Genus Aspergillus

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- Aspergillus is a filamentous, ubiquitous fungus found in nature. It is commonly isolated from soil, plant debris, and indoor air environments.
- Aspergillus is a very large genus containing more than 250 species, which are currently classified into seven subgenera that are in turn subdivided into several sections comprised of related species.

- The opportunistic mold Aspergillus is the etiologic agent responsible for a variety of infections and conditions referred to as **aspergillosis.** These manifestations include a spectrum of diseases from allergic responses to the organism (allergic bronchopulmonary aspergillosis), to colonization with Aspergillus spp. (aspergilloma or fungus ball and other superficial conditions, such as external ear colonization) and invasive infection (invasive pulmonary aspergillosis and other clinical syndromes of tissue invasion).

The most common Aspergillus SPP are:

1. *Aspergillus fumigatus complex*……..90% the Most Common type
2. *A. flavus complex*
3. *A. niger complex*
4. *A. terreus complex*

Other less common pathogenic SPP are :

A. Nidulans complex

A. vitis complex

A. versicolor complex

- Most species of Aspergillus reproduce asexually, but a teleomorph, or sexual form, has been identified for pathogenic species, including *Aspergillus fumigatus* with its recently described teleomorph *Neosartorya fumigata*,
- *Aspergillus nidulans* (teleomorph *Emericella nidulans*),
- *Aspergillus vitis* (teleomorph *Eurotium amstelodami)*

Aspergillus fumigatus Complex

- The most common pathogen in the section Fumigati is the most frequent species to cause invasive aspergillosis, comprising up to 90% of the isolates.
- Thermotolerance permits a wide range of suitable host conditions upto 50.
- **Macroscopically:** Colonies are a graygreenish color with velvety to powdery the reverse is white tan.
- **Microscopically:** Hyphae are septate and hyaline with columnar conidial heads. Conidiophores are smooth-walled, uncolored, and uniseriate with closely compacted phialides only on the upper portion of the vesicle. Conidia are smooth to finely roughened and are 2–3 mm in diameter.

