually single with an irregular sharply defined margins. Some times the lesions look like a cluster of confluent spores rather than a single, pigmented macule. Pigmentation range from brown to brownish black, greenish brown, or black and is often uneven within the lesion. There is no induration, erythema , or elevation. Figure(16,17)

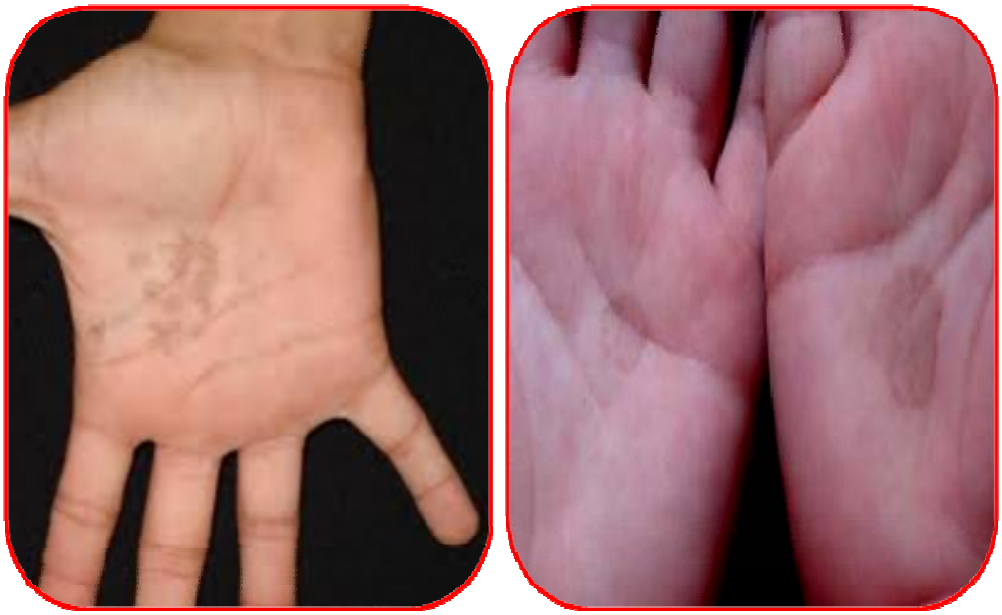


Figure 16: Tinea nigra
(dermatology-s10.cdib.org)

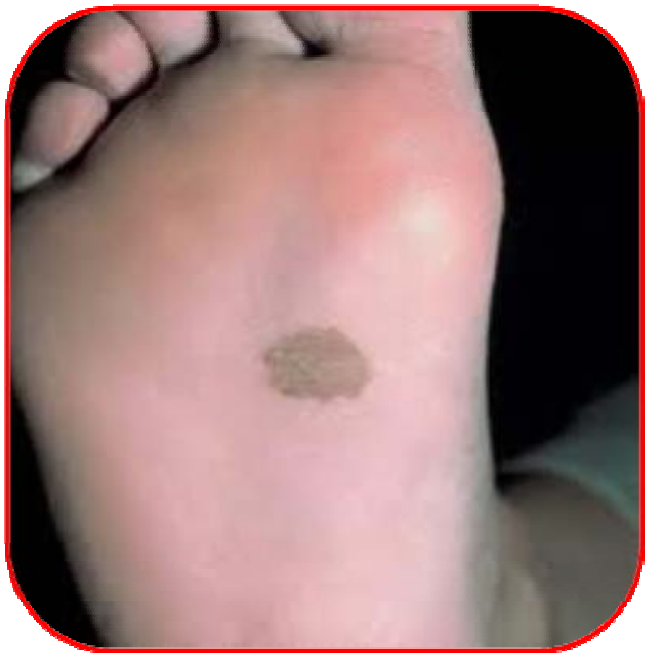


Figure 17: Tinea nigra in the sole of foot
(husatorm.com)

Treatment:

Treatment consists of topical application of dandruff shampoo, which contains selenium sulfide, over the skin. Topical antifungal imidazoles may also be used, such as Ketoconazole. This is the same treatment plan for tinea or pityriasis versicolor.

Diagnosis:

Diagnosis of tinea nigra causing fungus is made on microscopic examination of skin scrapings, mixed with potassium hydroxide (KOH). The KOH lyses the non fungal debris.

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Chapter 2

Subcutaneous Mycoses

Lobomycosis (Lobo's disease)

Lobomycosis, (1) also known as (Jorge) Lobo's disease or lacaziosis, (2) is a blastomycosis, a fungal infection of the skin caused by *Lacazia loboi* (formerly named *Loboa loboi*), (3) and discovered by Brazilian dermatologist Jorge Lobo. Other names which were given to the disease are: keloidal blastomycosis, Amazonian blastomycosis, blastomycoid granuloma, miraip and piraip. These last two names were given by natives of the Amazon and mean that which burns.

This disease is usually found in humans, (4) and bottle-nosed dolphins. Figure(1)

Figure 1 Lobomycosis in bottle-nosed dolphins (accessience.com)

Clinical manifestations:

Lobomycosis is an extremely indolent subcutaneous infection that usually begins as well, circumscribed, indurated asymptomatic papule. Sites of initial lesions are, leg, auricle, arm face neck, gluteal region, lumbosacral region. As the infection develops over the years, the initial lesion increase in size and most often, new lesions appear.

The disease described in five different forms: Infilterative, keloidal, gommatous, verrucoid and ulcerative. The most typical features of the disease that would suggest it's diagnosis are: Sharp, lobulated margins of the lesions, lobulated , indurated surface, smooth, lack of attachment of underlying muscles and bones and lack of local or systemic symptoms. Figure(2)

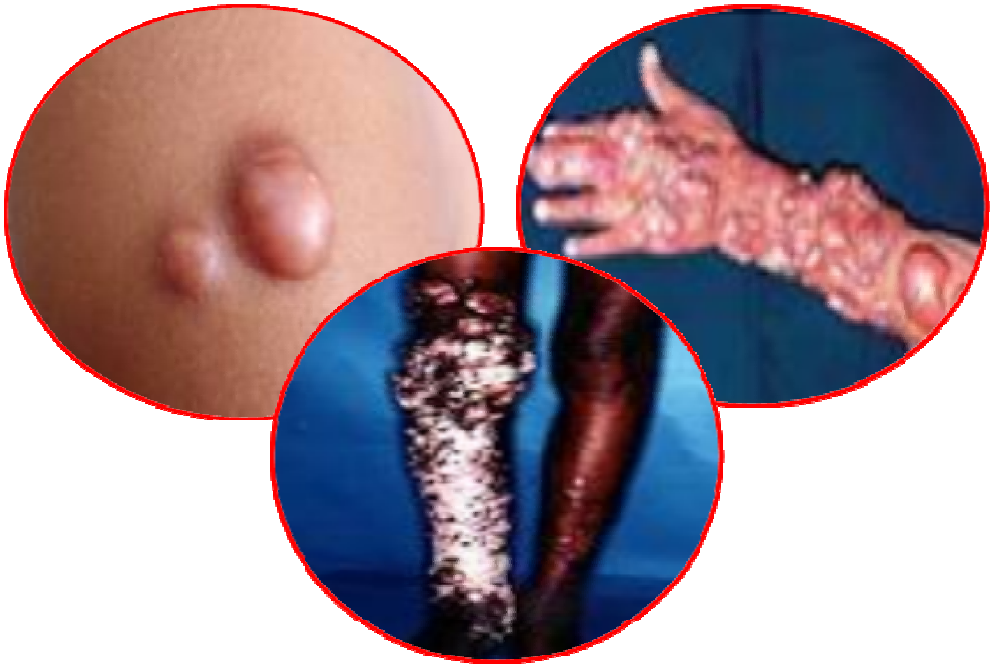


Figure 2: Different types of lobomycosis in different sites of the body, left picture(dermatlas.med.jhmi.edu, middle (elsvierimages.com), right picture: (dermamine.com)

Treatment:

Surgical excision or cryosurgery is the treatment of choice. (5) Treatment with antifungals has been considered ineffective, but the use of clofazimine and dapsone in patients with leprosy and lobomycosis has been found to improve the latter. This treatment regimen, with concomitant itraconazole, has been used to prevent recurrence after surgery. (6)

Diagnosis:

Diagnosis of Lobo's Disease is made by taking a sample of the infected skin (a skin biopsy) and examining it under the microscope. *Lacazia loboi* is characterized by long chains of spherical cells interconnected by tubules. The cells appear to be yeast-like with a diameter of 5 to 12 µm. Attempts to culture *L. loboi* have so far been unsuccessful.

Mycetoma (Madura foot, Maduramycosis)

Mycetoma is a chronic subcutaneous infection caused by actinomycetes or fungi. This infection results in a granulomatous inflammatory response in the deep dermis and subcutaneous tissue, which can extend to the underlying bone. Mycetoma is characterized by the formation of grains containing aggregates of the causative organisms that may be discharged onto the skin surface through multiple sinuses. Mycetoma was first described in the mid 1800s and initially named Madura foot, after the region of Madura in India where the disease was first identified.

Mycetoma caused by microaerophilic actinomycetes is termed actinomycetoma, and mycetoma caused by true fungi is called eumycetoma. Figure(3)

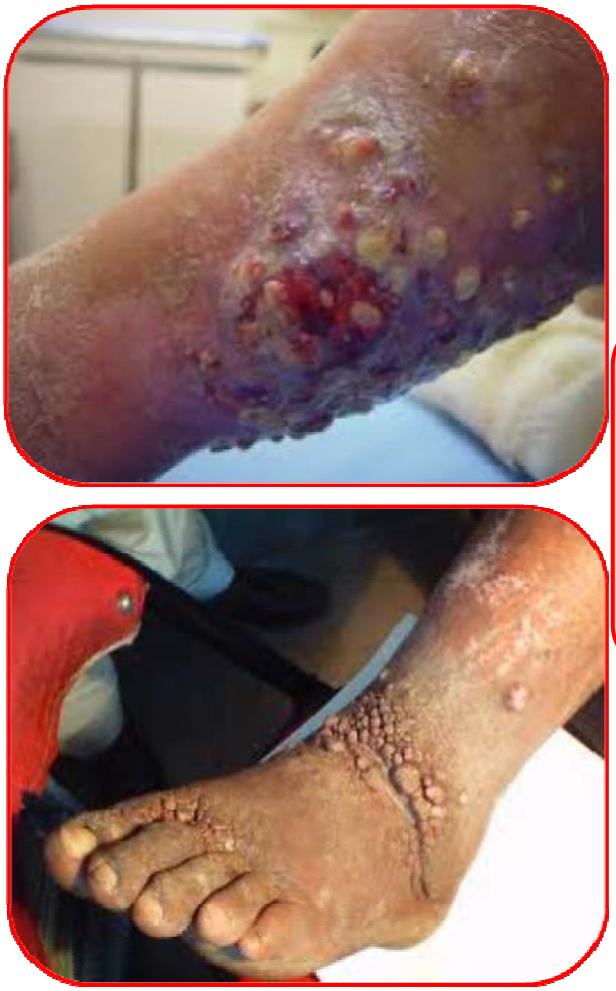


Figure 3
Mycetoma
(Madura foot)
above picture
(dermamine.com)
below picture
(dermatlas.med.jhmi.edu)

These conditions are to be differentiated from actinomycosis, which is an endogenous suppurative infection caused by *Actinomyces israelii* or other species of *Actinomyces* or related bacteria, affecting the cervical-facial, thoracic, and pelvic sites (the latter is usually associated with the use of intrauterine devices). The branching bacteria that cause

actinomycosis are non–acid-fast anaerobic or microaerophilic bacteria. These bacteria are smaller than 1 µm in diameter, smaller than eumycotic agents. On the other hand, the agents that cause actinomycetoma are always aerobic and are sometimes weakly acid-fast.

The term mycetoma can also be found incorrectly referring to a fungus ball in a preexisting cavity in the lung or within a paranasal sinus, most often caused by *Aspergillus* species. (7)

More than 20 species of fungi and bacteria can cause mycetoma. The ratio of mycetoma cases caused by bacteria (actinomycetoma) to those caused by true fungi (eumycetoma) is 197:67.

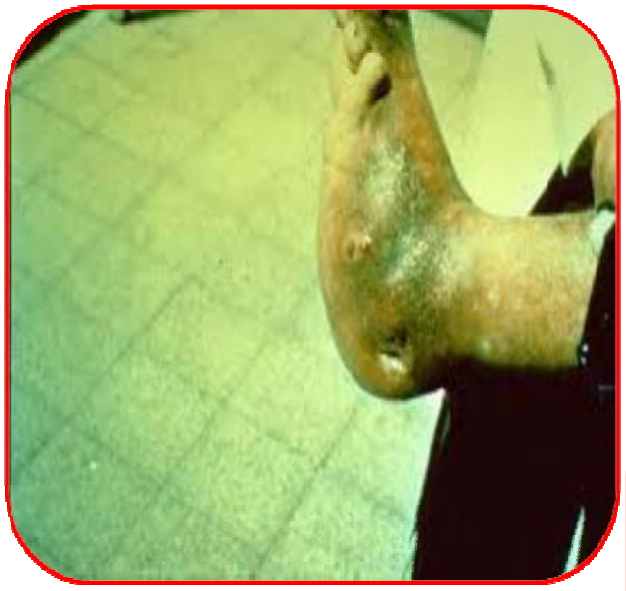
Clinical manifestations:

Mycetoma is a chronic, superficial infection of the subcuataneous tissues and contiguous bones. The lesions seems to begin at a site of minor trauma and continuous to spread locally over months and years.

The single most common site is the foot. Location of extra-pedal lesions depends upon what part of the body is subject to traumatic inoculation with the etiologic agent. Typical areas are: leg, hand, knee, buttock, arm, thigh, head, or neck. Carrying bundles of wood on the head and shoulders may lead to head and neck mycetoma. (8)

The initial lesion is a small subcutaneous swelling. On the foot, this lesion is often between the first and second metatarsal bones, either on the dorsum or plantar surface. The area is a few millimeters in diameter, firm, rubbery, or

painless. Figure(4) Over the ensuing months, the infection spreads along subcutaneous facial planas, creating a large indurated area. Numerous small abscess then form, generally connected by sinus tracts extend to the skin surface. They appear as soft or visculated areas that open to discharge contains the charactristic grains. Figure(5)



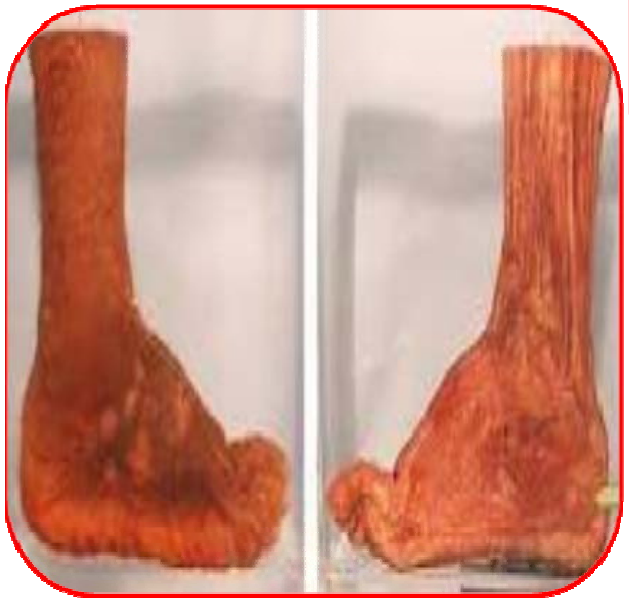


Figure 4
Mycetoma of the foot
above picture
(cdc.govx)
below picture
(health.act.gov.au)

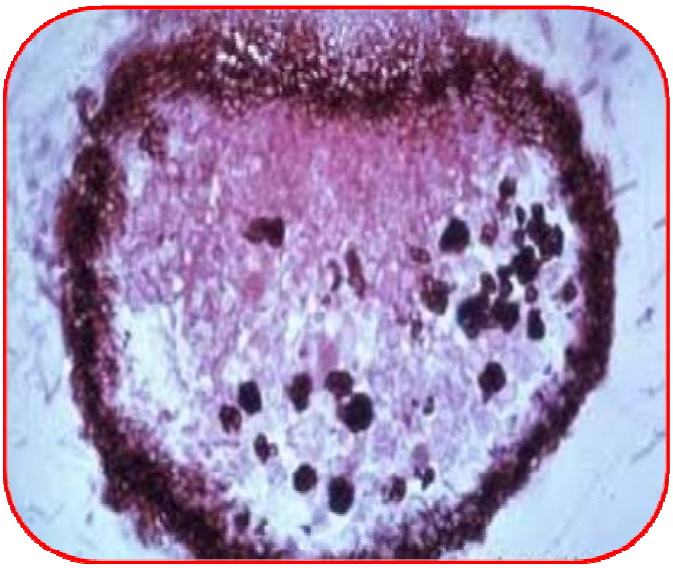


Figure 5: Grain of *Actinomadura madurae*. A large grain in tissue, a low power examination of an HandE-stained section (doctorfungus.org)

Once a sinus tract has begun to drain, it may continue to drain for long periods close spontaneously. Invasion of contiguous bone is not accompanied by pain on weight bearing, except in advanced cases.

Muscles, nerves and tendons are resistant to invasion. (9) Figure(6)

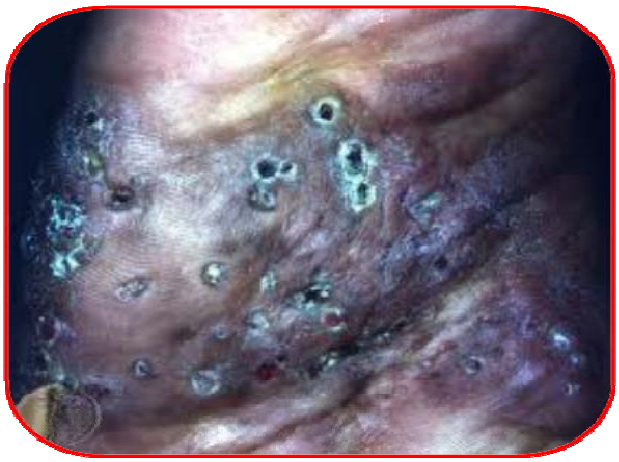


Figure 6
Mycetoma of the sole of foot caused by *Nocardia brasiliensis* (dermenetenz.org)

No fever, leukocytosis, anaemia, weight loss or systemic abnormalities accompanied the disease. Regional hymph nodes may be enlarged but rarely contain the agent. The infection itself can be fatal in some cases, such as thoracic mycetoma penetrating the pleura or cranial mycetoma. Actinomycetoma (i.e in *S.somaliensis*) is more malignant than eumycetoma because it can invade muscles. The other difference is causing either a ((moth-eaten)) radiologic appearance or numerous small lesions in contiguous bones, while this of eumycetoma may have a well-defined capsule.

Therapy:

Patients with actinomycetoma have often been helped and sometimes cured with appropriate antibacterial agents. (10, 11, 12) The best regimen seems to have included sulfonamide or dapsone. Trimethoprim-sulfamethaxazole combination may give good results. Streptomycin is another drug of choice. The response of eumycetoma to treatment has been disappointing. Ketocoazole may give good results in the case of *M. mycetomatis*. Amphotericin-B is another drug of choice.

Surgical excision of small, well-encapsulated eumycetoma lesion is useful. Amputation may have required in extensive eumycetoma.

Lab. DX:

Direct examination:

Microscopical examination of pus, exudates, or biopsy material reveals the presence of grains, which are the primary

diagnostic indicators. (13) Observation of the size, shape, color, and consistency of the grains may often lead the investigator to the identification of the fungal spp. Grains can be mounted in a drope of 10% NAOH on a slide and crushed under a cover glass. The size of hyphae, septation, morphologic features and pigment formation in hyphal walls will allow differentiation between eumycetes and actinomycetes. To prepare the grains for Gram staininig, the grains should be crushed between 2 slides and then heat fixed. The actinomycetes grains have a Gram +ve narrow filaments. Albert's stain used for eumycotic grains.

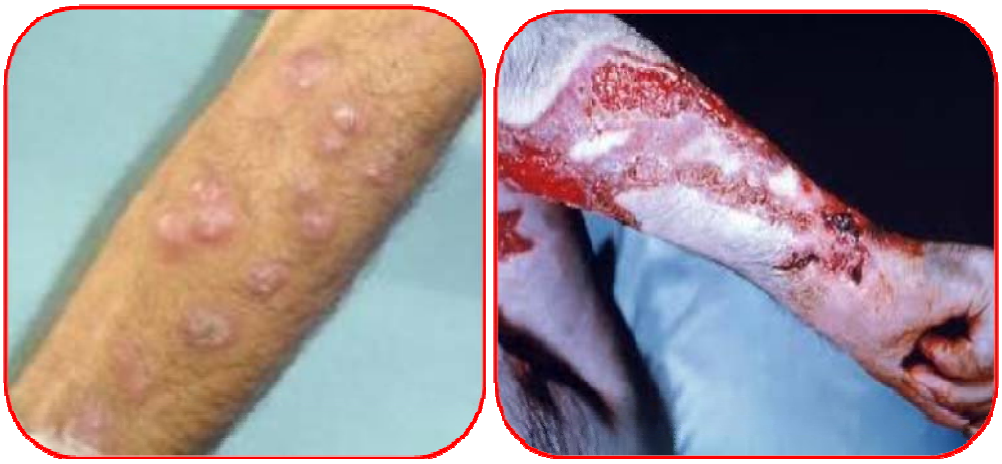
Direct culture:

When direct examination of the grains reveals evidence of eumyctes, additional grains can be washed in saline containing an antibiotics and plated on duplicate plates of modified Sabouraud's agar with antibiotics such as gentamycin sulfate (400µg/ml), Penicillin G (20µg/ml) and Streptomycin (40 µg/ml) or Chloramphenicol (50µg/ml). The medium should not contain Cyclohexamide. Incubation should be held for at least 6-8 weeks. If the direct examination of the grains shows evidence of actinomycotics agents, the culture media should not contain antibiotics. Blood, Brain-Heart infusion, Loweenstein agar, or modified Sabouraud' s agar with 0.5% Yeast extract are suitable for the isolation of the actinomycetes. The grains should be washed several times in sterile saline, crushed with a sterile glass rods and then streaked on the media. Incubation should be at (37 and 25)^{o}C.

Sporotrichosis

Sporotrichosis (also known as "Rose gardener's disease"(14))is a disease caused by the infection of the fungus *Sporothrix schenckii*. (15) This fungal disease usually affects the skin, although other rare forms can affect the lungs, joints, bones, and even the brain. Because roses can spread the disease, it is one of a few diseases referred to as rose-thorn or rose-gardeners' disease. (16)

Because *S. schencki* is naturally found in soil, hay, sphagnum moss, and plants, it usually affects farmers, gardeners, and agricultural workers. (15) It enters through small cuts and abrasions in the skin to cause the infection. In case of sporotrichosis affecting the lungs, the fungal spores enter through the respiratory pathways. Sporotrichosis can also be acquired from handling cats with the disease; it is an occupational hazard for veterinarians. Figure(7)



A **B**

Figure 7: Sporotrichosis. A, lymphangitic spread from a primary digital lesion up to the dorsal surface of the forearm. B, large ulcerative lesion of the forearm, A(dermrounds.com), B(commons.wikimedia.org)

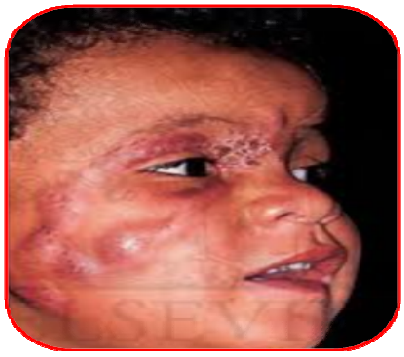
Sporotrichosis progresses slowly - the first symptoms may appear 1 to 12 weeks (average 3 weeks) after the initial exposure to the fungus. Serious complications can also develop in patients who have a compromised immune system.

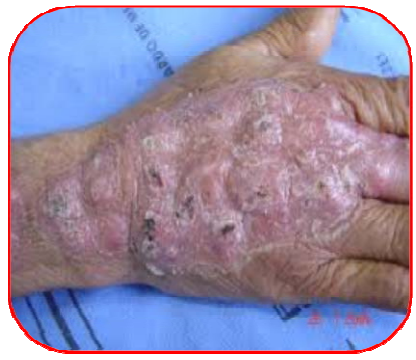
Clinical manifestations:

1. Cutaneous infection:

Disease arising at sites of minor trauma begines as asmall, erythematous painless papule that enlarges over days or weeks mainly discharging glairy pus.

systemic symptoms are present. Lesion may remain single, but in the face of children, discrete nodules spread to proximal site due to spreading through lymphangitic channels. In some cases single cutaneous lesion appear and remain the only infected site with out spreading through lymphangitic channales forming chronic lesions named plaques or fixed lesions which may persist for years. Skin lesions may also result from hematogenous dissemination. Figure(8)





A **B**

Figure 8: Sporotrichosis. A, multiple lesions in the face of children (elsevierimages.com). B, Large crusted lesion of the hand, (dermatlas.med.jhmi.edu)

2. Pulmonary sporotrichosis:

The typical cases with pulmonary sporotrichosis are predominant in male than in female the ratio is 6:1. Average age of infection is 46 years old. (17) Symptoms are: Cough, low grade fever, weight loss, and upper lobe lesions. Hemoptysis occurred in 18% of cases, fatal cavitation also was shown. (18). Figure(9)

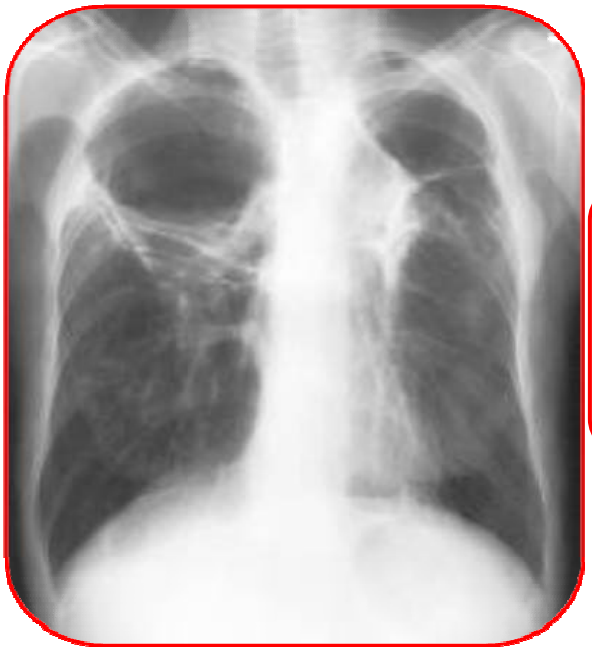


Figure 9
Pulmonary sporotrichosis, you can see bilateral upper cavities (microbiology.book.org)

3. Osteoarticular sporotrichosis:

The majority of patients present with indolent onset of stiffness pain in a large joint. Almost all cases of arithritis involve major peripheral joints: Knee, elbow, ankle, or wrist. In untreated patients, additional joints may involved, and the skin over the infected joints may appear erythematous and may develop draining sinus. Destruction of the joint cartilage continuous until all mobility is lost.

4. Other forms of hematogenous sporotrichosis:

Patients may present with multiple skin lesions from hematogenous spread, with or with-out lung or bone lesions. Endophthalmitis has occurred rarely. A few patients have presented with brain abscess or chronic meningitis. Meningitis is indolent associated with hypoglycorrhachia elevated C.S.F protein and variable or absent mononuclear cells. Hydrocephalus has complicated several cases.

Although patients who are immunological intact may develop hematogenous disseminated fatal infection, immunesuppressed patients have a high chance to develop hematogenous dissemination, particularly to skin and bones.

Diagnosis:

Sporotrichosis is a chronic disease with slow progression and often subtle symptoms. It is difficult to diagnose, as many other diseases share similar symptoms and therefore must be ruled out.

Patients with sporotrichosis will have antibody against the fungus *S. schenckii*, however, due to variability in sensitivity and specificity, it may not be a reliable diagnosis for this disease. The confirming diagnosis remains culturing the fungus from the skin, sputum, synovial fluid, and cerebrospinal fluid.

Cats with sporotrichosis are unique in that the exudate from their lesions may contain numerous organisms. This makes cytological evaluation of exudate a valuable diagnostic

tool in this species. Exudate is pyogranulomatous and phagocytic cells may be packed with yeast forms. These are variable in size, but many are cigar-shaped. Figure(10)

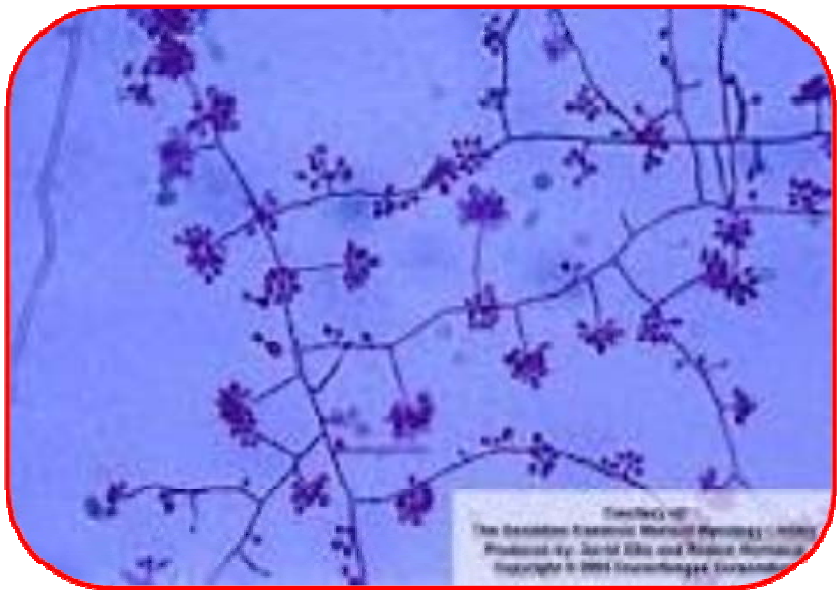


Figure 10: Sporotrichosis, elliptical to oblong produced densely on the hyphae and the conidiophores, stained with lactophenol cotton blue (x280) (healthinplainenglish.com)

Treatment:

Treatment of sporotrichosis depends on the severity and location of the disease. The following are treatment options for this condition. (19)

Saturated potassium iodide solution:

Although its mechanism is unknown, application of potassium iodide in droplet form can cure cutaneous sporotrichosis. This usually requires 3 to 6 months of treatment.

Itraconazole (Sporanox) and fluconazole:

These are antifungal drugs. Itraconazole is currently the drug of choice and is significantly more effective than fluconazole. Fluconazole should be reserved for patients who cannot tolerate itraconazole.

Amphotericin B:

This antifungal medication is delivered intravenously. Many patients, however, cannot tolerate Amphotericin B due to its potential side effects of fever, nausea, and vomiting.

Lipid formulations of Amphotericin B are usually recommended instead of Amphotericin B deoxycholate because of a better adverse-effect profile. Amphotericin B can be used for severe infection during pregnancy. For children with disseminated or severe disease, Amphotericin B deoxycholate can be used initially, followed by itraconazole (20).

In case of sporotrichosis meningitis, the patient may be given a combination of Amphotericin B and 5 - fluorocytosine / Flucytosine.

Newer Azoles:

Several studies have shown that posaconazole has in vitro activity similar to that of Amphotericin B and itraconazole; therefore, it shows promise as an alternative therapy. However, voriconazole susceptibility varies. Because the

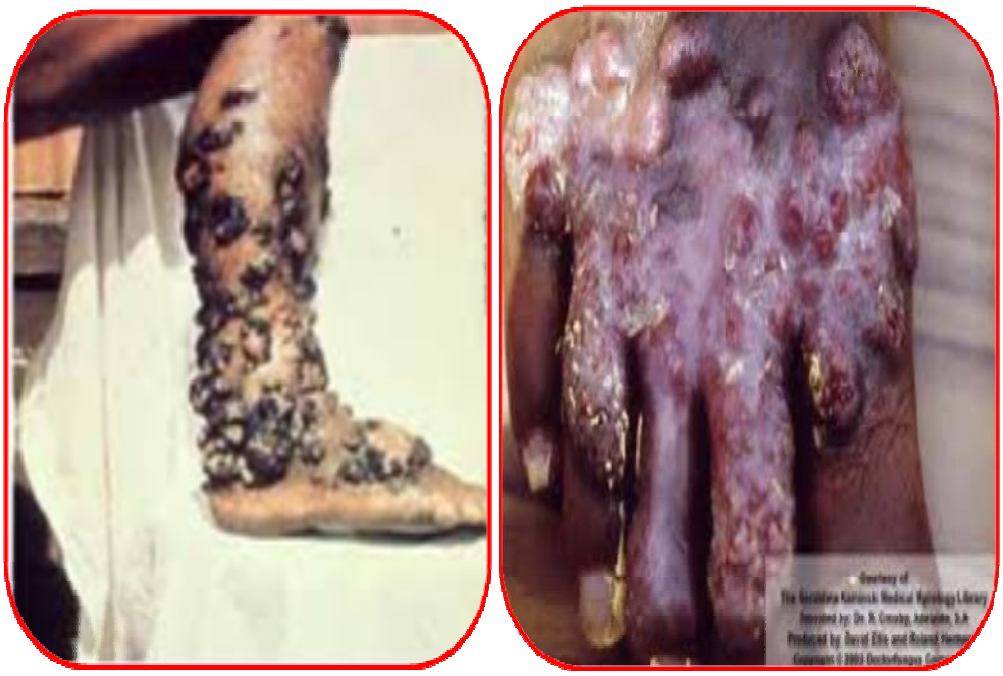
correlation between in vitro data and clinical response has not been demonstrated, there is insufficient evidence to recommend either posaconazole or voriconazole for treatment of sporotrichosis at this time. (20)

Surgery:

In cases of bone infection and cavitatory nodules in the lungs, surgery may be necessary.

Chromoblastomycosis (Chromomycosis)

Chromoblastomycosis (also known as "Chromomycosis," "Cladosporiosis," "Fonseca's disease," "Pedroso's disease," "Phaeosporotrichosis,"(21)"Verrucous dermatitis") is a long-term fungal infection of the skin (22) and subcutaneous tissue (a chronic subcutaneous mycosis). (23) The infection occurs most commonly in tropical or subtropical climates, often in rural areas. It can be caused by many different type of fungi which become implanted under the skin, often by thorns or splinters. Chromoblastomycosis spreads very slowly; it is rarely fatal and usually has a good prognosis, but it can be very difficult to cure. There are several treatment options, including medication and surgery. Figure(11)



A B

Figure 11: Chromoblastomycosis
A, a raised lesion of the lower part of the leg including foot showing a cauliflower-like surface (drugdiscoveryopinion.com).
B, crusty lesion on the hand(mold.ph)

Clinical manifestations:

Lesions of chromoblastomycosis cause few symptoms, starting with small lesion enlarge slowly, and come to medical attention after the infection which has been present for anywhere from 1 month to 20 years. (24) Lesion tend to arise in exposed areas of the skin among people exposed to minor trauma such as cuts and thorns. The majority of lesions are in the lower extremities. Figure(12) Other locations of lesions include the dorsum of the hand, wrist , elbow, other areas of the arm, buttock, neck, shoulders and face.

Lesions of the mucous membrane have been rare. Lesions of chromoblastomycosis may single or may be spread by autoinoculation or lymphatic drainage to other areas of the skin. The most common appearance is a verrucous plaque or nodule. Ulcerated or crusted lesions can also be seen. In the absence of secondary infection, there is no pain, but itching noted.

Complications of chromoblastomycosis include carcinomatous degeneration, elephantiasis of the effected extremity and secondary infection. Systemic symptoms such as fever, weight loss, or malaise are absent. Figure(13)

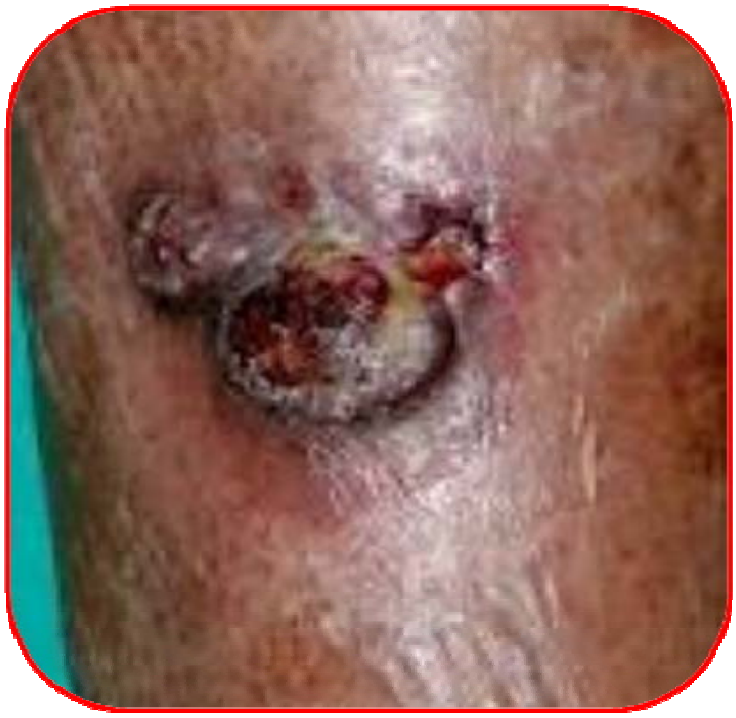


Figure 12
A single lesion with a sharp border on the leg
(dermamin.com)

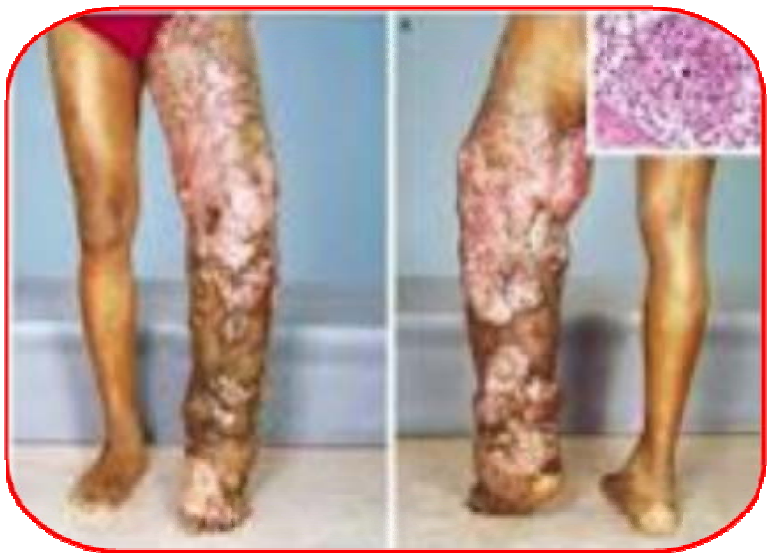


Figure 13
Elephantiasis as a complication of chromoblastomycosis
(nejm.org)

Diagnosis:

The most informative test is to scrape the lesion and add potassium hydroxide (KOH), then examine under a microscope. (KOH scrapings are commonly used to examine fungal infections.) The pathognomonic finding is observing Medlar bodies, sclerotic cells. Scrapings from the lesion can also be cultured to identify the organism involved. Blood tests and imaging studies are not commonly used.

On histology, chromoblastomycosis manifests as pigmented yeasts resembling "copper pennies." Special stains, such as periodic acid schiff and Gömöri methenamine silver, can be used to demonstrate the fungal organisms if needed. Laboratory diagnosis of chromoblastomycosis is easily accomplished because the sclerotic bodies can usually be seen by direct examination of the crusts and exudates of the lesions. (25)

After they are digested in hydroxide, superficial crusts, skin scrapings, aspirated pus, and biopsy material are examined under the microscope. Thick-walled round to oval brown cells with are seen in pus or biopsy specimens of epidermal and subcutaneous tissues. Figure(14).

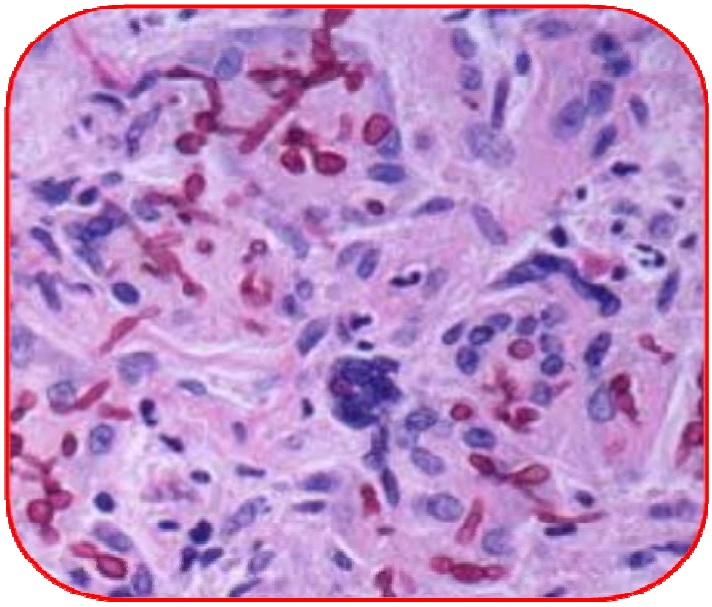


Figure 14: Chromoblastomycosis
Asclerotic body in a process of septation. HandE (X650)
(eyepathologist.com)

Isolation of the culture:

Crusts, pus, and biopsy materials are cultured for isolation of the fungus. Usually, the specimens is heavily contaminated with bacteria, and therefore the medium should contain chloramphenicol or other antibacterial antibiotics and should be incubated at room temperature or 30^{o}C for four weeks because this fungi is slow growing. Figure(15).

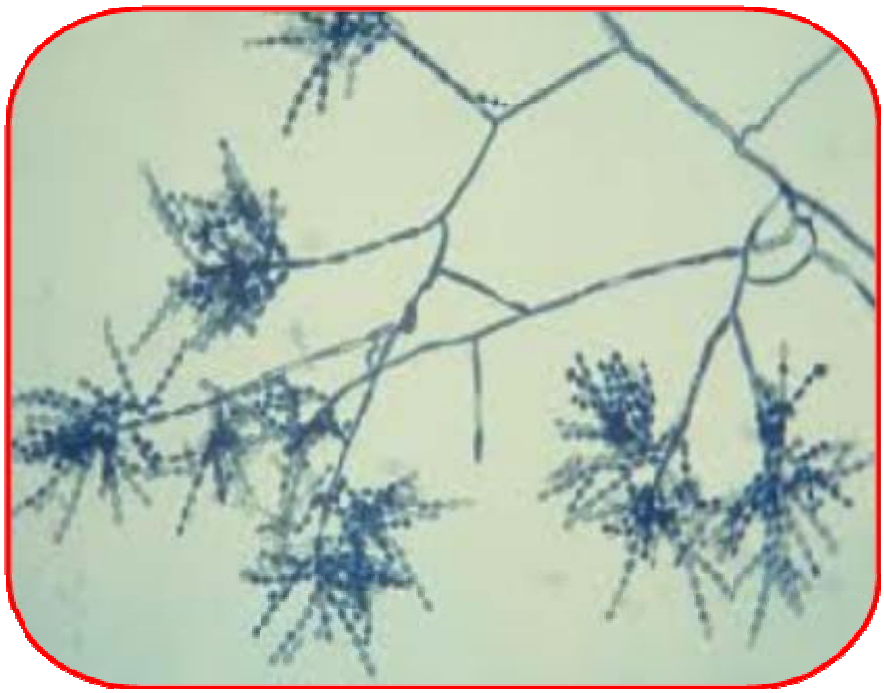


Figure 15
Fonesecaea pedrosoi*, *Rhinocladiella
type of sporulation (X300)
(microbiologybook.org)

Treatment:

Chromoblastomycosis is very difficult to cure. There are two primary treatments of choice. Itraconazole, an antifungal azole, is given orally, with or without flucytosine (5-FC).

Alternatively, cryosurgery with liquid nitrogen has also been shown to be effective. Other treatment options are the antifungal drug terbinafine, (26) an experimental drug posaconazole, and heat therapy. Antibiotics may be used to treat bacterial superinfections. Amphotericin B has also been used.

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Chapter 3

Deep Mycosis
(Systemic Mycosis)

Aspergillosis

Aspergillosis is the name given to a wide variety of diseases caused by fungi of the genus *Aspergillus*. The most common forms are allergic bronchopulmonary aspergillosis, pulmonary aspergilloma and invasive aspergillosis. Most humans inhale *Aspergillus* spores every day. Aspergillosis develops mainly in individuals who are immunocompromised, either from disease or from immunosuppressive drugs, and is a leading cause of death in acute leukemia and hematopoietic stem cell transplantation. Conversely, it may also develop as an allergic response. The most common cause is *Aspergillus fumigatus.*

Aspergillosis mean any illness other than mycotoxicosis caused by *Aspergillus* spp. There are three main forms of diseases caused by the Asperglli:

1. Allergic response to inhaled aspergilli.

2. Colonization of air spaces within the body. and

3. Tissue invasion by the fungus.

***A. fumigatus* is the most common ause of both invasive and non invasive aspergillosis worldwide and cause aspergilloma (fungus ball).**

***A. flavus* the second most ommon spp. isolated from lesions originating in the nasal sinuses. *A. niger* is a third most common cause of invasive pulmonary aspergillosis. (1)**

Clinical manefistations:

1. Allergic bronchopulmonary aspergillosis (ABPA):

It is a syndro defined by the following: Asthma history of transient pulmonary infiltrate, central broncheictasis, immediate utaneous hypersensitivity, reaction to *Aspergillus* antigens. and serum antibodies, sufficient to give a positive agar precipitin test with *Aspergillus* antigens, in addition to elevation of IgE serum concentration and specific IgE and IgG antibodies. to *Aspergillus* antigens, with eosinophilia in the peripheral blood. ABPA divided into (5) stages: Acute, and fibrosis. (2)

2. Fungus ball of the paranasal sinuses:

It is a growth of fungal mass within the sinus cavity, with-out invasion of the sinus mucosa. The maxillary sinus is the usual location for the fungus ball, but occasionally the ethmoid, sphenoid, or frontal sinus are involved. *A. fumigatus, A. flavus* are the species usually associated with fungus ball. Typically patients have a long history of nasal congestion, postnasal drip, and intermittent feelings of congestion, or pain over the sinus.

3. Invasie Aspergillosis of the paranasal sinus:

Invasive aspergillosis of paranasal sinuses may be acute or chronic. Acute infection occure in severely immunesuppressed patients and is first seen as fever with or with-out the followings: Symptoms of sinusitis or rhinitis, skin lesions over

the nose or sinus, signs of invasion into the orbit or a necrotic lesion of the hard palate or nasal turbinate. (3,4)

4. Endobronhial colonization:

Saprophytic colonization of bronchial tree occurs in patients with preexisting damage to lung architecture such as carcinoma, TB, histoplasmosis, sarcoidosis, recurrent bacterial pneumonia or lung abscesses. It is difficult to ascertain wither the fungus is contributing to the patient cough, sputum production or occasionally, hemoptysis. Figure(1)

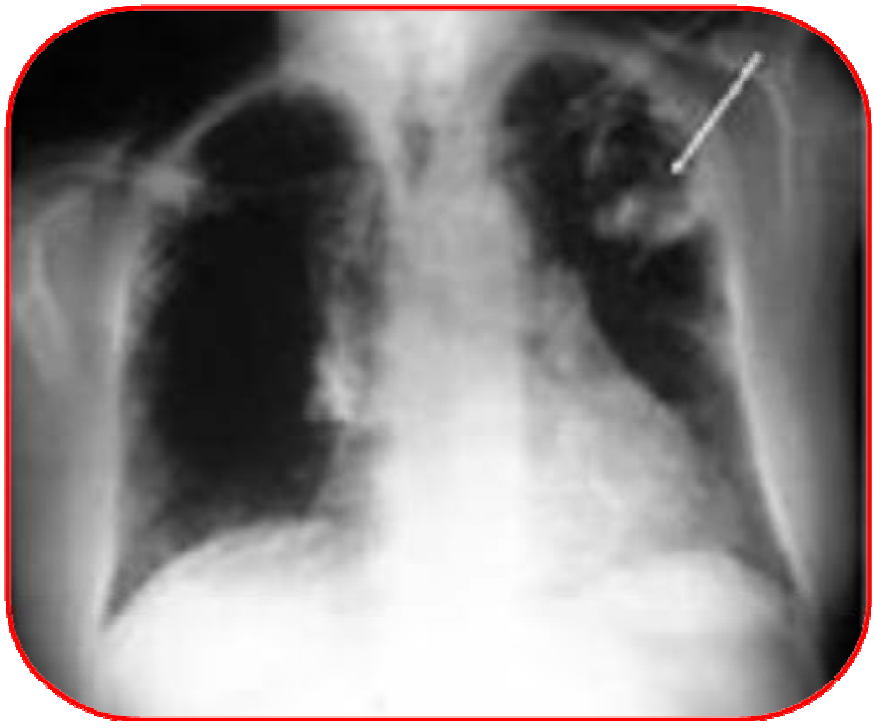


Figure 1: Chest radiograph in aspergillosis
Fungus ball in a patient with a prior bacterial lung abscess
(tjgmtqq.wordpress.com)

5. Invasive pulmonary Aspergillosis:

Invasive pulmonary aspergillosis in immunesuppressed patients is typically manifested as acute pneumonia. (5,6) Unless immunesuppression is rapidly decrease, patients usually die within 2 or 3 weeks.

Vascular invasion by fungal hyphae causes infarction of distal tissues. Pulmonary infection crosses into contiguous structures e.g, across the diaghram into the stomach or liver, or from lung into the pericardium, heart, or superior vena cava. Hematogenous dissemination occurs in about one third of cases. Symptoms begin with fever, which is followed within a day or two by pneumonia. Pleural pain and scanty hemoptysis may suggest pulmonary infarction. Cough, sputum production and pleural effusion are either abscent or minimal.

6. Hematogenously disseminated aspergillosis:

Presenting symptoms or signs of aspergillosis may originate from hematogenous dissemination, usually in patients with sever immunesuppressed or intra venoud drugs addiction. Occular lesions may present as indolent endophthalmitis, or as sudden monocular blindness from ischemic optic neuropathy. (7) Cerebral foci are infarcts or abscesses, usually single. Meningitis is rare. Metastatic skin lesions resemble ecthyma gangrenosum, forming erythematous macules with progressive central infarction. Figure(2) Although hematogenous bone lesions are rare, vertebral

osteomyelitis seems to predominant. Aspergilli endocarditis may occurred after cardiac surgery. Figure(3)

In addition to the symptoms, an X-ray or computerised tomography (CT) scan of the infected area provides clues for making the diagnosis. Whenever possible, a doctor sends a sample of infected material to a laboratory to confirm identification of the fungus.

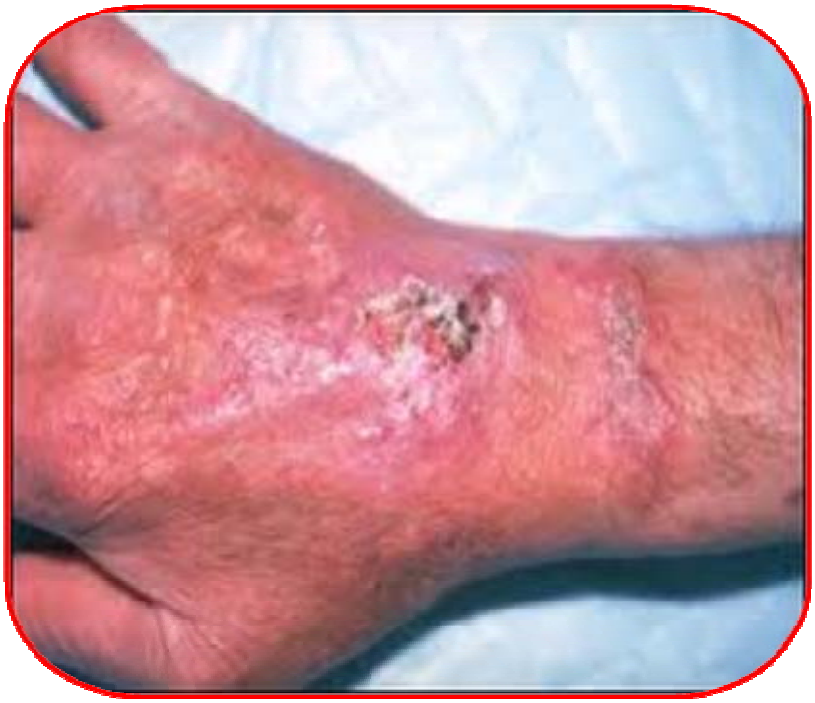


Figure 2: Aspergillosis
An erythematous macule on the hand of a patient with hematogenous disseminated aspergillosis (bryanking.net)

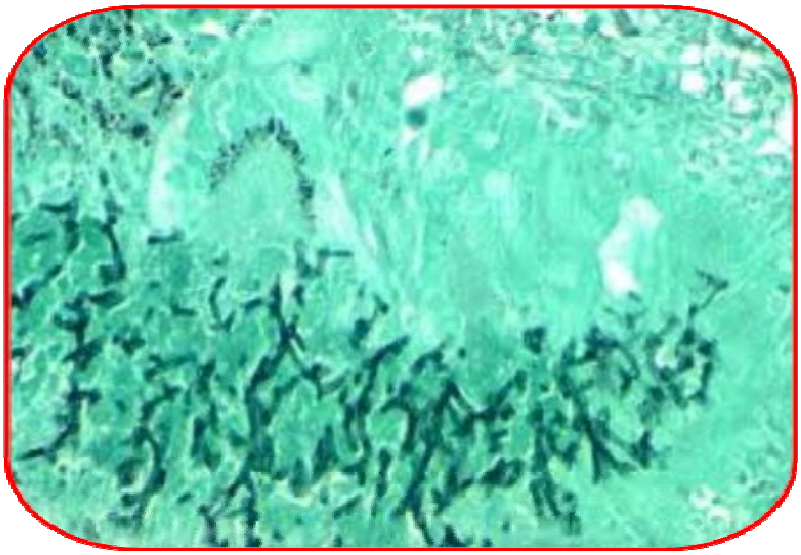


Figure 3: *Aspergillus* endocarditis
***Aspergillus* hyphae in tissue of patient with endocarditis; GMS(X240) (granuloma.homestead)**

Diagnosis:

On chest X-ray and CT, pulmonary aspergillosis classically manifests as a halo sign, and, later, an air crescent sign. (8) In hematologic patients with invasive aspergillosis, the galactomannan test can make the diagnosis in a noninvasive way.

On microscopy, *Aspergillus* species are reliably demonstrated by silver stains, e.g., Gridley stain or Gomori methenamine-silver. (9) These give the fungal walls a gray-black colour. The hyphae of *Aspergillus* species range in diameter from 2.5 to 4.5 µm. They have septate hyphae, (3) but these are not always apparent, and in such cases they may be mistaken for Zygomycota. *Aspergillus* hyphae tend to have dichotomous branching that is progressive and primarily at acute angles of about 45°.

Treatment:

The current treatments include voriconazole and liposomal Amphotericin B. Newer findings suggest use of mild oral steroids for a longer period of time, preferably for 6-9 months in aspergillosis in pulmonary segment.

Other drugs used, such as Amphotericin B, caspofungin (in combination therapy only), flucytosine (in combination therapy only) or itraconazole, (10,11) are used to treat this fungal infection. However, a growing proportion of infections are resistant to the triconazoles. (12)

Blastomycosis

(North American Blastomycosis, Checago disesase)

The term "South American blastomycosis" is sometimes used to describe infection with *Paracoccidioides brasiliensis*, (13) though the term Paracoccidioidomycosis is more frequently used to describe this condition. Blastomycosis (also known as "North American blastomycosis," "Blastomycetic dermatitis," and "Gilchrist's disease"(14) is a fungal infection caused by the organism *Blastomyces dermatitidis*. Endemic to portions of North America, blastomycosis causes clinical symptoms similar to histoplasmosis. (15)

Clinical manifestations:

1. Pulmonary blastomycosis:

The lung is the organ most commonly infected. (16) The onset is usually indolent. Occasionally, patients have an acute onset, with fever, productive cough and pleuritic chest pain. The chest radiograph shows single or multiple nodular or patchy infiltrates. Many patients with an acute onset of blastomycosis or blastomycotic pneumonia improve spontaneously after 2-12 weeks of symptoms. Figure(4)

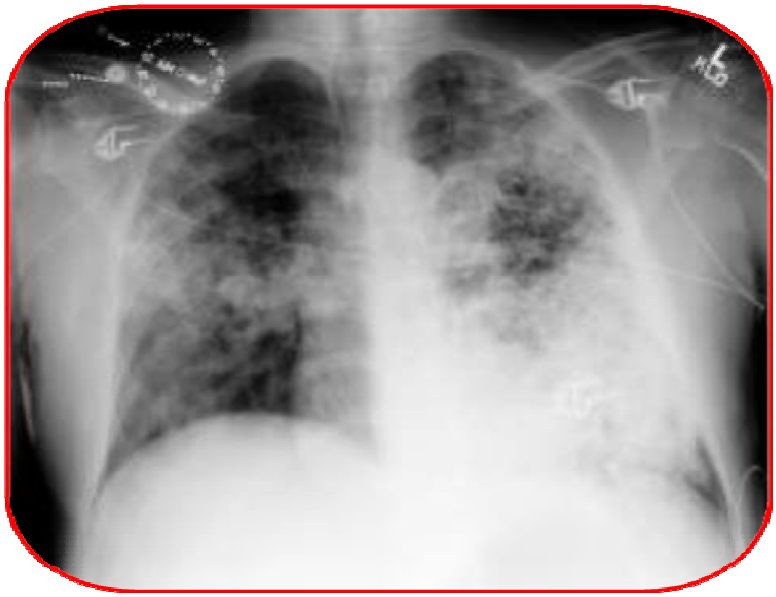


Figure 4: Chest radiograph of pulmonary blastomycosis
A symptomatic pneumonitis in a young male
(bestpractic.bmj.com)

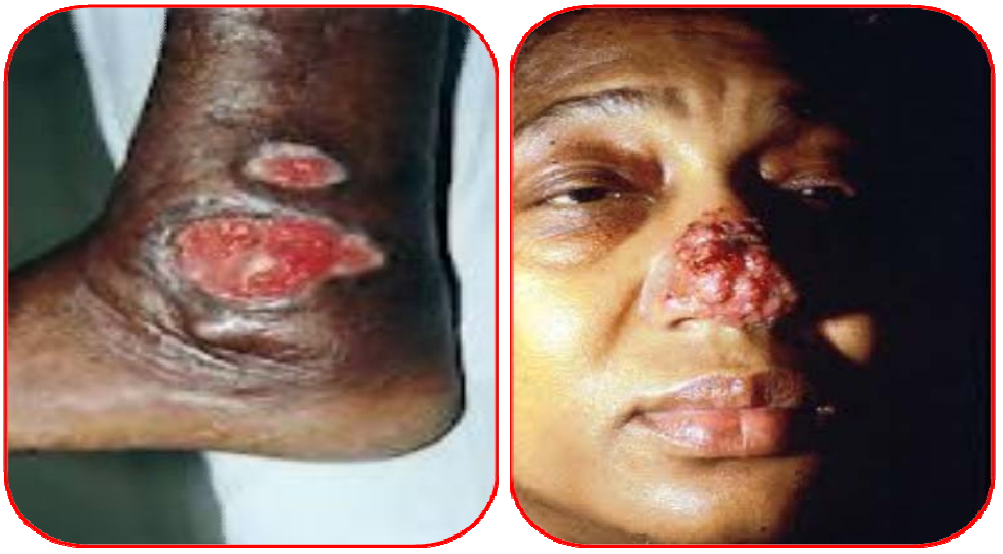
Although clinical and radiologic cure may be complete, some patients will appear months or years later with no lung disease but extra-pulmonary spread. Symptoms have been present for weeks, months or even years before diagnosis.