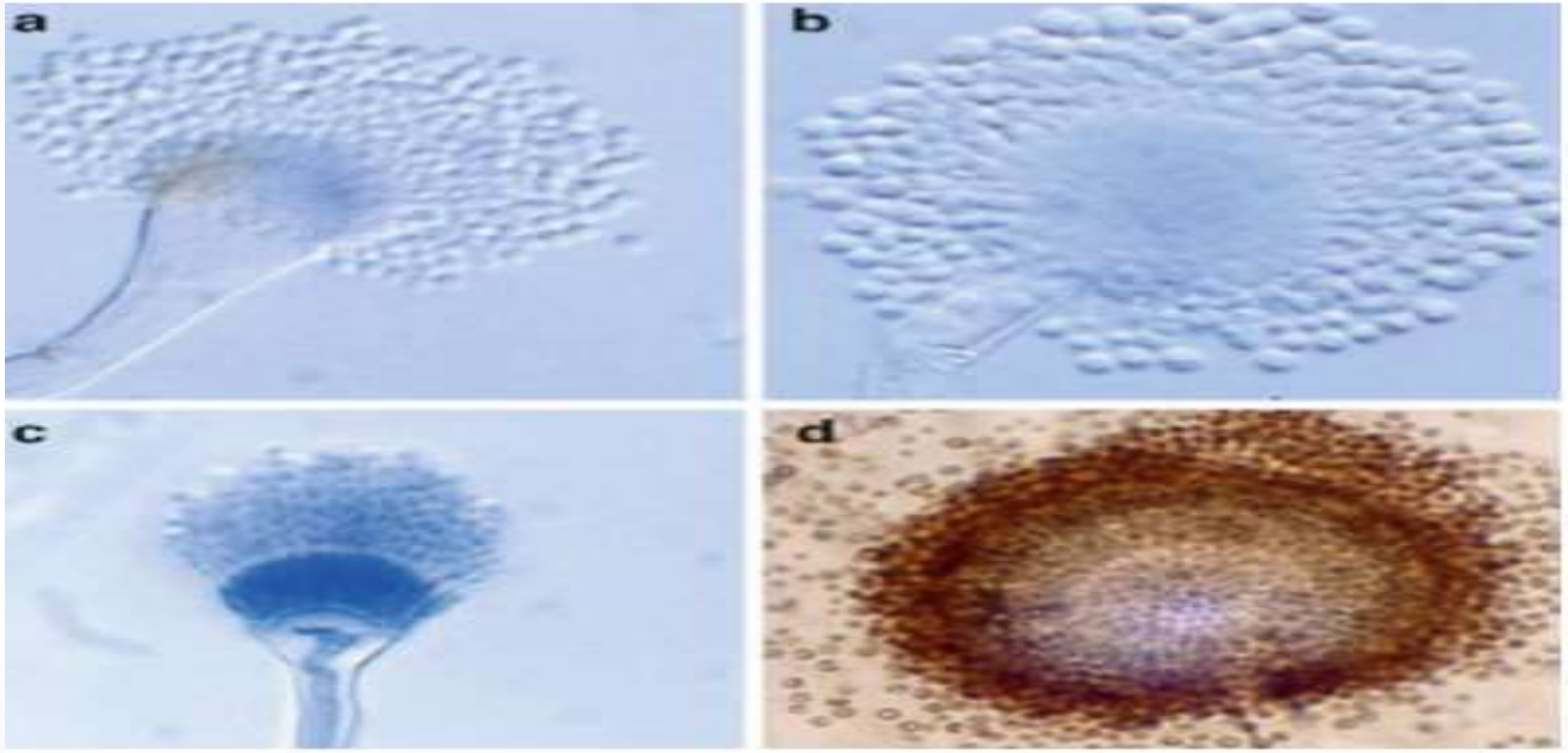


Fig.1 Microscopic characteristics of *Aspergillus* species fruiting structures. (a) *Aspergillus fumigatus* (b) *A. flavus* (c) *A. terreus* (d) *A. niger*