Symptoms are cough, weight loss chest pain, skin lesion, fever, hemoptysis, localized swelling, and veriety of less common symptoms. Laryngeal blastomycosis resemble squamous cell carcinoma both visually and histopathologically. Cavitation may appear.

Even a patient with a previously indolent disease may suddenly develop a widespread interstitial pattern and die in as a little as 2 weeks.

2. Cutaneous blastomycosis:

Lesions are most commonly located on the face, extremities, neck, and scalp. Figure(5,6). Although the location in the exposed surfaces might suggest inoculation, most lesions arise from hematogenous seeding. (17). Figure(7)



A **B**

Figure 5: Cutaneous blastomycosis

A, Blastomycosis of the leg.Typical lesions include a well-demarkated, rolled border with ulceration(medicalpicturesinfo.com).

B, Blastomycosis of the nose. Typically include crusted center Ulceration (microblog.me.UK)

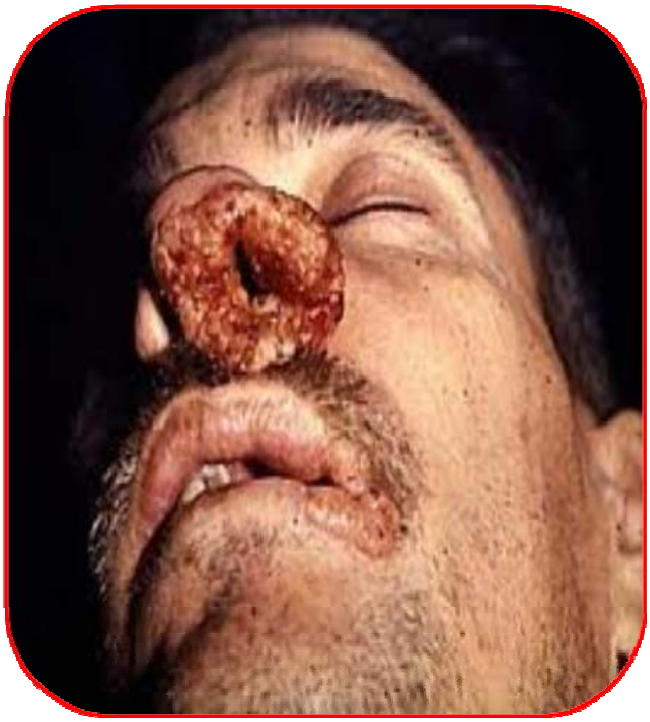


Figure 6
Cutaneous blastomycosis. Verrucous nasal lesion (picturesdepot.com)

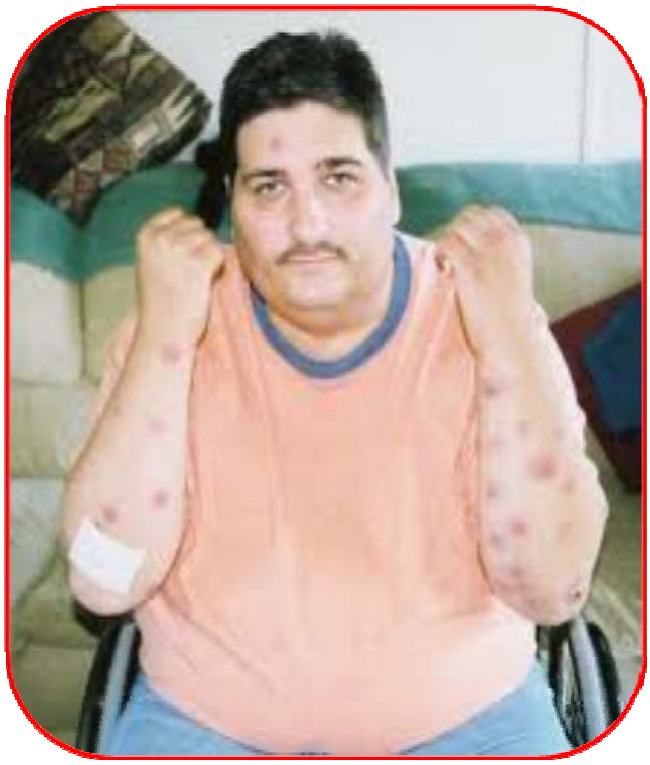


Figure 7
Cutaneous blastomycosis. A hematogenous disseminated cutaneous blastomycosis (blastomycosis.cax)

Occasionally, blastomycotic skin lesions have occurred at the site of previous trauma. This event raises 3 possibilities:

a. Local inoculation of etiologic agent has occurred.

b. Hematogenous seeding preferentially has occurred in area of trauma. or

c. Trauma has simply called attention to a minimally symptomatic hematogenous lesion.

There are cases have arisen from the bite of an infected dog. Inoculated lesions begin 1-5 weeks after injury with a slightly tenser, red or purplish nodule at the site of inoculation. Hematogenous skin lesions usually arise as indolent, well-circumscribed, erythematous nodules that are non-tender or only very slightly tender. Local adenopathy is uncommon. The papule enlarge over weeks or months, developing either a verrocous or ulcerated surface. After months or years, central healing with extensive fibrosis can occur in untreated cases. *Blastomyces* may cause well-circumscribed indurated lesion in the mucosa of the nose, larynx or mouth. Like skin lesions, mucosal blastomycosis is relatively painless.

3. Osteoarticular blastomycosis:

Osteomyelitis is found in 14-60% of all cases. The most common sites are the vertebrae, skull, ribs, and the distal half of the extremities, but almost any bone may be infected and multiple bony lesions are often found. (18) Radiography shows a sharply defined area of osteolysis.

Whereas symptoms leading to medical attention are usually cutaneous, pulmonary or systemic. Osseous lesions may produce symptoms by development of abscesses in adjacent soft tissues, by spread to contiguous joints, or by vertebral collapse. Blastomycotic arithritis is not rare. Joints disease first appears as swelling, pain, and limited motion in an elbow, knee, or ankle.

4. Genitourinary tract infection:

B.dermatitidis **can be cultured from centrifuged urine sediment in approximately forth of cases. (19) The source is usually the prostate, epididymis, or kidney. Pateints with proststitis may complain of difficulty initiating urination and dysuria. Epididimytis presents as scrotal swelling and the infection often spreads to the adjacent testes or along the spermatic cord. Endometrial blastomycosis rarely occure.**

5. Central nervous system infection:

Hematogenous spread to the brain has been reported to occur in 3-10% of cases. (20) The dominant neurological manifestations are: Meningitis, brain abscesses, spinal epidural lesion and cranial epidural lesions. Abscesses or granuloma in the brain were multiple whereas spinal cord granulomas or abscesses typically were single.

6. Involovment of other organs:

Blastomycotic lymphadenitis resembles that due to TB. Adrenal lesions are common. Intraocular infection has included focal choroiditis and endophthalmitis.

7. Systemic symptoms:

Fever is characteristically high in acute pulmonary blastomycosism, is low grade or abscent early in the course of disseminated disease, and become symptomatic again during sever illness. (19) Malaise and weight loss also present in systemic complications.

8. Involvement of other organs:

Blastomycotic lymphadenitis resemble that due to TB. Adrenal lesions are common. Intraocular infection has included focal endophthalmitis. (21)

Diagnosis:

Once suspected, the diagnosis of blastomycosis can usually be confirmed by demonstration of the characteristic broad based budding organisms. (22). Figure(8) in sputum or tissues by KOH, cytology, or histology. Tissue biopsy of skin or other organs may be required in order to diagnose extra-pulmonary disease. Commercially available urine antigen testing appears to be quite sensitive in suggesting the diagnosis in cases where the organism is not readily detected. While culture of the organism remains the definitive diagnostic standard, its slow growing nature can lead to delays in treatment of up to several weeks.

However, sometimes blood and sputum cultures may not detect blastomycosis, (23) lung biopsy is another option, and results will be shown promptly.

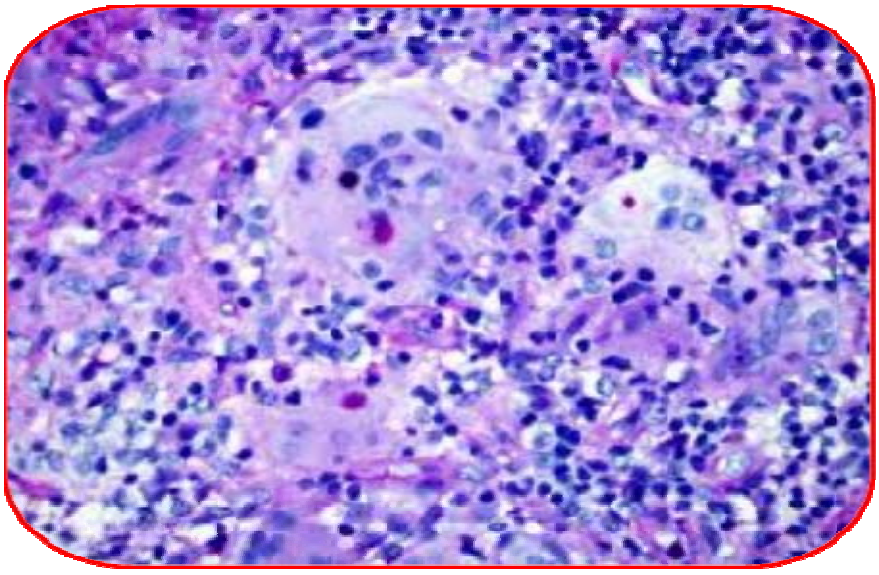


Figure 8: Blastomycosis of the lung. Microabscess of the lung with neutrophilic exudates and *Blastomyces* cells in the center (sarcomaimage.com)

Treatment:

Itraconazole given orally is the treatment of choice for most forms of the disease. Ketoconazole may also be used. Cure rates are high, and the treatment over a period of months is usually well tolerated. Amphotericin B is considerably more toxic, and is usually reserved for immunocompromised patients who are critically ill and those with central nervous system disease. Fluconazole has also been tested on patients in Canada.

Candidiasis (Moniliasis, Thrush, Candidosis)

Candidiasis or thrush is a fungal infection (mycosis) of any of the *Candida* species (all yeasts), of which *Candida albicans*

is the most common. (24,25) Also commonly referred to as a yeast infection, candidiasis is also technically known as candidosis, moniliasis, and oidiomycosis. (26)

Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life-threatening diseases. *Candida* infections of the latter category are also referred to as candidemia and are usually confined to severely immunocompromised persons, such as cancer, transplant, and AIDS patients as well as non-trauma emergency surgery patients. (27)

Superficial infections of skin and mucosal membranes by *Candida* causing local inflammation and discomfort are common in many human populations. (25,28,29) While clearly attributable to the presence of the opportunistic pathogens of the genus *Candida*, candidiasis describes a number of different disease syndromes that often differ in their causes and outcomes. (25,28)

Clinical manifestations:

Pulmonary and hepatosplenic candidiasis are manifestations of hematogenous dissemination, which refer to the later one as (chronic disseminated candidiasis). Thrush is an acceptable synonym for cutaneous, oropharyngeal or vulvovaginal candidiasis or even candidiasis as a name.

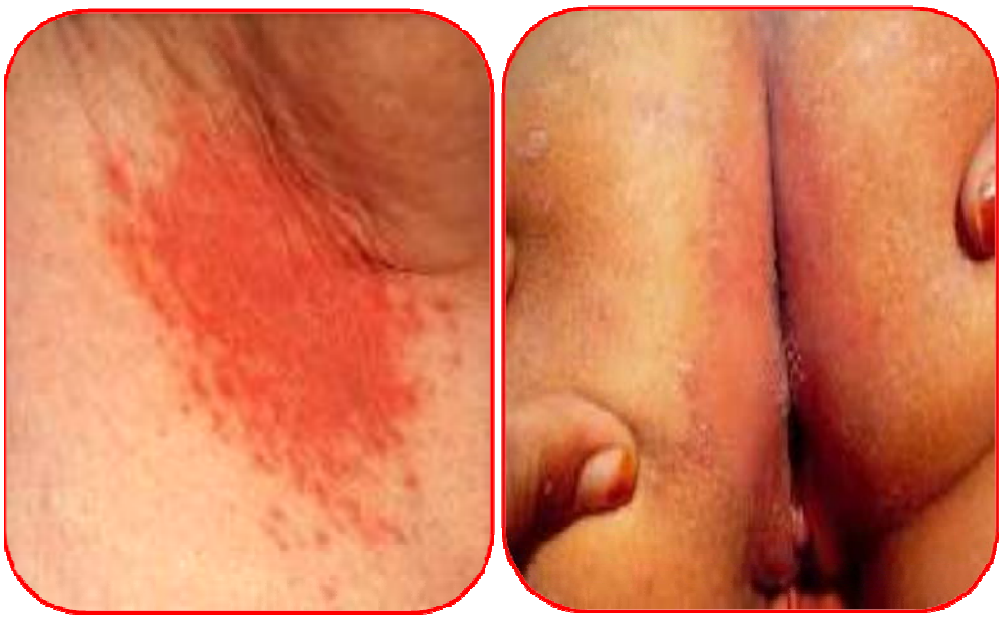
I. Superficial candidiasis:

1. (Infection of the skin, hair, nail, and mucous membrane).

Cutaneous candidiasis is a common cause of diaper rash in infants. Figure(9) A moist, macular erythematous rash is most marked in the intertriginous areas of the gluteal crease, perineum and inguinal folds.

Figure 9
Sever *Candida* diaper rash (dermnet.com)

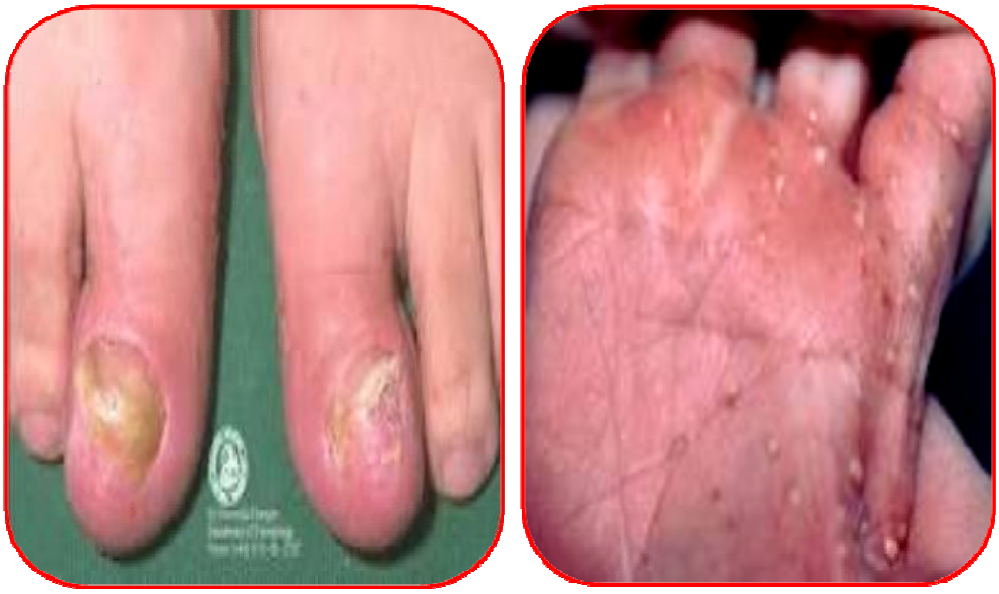
A similar distribution of intertrigo occurs in the elderly and obese adults, particularly in DM patients or after receiving antibiotic therapy. Macular erythematous patches may also be seen on the thigh or buttocks outside the confluent intertriginous areas. Figure(10)



A B

Figure 10: Candidiasis. A, Intertrigo in the axially of elderly patient (medartfx.com) B, Erythematous patches on the buttock of a child (bdigital.ces.edu.com)

In women, the infra-mammary folds may also be infected. Frequent exposure of the hands to water, result in candidiasis of the hands and fingernails. Figure(11) The areas of the hands most prone to infection are the digital folds, this microorganism also cause paronychia. Oropharyngeal candidiasis occurs in infants, patients with DM, those receiving antibacterial antibiotics and AIDS patients. (30) White patches appear on the buccal mucosa and, less commonly, on the gums, tonsillar area, Tongue and palate. Figure(12) Angular cheilitis, which is the fissuring at the corners of the mouth, commonly accompanies oropharyngeal thrush and may be the only complaint. Figure(13)



A B

Figure 11: Superficial candidiasis. A, *Candida* onychomycosis(html.rincondelvago.com). B, multiple papules distributed in the palm of hand(emedicinehealth.com)

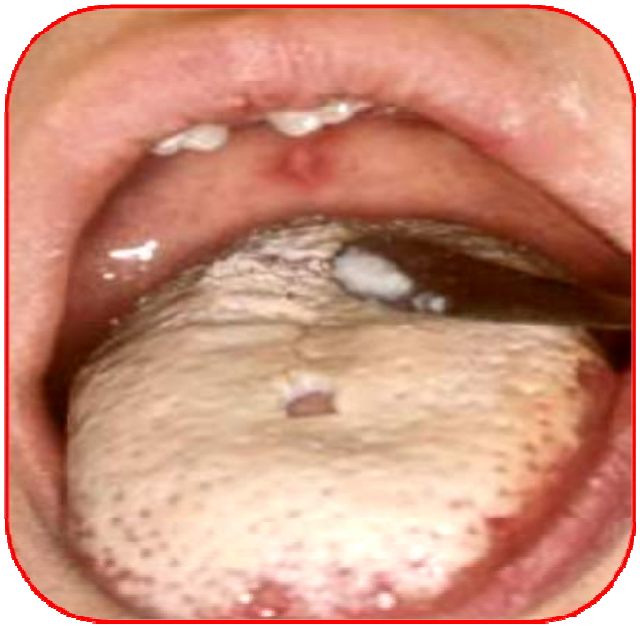


Figure 12: Extensive *Candida* glossitis in a child with oral candidiasis (drugster.info)

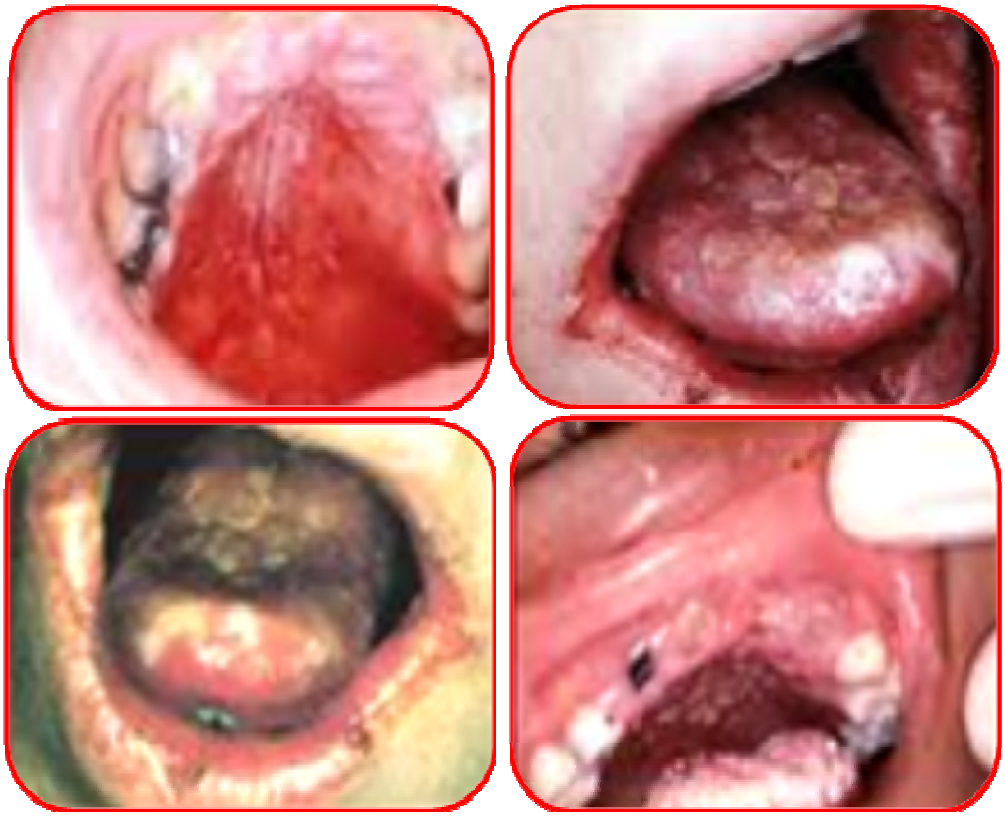


Figure 13: Angular cheilitis with oropharyngeal candidiasis (painconsortium.gov)

Vulvovaginal candidiasis occurs most commonly in post puberetal women who have DM, have taking systemic antibacterial agents are in the third trimester of pregnancy, or are sexually active. Vaginal discharge which may be curd like, itching, burning, and dyspauria are the most common symptoms. On examination, vulvar erythema and edema may be seen.

Chronic mucocutaneous candidiasis is the disease restricted in distinct entity of children. Beginning in the infancy or later in childhood, oral thrush is noted, often following antibiotic therapy.

Infection persist in the mouth and spread slowly to the skin, nails, hair, esophagus, and in female to the vagina. Figure(14) Skin lesions are begin to appear on the scalp, hand, arms, and anywhere. The cause of mucocutaneous candidiasis is unknown. Some of these patients have an inherited autosomal recessive disease, called (autoimmune polyendocrinopathy-candidiasis ectodermal dystrophy syndrome).

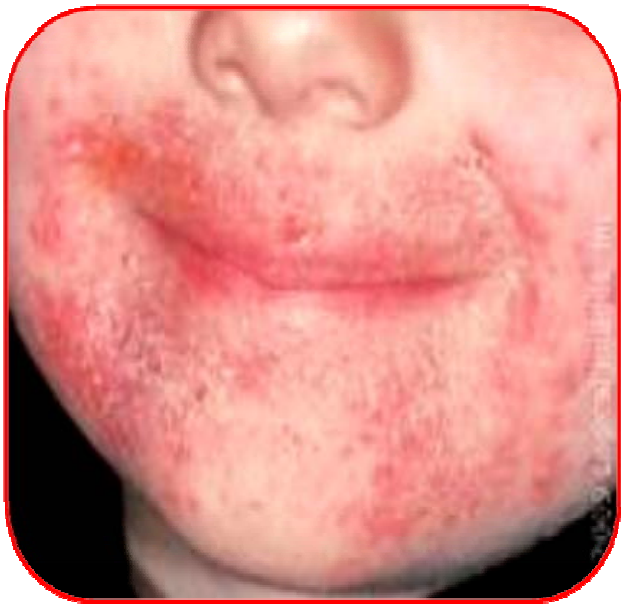


Figure 14
A child with cutaneous Candidiasis (skinsight.com)

2. Ocular candidiasis:

***Candida* keratoconjunctivitis is a complication of long-term use of corticosteroid eye drops. Additional risk factors are breaks in the corneal epithelium, such as those caused by bullos keratopathy or corneal ulcers due to herpes simplex. Findings include conjunctival erythema, cheesy discharge in the conjunctival sac and progressive corneal ulceration.**

II. Deep candidiasis:

1. Esophagitis:

Candida **esophagitis may be completely asymptomatic, or may cause burning in the substernal area, epigastrium, or throat. Pain in the substernal area is worse on swallowing. A sense of obstruction sometimes is noted when meat or other solid foods are swallowed.**

In esophagoscopym white mucosal plaques resembling oral thrush is notable in the distal third of the esophagus. Figure(15) Complications of *Candida* esophagitis include bleeding, perforation, and with chronisty lead to stenosis. (31,32)

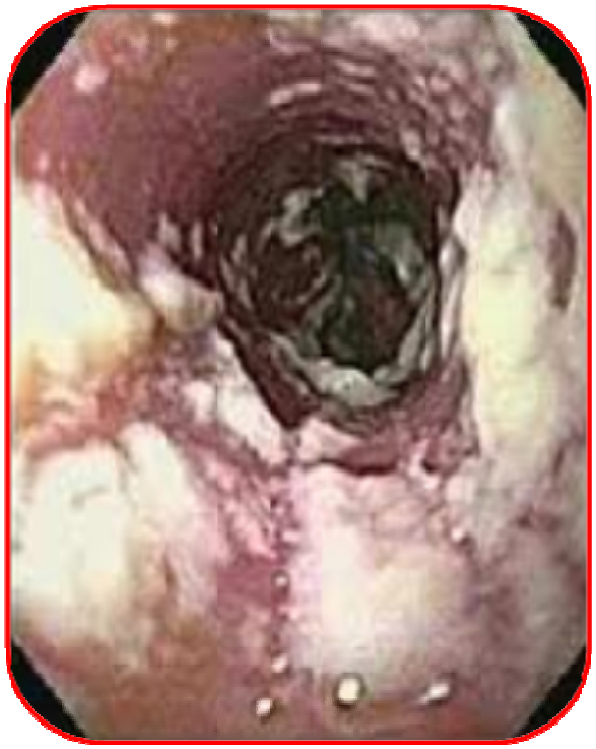


Figure 15
Candida **esophagitis. Esophagoscopy reveals white patches over infected esophagus (gastrointestinalatlas.com)**

2. Gastrointestinal candidasis:

The stomach is second only to the esophagus as a site of gastrointestinal candidiasis. Invasion of superficial gastric erosions by *Candida* is not rare. (33) Symptoms mainly attributed to underlying gastritis than to *Candida*. However, patients with acute leukemia or other hematologic malignant diseases may have numerous ulcerations of the stomach and less commonly, of the duodenum and intestine. (34) In these immunosuppressed patients *Candida* is deeply invasive and spread hematogenously to the liver, spleen and other organs. Fever is the only manifestations. Occult blood which may be found in the stool of these patients, most of whom are thrombocytopenic.

3. Peritonitis and intra-abdominal abscesses:

***Candida* peritonitis is uncommon complications of chronic ambulatory peritoneal dialysis. Presenting symptoms are abdominal pain and tenderness, with or with-out nausea, vomiting, or low grade fever. *Candida* is an occasional cause of cholysistitis, biliary tract diseas, or pancreatic abscess, usually in patients who are elderly or immunosuppressed.**

4. Respiratory tract candidiasis:

Candidal laryngitis may occur in the absence of oropharyngeal or esophageal candidiasis, presenting as hoarseness in a patient receiving antibiotics. (35) Sore throat and dysphagia are more readily attributable to accompanying pharyngitis or esophagitis.

On endoscopy, shallow alceration or gray membrane are seen on an erythematous larynx. Pulmonary candidiasis is more of an autopsy finding than a clinical entity. Pulmonary lesions arise from hematogenous seedings.

5. Urinary tract candidiasis:

Candida **in cultures of female voided urine usually are contaminants from the vulva or vaginal secretions. Colonization of the bladder most commonly is a complication of prolonged catheterization of the bladder in a patient receiving antibiotics. A bladder can also act as foreign body. Other conditions predisposing patients to bladder colonization are DM and diseases that lead to incomplete bladder empyting such as diabetic neurologic bladder, chronic obstruction from from prostatic hypertrophy, or pelvic irradiation for cervical carcinoma.**

Pyuria in the presence of candiduria can signify bladder candidiasis, but it is more often due to the underlying diseases of bladder. Considering that the kidney receive about a fourth of the cardiac output, it is not surprising that hematogenous candidiasis is prone to cause renal abscesses.