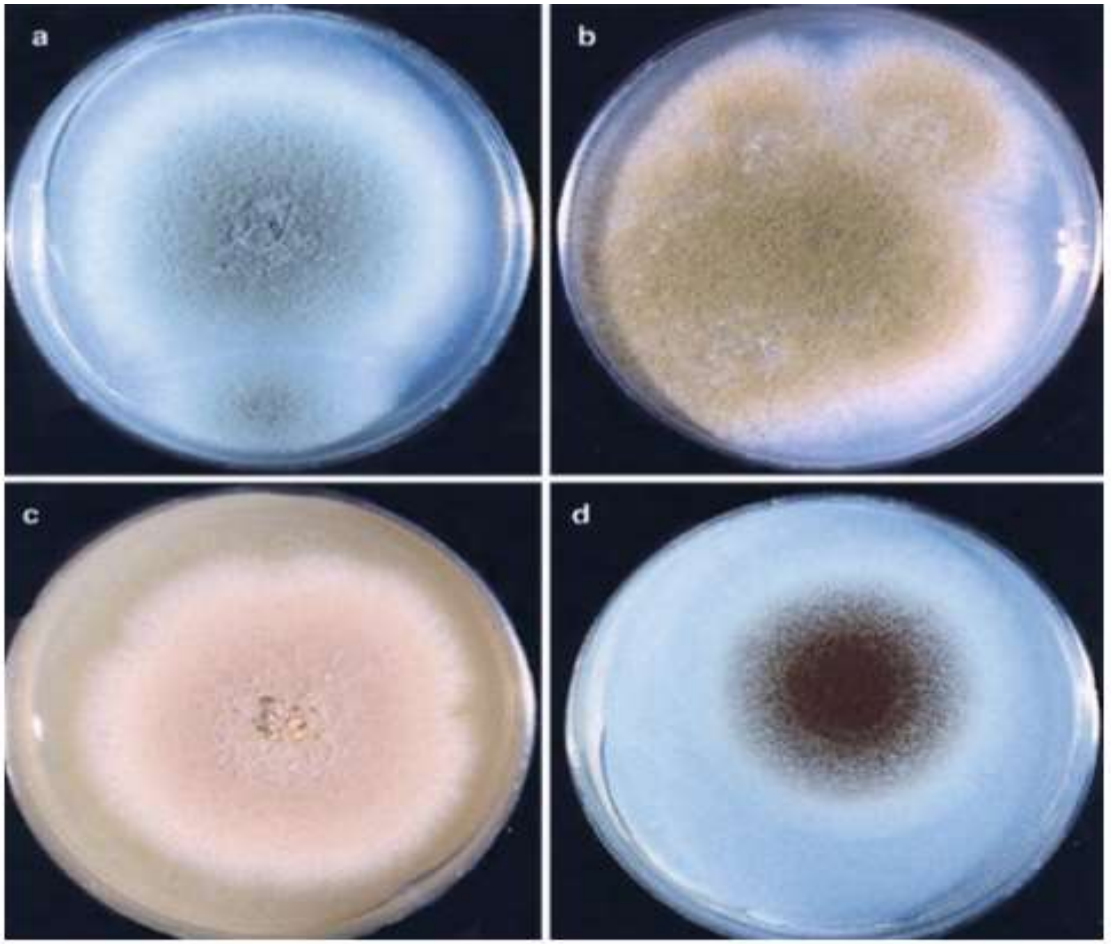
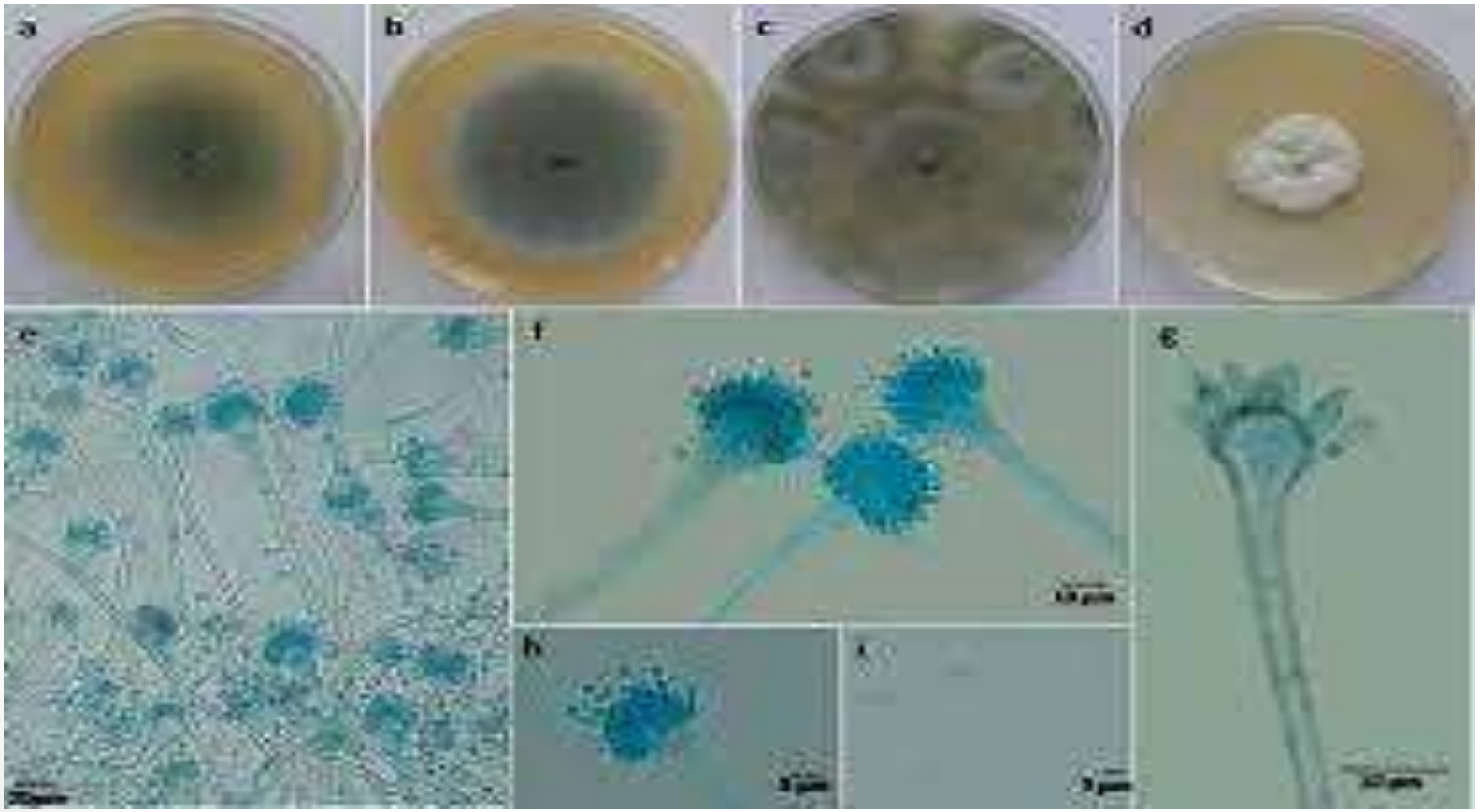