6. Hematogenously disseminated candidiasis:

Approximately 10-15% of cases of septicemia seen in tertiary care hospitals are caused by ***Candida*** **spp. Factors predisposing patients to hospital – acquired candidemia include intra venous (IV) catheters, administration of**

antibacterial antibiotics, urinary catheters, surgical procedures, corticosteroid therapy, neutropenia, and severe burns. (36) *Candida albicans* is most common, but *C. tropicalis* accounting for (third to fourth) of the *Candida* isolates from the blood of neutropenic patients. (37)

***Candida parapsilosis* is the next most common, tending to enter yhe circulation from a cutaneous source, such as IV catheters or heroin injections.**

Symptoms of *Candida* sepsis are like those of bacterial one, with fever, or in the most severe cases shock or disseminated intravascular coagulation (DIC). The concentration of *Candida* in the circulation may be small and candidemia may be difficult to detect in immunosuppressed patients, despite rapid deterioration and death. In more immunological intact patients, high levels of candidemia may persist for several days with-out causing shock or death. *Candida* in patients with acute leukemia in relapse is likely to seed the liver, spleen, and kidneys, causing an entity called hepatosplenic candidiasis.

a. Disseminated candidasis in neonates:

Chorioamnionitis may arise from prolonged rupture of the membrane and may cause a diffuse erythematous rash and diffuse pulmonary infiltrates in the newborns.

Neonates with long term antibiotics or parentral nutrition by umbilical or IV catheters are prone to candidemia. Early

clinical manefistations of candidemia in very low birth-weight neonates include temperature instability, abdominal destination, respiratory failure and hyperglycemia. Candidemia in neonates has a strong propensity to cause meningitis, endocarditis, renal cortical abscesses, and arthritis of the knee. (38,39)

b. Chronic disseminated (hepatosplenic) candidiasis:

This entity occurs in patients with severe neutropenia, (40) from acute leukemia. The causative agents are *C.albicans*, or *C. tropicalis*.

The portal of entry for *Candida* may be gastrointestinal ulcerations or indwelling I.V catheters. The first evidence of this disease is fever that returns when Amphotericin B therapy is discontinued or fever that fails to disappear when the neutrophil count returns to normal.

c. Disseminated candidiasis due to heroin abuse:

Between 2-8 hrs after a heroin injection, patients have a sudden onset of high fever, chills, myaligia, headache and sweating. After 1-4 days, painful nodules or pustules appear on the scalp and other hairy areas. *Candida* may grow into the hair shafts, causing purulent folliculitis. Impure (brown) heroin has been associated with this syndrome. (41)

d. Suppurative phlebitis:

Catheters in peripheral veins may lead to supperative infection of the veins extending proximally from the catheter insertion site.

Prolonged administration of antibiotic through a peripheral catheter, underlying illness and inadequate catheter care the predisposing factors. Fever and candidemia are uniformly present.

Most patients with peripheral phlebitis have an erythematous tender cord over the vein. *Candida* can also cause phlebitis when deep veins, such as subclavian or internal carotid, are catheterized.

e. Endocarditis and pericarditis:

Candidemia can cause subacute endocarditis, particularly in patients with a previously abnormal native valves or prosthetic heart valves. (42) IV drug abuse and IV catheters are most common predisposing causes. Figure(16) Fever, embolic phenomena and cardiac failure occur, just as in subacute bacterial endocarditis.

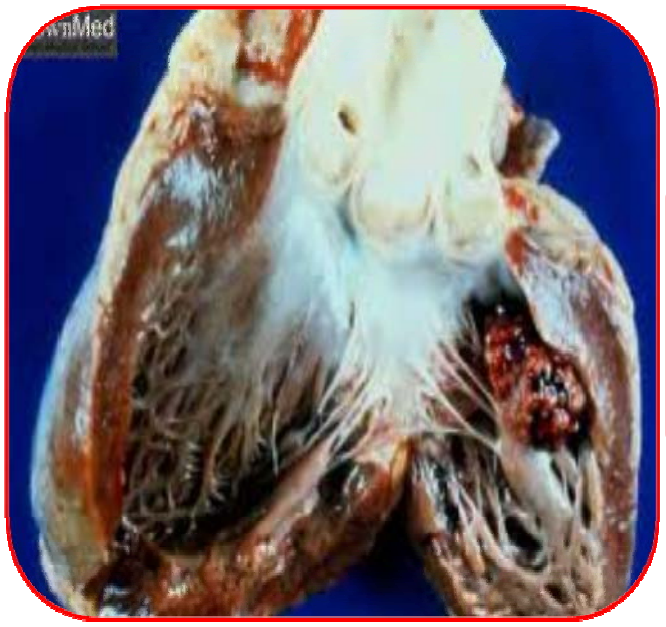


Figure 16
Severe *Candida* endocarditis. View from the left atrium of opened heart (brown.edu)

f. Arthritis:

Prosthetic, (43) or rheumatoid, (44) joints are prone to infected by *Candida*, either by hematogenous spread or by inadvertent direct inoculation, (45) during joint surgery or intra-articular corticosteroid injection. Figure(17)

Arthritis may also be a late sequel of candidemia in neonates or neutropenic patients. Infection of the knee, which is the most common site, causes pain on weight bearing or on full extension.

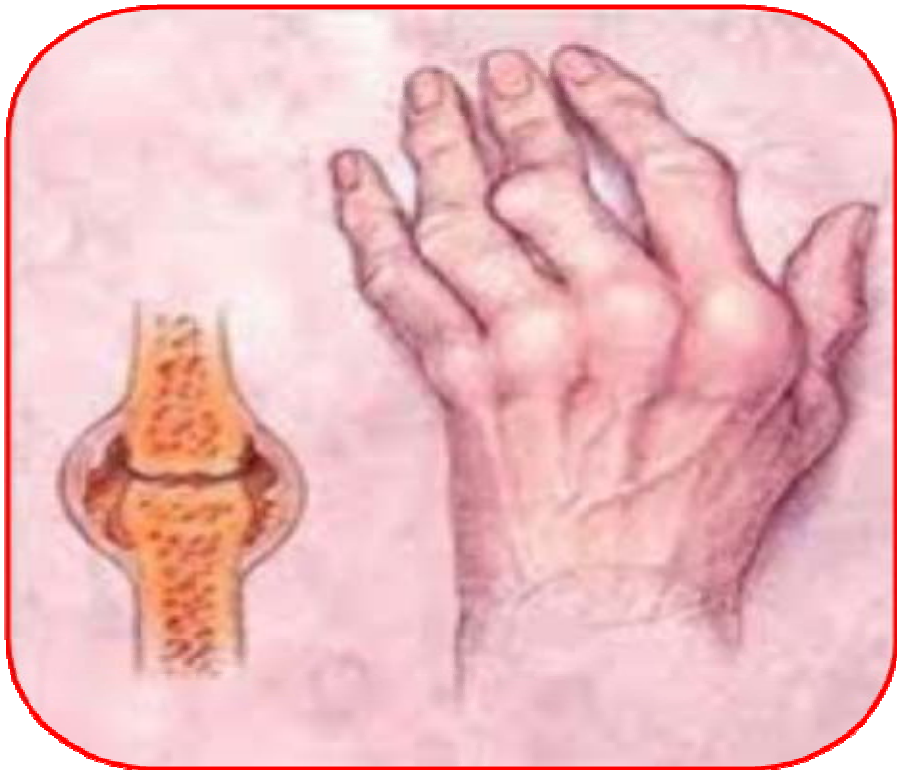


Figure 17
***Candida* rheumatoid Arthritis**
(candidayeastcleanse.com)

g. Osteomyelitis:

Bone is infected hematogenously, with the occasional exception of sternal infection complicating median sternotomy. (46). Figure(18) The indolent onset of fever and back pain herald the onset.

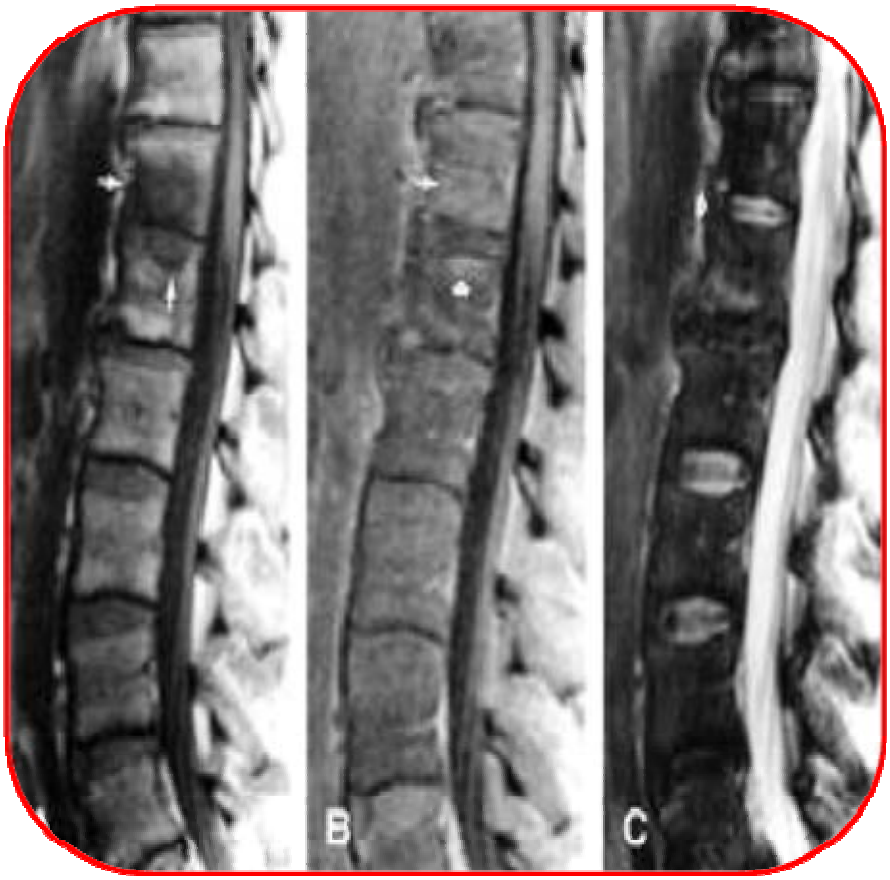


Figure 18
***Candida* vertebral osteomyelitis.**
Note the involvement of the disk and both adjacent vertebral bodies (arrowed) (ajnr.org)

h. Endophthalmitis:

When careful examination of the eye are done, 10-37% of adults with candidemia have evidence of endophthalmitis. (47) Although ocular examination becomes abnormal within two weeks of the fungemia.

Small sharply outlined white retinal lesions in the posterior pole are the earliest sign. Figure(19)

Figure 19: Ocular candidiasis. Foccal retinal lesions of hematogenous *Candida* endophthalmitis (timm.main.teikyo-U.ac.jp)

Retinal lesions progressively enlarge, and the inflammation spread to the overlying vitreous humor. With the development of the disease, the patients complain of (floaters) or cloudy vision. Once the vitreous or aqueous humor has

become cloudy, progression to blindness is used unless effective therapy is given. (48)

Endophthalmitis is a rare complication of ocular surgery, such as intraocular lens implantation.

i. Central nervous system candidiasis:

***Candida* meningitis is seen in low-birth weight neonates with candidemia and in patients with hematogenous malignancies, complicated neurosurgery, or intra-cerebral prosthetic devices such as ventriculo-peritoneal shunt. An indolent course with little or no fever is common. Hydrocephlus can result from chronic mengitis or shunt obstruction. Patients who die of disseminated candidiasis may have multiple small intracerebral lesions. Rarely, a solitary intracerebral granuloma is found as a late sequel of candidemia.**

Diagnosis:

Diagnosis of a yeast infection is done either via microscopic examination or culturing.

For identification by light microscopy, a scraping or swab of the affected area is placed on a microscope slide. A single drop of 10% potassium hydroxide (KOH) solution is then added to the specimen. The KOH dissolves the skin cells but leaves the *Candida* cells intact, permitting visualization of pseudohyphae and budding yeast cells typical of many *Candida* species.

For the culturing method, a sterile swab is rubbed on the infected skin surface. The swab is then streaked on a culture medium. The culture is incubated at 37 °C for several days, to allow development of yeast or bacterial colonies. The characteristics (such as morphology and colour) of the colonies may allow initial diagnosis of the organism that is causing disease symptoms. (49)

Treatment:

In clinical settings, candidiasis is commonly treated with antimycotics, the antifungal drugs commonly used to treat candidiasis are topical clotrimazole, topical nystatin, fluconazole, and topical ketoconazole.

For example, a one-time dose of fluconazole (150-mg tablet taken orally) has been reported as being 90% effective in treating a vaginal yeast infection. This dose is only effective for vaginal yeast infections, and other types of yeast infections may require different dosing. In severe infections Amphotericin B, caspofungin, or voriconazole may be used. Local treatment may include vaginal suppositories or medicated douches. Gentian violet can be used for breastfeeding thrush, but when used in large quantities it can cause mouth and throat ulcerations in nursing babies, and has been linked to mouth cancer in humans and to cancer in the digestive tract of other animals. (50)

Chlorhexidine gluconate oral rinse is not recommended to treat candidiasis, (51) but is effective as prophylaxis, (52) chlorine dioxide rinse was found to have similar *in vitro* effectiveness against *Candida*. (53)

C. albicans **can develop resistance to antimycotic drugs. (54) Recurring infections may be treatable with other anti-fungal drugs, but resistance to these alternative agents may also develop.**

Coccidoidomycosis
(Coccidoidal granuloma, Vally fever, Desert rheumatism, California disease)

Coccidioidomycosis [pronounced: kok-siddee-oydo-my-cohssiss](commonly known as "Valley fever", (55) as well as "California fever", "Desert rheumatism", and "San Joaquin Valley fever") is a fungal disease caused by *Coccidioides immitis* or *C. posadasii*. (56) It is endemic in certain parts of Arizona, California, Nevada, New Mexico, Texas, Utah and northwestern Mexico. (57)

C. immitis **resides in the soil in certain parts of the southwestern United States, northern Mexico, and parts of Central and South America. (58) It is dormant during long dry spells, then develops as a mold with long filaments that break off into airborne spores when the rains come. The spores, known as arthroconidia, are swept into the air by disruption of the soil, such as during construction, farming, or an earthquake. (59) Infection is caused by inhalation of the particles. The disease is not transmitted from person to person. *C. immitis* is a dimorphic saprophytic organism that grows as a mycelium in the soil and produces a spherule form in the host organism.**

Clinical manefistations:

1. Primary coccidoidomycosis:

The onset of symptoms usually begins 10-16 days following exposure, the extremes being 7-28 days. (60) The most common symptoms is fever, usually accompanied by pleuritic or dull, aching chest pain. (61)

Cough of some degree is usually present and may be either dry or productive of white, purulent, or blood streaked sputum. More dramatic and persistent is the rash of erythema nodosum, or erythema multiform. These cutaneous manifestations appear at any time from several days to 3 weeks after the onset of respiratory symptoms. Figure(20) Erythema nodosum, most likely to occur in female, appears bright red, painful nodules over both pretibial areas. In a third of cases, are accompanied by arthralgia in one or more joints, particularly the ankle or knee. This symptom complex gave rise to the old name (desert rheumatism).

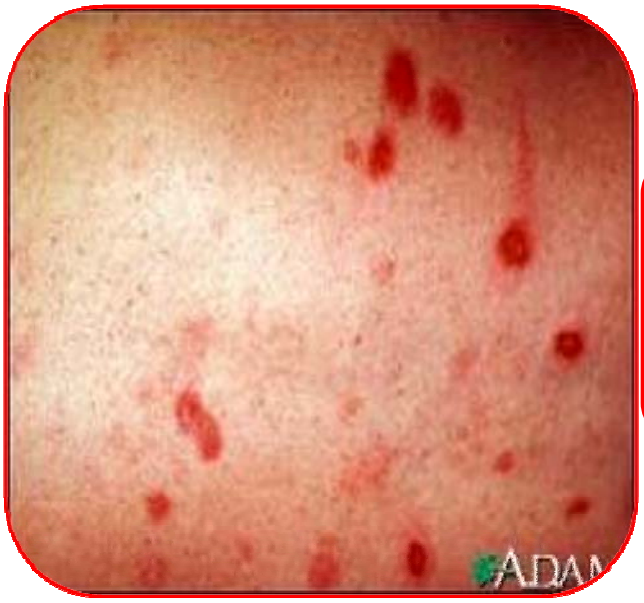


Figure 20
Erythematous skin lesion associated with acute primary pulmonary coccidoidomycosis (perridermatology.com)

2. Pulmonary residual of primary coccidoidomycosis:

Many cases of coccidoidal pneumonia resolve by forming a dense, spherical nodules in the area of infiltrate. Coccidoidoma (coccidoidal multiple nodules) are benign, but some solitary nodule may be cancerous can lead to unnecessary thoractomy. The most common symptoms were hemoptysis, cough when present, is dry , while fever, weight loss, and night sweats are not encountered. Extention of the cavity to the pleura can cause bronchopleural fistula m pneumothorax, and coccidoidal empyema, chest pain, dyspnea, and low grade fever may herald the appearance of such complications.

3. Disseminated coccidoidomycosis:

The most frequently fatal complication of coccidoidomycosis is hematogenous dissemination. Dissemination is more likely to occur in nigroes, filipians, and immunesupressed patients. (62) Dissemination is more indolent in onset, heralded by the appearance of skin lesions, figure(21), subcutaneous abscesses, bone lesions, or chronic meningitis.

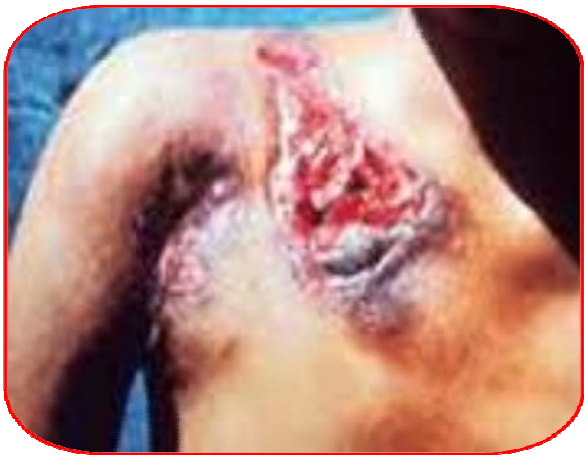


Figure 21
Cutaneous lesions of coccidoidomycosis. Massive lesion with peripheral healing. (bioidea.net)

Meningitis occur in third to half the cases of disseminated coccidoidomycosis either alone or along with infection of other extra-pulmonary organs. Lymphadinitis is a common sequel in all forms of coccidoidomycosis, including disseminated diseases.

At autopsy, lesions are often present in the liver, kidneys, (63) and adrenal but clinical disease is rarely seen in these sites.

Diagnostic tests:

Fungal infection can be demonstrated by microscopic detection of diagnostic cells in body fluids, exudates, sputum and biopsy-tissue. Figure(22) With specific nucleotide primers *C. immitis* DNA can be amplified by PCR. It can also be detected in culture by morphological identification or by using molecular probes that hybridize with *C. immitis* RNA. An indirect demonstration of fungal infection can be achieved also by serologic analysis detecting fungal antigen or host antibody produced against the fungus.

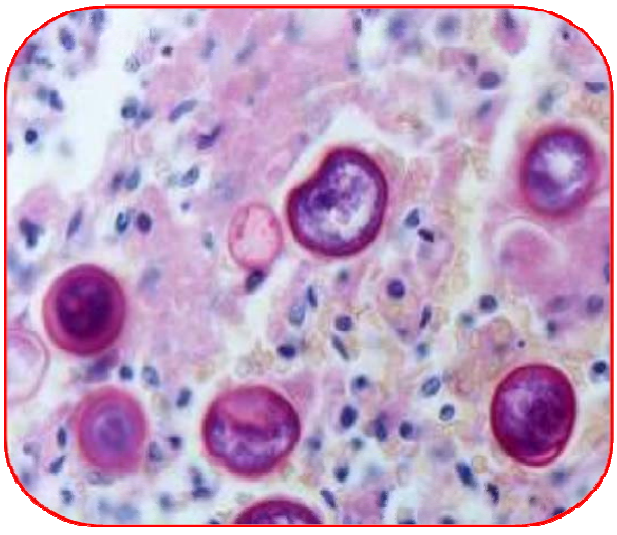


Figure 22 Morphology of *Coccidoidis immitis* cells in human tissue. Young spherules with HandE staining(X1000) (pawprintshemagazine. com)

Treatment:

There are no published prospective studies that examine optimal antifungal therapy for coccidioidomycosis. Mild cases often do not require treatment. Oral Fluconazole and intravenous Amphotericin B are used in progressive or disseminated disease, or in which patients are immunocompromised. Alternatively, itraconazole or ketoconazole may be used. (64) Posaconazole and voriconazole have also been used.

Cryptococcosis
(Torulosis, European Blastomycosis, Busse - Buscke disease)

Cryptococcosis, or cryptococcal disease, is a potentially fatal fungal disease. It is caused by one of two species; *Cryptococcus neoformans* and *Cryptococcus gattii*. These were all previously thought to be subspecies of *C. neoformans*, but have now been identified as distinct species.

Cryptococcosis is believed to be acquired by inhalation of the infectious propagule from the environment. Although the exact nature of the infectious propagule is unknown, the leading hypothesis is the basidiospore created through sexual or asexual reproduction.

Cryptococcosis is a defining opportunistic infection for AIDS. Other conditions which pose an increased risk include certain lymphomas (e.g. Hodgkin's lymphoma), sarcoidosis, and patients on long-term corticosteroid therapy.

Distribution is worldwide in soil. (65) The prevalence of cryptococcosis has been increasing over the past 20 years for many reasons, including the increase in incidence of AIDS and the expanded use of immunosuppressive drugs.

In humans, *C. neoformans* causes three types of infections:

- **Wound or cutaneous cryptococcosis**
- **Pulmonary cryptococcosis, and**
- **Cryptococcal meningitis.**

Although the most common presentation of cryptococcosis is of *C. neoformans* infection in an immunocompromised person (such as patients with AIDS), the *C. gattii* is being increasingly recognised as a pathogen in presumptively immunocompetent hosts, especially in Canada and Australia. This may be due to rare exposure and high pathogenicity, or to unrecognised isolated defects in immunity specific to this organism.

Clinical manefistations:

1. Meningiencephalitis:

Infection of the brain and meninges is the most common clinical manifestations of cryptococcosis and the most common cause of death.

Symptoms depend on the rapidity of onset. The indolent cases note headache, initially relieved by analgesics. Pain is dull, bilateral, and fairly diffuse. Nausea, dizziness, irritability, decrease comprehension, impaired memory, gait ataxia gradually supervene. Figure(23,24)

Changing personality and mental acuity are reported by family members, but not by the patient himself. As infection progress, lassitude, blurred vision, double vision or a blind spot (scotoma) in the visual field may be noted. The onset of coma may be sudden, sometimes accompanied by respiratory arrest. Fever is often low grade or absent until late in the course of infection.

At the other extreme, patients may deteriorate and die within as little as two weeks of onset.

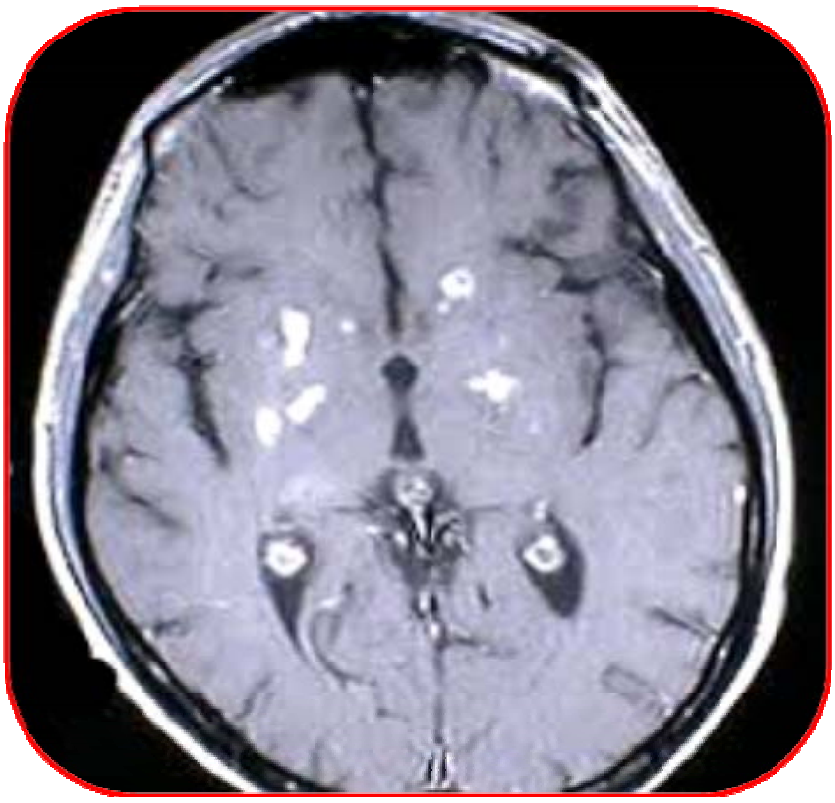


Figure 23: Cryptococcal meningoencephalitis. A computed tomographic section through a brain of patient with meningoencephalitis (med.uc.edu)

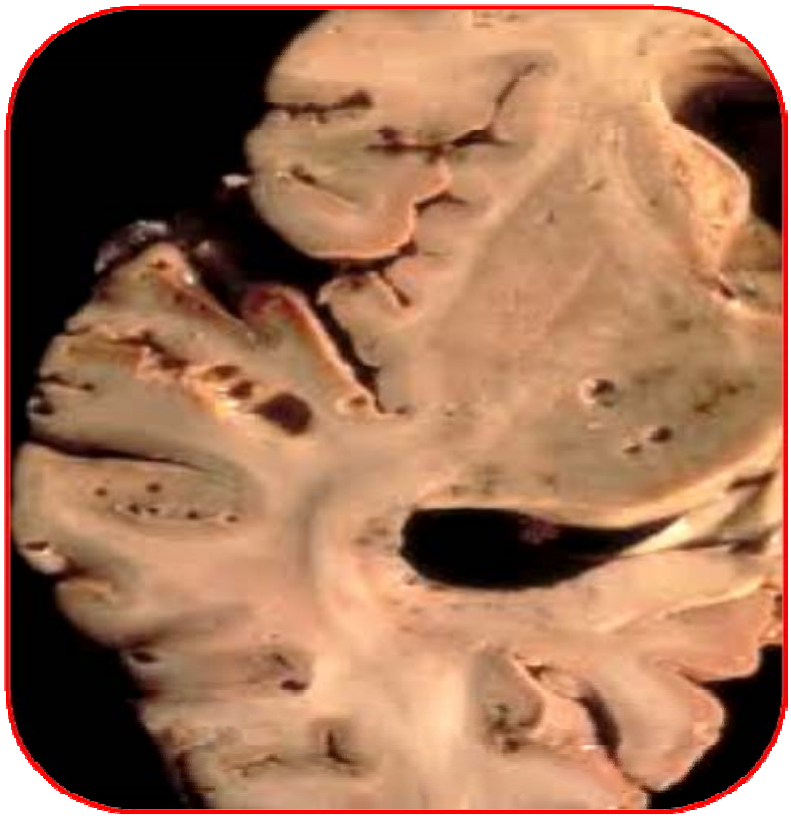


Figure 24: Cut surface of the brain showing cystic Cluste Cryptococci (neuropathologyweb.org)

2. Pneumonia:

Pulmonary cryptococcosis has many infestations. Patients with preexistence lung diseases may have asymptomatic colonization of their bronchial tree and have *C. neoformans* cultured from their sputum over many years. Normal patients may experience a self-limited pneumonia. Figure(25) The onset is indolent and symptoms, present in only half the cases, are dry cough, dull chest pain, and little or no fever. Central cavitations are found occasionally. (66) Calcification is rare.

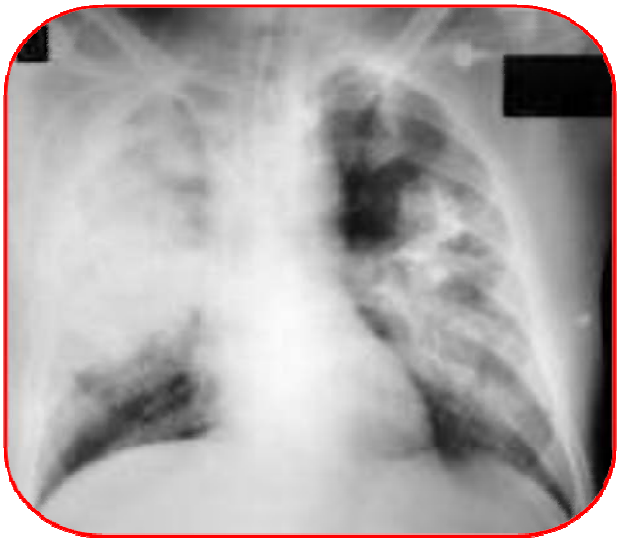


Figure 25
Chest x-ray sudy of a patient with unilateral cavity due to pulmonary cryptococcosis (thorax.highwire.org)

3. Skin lesions:

Hematogenous lesions to the skin occur in about 10% of patients with cryptococcosis, being more common in immunosuppressed patients. (67). Figure(26) The lesion start with painless papule, as the lesion become several centemeteres in diameter, the center often becomes shiny and flat.

Figure 26: Cutaneous cryptococcosis. Maculopapular lesions In an AIDS patients resembling molluscum contagiousum.Left picture (ftguoline.org), right picture (scielo.br)

With progression the center become depressed and usually ulcerated, draining a thin exudates that contains numerous causative agents. Secondary infection is not notable.

4. Bones and joints involvement:

Skeletal cryptococcosus has occurred in about 5% of reported cases. (68) The involved bones as follows: Pelvis, vertebrae, skull, ribs, with lesser No. in other bones. Figure(27) X-rays show one or more well-circumscribed areas of osteolysis. A (cold abscess) occurs in time, with sanguine-purulent exudates accumulating in the adjacent soft tissues. This exudates contains numerous cryptococci.

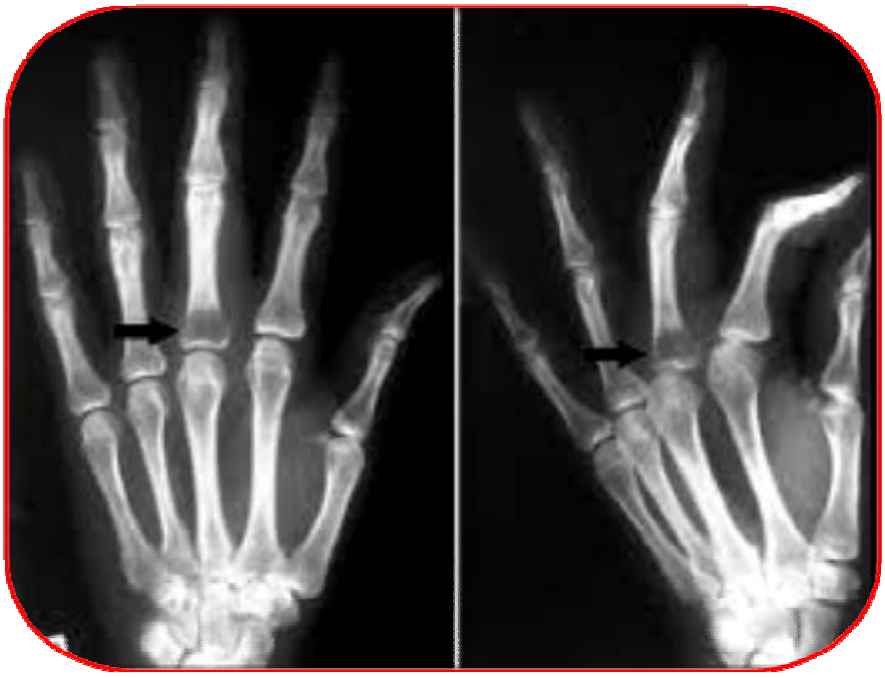


Figure 27:Cryptococcal bone lesions. X-rays study of metatarsal bones shows well-circumscribed areas of osteolysis (arrows) (ijpmonline.org)

5. Eye lesions:

Ocular manifestations of cryptococcosis include optic atrophy, extra-ocular motor palsy, scotoma, chorioretinitis. Blindness is one of the permenant sequel of the disease. (69). Figure(28)

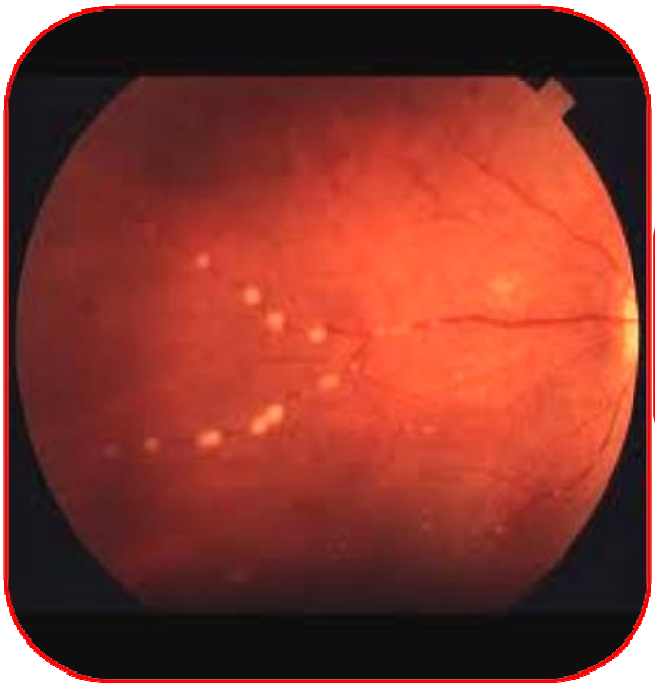


Figure 28
Retinography shows cryptococcal retinal lesions (eyepathologist.com)

6. Other sites of cryptococcosis:

***C. neoformans* is often cultured from the urine of patients with disseminated infection, but the site within the urinary tract is clinically occult. Occasionally, signs of pyelonephritis, (70) or prostatitis are present. Adrenal cortical lesions are common at autopsy.**

Endocarditis on native or prosthetic valves, hepatitis, draining lymph nodes, sinusitis and localized esophageal lesions, (71) are the other clinical manifestations. Figure(29)

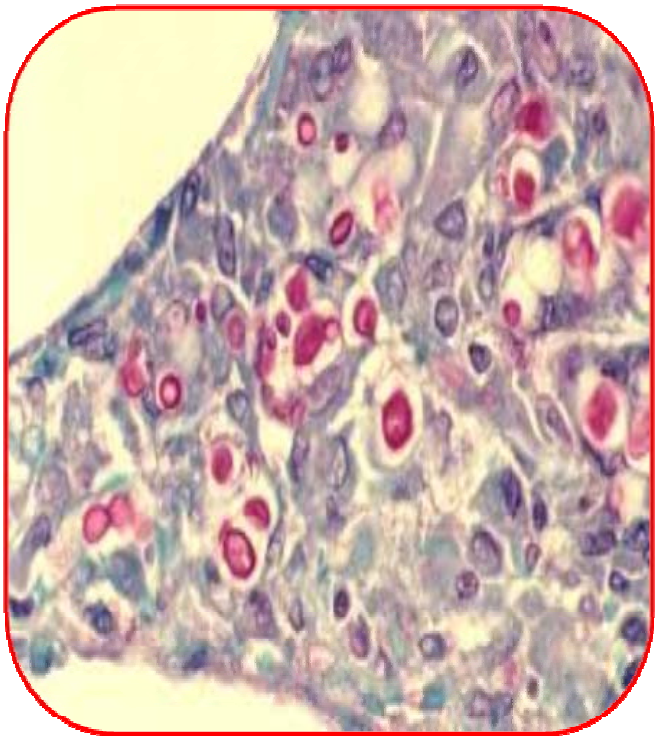


Figure 29
A section of liver infected with *Cryptococcus neoformans* stained with HandE. (X225) (en.wikipedia.org)

Diagnosis:

Symptoms include fever, fatigue, chest pain, dry cough, swelling of abdomen, headache, blurred vision and confusion. (64)

Detection of cryptococcal antigen (capsular material) by culture of CSF, sputum and urine provides definitive diagnosis. Blood cultures may be positive in heavy infections.

Cryptococcosis can rarely occur in the immunocompetent person without HIV, when it usually goes undiagnosed. Less than 250 cases in all are reported in the medical literature, the majority diagnosed postmortem. (72)

Treatment:

Treatment options in non-AIDS patients who have reduced immune-system function is not well studied. Intravenous Amphotericin B combined with oral flucytosine may be effective. Every attempt should be made to reduce the amount of immunosuppressive medication until the infection is resolved.

AIDS patients often have a reduced response to Amphotericin B and flucytosine, therefore after initial treatment as above, oral fluconazole can be used. (64) The decision on when to start treatment for HIV is not yet settled, although one small, under-powered trial suggested that delaying the start of treatment for 10 weeks may be beneficial in avoiding deaths from Immune reconstitution inflammatory syndrome IRIS (immune reconstitution inflammatory syndrome). (73)

Histoplasmosis
(Darling's diseases, Ohio Vally disease)

Histoplasmosis (also known as "Cave disease", "Darling's disease","Ohio valley disease", "Reticuloendotheliosis", (74) "Spelunker's Lung" and Caver's disease) is a disease caused by the fungus *Histoplasma capsulatum*. Symptoms of this infection vary greatly, but the disease primarily affects the lungs. (75) Occasionally, other organs are affected; this is called disseminated histoplasmosis, and it can be fatal if left untreated. Histoplasmosis is common among AIDS patients because of their suppressed immune system. (76)

Clinical manifestations:

Although there is no uniformly accepted system for categorizing the various manifestations of classic histoplasmosis, the disease is so heterogeneous.

1. Acute pulmonary histoplasmosis:

The full spectrum of this disease is best recognized from outbreaks were the milder cases can be accepted on an epidemiologic bases.

The usual symptoms include fever, malaise, weakness, retrosternal or pleuritic chest pain, headache, cough, myalgia, chills, nausea, anorexia, and weight loss. (77,78)

Pneumonia may be in one lobe but is more commonly a patchy or finely nodular bilateral infiltrate. Figure(30) In severe cases, a diffuse hazy infiltrate with nodular areas may appear. In mild cases, hilar adenopathy can be the only radiologic abnormality. The natural course of acute pulmonary histoplasmosis is one of complete, spontaneous resolution. Pleural effusion resolve over several weeks.

Healing of infiltrate due to acute pulmonary histoplasmosis may progress causing of a round, sharply outlined pulmonary nodules named histoplasmoma.

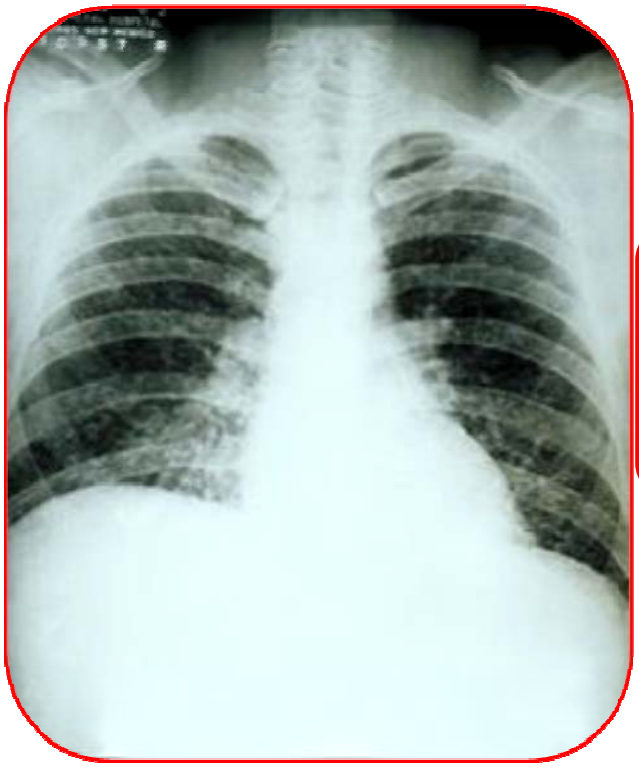


Figure 30
Chest X-ray with a patient with acute Histoplasmosis (depts..washington.edu)

2. Mediastinitis and pericarditis:

Lymphatic extension of acute pulmonary histoplasmosis may cause hilar or mediastinal lymphadenitis that continuous along beyond resolution of pneumonitis. Enlarge nodes are usually asymptomatic but dry cough may be present. (79) Massive adenopathy may appear in the hilar or right paratrachial area on the chest radiography.

Pericarditis probably arises when inflammation extends from subcarinal nodes to contagious pericardium. In the most severe cases of mediastinal granuloma due to histoplasmosis, granulomatous inflammation with central areas of caseation may replace entire mediastinal lymph nodes, extend to contiguous tissues, and cause fibrosing mediastinitis.

Fibrosing mediastinitis is one cause of superior vena cava syndrome. Oclusion of superior vena cava develops gradually, allowing development of extensive collateral circulation through the azygos and lateral thoracic veins. (80)

3. Chronic pulmonary histoplasmosis:

Chronic pulmonary histoplasmosis occurs in adults with preexisting chronic bronchitis, cause disease initially in the upper lobes, tends to cavitate, can progress to fatal pulmonary failure, and exhibits little or no tendency to disseminate beyond the lung and contiguous lymph nodes. Chronic pulmonary histoplasmosis is largely and disease of middle-agged men. The ratio of men to women is from 10:1 to 20:1. Black persons are at lesser risk than white ones. (81) Symptoms of chronic pulmonary histoplasmosis include cough or increased cough, sputum, chest pain, dyspnea, malaise weakness, fever, weight loss, and fatigability. Hemoptysis, increasingly sever cough, and dyspnea become compelling reasons for the patients to seek attention, with fever and weight loss. (81)

4. Cavitary pulmonary histoplasmosis:

This entity occurs among the same patients described as having chronic pulmonary hitoplasmosis. Figure(31) Significant risk factors for developing cavitary diseases were increasing age, male sex, white race, and preexisting lung diseases.

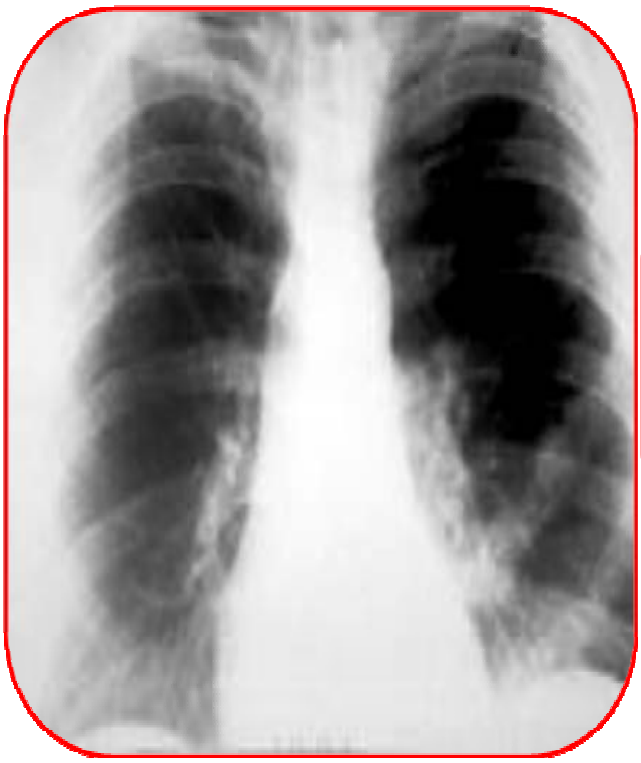


Figure 31
Cavitary pulmonary nodules in chest Radiograph (rightdiagnosis.com)

The most prominent symptoms are: Cough, sputum, dyspnea , weight loss, fever, chest pain, hemoptysis, and weakness. Untreated cavitary pulmonary histoplasmosis progresses gradually, insidiously over many years. Pulmonary lesions are more common in the right upper lobe than in the left upper one. In about one fourth the patients, lesions are bilateral. (82)

5. Mucutaneous inoculation:

Few cases have been reported in which lab. workers accidentally was injected with *H. capsulatum* while handling infected tissue or culture. (83) It is a sexually transmitted disease. (84). Figure(32)

Figure 32
Mucocutaneous lesions of histoplasmosis (anageu.ucdavis.edu)

6. Hematogenously disseminated histoplasmosis:

This rare complication of histoplasmosis assumes importance because it is potentially lethal and difficult to diagnose. Clinically apparent dissemination of histoplasmosis occur in several distinct group.

A largest group, the the immunesuppressed individuals such as in the case of malignant hematological diseases, supraphsiological doses of adrenal corticosteroids, and AIDS. (85,86) The other group, which are the normal one, but at the extreme of age. Age less than 1 year and age greater than 54 years, are at risk factors for dissemination in particularly in males. (87)

Symptoms are fever, malaise, military pulmonary infiltrates, hepatosplenomegaly, and lymphadenopathy.

Adrenal insufficiency, caused by caseous destruction of both adrenal glands, is one of hematogenous dissemination complicated cases. CNS manifestations occur in 8-29% of patients and can be sorted into three groups:

1. **About 40% have chronic meningitis along with other manifestations of disseminated infection.**
2. **About 25% have only chronic meningitis. and**
3. **The remainder have intra-cerebral lesions with or with-out meningitis or disseminated infection. (88,89)**

Mucous membrane lesions can occurred in acute dissemination but are more common in more indolent cases, they are found in up to two thirds of the chronic cases. (87) Endocarditis, gastrointestinal histoplasmosis, cutaneous lesions on face, (90) penis, vagina, (91) vulva, or other sites, (92), figure(33,34), tendonitis arthritis, solitary bone lesions, chorioretinitis, (93) peritonitis, (94) hypercalcemia, (95) and orcihtis are the other complications due to disseminated histoplasmosis.

Ocular histoplasmosis syndrome is occurred as a completeness of disseminated histoplasmosis but there is no cases reported of active infection with *H. capsulatum*. (96). Figure(35)

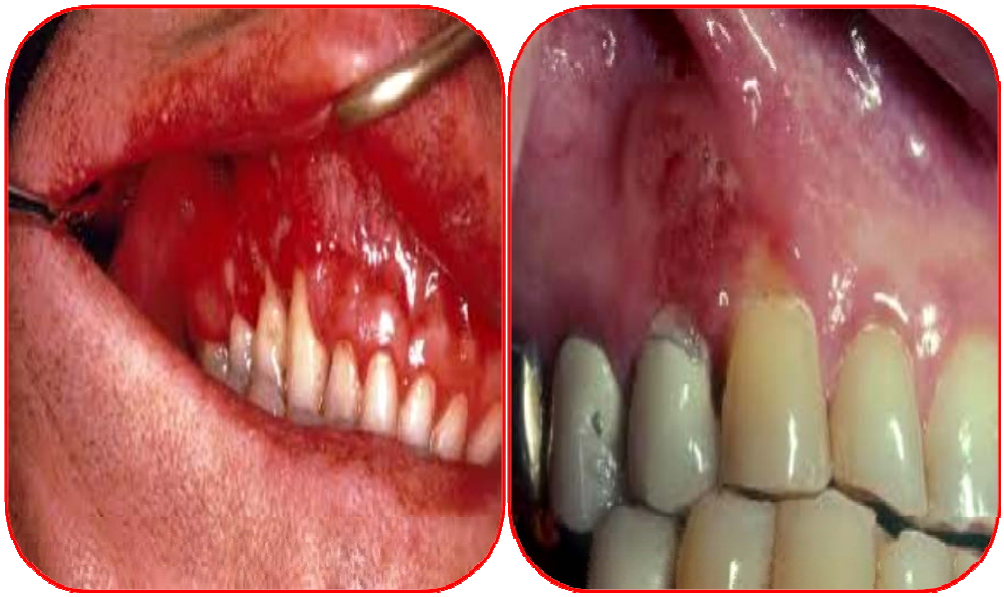


Figure 33: Disseminated histoplasmosis. Gingival ulceration. Periodonitis, left picture (botany.hawaii.edu), right picture (dermamin.com)

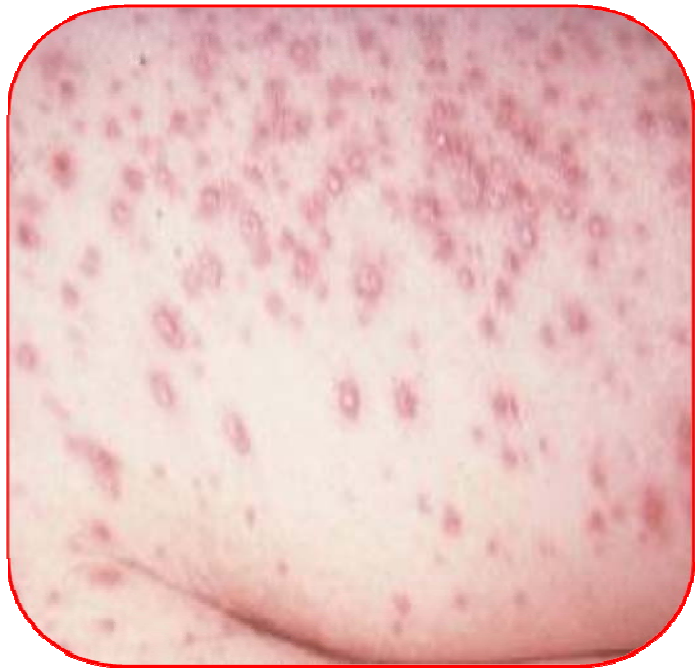


Figure 34: Cutaneous lesions in a patient with disseminated histoplasmosis (webmed.com)

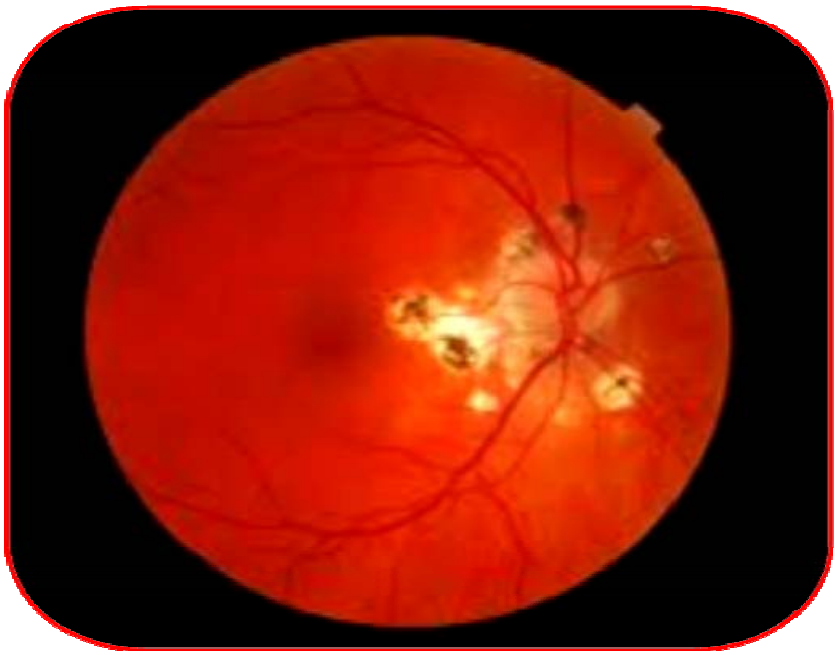


Figure 35: A retinography showing multiple ocular lesion in a patient with disseminated histoplasmosis (drugster.info)

Diagnosis:

Histoplasmosis can be diagnosed by samples containing the fungus taken from sputum, blood, or infected organs. It can also be diagnosed by detection of antigens in blood or urine samples by ELISA or PCR. It can also be diagnosed by a test for antibodies against *Histoplasma* in the blood. *Histoplasma* skin tests indicate whether a person has been exposed, but do not indicate whether they have the disease. (75) Formal histoplasmosis diagnoses are often confirmed only by culturing the fungus directly. (76) Cutaneous manifestations of disseminated disease are diverse and often present as a nondescript rash with systemic complaints. Diagnosis is best established by histopathologic examination with appropriate stains for fungal organisms. (97)

Treatment:

Antifungal medications are used to treat severe cases of acute histoplasmosis and all cases of chronic and disseminated disease. Typical treatment of severe disease first involves treatment with Amphotericin B, followed by oral itraconazole. (98) Treatment with itraconazole will need to continue for at least a year in severe cases. (99)

In many milder cases, oral itraconazole or ketoconazole is sufficient. Asymptomatic disease is typically not treated. Past infection results in partial protection against ill effects if reinfected.

Human Protothecosis

Protothecosis is a disease found in dogs, cats, cattle, and humans caused by a type of green alga known as *Prototheca* that lacks chlorophyll. It and its close relative *Helicosporidium* are unusual in that they are actually green algae that have become parasites. (100) The two most common species are *Prototheca wickerhamii* and *Prototheca zopfii*. Both are known to cause disease in dogs, while most human cases are caused by *P. wickerhami*. (101)

***Prototheca* is found worldwide in sewage and soil. Infection is rare despite high exposure, and can be related to a defective immune system. (103) In dogs, females and Collies are most commonly affected. (103) The first human case was identified in 1964 in Sierra Leone. (104)**

Clinical Findings:

The occurrence of protothecosis can be local or disseminated and acute or chronic, with the latter being more common. Protothecosis has been classified in three clinical forms, namely, (i) cutaneous lesions, (ii) olecranon bursitis, and (iii) disseminated or systemic infections. (105,106)

At least half of protothecosis cases are simple cutaneous infections, and the majority of these infections occur in individuals who are compromised by immunosuppressive therapy. Individuals presenting with olecranon bursitis are usually not immunocompromised but report penetrating or nonpenetrating trauma to the affected elbow. (107, 108) Dissemination occurs in individuals who are severely immunocompromised. Uncommon presentations, such as urinary tract protothecosis, (109) colpitis, (110) respiratory tract protothecosis, (111) choroiditis, (112) intestinal protothecosis, (113) ungual infections, (114) and meningitis, (115), have been documented in the literature. Also, three cases with lung involvement have been reported, including one case of probable and two cases of autopsy-proven pulmonary infection. (116)

The chronic presentation of protothecosis is typical for skin lesions and olecranon bursitis, yet in one patient protothecal meningitis persisted for more than 6 years despite treatment with various antifungal agents. (115) Acute and fatal

infections are rare and usually occur in severely immunosuppressed patients. (117) In one case, the use of infliximab for treatment of steroid-refractory graft-versus-host disease likely played a role in the fatal outcome of protothecosis. (118)

Cutaneous Infections:

Cutaneous protothecosis includes cases of infection coincident with trauma and consequent to defects in skin and mucosal surfaces (such as postoperative wounds) but also encompasses situations with no clear compromise of mucosal integrity. (119) Manifestations develop slowly and usually show no spontaneous dissolution. (120)

The most common presentation of cutaneous protothecosis is usually a vesiculobullous and ulcerative lesion with purulent discharge and crusting. (121) However, the spectrum of cutaneous lesions can take various other forms, including erythematous plaques, pustules, papules, nodules, verrucous lesions, pyodermic and herpetiform lesions, vesicles, ulcers, and hypopigmented or atrophic lesions. (122) Manifestations of postoperative infection include nodular lesions, synovitis, tendosynovitis, and chronically draining wounds. (123). Figure(36)

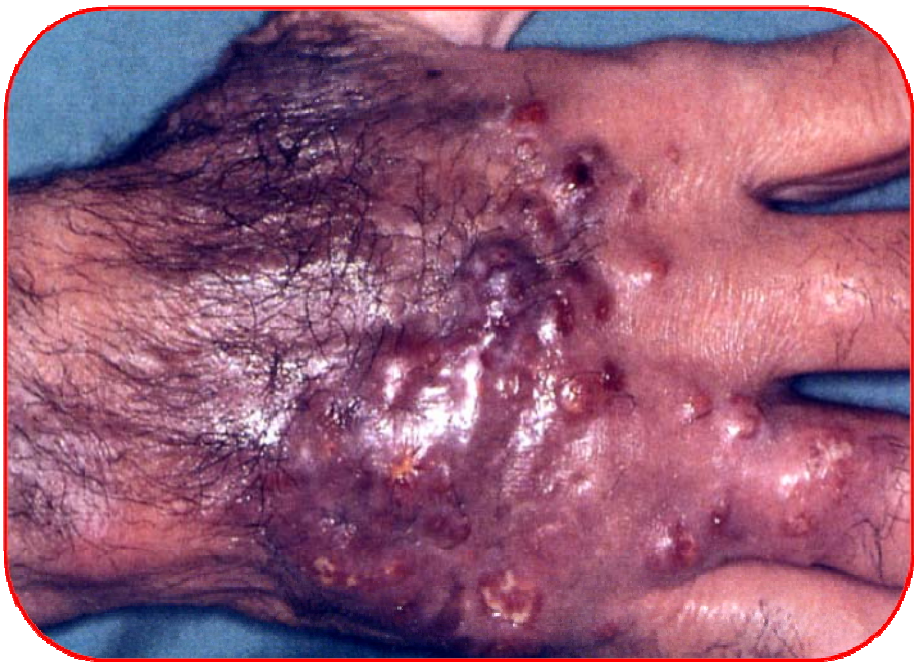


Figure 36: Cutaneous protothecosis of hand (fujita-hu.ac.jp)

It is believed that the incubation period is several weeks and that the algae penetrate the skin following posttraumatic damage. (124) The lesions generally remain localized; immunocompromised patients, particularly those with cellular immunodeficiency, show a trend toward dissemination. (125) The cutaneous lesions are located mainly in exposed areas, such as the extremities and the face. Over one-half of documented cases of protothecosis concern cutaneous or subcutaneous manifestations, which are often preceded by skin or wound infections. (126)

The first human case of protothecosis was diagnosed in 1964 on the foot of a barefoot rice farmer from Sierra Leone. (127) The lesion began on the inner side of the right foot as a depigmented area that had been injured several times by the

patient's walking barefoot. Within 3 years, it had become a papule with a raised edge covering two-thirds of the foot. In tissue sections and culture, the grouped, rounded bodies of *Prototheca* species were observed. A skin biopsy showed hyperkeratosis and pseudoepitheliomatous hyperplasia. The organism was seen in the epidermis and in the papillary and reticular dermis. Because *Prototheca* species had been noted to be sensitive to pentamidine, a total of 4.9 g was given to the patient, but without benefit. At last report, the lesion was advancing and the organism was found in the lower femoral lymph nodes. The isolate was named *P. segbwema* after the location of the hospital in which the organism was isolated, but the name has been suggested to be synonymous with *P. zopfii*. Over the following years, the number of documented cases of protothecosis rose continuously, with about four new cases being diagnosed every year over the past decade.