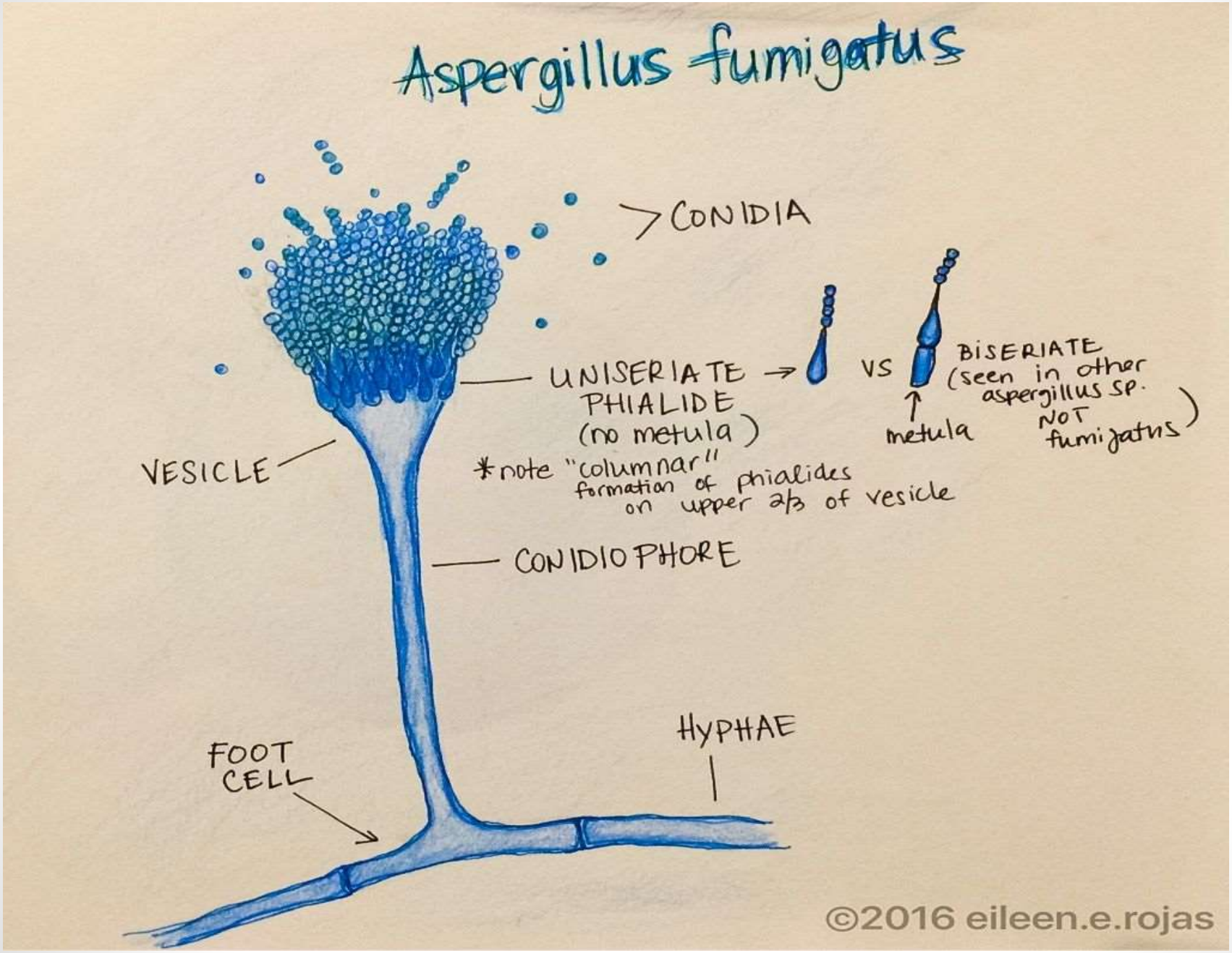