Olecranon Bursitis:

Infections of the bursa subcutanea olecrani, which are generally preceded by injuries or grazing of the elbow, are clinically significant in this respect. (128,129) The reason for the predilection for the olecranon bursa as a site of *Prototheca* infection is unclear but may reflect the predisposition of this area to repeated trauma. Signs and symptoms appear gradually several weeks following the trauma and include mild induration of the bursa accompanied by tenderness, erythema, and production of variable amounts of serosanguinous fluid. (130). Figure(37)

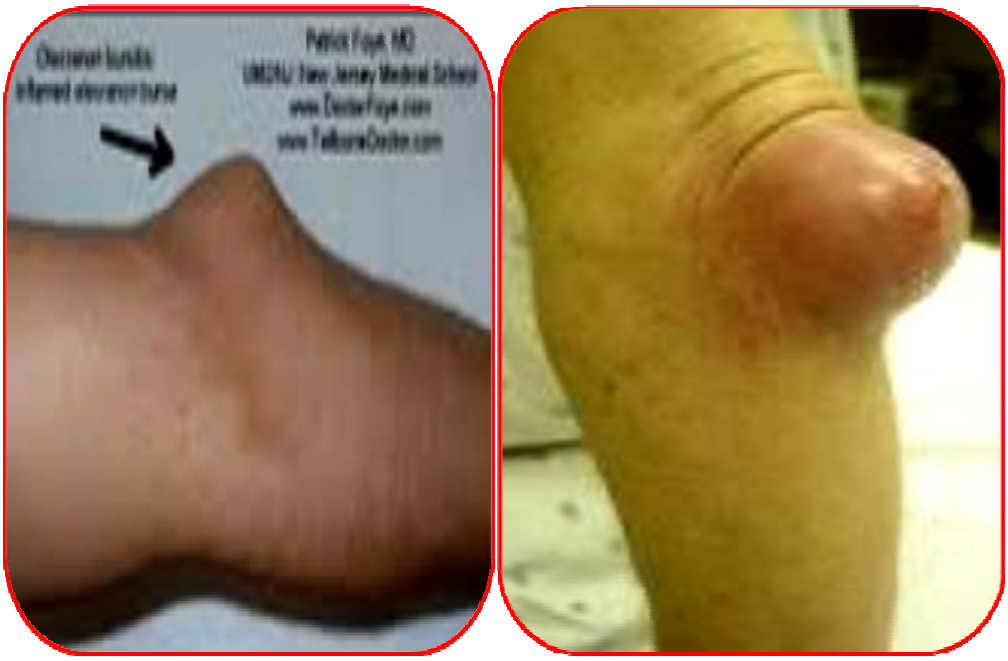


Figure 37: Protothecosis. Olecranon bursitis (emedicine.medscape.com)

Reports of olecranon bursitis have also included cases of wound contamination, such as when a *Prototheca* species was probably introduced into a preexisting wound during cleaning of a contaminated tank, cases without penetrating trauma, and cases occurring in the setting of chronic bursitis.

Systemic Infections:

Disseminated protothecosis occurs in individuals undergoing cancer treatment, (130) or solid organ transplantation or in those with AIDS. The organs most commonly affected in dissemination are the skin, subcutaneous tissue, gut, peritoneum, blood, and spleen. Overall, 23 cases of disseminated infection have been described. The dissemination cases were observed in

immunocompromised individuals; in all but two cases, the species involved was *P. wickerhamii*.

What may be termed a disseminated opportunistic infection by a *Prototheca* species was first reported by Klintworth *et al*. (131) in 1968. The patient was diabetic and had widespread metastases of breast cancer; *P. wickerhamii* was isolated from several ulcerating papulopustular lesions on the leg. It was concluded that the patient died of the carcinoma, but no autopsy was performed. The first clear case of multiorgan systemic protothecal infection was described by Cox *et al*. in 1974. (132) The patient was a 29-year-old man who had an unknown defect in cellular immunity. Multiple lesions were found in the peritoneal cavity, lymph nodes, skin, and blood. A similar case of visceral protothecosis was later described in 1990 by Chan *et al*. (133); the infection mimicked sclerosing cholangitis. The patient had multiple peritoneal nodules that resembled metastatic cancer but were in fact manifestations of protothecosis. The authors recommended that protothecosis be considered in the differential diagnosis of hepatic and biliary inflammatory diseases of uncertain etiology.

Four catheter-related cases of protothecosis included three episodes of peritonitis complicating continuous ambulatory peritoneal dialysis, (134) and one episode of mixed infection of a Hickman catheter with *P. wickerhamii* and *Torulopsis* sp. Central venous catheter-related algemia has been reported, with accompanying fever, chills, and sepsis syndromes. In the meantime, several other cases of disseminated protothecosis

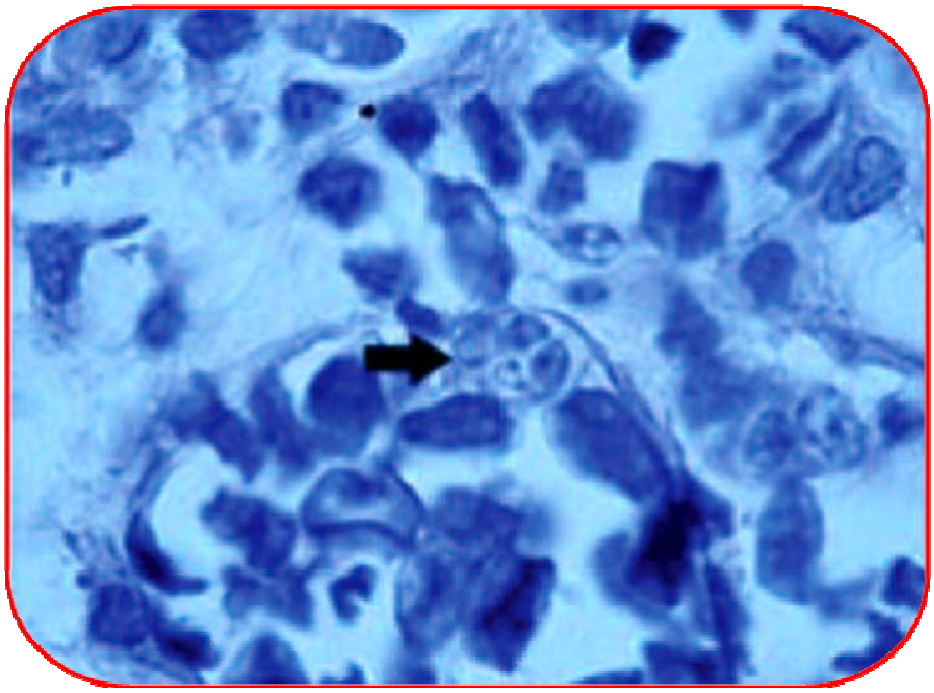
have been described. Recovery of *Prototheca* spp. from the blood occurred in 47% (n = 11) of cases with dissemination. The manual lysis centrifugation method was superior to other methods in detecting *P. wickerhamii* in blood from HIV patients. (135) Not that uncommonly, *Prototheca* spp. are associated with copathogens, such as *Candida glabrata*, *Staphylococcus aureus*, (136) herpes simplex virus, *Enterococcus faecalis*, *Leuconostoc* spp., *Klbsiella pneumoniae* , *Cryptococcus* spp. , *Pseudomonas aeruginosa*, and *Escherichia coli.*

Diagnosis:

Protothecosis is generally not suspected clinically, and patients are subjected to various treatment modalities for long periods without satisfactory results. The definitive diagnosis of infection usually depends on morphological identification of the organisms in wet slide preparations of cultures and/or direct identification in tissue specimens. *Prototheca* species react to the periodic acid-Schiff stain (PAS) and reveal yeast-like colonies on Sabouraud dextrose agar but differ from fungi as they lack glucosamine in their cell walls. (137)

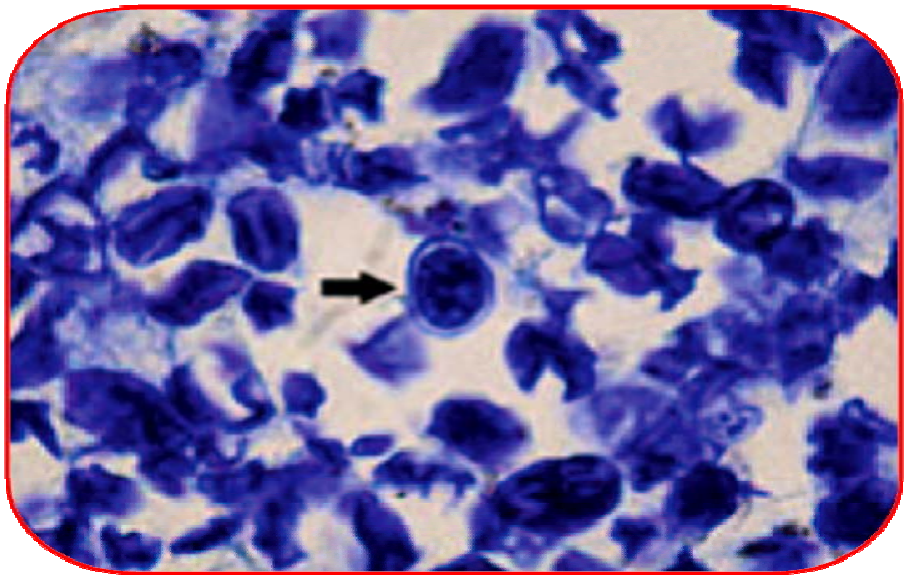
Histopathologic studies describe a variety of host tissue responses, ranging from severe granulomatous necrosis to a total absence of inflammatory changes. (138) Microbiological tests : The failure to isolate *Prototheca* species may be explained by the fact that they are readily overgrown by bacteria and fungi when culture is attempted from contaminated sources.

Media that may be useful include beef infusion broth, blood agar, and brain heart infusion agar. The combination of flucytosine, (139) and potassium hydrogen phthalate inhibits most bacteria and fungi (isolation media). Growth is optimized between 25 and 37°C, and organisms usually proliferate within 48 h as soft, wet, yeast-like, white-to-light-tan colonies. Figure(38)



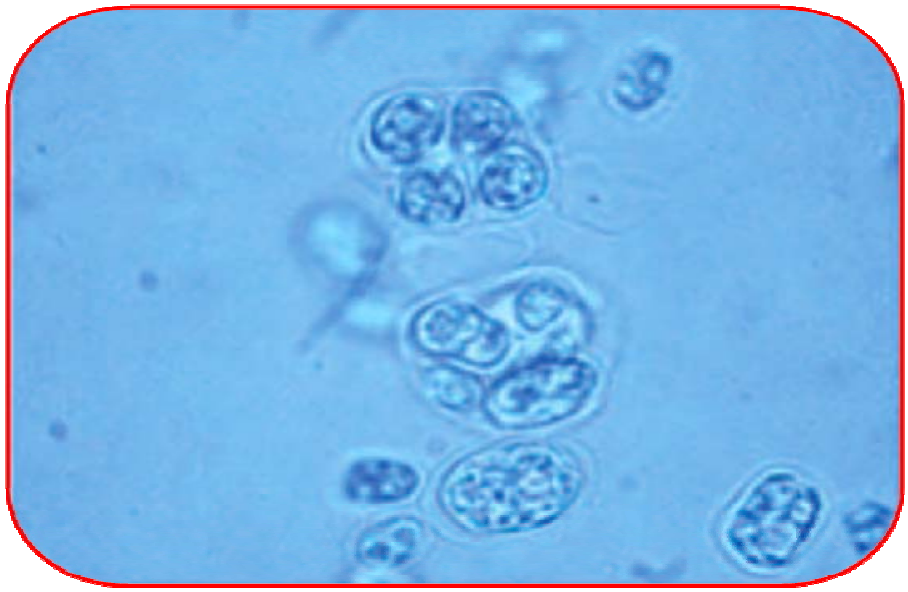
A

Figure 38. A:
Histopathology by hematoxylin and eosin staining of cutaneous protothecosis shows morula-like structures (large arrow) and endospores (small arrow)
(cmr.asm.org)



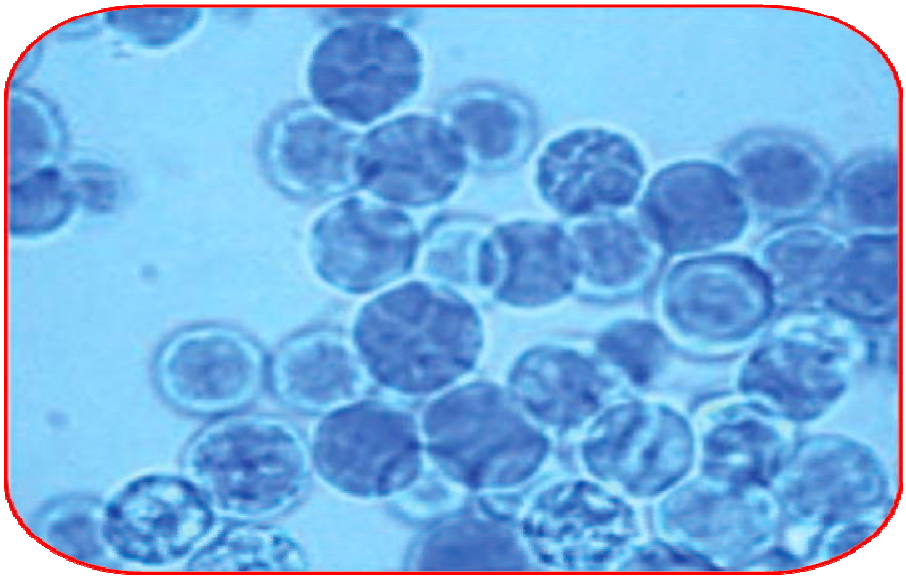
B

Figure 38. B: Typical morphology is best observed with PAS(cmr.asm.org)



C

Figure 38. C: Wet mount preparation with lactophenol cotton blue shows *P. zopfii*, with asymmetrical morula-like structures. (cmr.asm.org)



D

Figure 38. D: Wet mount preparation with lactophenol cotton blue shows *P. wickerhamii*, with symmetrical morula-like structures (cmr.asm.org)

E

Figure 38. E: Smooth, creamy, white, yeast-like colonies of *P. zopfii* on Columbia blood agar (cmr.asm.org)

Treatment:

Treatment of protothecal infections remains controversial, and various treatment regimens have been attempted, but there has been no consistency in the clinical responses. Data on potential therapy are drawn from isolated case reports, limited case series, and in vitro studies. No prospective clinical studies have been published comparing specific treatments for protothecosis. Antifungals such as ketoconazole, itraconazole, fluconazole, conventional Amphotericin B, and liposomal Amphotericin B are the most commonly used drugs to date. Among them, Amphotericin B displays the best activity against *Prototheca* spp. Usually, treatment involves medical and surgical approaches; treatment failure is not uncommon. Infection is indolent, with no apparent tendency toward self-healing. (140)

Successful options for cutaneous lesions have included total excision, (141) topical therapy with Amphotericin B, (142) ketoconazole, (143) itraconazole, (144) fluconazole, (145) transfer factor, topical Amphotericin B with systemic tetracyclines, (146) systemic Amphotericin B, with or without excision, (147) and oral tetracyclines. Failed treatments involved tetracycline, itraconazole, (148) fluconazole, flucytosine, and ketoconazole. (149) Various success rates were reported for systemic penicillin, griseofulvin, and emetine as well as for topical therapies such as peroxide, chlorhexidine, potassium permanganate, copper sulfate, picric acid, ammonium compounds, Castellani's paint, and potassium iodide.

Excision of infected small localized tissue may be acceptable in superficial infections, as evidenced by the success of this approach in several previously reported cases. Persistent or deeper infection may require systemic therapy plus excision. According to Boyd et al., patients with protothecosis should receive intravenous amphotericin B with oral tetracycline. If oral therapy is indicated, an azole antifungal agent should be considered. (150) However, the data on azoles are inconsistent. Itraconazole (400 mg/day for 6 weeks) failed as therapy, and treatment with fluconazole at 200 mg/day improved the patient's condition. Fluconazole appears to provide clinical efficacy somewhat superior to that of itraconazole despite the high MICs obtained *in vitro*. In accordance with the results of drug susceptibility tests, one patient was treated with amikacin combined with tetracycline and responded well to this therapy. (151) The duration of treatment Successful treatment of olecranon bursitis has focused on bursectomy; repeated drainage has failed. Drainage coupled with local instillation of amphotericin B has been curative. Breakthrough infections with fluconazole , voriconazole, (152) or itraconazole treatment have been observed and display the moderate activity of azole drugs against *Prototheca* species.

Fungal opportunistic infections in HIV/AIDS patients

Ever since its discovery, the human immunodeficiency virus (HIV) has emerged as a global disaster. Around the world acquired immunodeficiency syndrome (AIDS) has led to more than 20 million deaths. Over 33 million people are living with HIV today, fig(39) and by 2010 it is estimated that more than 40 million children will have one or both parents dead from AIDS. People in productive age groups are predominantly affected by AIDS and hence in some countries the impact of AIDS has led to a major decrease in gross national product. To date the only tool available to ascertain the presence of HIV in an otherwise healthy-looking individual is a laboratory assay. (153)

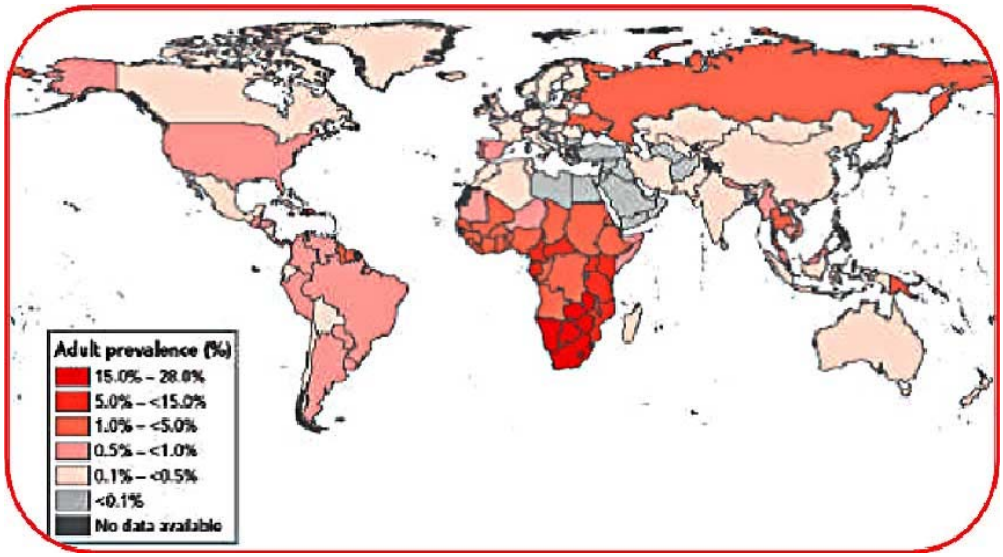


Figure. 39: A global view of HIV infection
33 million people [30–36 million] living with HIV, 2007
Source: UNAIDS, 2008 Report on the global AIDS epidemic

HIV/AIDS in the South-East Asia Region

The South-East Asia (SEA) Region bears the second highest burden of HIV/AIDS, with 3.6 million people estimated to be living with the virus. There is wide variation in the number of people living with HIV in countries in this Region.

There is a wide variation in the number of people living with HIV from less than 100 in Maldives to 2.4 million in India.

HIV and opportunistic infections

HIV does not kill those who are infected directly. Instead, it weakens the body's ability to fight disease. Infections which are rarely seen in those with normal immune systems can be deadly to those with HIV.

People with HIV can get many infections (known as opportunistic infections, or OIs). Many of these illnesses are very serious and require treatment. Some can be prevented.

OIs are caused either by organisms of low or no virulence which are nonpathogenic in individuals with an intact immune system, or by known pathogens which present in a different way than usual in immunodeficient individuals, e.g. in the form of increased virulence, recurrence, multidrug resistance or atypical presentation.

The spectrum of OIs has been found to vary from continent to continent and region to region. With the unprecedented increase in the number of AIDS cases, OIs are also increasing. Several of these OIs are recognized as case-defining entities in HIV/AIDS patients(Table1).

Table 1: WHO clinical staging of HIV/AIDS for adults and adolescents with confirmed HIV infection

Clinical Stage 1

*Asymptomatic.

*Persistent generalized lymphadenopathy.

Clinical Stage 2

*Moderate unexplained weight loss (<10% of presumed or measured body weight).

*Recurrent respiratory tract infections (sinusitis, tonsillitis, bronchitis, otitis media, pharyngitis).

*Herpes zoster.

*Angular cheilitis.

*Recurrent oral ulceration.

*Papular pruritic eruptions.

*Seborrhoeic dermatitis.

*Fungal nail infections.

Clinical Stage 3

*Unexplained severe weight loss (>10% of presumed or measured body weight).

*Unexplained chronic diarrhoea for longer than one month.

*Unexplained persistent fever (intermittent or constant for longer than one month).

*Persistent oral candidiasis.

*Oral hairy leukoplakia.

*Pulmonary tuberculosis.

*Lymph node TB.

*Severe bacterial infections (e.g. pneumonia, empyema, pyomyositis, bone or joint infection, meningitis, bacteraemia).
*Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis.
*Unexplained anaemia (<8 g/dl), neutropenia (< 0.5 x 109 /L) and/or chronic thrombocytopenia (< 50 X 109 /L3).

Clinical Stage 4

*HIV wasting syndrome.
*Pneumocystis pneumonia (caused by *Pneumocystis jiroveci*).
*Recurrent severe bacterial pneumonia.
*Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration or visceral at any site).
*Oesophageal candidiasis (or candidiasis of trachea, bronchi or lungs).
*Extrapulmonary tuberculosis.
*Kaposi's sarcoma.
*Cytomegalovirus infection (retinitis or infection of other organs).
*Central nervous system toxoplasmosis.
*HIV encephalopathy.
*Extrapulmonary cryptococcosis (including meningitis).
*Disseminated non-tuberculous mycobacteria infection.
*Progressive multifocal leukoencephalopathy.
*Chronic cryptosporidiosis.
*Chronic isosporiasis.
*Disseminated mycosis (extrapulmonary histoplasmosis or coccidiomycosis).

*Recurrent septicaemia (including non-typhoidal *Salmonella*).
*Lymphoma (cerebral or B cell non-Hodgkin).
*Invasive cervical carcinoma.
*Atypical disseminated leishmaniasis.
*Symptomatic HIV-associated nephropathy or HIV-associated cardiomyopathy.

The early diagnosis of these infections is vital for better management and preventive measures. OIs can be bacterial, viral, parasitic or fungal, and it is in this context that the establishment and strengthening of existing mycology laboratories gains paramount importance. The ability to easily, cheaply and quickly diagnose these and other potential OIs is crucial.

Opportunistic fungal infections

Even though all fungal infections are "opportunistic", i.e. they cause disease in a host whose resistance is lowered, the true "opportunistic" fungal infections only infect those who are already sick or immunocompromised. (154)

Opportunistic fungal infections such as mucocutaneous candidiasis, pneumocystosis, cryptococcosis and histoplasmosis are the most common AIDS-defining conditions in HIV-positive individuals. Other fungal infections like coccidioidomycosis and Penicilliosis marneffei are usually seen in geographically restricted areas, the latter being reported frequently from northeast India.

Blastomycosis and paracoccidioidomycosis have been reported to cause severe and disseminated disease in AIDS patients, though there has been no significant increase in the number of infections occurring in such patients. Unlike the above diseases, which occur primarily due to the defect in cell mediated immunity, aspergillosis and zygomycosis are now being increasingly encountered in advanced AIDS cases with neutropenia. Neutropenia in these patients also increases the risk of disseminated candidiasis, and invasive infections due to miscellaneous hyaline and dematiaceous (melanized) fungi. In Asia, paracoccidioidomycosis has only been reported from Japan, and only two authentic cases of coccidioidomycosis were reported from India (both were imported cases from an endemic area). (155)

Antiretroviral therapy (ART) can lower the incidence of OIs in patients with AIDS. However, this therapy has not been available to most HIV positive individuals due to its cost. Moreover, patients are not diagnosed in the early stages in a substantial number of cases. Hence there has been no let-up in incidence of the often fatal, invasive mycotic infections in the SEA Region, unlike the change in prevalence of these infections in developed countries.

CD4 T lymphocytes and fungal infections

CD4 counts, a useful prognostic marker in HIV/AIDS patients, also have critical levels below which certain invasive fungal infections start appearing frequently (Table 2).

Table 2: Relationship between CD4 T lymphocytes and fungal OIs

CD4 count	Opportunistic fungal infection
<350 cells/µl	Mucocutaneous candidosis
<200 cells/µl	Pneumocystosis
<150 cells/µl	Histoplasmosis
<100 cells/µl	Cryptococcosis and *Penicilliosis marneffei*
<50 cells/µl	Aspergillosis and zygomycosis

Clinical criteria provide only a presumptive diagnosis of fungal infection. Usually there are few specific signs and symptoms indicating fungal infections. The lesions and radiological findings are sometimes characteristic enough (crescent sign and halo sign in invasive aspergillosis), (156) to strongly suggest a fungal etiology but the accompanying immunosuppresion frequently results in atypical presentations. In most circumstances, early diagnosis considerably increases the chance of successful treatment. Thus, it is important that the possibility of fungal infection be considered from the outset so that appropriate mycological investigations can be carried out.

Diagnostic mycology is also rapidly moving into the mainstream of clinical medicine as a result of the convergence of several independent developments. First, dramatic progress in antifungal therapeutics has increased the need for

specific viral diagnosis. Second, the number of patients at risk of accruing opportunistic viral infections has expanded greatly as a result of the HIV/AIDS epidemic. Finally, modern management of HIV infection is providing a new paradigm for the integration of molecular techniques into management of chronic fungal infections. All these developments are not only increasing the use of diagnostic mycology techniques but have enabled the detection of a variety of fungal OIs. These guidelines are therefore intended to provide the laboratory techniques in diagnosis of fungal OIs at different levels of health laboratories.

Common opportunistic fungal infections in HIV/AIDS

Candidiasis

Candidiasis is commonly an endogenous opportunistic infection. Occasionally exogenous acquisition has also been proven. Of the causative agents, the most common species is *Candida albicans*. Other non-albicans *Candida* species, especially *C. tropicalis* are increasingly being reported. Candidiasis is the most common fungal infection found in HIV/AIDS patients. Extensive esophageal candidiasis is an AIDS-defining infection. But oral candidiasis, unless very extensive and causing symptoms unequivocally, is not diagnostic of AIDS. It is of prognostic value only as its presence indicates progression of immunodeficiency. Vulvovaginal candidiasis, though not unequivocally shown to occur more frequently in AIDS patients, nevertheless affects a

considerable proportion of HIV-positive women with extensive disease. In advanced AIDS cases, with neutropenia and very low CD4 counts, disseminated candidiasis is certainly a possibility.

Clinical considerations:

Clinical manifestations include:

- **oropharyngeal candidiasis – up to 90% of untreated, advanced HIV cases develop this disease, with more than 60% getting >1 episode per year.**
- **esophageal candidiasis – 10%-20% of HIV cases have esophageal candidiasis (the most common cause of esophageal disease in these patients).**
- **vaginal candidiasis – 27%-60% women of childbearing age develop this disease, and the rates are similar for HIV-infected and non-infected patients.**
- **non-healing extensive skin ulcers (seen in Indian patients).**
- **intertriginous candidiasis.**
- **hospital-acquired UTI.**
- **superinfection of bacterial abscesses/infections in those treated for prolonged period with broad-spectrum antibiotics.**
- ***Candida* lung infection (very rare).**
- **disseminated candidiasis (rare in HIV patients).**

Cryptococcosis

The etiologic agent is *Cryptococcus neoformans*, the only pathogenic species of the genus *Cryptococcus*. There are five serotypes (A, B, C, D and AD), and it exists in three varieties, var. *grubii* (A), var. *gattii* (B and C) and var. *neoformans* (D). The current thinking on varieties, serotypes and pathogenesis of *Cryptococcus neoformans* is depicted in the box below:

Cryptococcus neoformans - varieties, serotypes and pathogenesis

- **The encapsulated basidiomycete *Cryptococcus neoformans* was recently divided into 2 species *C. neoformans (Cn)* and *C. gattii (Cg)*.**
- **Cn consists of 2 varieties - *grubii (CnVG)* and neoformans *(CnVN)*, which are opportunistic pathogens and predominantly infect immunocompromised persons.**
- ***CnVG* is the major causative agent of cryptococcosis worldwide, except in central Europe, where *CnVN* infection is most prominent.**
- **In contrast, *Cg* is a primary pathogen, which predominantly infects immunocompetent persons.**

***Cg* was previously thought to be restricted to tropical and subtropical climates with a special ecologic niche on Eucalyptus trees.**

However, the recent outbreak of *Cg* infection in healthy humans and animals in the temperate climate of Vancouver in Canada, and its isolation from several species of trees other than Eucalyptus have raised the strong possibility that this fungus might have broader geographic distribution.

- **The mechanisms underlying pathogenic and environmental differences between *Cn* and *Cg* are not known.**
- **Within *Cn* species, *CnVN* infections are more likely to display skin involvement and to afflict older patients.**
- ***CnVG* infections are reported to have a higher mortality rate.**
- **In contrast, infections caused by *Cg* result in a lower mortality rate but are frequently complicated by neurologic sequelae and require surgery and prolonged therapy.**

Cryptococcosis generally begins with primary pulmonary invasion. In immunocompetent individuals, it remains as inapparent subclinical infection, but in immunosuppressed patients it spreads and occasionally becomes disseminated. *C. neoformans* has a predilection for the central nervous system (CNS), and patients mostly present with symptoms of chronic meningitis. In the present era of AIDS pandemic, WHO has considered cryptococcal meningitis as one of the AIDS-defining infections. Incidence varies from 4%-7% in African patients to <1% in the developed world. Introduction of fluconazole and ART at reduced cost in developing countries like Thailand and Brazil has helped to reduce incidence in those countries. The effects are yet to be observed in other countries of the SEA Region.

Clinical considerations:

CNS cryptococcosis/Disseminated cryptococcosis Clinical manifestations include:

- **onset is insidious; symptoms are present for weeks**
- **headache with minimal or no neck rigidity**
- **fever of unknown origin**
- **drowsiness and alteration in sensorium, with advancement of disease process**
- **chronic meningitis**
- **may result occasionally in cerebral cryptococcal granuloma**
- **May be associated with visual loss (rapid or slow), osteolytic**

bony lesions, secondary cutaneous cryptococcosis, cryptococcemia and cryptococciuria – called disseminated cryptococcosis. In recent years a few cases of cryptococcal peritonitis have also been reported.

Pulmonary cryptococcosis:

- **Often asymptomatic.**
- **When symptomatic, patients have self-limiting pneumonitis, mild fever, rare pleural effusion, hilar adenopathy and calcification.**

Pneumocystis jiroveci infections

Pneumocystis carinii **was officially recognized as a fungus in 2001, and renamed as *Pneumocystis jiroveci*, though the disease continues to be referred as PCP (*Pneumocystis carinii* pneumonia). PCP is the most common AIDS-defining condition, though compared to the developed world it is less commonly reported from South-East Asia. It is nonetheless a major OI in HIV-infected individuals in the Region. The likelihood of developing PCP in AIDS patients increases as the CD4 count falls below 200/ml. Thrush, persistent fever and presence of other AIDS-defining OIs are other independent risk factors for PCP in these patients. (154)**

Most healthy adults have been exposed to *Pneumocystis jiroveci* at some point of time in life, though asymptomatic carriage is quite common. The lifecycle of *Pneumocystis*, though incompletely understood, contains at least two stages – the cyst and the trophozoite. The walls of the cyst forms take up the GMS and Toluidine Blue O stain, round-, cup- or typically "deflated-ball"-shaped. In Giemsa stain the trophozoites are also seen and the cysts show eight sporozoites within them.

Clinical considerations:

- **Clinical presentation is often insidious with slow but steady progression of fatigue, fever, chills, sweats and exertional dyspnoea. The features are characteristic of atypical pneumonia. Circumoral, peripheral and mucus membrane cyanosis may be apparent in extensive infection.**

➢ **Extrapulmonary pneumocystis has been reported in patients with advanced HIV disease, particularly in the setting of aerosolized pentamidine prophylaxis. Orbit, thyroid, skin, ears, adrenals, kidneys, bone marrow and lymph nodes are among the organs affected.**

Histoplasmosis

Histoplasmosis is an intracellular mycotic infection of the reticuloendothelial system, caused by inhalation of microconidia of the fungus *Histoplasma capsulatum*. Approximately 95% of cases of histoplasmosis are subclinical. Five percent of the cases have chronic progressive lung disease, chronic cutaneous or systemic disease or an acute fulminating fatal systemic disease. It is being increasingly reported in HIV/AIDS patients from endemic areas and the majority represents progressive disseminated disease. (156)

Two varieties of *H. capsulatum* are recognized in human diseases, depending on the clinical disease: var. *capsulatum* and var. *duboisii* (the African type). *H. capsulatum* var. *duboisii* causes disease restricted to Africa and Madagascar, and no case has been described from South-East Asia. Histoplasmosis due to var. *capsulatum* has a worldwide distribution.

Clinical considerations:

All stages of this disease may mimic tuberculosis. In some clinical situations, it can be confused with sarcoidosis.

- **Since this intracellular pathogen resides in macrophages, the ideal specimen for direct demonstration and culture is bone marrow. The organism is best grown when inoculated in the medium at the bedside immediately after collection from the patient.**

- **In patients with AIDS, histoplasmosis presents as a progressive disseminated infection in 95% of cases. The majority of such cases have CD4 counts <150 cells/ml and a median cell count of 50 cells/ml.**

- **Patients with disseminated disease usually present with fever, weight loss and malaise over a period of several weeks. In about half, respiratory symptoms are reported.**

- **Hepatosplenomegaly and generalized lymphadenopathy are encountered in 15%-30% of cases.**

- **A syndrome representing septicemia has been well documented. The patients usually present with hypotension, acute respiratory distress syndrome (ARDS), hepatic failure, renal failure and disseminated intravascular coagulation. It represents a later manifestation of disseminated histoplasmosis and can occur in 10%-15% cases of disseminated histoplasmosis in AIDS.**

- **Mucocutaneous involvement is very common in patients from South-East Asia, particularly India.**

- **Neurologic complications have been reported in up to 20% of cases. These include encephalopathy, lymphocytic meningitis and focal pulmonary lesions in the brain and spinal cord. Signs and symptoms include headache, fever, altered mental status and focal neurologic findings.**

- **Gastrointestinal and osteo-articular lesions are also found. Adrenal insufficiency may be a presentation of disseminated histoplasmosis.**

- **Patients who experience immunologic improvement following anti-retroviral therapy may demonstrate atypical pathology. This has been referred to as immune reconstitution inflammatory syndrome and is characterized by focal inflammatory histology, elevation of hepatic enzymes, hepatic abscesses, lymphadenopathy, arthritis, uveitis and intestinal obstruction. (157)**

Penicilliosis marneffei

The dimorphic fungus *Penicillium marneffei* causes the disease, which is an emerging systemic mycosis in AIDS patients. *Penicillium marneffei* is endemic in Thailand, Northeastern India (Manipur and Nagaland states), southern China, Hong Kong, Vietnam and Taiwan. Bamboo rats and soil are considered the reservoir of the disease though the agent has never been isolated from soil except near bamboo rat burrows. (158)

Penicilliosis marneffei, after tuberculosis and cryptococcosis, is the third most common opportunistic infection in patients with AIDS in the South-East Asia Region, and is therefore considered an AIDS-defining illness. Persons affected by penicilliosis usually have AIDS with low CD4 counts, typically <100/µl. The average CD4 count at presentation is 63.5/µl.

Clinical considerations:

Various types of manifestations include:

- **fever of unknown origin, loss of weight, generalized lymphadenopathy, anemia.**
- **hepatomegaly with or without splenomegaly.**
- **pneumonitis: cough and dyspnea occur in about 50% of cases, sometimes with hemoptysis.**
- **skin lesions - characteristic generalized papular eruptions, central umbilicated papules resembling those of molluscum contagiosum, or acne like lesions and folliculitis over face, trunk, and extremities.**
- **pharyngeal and palatal lesions also can be seen.**
- **subcutaneous nodules may be seen.**
- **chest radiographic abnormalities typically manifest as diffuse reticulonodular infiltrates, and cavitations. (158)**

Invasive aspergillosis (IA)

Exposure to *Aspergillus* is universal, but aspergillosis is uncommon unless the host immune defense is compromised. The aspergilli producing infection are many but more than 95% of disease is caused by *A. fumigates* and *A. flavus*; the latter is especially more common in the tropics. The disease usually occurs when phagocytic host defenses by granulocytes and macrophages are quantitatively or functionally suppressed. Despite the severe immunosuppression that

results from advanced HIV infection, there are relatively few cases of aspergillosis in patients with HIV disease. The overall incidence varies from 1.1 to 3.5 episodes per 1,000 person-years. The incidence of aspergillosis in AIDS patients is significantly high in the following settings: age =35 years old, men who have sex with men, white blood count <2,500 cells/µl, CD4 count <100/µl, prior history of an OI, and prescribed medications associated with neutropenia. (156)

In patients with AIDS, aspergillosis is usually restricted to the lungs with a variety of distinct manifestations, including thick-walled cavitary disease of the upper lobes, diffuse unilateral or bilateral infiltrates, ulcerative tracheobronchial disease, and obstructive bronchitis. The diagnosis is made often upon postmortem and relies on the histologic or microbiologic identification of the fungus in infected tissue.

Clinical considerations:

- **Pulmonary – most common site of involvement.**

(1) Invasive pulmonary aspergillosis – fever, dyspnoea, cough, chest pain, hemoptysis, thick-walled cavities on chest X-ray, mainly in the upper lobes, pulmonary infiltrates – bilateral diffuse or nodular; pseudo-membranous trachea – bronchitis, extensive bronchial hemorrhage.

(2) Noninvasive, obstructing bronchial aspergillosis – fungal plugs block the airways, leading to breathlessness, cough and chest pain. Chest X-ray shows hazy infiltrates, representing segmental or lobar atelectasis.

- **Extrapulmonary aspergillosis – CNS aspergillosis is very uncommon in AIDS patients and may present as focal abscesses or hemorrhagic or mycotic aneurysms. In rare cases it may present as a focal neurologic deficit or intracerebral spaceoccupying lesion. The lesion usually extends from the sinuses; in fact it is merely an extension of the mycotic disease into the contiguous cranial cavity.**

- **Very rarely, unusual manifestations in the AIDS patients include endocarditis and myocarditis, esophagitis, lymphadenitis, musculoskeletal and skin abscess, liver and spleen involvement, and renal and pancreatic abscesses.**

Zygomycosis

Though the disease is rare in AIDS patients, there has been an increase in the number of cases of zygomycosis in recent years, especially in tropical countries. Advanced AIDS with neutropenia is now recognized as a risk factor for acquiring zygomycosis, a disease caused by Zygomycetes belonging to the order Mucorales. *Rhizopus oryzae, Rhizopus rhizopodiformis, Rhizomucor pusillus, Absidia corymbifera, Apophysomyces elegans, Mucor circinelloides, Cokeromyces recurvatus* and *Saksenaea vasiformis* are the commonly recognized human pathogens causing this fatal disease. Fungi invade vascular tissues and cause extensive necrosis in these cases, and disseminated disease is common in the immunosuppressed. These fungi are characterized by wide (5-20 µm diameter), aseptate or sparsely septate, coenocytic

hyphae with rightangled branching along with a predominantly necrotic and acute neutrophilic tissue reaction. In culture, these fungi show unrestricted cottony mycelia mostly white to grey and black in colour. (159)

Clinical considerations:

Zygomycosis may affect any or more than one organ complex and the disease is categorized as:

1. Rhino-orbito-cerebral (most common and carries a poor prognosis) – nasal congestion, dark-blood-tinged rhinorhea, epistaxis, sinus tenderness, retro-orbital headache, fever, malaise, facial swelling, blurred vision, lacrimation, chemosis, proptosis, loss of vision. Examination of nasal cavities may reveal a black eschar on septum or turbinates.

2. Cutaneous – acute inflammation, tissue swelling, pus formation, progresses to necrotizing-fascitis-like lesion.

3. Pulmonary – hemoptysis, fever and cough – fatal and frequently diagnosed post mortem.

4. Gastrointestinal – necrotizing lesions.

5. Renal – though isolated renal disease is yet to be seen in patients with AIDS, the disease is prevalent in South - East Asia.

6. Cerebral – as a part of rhino-cerebral disease, or disseminated disease or isolated cerebral zygomycosis. Isolated disease presents as space-occupying lesion, encephalopathy or strokelike symptoms. Unlike in the west,

where isolated disease mainly occurs among injecting drug users, in South-East Asia it occurs mainly in immunocompetent individuals or diabetics.

7. Disseminated – where more than one organ system is involved.

Blastomycosis

Blastomycosis, caused by a dimorphic fungus, *Blastomyces dermatitidis,* is a systemic fungal infection endemic in North America, but cases have been reported worldwide in Africa and the Middle East as well as South-East Asia. Frequently associated with point-source exposure, it occurs more commonly in men than in women, which may reflect a larger number of male occupational exposures. However, in more recent reports, occupational exposure accounts for a smaller proportion of cases, presumably as recreational exposures increase. Immunosuppressed patients typically develop infection following exposure to the organism, but reactivation may also occur. (160)

Blastomycosis is uncommon among people infected with HIV and is not recognized as an AIDS-defining illness. In the setting of AIDS or other marked immune suppression, the disease is usually more severe with multiple-system involvement, including the CNS, and progresses rapidly to a fatal course.

Clinical considerations:

- **Acute disease typically presents as flu-like illness characterized by fever, malaise, fatigue, weight loss, and pulmonary involvement.**
- **Rarely, pneumonia with fever, chills, purulent sputum and hemoptysis may occur. ARDS may occur very rarely.**
- **Individuals with underlying lung disease may develop chronic pneumonia with symptoms lasting 2-6 months: weight loss, night sweats, fever, chest pain, and productive cough mimicking tuberculosis.**
- **Cutaneous blastomycosis is very common – lesions vary from nodules to verrucous lesions and may become ulcerative, mimicking squamous cell cancer and keratoacanthoma.**
- **Prostatitis, female genital tract infections, osteolytic lesions involving vertebrae, skull, ribs and long bone may also be seen.**
- **Disseminated disease has been found in the brain, skeletal system, prostate, myocardium, pericardium, sinuses, pituitary, and adrenal glands and also invades the reticuloendothelial system. Epidural or cranial abscesses as well as meningitis occur in up to 40% of patients with AIDS and chronic blastomycosis.**

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Chapter 4

Mycotoxicosis

Mycotoxin

A mycotoxin (from Greek μύκης , mykes, mukos) "fungus" and Latin (toxicum) "poison", (1,2) is a toxic secondary metabolite produced by organisms of the fungus kingdom, commonly known as molds. (3,4) The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops.(3) One mold species may produce many different mycotoxins and/or the same mycotoxin as another species.(5)

Overview

Most fungi are aerobic (use oxygen) and are found almost everywhere in extremely small quantities due to the minute size of their spores. They consume organic matter wherever humidity and temperature are sufficient. Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high. The reason for the production of mycotoxins is not yet known; they are necessary for neither growth nor the development of the fungi. (6) Because mycotoxins weaken the receiving host, the fungus may use them as a strategy to better the environment for further fungal proliferation. The production of toxins depends on the surrounding intrinsic and extrinsic environments and the toxins vary greatly in their severity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms. (7) Some of the health effects found in animals and humans include death, identifiable diseases or health problems, weakened immune systems without specificity to a toxin, and as

allergens or irritants. Some mycotoxins are harmful to other micro-organisms such as other fungi or even bacteria; penicillin is one example. (8) It has been suggested that mycotoxins in stored animal feed are the cause of apparent sex change in hens. (9)

Mycotoxins can appear in the food chain as a result of fungal infection of crops, either by being eaten directly by humans or by being used as livestock feed. Mycotoxins greatly resist decomposition or being broken down in digestion, so they remain in the food chain in meat and dairy products. Even temperature treatments, such as cooking and freezing, do not destroy mycotoxins.

Although various wild mushrooms contain an assortment of poisons that are definitely fungal metabolites causing noteworthy health problems for humans, they are rather arbitrarily excluded from discussions of mycotoxicology. In such cases the distinction is based on the size of the producing fungus and human intention. (10) Mycotoxin exposure is almost always accidental whereas with mushrooms improper identification and ingestion causing mushroom poisoning is commonly the case. Ingestion of misidentified mushrooms containing mycotoxins may result in hallucinations. The cyclopeptide-producing Amanita phalloide is well known for its toxic potential and is responsible for approximately 90% of all mushroom fatalities. (11) The other primary mycotoxin groups found in mushrooms include: orellanine, monomethylhydrazine, disulfiram-like, hallucinogenic indoles, muscarinic, isoxazole, and gastrointestinal (GI)-

specific irritants. (12) The bulk of this article is about mycotoxins that are found in microfungi other than poisons from mushrooms or macroscopic fungi. (10)

Many international agencies are trying to achieve universal standardization of regulatory limits for mycotoxins. Currently, over 100 countries have regulations regarding mycotoxins in the feed industry, in which 13 mycotoxins or groups of mycotoxins are of concern. (13) The process of assessing a need for mycotoxin regulation includes a wide array of in-laboratory testing that includes extracting, clean-up and separation techniques. (14) Most official regulations and control methods are based on high-performance liquid techniques (e.g., HPLC) through international bodies. (14) It is implied that any regulations regarding these toxins will be in co-ordinance with any other countries with which a trade agreement exists. Many of the standards for the method performance analysis for mycotoxins is set by the European Committee for Standardization (CEN). (14) However, one must take note that scientific risk assessment is commonly influenced by culture and politics, which, in turn, will affect trade regulations of mycotoxins. (15)

Food-based mycotoxins were studied extensively worldwide throughout the 20th century. In Europe, statutory levels of a range of mycotoxins permitted in food and animal feed are set by a range of European directives and Commission regulations. The U.S. Food and Drug Administration has regulated and enforced limits on concentrations of mycotoxins in foods and feed industries since 1985. It is through various

compliance programs that the FDA monitors these industries to guarantee that mycotoxins are kept at a practical level. These compliance programs sample food products including peanuts and peanut products, tree nuts, corn and corn products, cottonseed, and milk. There is still a lack of sufficient surveillance data on some mycotoxins that occur in the U.S., which is due largely to the lack of reliable analytical methods. (16)

Major groups

Aflatoxin

Aflatoxins are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus*. (17) The umbrella term aflatoxin refers to four different types of mycotoxins produced, which are B1, B2, G1, and G2. (18) Figure(1) Aflatoxin B1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as liver cancer, in many animal species. Aflatoxins are largely associated with commodities produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios and maize. (17,18).

During the 1950s ((moldy feed toxicosis)) was recognized as a serious livestock problem that took several clinically distinct forms.

The discovery of aflatoxin produced by *A. flavus* and *A. parasiticus* was not in fact made until 1960 duing the investigation in the UK of a dramatic form of moldy feed

toxicosis which become known as ((Turkey-x disease)). The earliest signs of disease were: anorexia, lethargy, and muscular weakness, within only a few days the animal died. At autopsy hemorrhages were seen in the liver, which was also necrotic, kidney were often engorged. Histopathological examination revealed paranchymal cell degeneration and extensive proliferation of bile duct epithelial cells. All these symptoms occur in the acute toxication, chronisity of this toxin lead to an carcinogenic effect. (17)

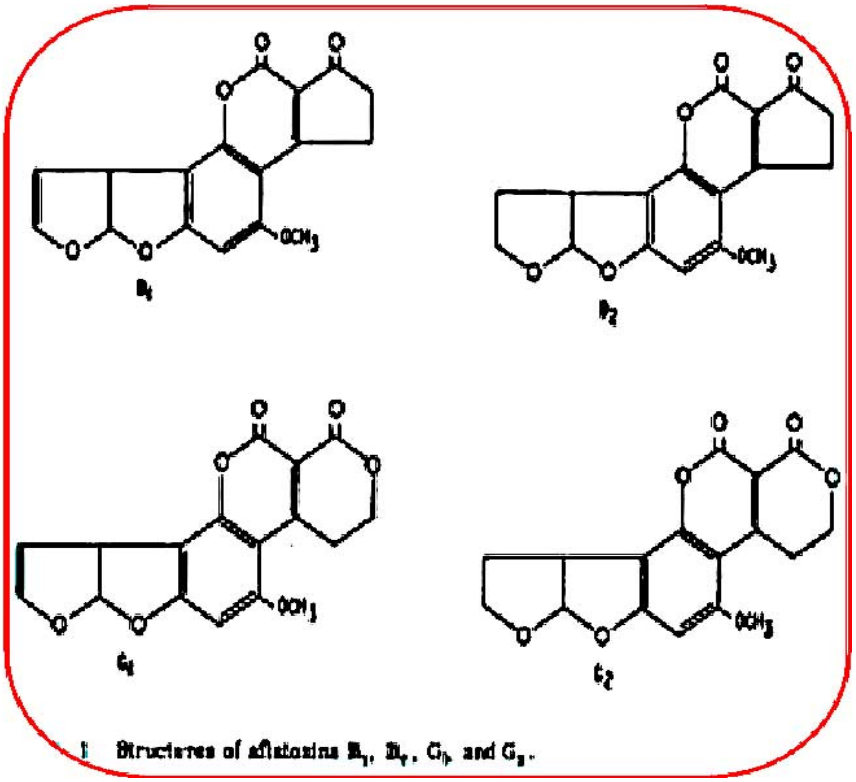

Figure 1: Structure of aflatoxin

Creatures known to be affected by aflatoxin and related compounds are man, cattle, sheep, swine, poultry, horses, doges, lab. animals, and even fish. It have an antibacterial

activity to different types of bacteria in particularly *S. typhimurium*, that killed by 1.5 nmol of aflatoxin B_1 in the culture media. The toxin effect on proteins, lipids, carbohydrate metabolism, interaction with nucleic acids and enzymes, interruption of electron transport in the terminal respiratory chain, it is labilizing action on cellular and subcellular membrane, and many biological effects notably the carcinogenic, mutagenic, and teratogenic activities. Natural ocurance of aflatoxin, in groundnuts and it's products, oilseed and it's products, edible nuts, cereals, dairy products, eggs, meat, ...etc. (18).

Ochratoxin

is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. Figure(2) All are produced by *Penicillium* and *Aspergillus* species. The three forms differ in that Ochratoxin B (OTB) is a nonchlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form Ochratoxin A. (19) *Aspergillus ochraceus* is found as a contaminant of a wide range of commodities including beverages such as beer and wine. *Aspergillus carbonarius* is the main species found on vine fruit, which releases its toxin during the juice making process. (20) OTA has been labeled as a carcinogen and a nephrotoxin, and has been linked to tumors in the human urinary tract, although research in humans is limited by confounding factors.

Ochratoxin A was initially found to be toxic compound because of acute liver injury caused in day-old duckl;ings.

Ochratoxin B was at first described as nontoxic, later on the scientist found that both compounds caused acute kidney damage in chickes that died. Ochratoxin C also is another compound that be toxic to animals. In rats and mice ochratoxin A cause necrosis of renal tubules and periportal liver cells.

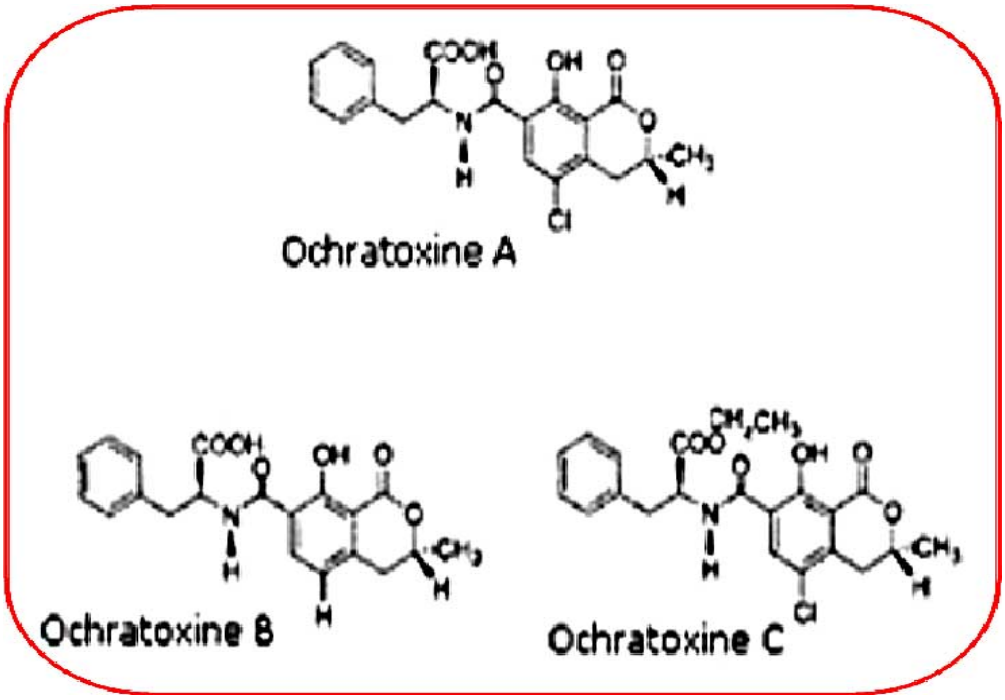

Figure 2: Structure of ochratoxin

Citrinin

Citrinin is a toxin that was first isolated from *Penicillium citrinum*, but has been identified in over a dozen species of *Penicillium* and several species of *Aspergillus*. Some of these species are used to produce human foodstuffs such as cheese (*Penicillium camemberti*), sake, miso, and soy sauce (*Aspergillus oryzae*). Citrinin is associated with yellow rice

disease in Japan and acts as a nephrotoxin in all animal species tested. Although it is associated with many human foods (wheat, rice, corn, barley, oats, rye, and food colored with Monascus pigment) its full significance for human health is unknown. Citrinin can also act synergistically with Ochratoxin A to depress RNA synthesis in murine kidneys. (10)

The most striking pathological changes caused by citrinin in experimental animals is kidney damage. Lethal doses administered IV to rabbits and guina pigs caused swelling of the kidneys and acute tubular necrosis. Such necrosis has also been observed in rats and pigs and it associated with growth depression and glucoseuria. Chronic kidney degeneration occurred in rabbets injected IV as sublethal doses.

Patulin

Patulin is a toxin produced by the *Penicillium expansum*, *Aspergillus*, *Penicillium*, and *Paecilomyces* fungal species. *P. expansum* is especially associated with a range of moldy fruits and vegetables, in particular rotting apples and figs. (21,22) Figure(3) It is destroyed by the fermentation process and so is not found in apple beverages, such as cider. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals. In 2004, the European Community set limits to the concentrations of patulin in food products. They currently stand at 50 µg/kg in all fruit juice concentrations, at 25 µg/kg in solid apple products used for direct consumption, and at 10 µg/kg for children's apple products, including apple juice. (21,22)

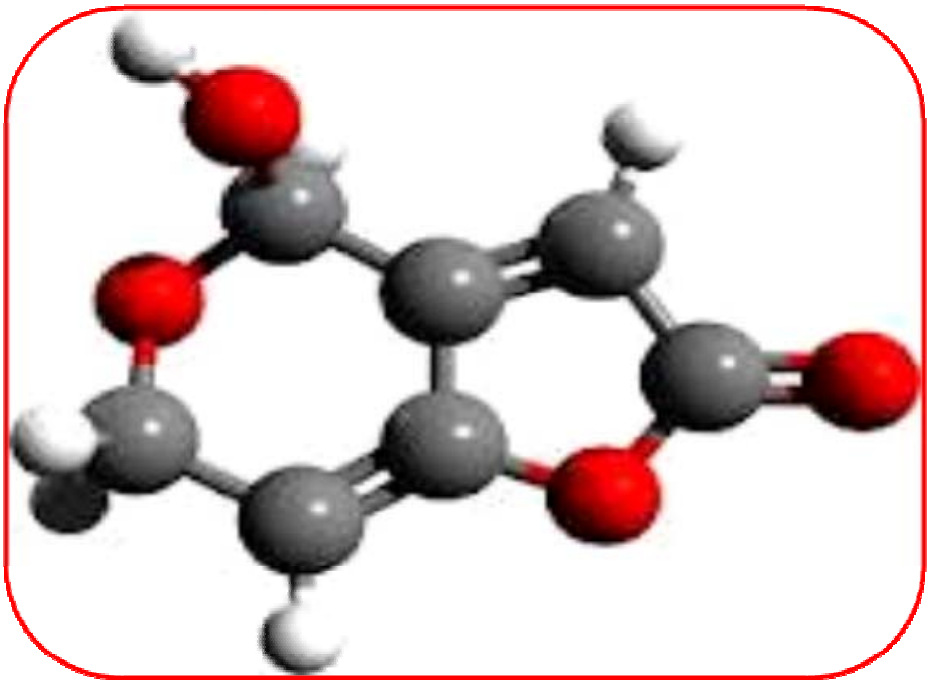

Figure 3: Structure of patulin

The antibiotic and mycotoxin now generally known as patulin was first isolated as a crystalline compound in 1942. *Penicillium patulum* is the fungus responsible for it's production. This toxin was isolated from a dry malt feed reported to have cause death of ((dairy cows)).