Fig. 2 Colony morphology of *Aspergillus* species. **(a)** *Aspergillus fumigatus* **(b)** *A. flavus* **(c)** *A. terreus* **(d)** *A. niger* (Colony photographs kindly provided by Dr. Deanna Sutton)

Aspergillus fumigatus




Aspergillus fumigatus
CONIDIA
UNISERIATE PHIALIDE (no metula)
VS
BISERIATE (seen in other aspergillus sp. NOT fumigatus)
metula
*note "columnar" formation of phialides on upper 2/3 of vesicle
VESICLE
CONIDIOPHORE
HYPHAE
FOOT CELL
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- Other “cryptic” members in the section Fumigati that are pathogenic in humans have been described, *including A. lentulus, A. novofumigatus*, and others. These species sporulate poorly and, in contrast to *A. fumigatus*, fail to grow at 50 °C but do grow at 10 °C
- *A. lentulus*, exhibit decreased antifungal susceptibility antifungal agents
- **Key Features**: Uniseriate and columnar conidial heads with the phialides limited to the upper two thirds of the vesicle and curving to be roughly parallel to each other.

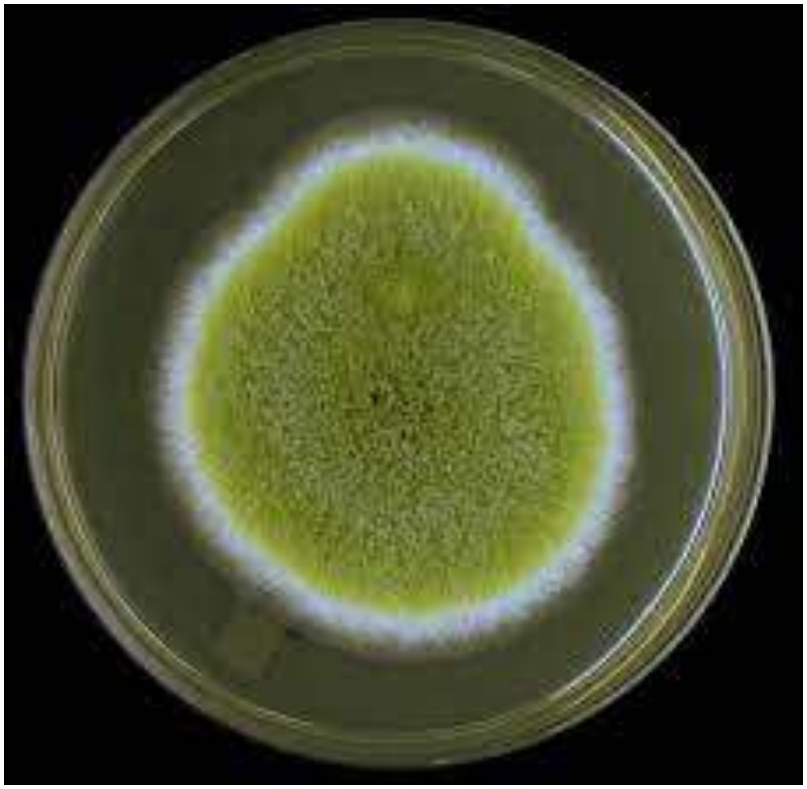
Aspergillus flavus Complex

- A worldwide distribution and normally occurs as a saprophyte in soil and on many kinds of decaying organic matter, however, it is also a recognized pathogen of humans and animals.
- *A. flavus* and other species in the section Flavi
- *A. flavus* is second only to *A. fumigatus* as the cause of human invasive aspergillosis
- RG-2 organism
- **Macroscopically:** colonies are granular to velvety, flat, white then yellow at first but quickly becoming yellow-green with age (lime green)
- Grow at a rapid rate.