The clinical symptoms observed: Ascending paralysis of the motor nerves, convulsions, and reflex excitement. Cerebral hemorrhage was found on dissection. Its toxic effects on animals found in autopsy of mice, rats, and rabbits including edema of the lung and brain, with congestion in the lungs, kidney, liver, and spleen. Patulin had a marked anti-diuretic effect in rats. Liver necrosis was observed in chicks. (22)

Patulin has been reported to produce nausea and stomach irritation on oral administration to human, although IV.

perfusion of 100 mg patulin was well tolerated. Application of ointment containing 1% patulin to the skin caused edema. In conection with studies on the possible effectiveness of patulin as a remedy of the common cold, dilute solutions of patulin sprayed into the nose and throat produced no ill effect.

Fusarium toxins

***Fusarium* toxins are produced by over 50 species of *Fusarium* and have a history of infecting the grain of developing cereals such as wheat and maize. (23,24)They include a range of mycotoxins, such as: the fumonisins, which affect the nervous systems of horses and may cause cancer in rodents; the trichothecenes, which are most strongly associated with chronic and fatal toxic effects in animals and humans; and zearalenone, which is not correlated to any fatal toxic effects in animals or humans. Some of the other major types of Fusarium toxins include: beauvercin and enniatins, butenolide, equisetin, and fusarins. (25)**

Penicillic acid

Penicillic acid was obtained in 1971 as amajor metabolite of *Penicillium olivino-viride*, figure(4) isolated from mixed feed associated with liver cirrhosis in pigs. It is produced histopathological lesions of the liver, kidney and thyroid in animals as a toxic effect. Generalized cell necrosis was observed in mouse liver. Penicillic acid also produced severe edema in rabbits skin. Also it's produce malignant, transplantable, local tumors in rats and mice injected subcutaneously. In contrast to it's carcinogenic properties,

penicillic acid also possesses anti-tumor activity. It possess an antibacterial (mainly bacteriostatic) and weak antifungal activity.

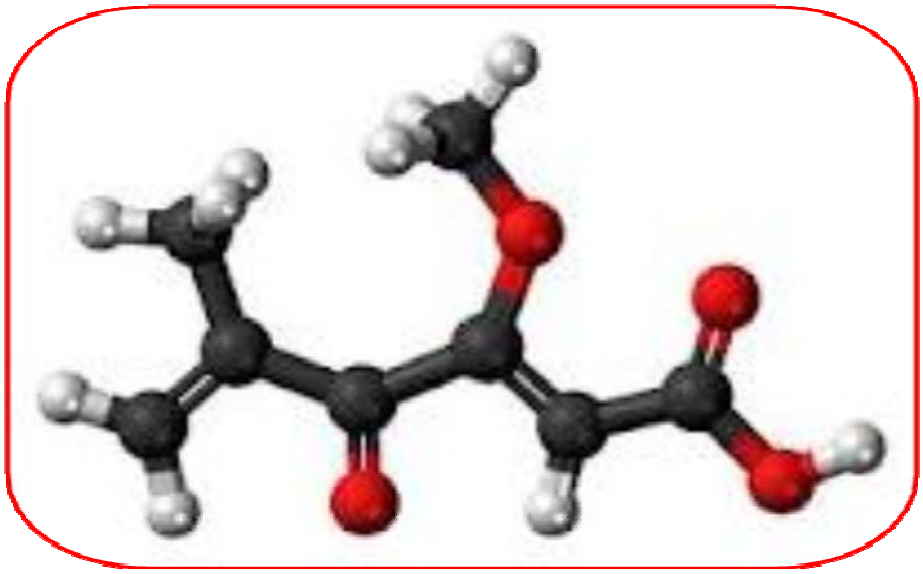

Figure 4: Structure of penicillic acid

Ergot

Alkaloids are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of *Claviceps*, Figure(5,6) which are common pathogens of various grass species. The ingestion of ergot sclerotia from infected cereals, commonly in the form of bread produced from contaminated flour, cause ergotism the human disease historically known as St. Anthony's Fire. There are two forms of ergotism gangrenous affecting blood supply to extremities and convulsive that affect the central nervous system. Modern methods of grain cleaning have significantly reduced ergotism as a human disease, however it is still an important veterinary problem. Ergot alkaloids have been used pharmaceutically. (10)

Figure 5
Claviceps purpurea
(aspnet.org)

Figure 6
Structure of Ergot
(phototimes.ru)

first who distinguished two forms of the disease in humans, gangrenous and convulsion due to this toxin. Barger attributed the convulsive form to predisposition induced by hypovitaminosis A. The toxicity of naturally occurring ergot alkaloids stems from their wide spectrum of pharmacological activity involving CNS stimulation, antagonism toward adrenaline and 5-hydroxystypamine, and peripheral action expressed through stimulation of smooth muscle whether in the uterus or arteriole walls. Stimulation of contraction of smooth muscle in vascular tissues can express itself as toxicity by causing: Anoxia, death of peripheral tissues, and dry gangrene. Therapeutically, the control by ergotamine of the diameter of cranial arteriole's lumen, prevent excessive amplitude of blood flow pulsations, minimizing the acute headache component of migraine. The contraction of uterine muscle which ergometrine evokes are useful in controlling postpartum hemorrhage… etc. Impairment of lactation was first observed clinically by ((Dodart))in nursing mothers with gangrenous ergotism. It appears that the principal route for excretion of recognizable alkaloid in the feces, possibly via the bile. Thus it seems probable that most ingested alkaloid is excreted, either free or combined in some conjugated complexes.The physiology of ergot alkaloid/animal interaction concern the disturbance of pituitary function through hypothalamous mediation.

Human health effects:

Mycotoxicoses is the term used for poisoning associated with exposures to mycotoxins. The symptoms of a

mycotoxicosis depend on the type of mycotoxin; the concentration and length of exposure; as well as age, health, and sex of the exposed individual. (10) The synergistic effects associated with several other factors such as genetics, diet, and interactions with other toxics have been poorly studied. Therefore it is possible that vitamin deficiency, caloric deprivation, alcohol abuse, and infectious disease status can all have compounded effects with mycotoxins. (10) In turn, mycotoxins have the potential for both acute and chronic health effects via ingestion, skin contact, and inhalation. These toxins can enter the blood stream and lymphatic system, they inhibit protein synthesis, damage macrophage systems, inhibit particle clearance of the lung, and increase sensitivity to bacterial endotoxin. (26)

In 2004 in Kenya, 125 people died and nearly 200 others were treated after eating aflatoxin-contaminated maize. (27) The deaths were mainly associated with homegrown maize that had not been treated with fungicides or properly dried before storage. Due to food shortages at the time, farmers may have been harvesting maize earlier than normal to prevent thefts from their fields, so that the grain had not fully matured and was more susceptible to infection.

Poisonous mushrooms

Approximately 140.000 species of mushrooms have already been catalogued all over the world Approximately 140.000 species of mushrooms have, about 2.000 being considered safe

for human consumption and about 700 have therapeutic properties. (28) A great variety of species was classified as poisonous and represents risks to health if ingested. Apart from mushrooms that contain psychoactive toxins, ingestion of toxic mushrooms is invariably accidental and caused by misidentification of species. (29,30)

There are some mushrooms that contain exceptionally powerful toxins that represent a real hazard to health even when ingested in small doses. Most toxins were well studied and are described in literature, such as amatoxins that are cytotoxic and cause harm to kidney and liver and orellanine that is nephrotoxic. (30) Some species are well utilized for food and medicine due to the presence of pharmacologically active substances and essential nutrients. Because of these properties, mushrooms were described as popular remedies in ancient oriental documents and some of them became ingredients in traditional medicine. (31,32,33) Even in species with beneficial properties toxic substances were already found. (34) Before using species of mushroom for human consumption it is necessary to characterize their toxicological profile because even in some edible species toxic substances have been identified. The toxicity studies consist of exposing species of mammal to a toxic agent during a specific period of time. (35) The aim of the present work is to review the most common intoxications caused toxic species and the toxic potential caused by edible and medicinal mushrooms.

Poisonous species of mushrooms:

Some species of mushrooms are known as toxic and in some countries many cases of mushroom poisoning are reported every year. In the year 1998 in France 1,675 cases of intoxications by mushrooms were reported and in this country alone it is estimated that 8- 10.000 cases are expected to be registered every year. Most of these accidents are due to incorrect identification of species that is often made by empirical and traditional knowledge. (30,36) A wide variety of toxic mushrooms belong to different genus that will be discussed bellow.

Genus *Amanita*

The family Amanitaceae (genus *Amanita*) is well known as having many toxic species. Amatoxins are present in species of Amanita genus such as: *Amanita phalloides, A. virosa, A. verna, A. ocreata, A. bisporigera, A. subalbiacea, A. tenuifolia and A. hygroscopica.* The family of amatoxin comprises a neutral component designated as alpha-amanitin, an acid one called betaamanitin, gamma and delta-amanitin and the nonpoisonous component amanullin from *A. phalloides* and amaninamine from *A. virosa.* (37) Other toxins also found in *Amanita* genus belong to the family of phallotoxin that includes phalloin, phalloidin, phallisin, phallacidin, phallacin and phallisacin.

Virotoxin is also found in this genus and are closed related the phallotoxins. (37) The specie *A. phalloides* is responsible for the majority of the fatalities caused by mushroom

poisoning. The toxic effects are caused by phallotoxin and amatoxin. Phallotoxin causes alterations of enterocytes cellular membrane, while amatoxin inhibits protein synthesis at a transcriptional level within enterocytes, hepatocytes and proximal renal tubular cells. After ingestion of *A. phalloides*, amatoxin causes necrosis of liver cells with mortality rates ranging from about 10% to 20%. Only a minority of patients need emergency liver transplantation. (38,39) Species with hallucinogenic effects are also found in amanita genus. *A. pantherina* and *A. muscaria* are well known toxic mushrooms that have been mistaken for the edible mushroom *A. rubescens.* Two dissociative constituents such as ibotenic acid (IBO) and muscimol (MUS) are responsible for the hallucinogenic effects. IBO is a powerful agonist of N-methyl-D-aspartic-acid (NMDA) receptor and MUS is a potent GABAA agonist. (40) The intoxications caused by *A. muscaria* for long time were believed to be due to muscarine, but it was demonstrated that this substance is present in small amounts. (41) *A. muscaria* and *A. pantherina* grow in North Ame - rica, Europe, Africa and Japan, in recent years it has been reported that young people in several countries have intentionally eaten *A. muscaria* to evoke hallucinations. (42) The most common symptoms of intoxication are motor depression, ataxia, changes in mood, perception and feelings, dizziness, euphoria, drowsiness, gastrointestinal disturbances and muscle twitches. (40,42,43) The pantherina-muscaria syndrome is atropine-like and in the number and severity of poisoning cases fatality is rare. In most cases recovery is complete after 24 hours. The treatment is mainly

symptomatic cholinestarase inhibitors may be recommended as it counteracts the effects of poisoning, benzodiazepinics or phenobarbitone can be used in case of seizures. (41,44) The treatment of patients intoxicated with species containing amatoxins includes detoxification, careful monitoring and sometimes liver transplantation is necessary. (37)

Genus *Clitocybe and Inocybe*

A particular syndrome that affected five people in the region of Savoie in France was later identified as intoxication caused by the mushroom *Clitocybe amoenolens*. First symptoms appeared 24 hours after ingestion. Patients presented paresthesia of the toes and fingers followed by paroxysmal burning pain lasting 2-3 hours, notably at night. A sensation of heat, numbness, oedema and local erythema are associated with crises. Symptoms are partially relieved with cold water, acetylsalicylic acid, morphine and clomipramine. Recovery is completely after 1-4 months. (45)

The administration of high dose of *C. amoenolens* in rats caused weight loss, locomotor disability and erythema of the toes. Examination of the sciatic nerves showed decreased axon density and neuronal fiber degeneration. (46)

The poisonous specie *C. acromelalga* can be confused with the edible one *Lepista inversa*. The substances pointed out as responsible for the symptoms are the acromelic acids A-E. Acromelic acid (ACRO) is a kainate analogue that is assumed to be involved in poisoning episodes. ACRO has two isomers, ACROA, which is the most potent and ACRO-B. ACRO-A

was demonstrated to have a powerful excitatory action on mechanosensitive unmyelinated afferents in skeletal muscle of the rat. (47) Species of genus Clitocybe also cause muscarinic syndrome. The species *C. dealbata, C. rivulosa, C. candicans, C. cerussata*, and *C. phyllophila* are described in literature as poisonous mushrooms due to the presence of muscarine in their chemical composition. Approximately 15 minutes to 2 hours after ingestion patient can present gastrointestinal problems, miosis, hypersecretion and in severe cases bradycardia and collapses. The treatment of this syndrome is symptomatic and atropine can be administered to counteract the effects of muscarine. (48)

There are approximately 40 species belonging do Inocybe genus in China, and they are known to be not edible. The species: I. *asterospora, I. fastigiata* f. subcandida, *I. gobeyi, I. lilacina, I. nappies, I. pallidicremea, I. patowillandii, I. radiate, I. repanda* and *I. rimosa* have toxic properties. They produce neurotoxic and psychotropic effects due to the presence of biogenic amines, muscarin, aeruginacin a thymethylammonium analogue of psylocibin which effects will be discussed later in this article. (49) Intoxications caused by members of this genus is similar to the ones caused by Clitocybe because the species contain muscarine.

Genus *Cortinarius*

The genus Cortinarius comprises between 2,000- 3,000 species of mushrooms that were considered as non-toxic until

1950. One hundred-and-thirty-five cases of intoxication caused by *C. orellanus* were described from 1953-1962 in Poland. Poisoning syndrome is characterized by a delayed acute tubulopathy that can progress to chronic renal insufficiency. (50) In several case reports it was demonstrated that the mushrooms *C. speciosissimus* and *C. orellanus* are nephrotoxic due to the presence of the cyclopeptide orellanine whose metabolites are supposed to be most active. In additional studies it was shown that the oxidation of orellanine in renal tissue may accumulate quinone compounds which bind covalently with biological structures leading to cell damage. (29) The symptoms of orellanine intoxication may appear between 2-20 days after ingestion. Initially people can experience nausea, vomiting and abdominal pain. This is followed by intense thirst, chills, polyuria or oliguria and possibly anuria. Hemodialysis may be necessary until renal function gradually improves. (50) Some species of genus Cortinarius can be confused with members of Psilocybe genus which is known as magic because the hallucinogenic properties. This fact has led to several cases of accidental intoxication because Psilocybe mushrooms are used for some people for recreational purposes. (51)

Genus *Gyromitra*

Species of genus *Gyromitra*, family *Helvellaceae* are really attractive to hunters and gourmets because of their taste. However, some species of *Gyromitra* contain a well known

toxin named gyromitrin, whereas other species are non-toxic. This is one of the reasons why intoxications occur, toxic and non-toxic species are sometimes difficult to distinguish because they are mixed-up. The other reason is that the toxin is water soluble and volatile, boiling for long time and drying allows ingestion without risk of poisoning, but if these procedures are not done properly intoxication may occur. (29) The third reason for intoxication is the confusion with species that are consumed frequently. The specie *G. esculenta* is known as false morels and is commonly confused with morels such as *Morchella esculenta* and *M. elata*. The toxin gyromitrin is the responsible for the effects of this specie. Intoxications have occurred not only by eating fresh false morels but also by the inhalation of vapors from cooking. (30,36) Intoxications caused by *G. esculenta* were reported by the Swedish Poisons Information Centre which handled 706 inquiries in the period of 1994-2002. Most common symptoms are gastrointestinal (vomiting and diarrhea) and neurological (vertigo, fatigue, tremor, ataxia, nystagmus). A few patients have developed mild to moderate liver damage and haemolysis. After ingestion gyromitrin is hydrolysed in stomach forming hydrazines that are cytotoxic, convulsants and irritating to mucous membranes. (29,52) The other effect of gyromitrin include carcinogenesis due to the hepatic metabolization that produces free radicals with mutagenic properties in animals and are also responsible for the hepatic problems. Symptoms of intoxication start 8-12 hours after ingestion. Treatment consists of monitoring the symptoms and administrating vitamin B6 intravenously considering that gyromitrin inactivates this vitamin. (53)

Genus *Psilocybe*

The use of psychoactive substances of fungal origin for recreational purposes has become an increasing problem in many countries all over the world. Species of genus *Psilocybe* are known due to their psychedelic effects caused by psilocybin. (54) Common psylocibin containing mushrooms are: *P. semilanceata, P. Mexicana, P. bohemica, P. cubensis* and *P. baeocistis.* (52) The symptoms of intoxication occur 30 minutes after ingestion of fresh or dried mushroom and start with anxiety, nausea, vertigo and asthenia, neurosensorial symptoms consists of visual problems, disorientation, motor incoordination and sympathomimetic symptoms consist of mydriasis, tachycardia and hypertension. Recovery is completely 4 to 12 hours after ingestion. The need of hospitalization is rare and in exceptional cases myocardial infarction may occur in adult patients while children may present hyperthermia, seizures and comma. (52)

Toxicity caused by commonly consumed mushrooms

Some species known as edible and medicinal also have substances that can cause harm to health, but the dose and magnitude of effects on humans must be carefully studied. Ostreolysin is a cytolytic protein that was isolated from mushrooms of the genus Pleurotus that was able to cause cytolytic pore formation when administered by intravenous route to rats. As a consequence it was observed blood pressure increase, cardiac ischemia, tachycardia, hypoxia and

elevated serum potassium. (55) The administration of the mushroom *Phellinus linteus* to rats bearing experimentally induced prostatic hyperplasia leads to an enlargement of prostate stroma which is involved in transforming growth factor-beta1 (TGF-β1) regulation. The prostate is known to be regulated by various growth factors. Among them the TGFs have been reported to play important role in prostate cell growth regulation. The administration of *P. linteus* increased the expression of TGF-β1 compared to animals treated with placebo. (56)

***Agaricus bisporus* is the most consumed mushroom world-wide but is has been pointed out as potentially carcinogenic due to the substantial amounts of aromatic hydrazines, an established class of direct-acting chemical carcinogens. Life-time administration of *A. bisporus* raw or baked to mice three days a week followed by balanced semi-synthetic diet for the remaining days, induced tumors in a number of tissues. The administration of the methanolic and aqueous extracts of this same mushroom is weakly mutagenic. The ethanolic extract of this mushroom is increased in the presece of fungal mammalian enzyme systems purified mushroom tyrosinase and rat hepatic citosol. (57,58)**

The specie *Pleucybella porrigens* popularly knownas Sugihiratake is a white mushroom widely distributed in the mountain areas of Japan and is commonly used as ingredient to various processed foods, but this was pointed out as hazardous due to the presence of substances analogous to vitamin D that are able to cause cryptogenic encephalopathy

in patients with renal failure. (59) The acute toxicity of *Agaricus silvaticus* was evaluated by administering the aqueous extract of this mushroom in the dose of 1.5 g/kg/day of body weight to adult male and female rats by gavage every 2 hours and 40 minutes, during a period of 24 hours, followed by a protocol of The National Health Surveillance Agency (ANVISA, Brazil). It was observed that not only the administration of A. sylvaticus aqueous extract but also the placebo, caused the temporary appearance of apathy, respiratory alterations and piloerection, that were slightly more persistent in the group treated with the fungus. Biochemical and hystopathological were not statistically significant among the groups. The administration of the *A. silvaticus* aqueous extract induced very low toxicity. (60) Species of genus Tricholoma especially *T. equestre (T. flavovirens),* known as yellow tricholoma, has been implicated in 12 human poisonings causing a delayed rhabdomyolysis severe enough to be fatal in 3 cases reported in France. The symptoms were muscular weakness, fatigue and myalgias within 24-72 hours after ingestion. The substance responsible for toxic effects was not identified.2 *T. equestre* is a wild mushroom considered in Europe as a delicacy. Toxicity is observed after a consumptionof considerable amounts of fresh mushroom which ranges from 100 to 400 g at 3 to 9 consecutive meals. (61) A neurological syndrome appears after the ingestion of the specie *Hapalopilus rutilans* that is considered edible. Common symptoms consist of visual disturbances, somnolence, hypotonia and hepatic and renal insufficiency. (62) Hepatic cytolysis and renal insufficiency were described in children. (49,63).

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Chapter 5

Antimycotic drugs

Antifungal medication

An antifungal medication is a medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others. Such drugs are usually obtained by a doctor's prescription or purchased over-the-counter.

Adverse effects

Apart from side-effects like liver-damage or affecting estrogen levels, many anti-fungal medicines can cause allergic reactions in people. For example, the azole group of drugs is known to have caused anaphylaxis.

There are also many drug interactions. Patients must read in detail the enclosed data sheet(s) of the medicine. For example, the azole antifungals such as ketoconazole or itraconazole can be both substrates and inhibitors of the P-glycoprotein, which (among other functions) excretes toxins and drugs into the intestines. (1) Azole antifungals also are both substrates and inhibitors of the cytochrome P450 family CYP3A4, (2) causing increased concentration when administering, for example, calcium channel blockers, immunosuppressants, chemotherapeutic drugs, benzodiazepines, tricyclic antidepressants, and macrolides.

Classes

Polyene antifungals:

A polyene is a molecule with multiple conjugated double bonds. A polyene antifungal is a macrocyclic polyene with a

heavily hydroxylated region on the ring opposite the conjugated system. This makes polyene antifungals amphiphilic. The polyene antimycotics bind with sterols in the fungal cell membrane, principally ergosterol. This changes the transition temperature (Tg) of the cell membrane, thereby placing the membrane in a less fluid, more crystalline state. As a result, the cell's contents including monovalent ions (K+, Na+, H+, and Cl-), small organic molecules leak and this is regarded one of the primary ways cell dies. (3) Animal cells contain cholesterol instead of ergosterol and so they are much less susceptible. However, at therapeutic doses, some amphotericin B may bind to animal membrane cholesterol, increasing the risk of human toxicity. Amphotericin B is nephrotoxic when given intravenously. As a polyene's hydrophobic chain is shortened, its sterol binding activity is increased. Therefore, further reduction of the hydrophobic chain may result in it binding to cholesterol, making it toxic to animals.

- **Amphotericin B**
- **Candicidin**
- **Filipin - 35 carbons, binds to cholesterol (toxic)**
- **Hamycin**
- **Natamycin - 33 carbons, binds well to ergosterol**
- **Nystatin**
- **Rimocidin**

Imidazole, triazole, and thiazole antifungals:

Azole antifungal drugs inhibit the enzyme lanosterol 14 demethylase; the enzyme necessary to convert lanosterol to

ergosterol. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth. (4)

Imidazoles:

- **Canesten (clotrimazole) antifungal cream**
- **Bifonazole**
- **Butoconazole**
- **Clotrimazole**
- **Econazole**
- **Fenticonazole**
- **Isoconazole**
- **Ketoconazole**
- **Miconazole**
- **Omoconazole**
- **Oxiconazole**
- **Sertaconazole**
- **Sulconazole**
- **Tioconazole**

Triazoles:

- **Albaconazole**
- **Fluconazole**
- **Isavuconazole**
- **Itraconazole**
- **Posaconazole**
- **Ravuconazole**
- **Terconazole**
- **Voriconazole**

Thiazoles:

- **Abafungin**

Allylamines:

Allylamines inhibit squalene epoxidase, another enzyme required for ergosterol synthesis:

- **Amorolfin**
- **Butenafine**
- **Naftifine**
- **Terbinafine**

Echinocandins:

Echinocandins may be used for systemic fungal infections in immunocompromised patients, they inhibit the synthesis of glucan in the cell wall via the enzyme 1,3-β glucan synthase:

- **Anidulafungin**
- **Caspofungin**
- **Micafungin**

Echinocandins are poorly absorbed when administered orally. When administered by injection they will reach most tissues and organs with concentrations sufficient to treat localized and systemic fungal infections. (5)

Others:

- **Benzoic acid - has antifugal properties but must be combined with a keratolytic agent such as in Whitfield's Ointment. (6)**

- **Ciclopirox - (ciclopirox olamine), most useful against Tinea versicolour, (7) Flucytosine or 5-fluorocytosine - an antimetabolite**
- **Griseofulvin - binds to polymerized microtubules and inhibits fungal mitosis**
- **Haloprogin - discontinued due to the emergence of more modern antifungals with fewer side effects. (8)**
- **Polygodial, (9,10) - strong and fast-acting in-vitroantifungal activity against Candida albicans.**
- **Tolnaftate (trade names Tinactin, Desenex and Aftate)**
- **Undecylenic acid - an unsaturated fatty acid derived from natural castor oil; fungistatic as well as anti-bacterial and anti-viral**
- **Crystal violet - a triarylmethane dye. Crystal violet has antibacterial, antifungal, and anthelmintic properties and was formerly important as a topical antiseptic. (11)**

Alternatives:

Research conducted in 1996 indicated that the following substances or essential oils had anti-fungal properties. (12)

- **Allicin - created from crushing garlic**
- **Citronella oil - obtained from the leaves and stems of different species of Cymbopogon (Lemon grass)**
- **Coconut oil - medium chain triglycerides in the oil have antifungal activities**
- **Iodine - Lugol's iodine**
- **lemon myrtle**
- **Neem Seed Oil**
- **Olive leaf**

- **Orange oil**
- **palmarosa oil**
- **patchouli**
- **Selenium - in dietary supplements or natural food sources, particularly Brazil nuts**
- **Tea tree oil - ISO 4730 ("Oil of Melaleuca, Terpinen-4-ol type")**
- **Zinc - in dietary supplements or natural food sources, including pumpkin seeds and chick peas**
- **Horopito (Pseudowintera colorata) leaf contains the anti-fungal compound polygodial. (9)**

Researchers published a study in 2009 indicating that carnivorous plants like the Venus flytrap contain compounds that may be useful in providing a new class of anti-fungal drugs for use in humans, for fungal infections that are resistant to current anti-fungal drugs. (13,14,15)

Mechanism of action

Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus fungal and human cells are similar at the molecular level. This makes it more difficult to find or design drugs that target fungi without affecting human cells. As a consequence, many antifungal drugs cause side-effects. Some of these side-effects can be life-threatening if the drugs are not used properly.

Anti-dandruff shampoos

- **Nizoral (ketoconazole) 2% shampoo**
- **Antifungal agents (such as ketoconazole) are often found in anti-dandruff shampoos. The antifungal drugs inhibit the yeast *Malassezia globosa* which encourages seborrhoeic dermatitis and tinea versicolor. Table(1)**

Table 1: Medical application of antimycotic agents

Agent	Trade name	Medical application
Ketoconazole	Nizoral, Fungoral and Sebizole	Preliminary findings, research and studies including the completion of a small controlled clinical trial have produced data suggesting that ketoconazole shampoo is effective as a hair loss treatment in men with androgenic alopecia. Larger controlled clinical studies are still needed to evaluate the ideal dosage, formulation, and to determine the routine of treatment for this condition, thus ketoconazole shampoo is not FDA approved for this indication(17)

Ciclopirox olamine	**Loprox**	**The cream and lotion form of this agent is used to treat fungal infections of the skin. The lacquer form is used as part of a treatment plan to treat fungal infections of the nails. The shampoo form of this medication is used to treat and prevent dandruff or to treat seborrhoeic dermatitis.**
Piroctone olamine	**Octopirox(18) and Nivea Complete Control**	**Piroctone olamine is sometimes used as an anti fungal agent, and it often used in dandruff shampoos in lieu of zinc. Piroctone Olamine is said to be less toxic than other anti-dandruff agents, often bypassing some of the normal FDA warnings, but still must be used with care, and only externally.**
Zinc pyrithione(19)	**Head and Shoulders, Johnson and Johnson ZP-11, Clinic All Clear, Pantene Pro V and Sikkai Powder**	**An antifungal and antibacterial agent first reported in the 1930s. Zinc pyrithione is best known for its use in the treatment of dandruff and seborrhoeic dermatitis. It also has antibacterial properties and is effective**

		against many pathogens from the streptococcus and staphylococcus class. Its other medical applications include treatments of psoriasis, eczema, ringworm, fungus, athletes foot, dry skin, atopic dermatitis, tinea, and vitiligo.
Selenium sulfide	Selsun Blue, Head and Shoulders and Vichy Dercos Anti-Dandruff Shampoo	Selenium sulfide is available as a 1% and 2.5% lotion and shampoo. In some countries, the higher strength preparations requires a doctors prescription. The shampoo is used to treat dandruff and seborrhea of the scalp, and the lotion is used to treat tinea versicolor, a fungal infection of the skin.
Tar(20)	Neutrogena T-Gel	Is effective as a therapeutic treatment to control scalp itching and flaking symptomatic of scalp psoriasis, eczema, seborrhoeic dermatitis and dandruff.
Tea tree oil(21)	Dr. Bronner's Castile Soap	Is used topically as an ingredient in creams, ointments, lotions, soaps,

		and shampoos. In addition to antifungal properties, tea tree oil has antiseptic, antibacterial, and antiviral actions. It is effective against bacteria, viruses, fungal infections, mites (such as scabies), and lice (such as head lice).

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