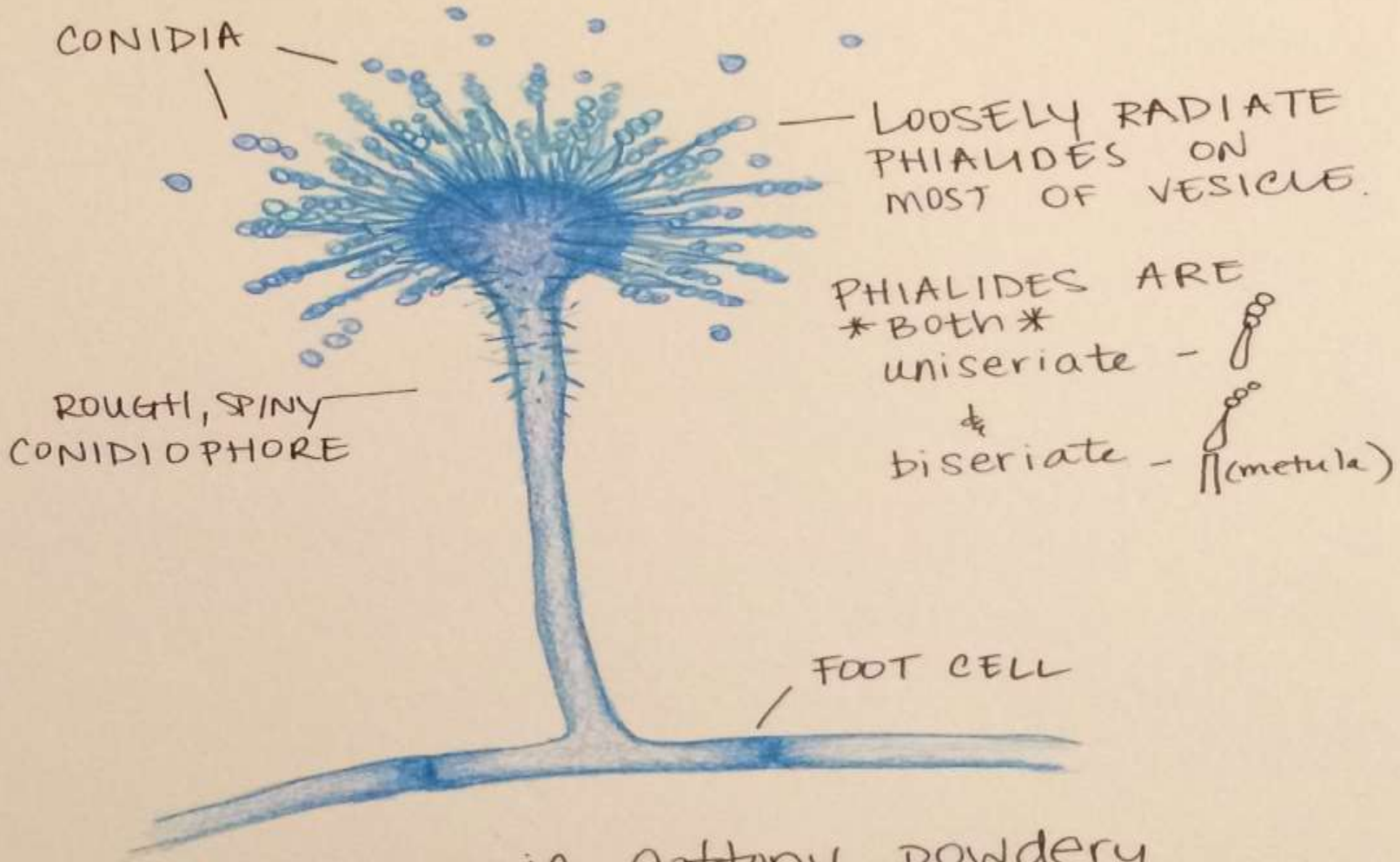
- **Microscopically:** This species is typically biseriate with rough conidiophores and smooth conidia 3–6 mm that serve to distinguish the species. Some isolates are uniseriate
- The organism is a common cause of sinusitis as well as invasive infection in immunosuppressed hosts.
- *Aspergillus flavus* is also responsible for a mycotoxicosis, as the species produces a potent aflatoxin

- **Key Features:** Spreading yellow-green colonies, rough-walled stipes (conidophore), mature vesicles bearing phialides over their entire surface

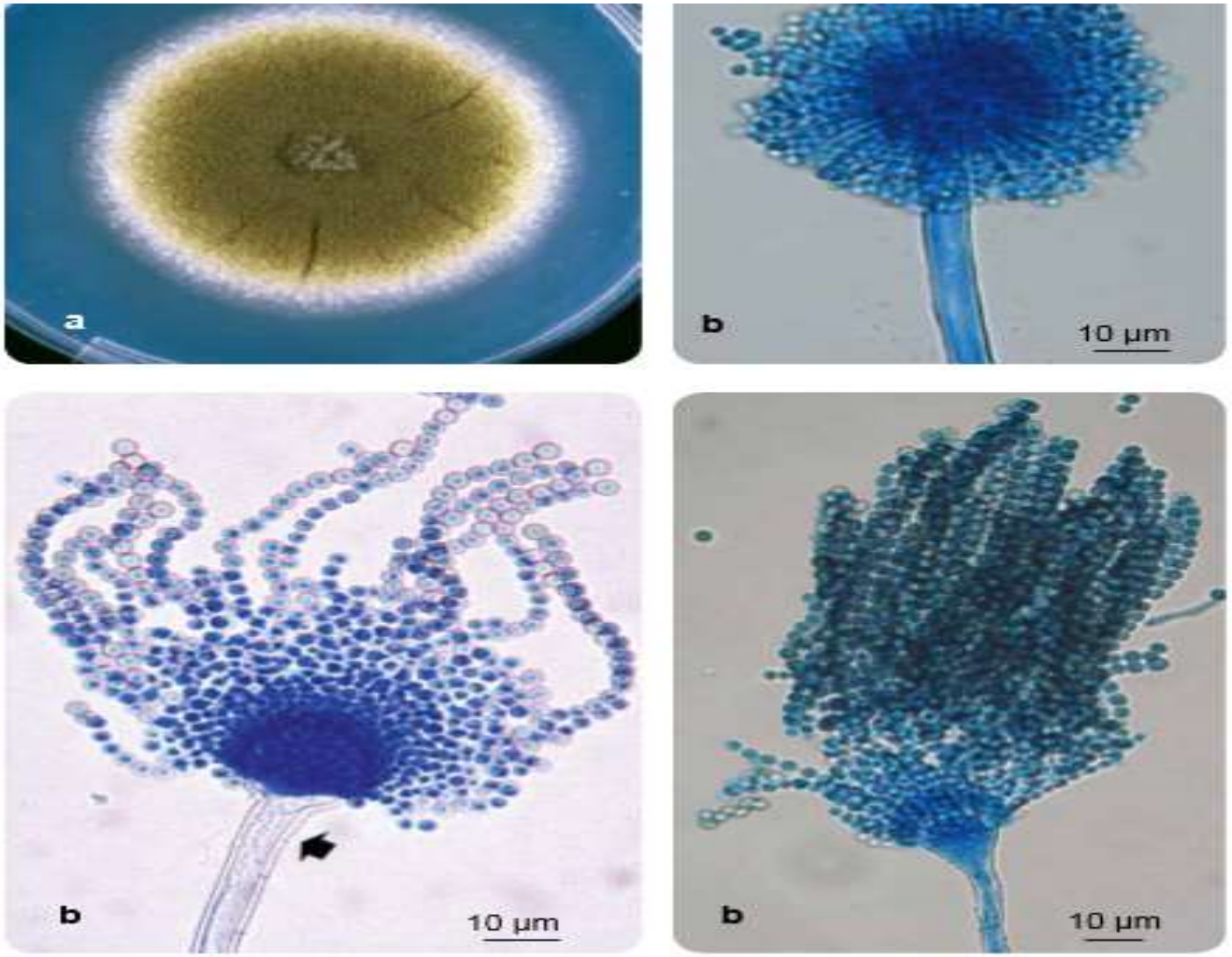
Aspergillus flavus



Aspergillus flavus



Macroscopic: Cottony, powdery
Lime Green

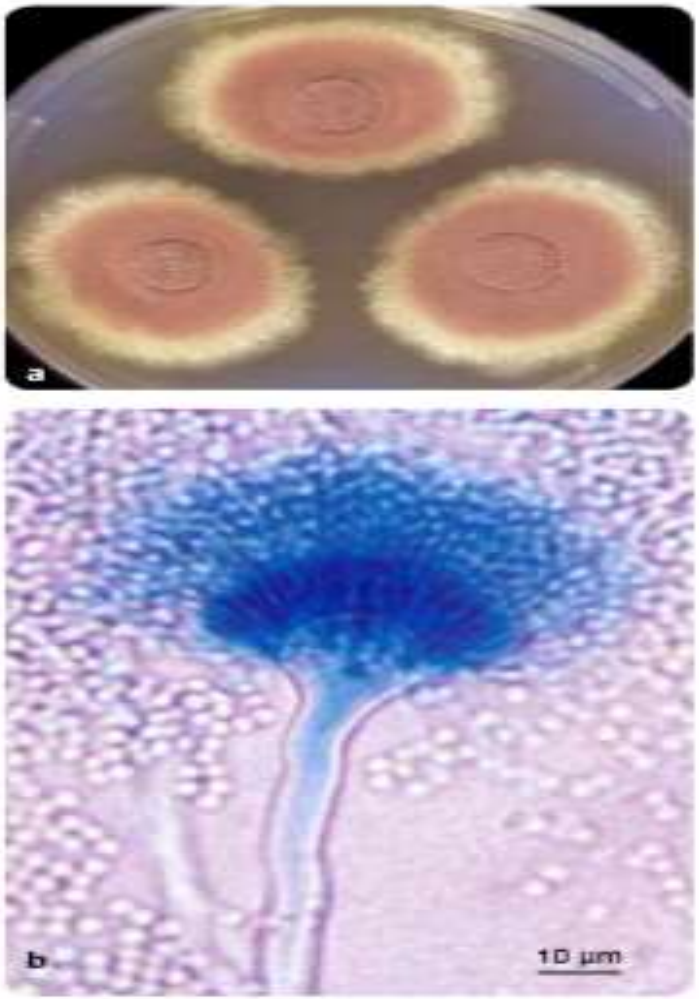


Aspergillus flavus (a) culture and (b) conidial heads.
Note: Rough-walled stipe near vesicle (arrow) and both uniseriate and biseriate conidial heads may be present.

Aspergillus terreus Complex

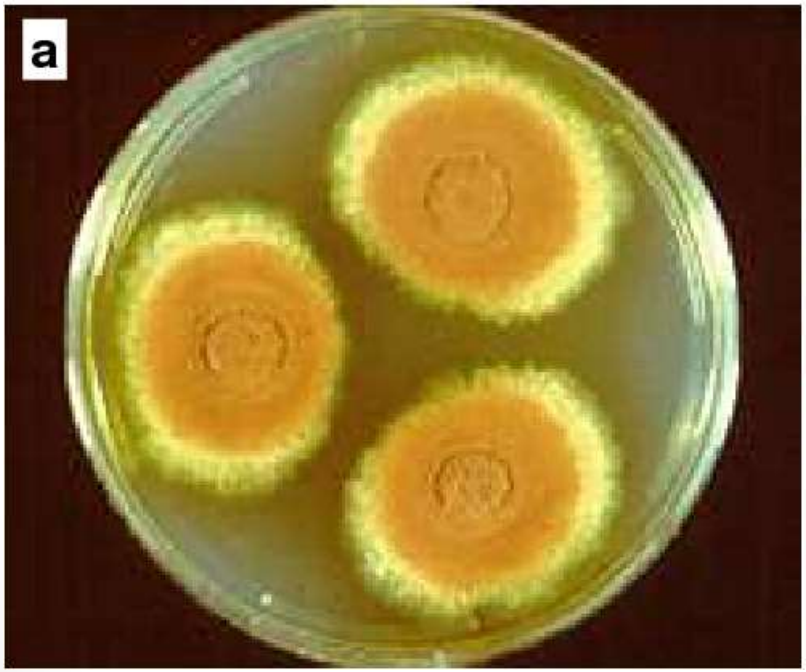
- Is common in tropical and subtropical habitats and has been increasingly reported as a cause of invasive infection in immunocompromised hosts
- **Macroscopically:** Colonies are buff to beige to cinnamon.
- **Microscopically:** Conidial heads are biseriate, columnar and compact. Conidiophores are smooth-walled and hyaline. Globose, sessile accessory conidia are frequently produced on submerged hyphae. Conidia are small (2–2.5 mm).
- **Key Features:** cinnamon-brown cultures, conidial heads biseriate with metulae as long as the phialides.
- Notable for its decreased susceptibility to amphotericin B

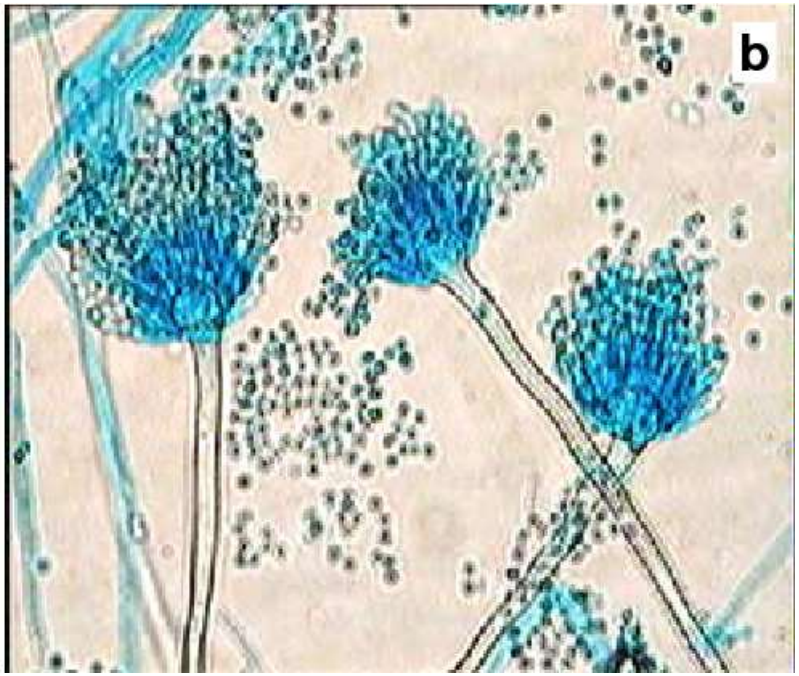
Aspergillus terreus Thom



Aspergillus terreus (a) culture and (b) conidial head morphology
Note: Conidial heads are biseriate.

Aspergillus terreus Complex





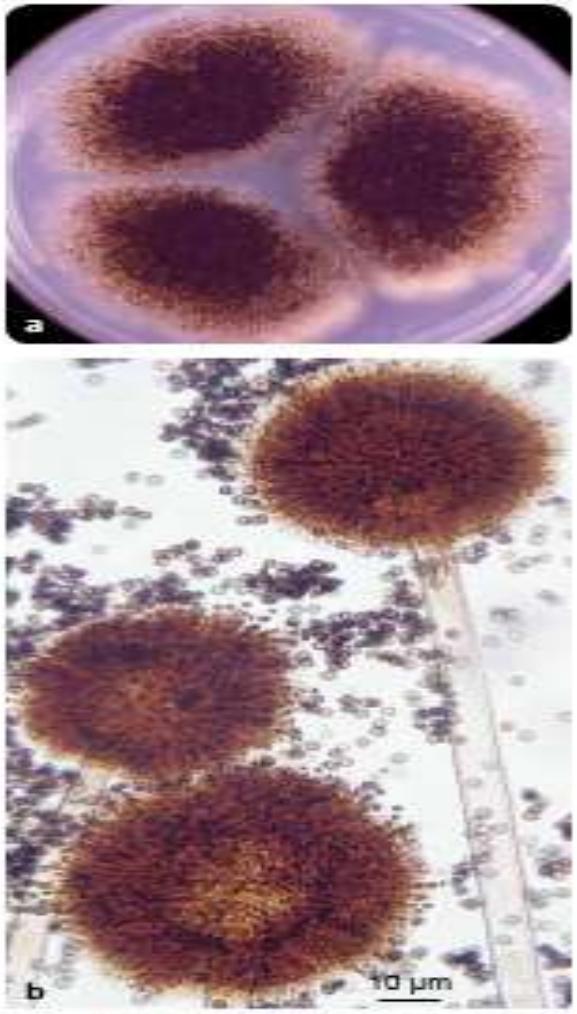
Aspergillus niger Complex

- Is one of the most common and easily identifiable species of the genus Aspergillus
- This species is very commonly found in aspergillomas and is the most frequently encountered agent of otomycosis. It is also a common laboratory contaminant
- **Macroscopically:** wooly colonies are initially white but quickly become black with the production of the fruiting structures. It grows rapidly with a pale yellow reverse side.
- **Microscopically:** Like other Aspergillus species, hyphae are hyaline and septate. Conidial heads are biseriate and cover the entire vesicle. Conidia are brown to black and are very rough (4–5 mm).

Aspergillus niger Complex

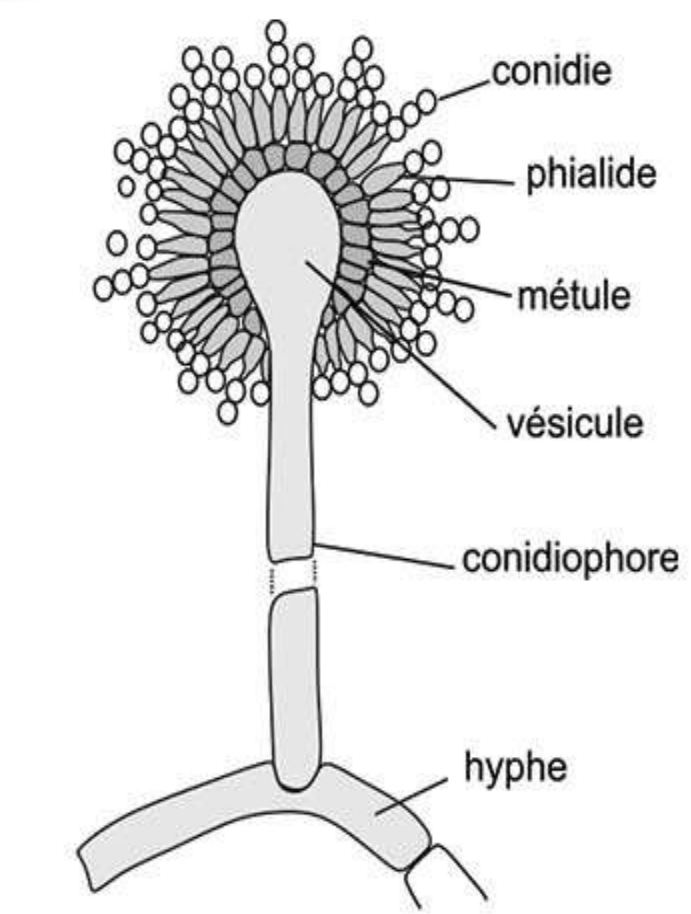
- **Key Features:** conidial heads are dark brown to black, radiate and biseriate with metulae twice as long as the phialides. conidia brown and rough-walled.
- It affects human and animals.

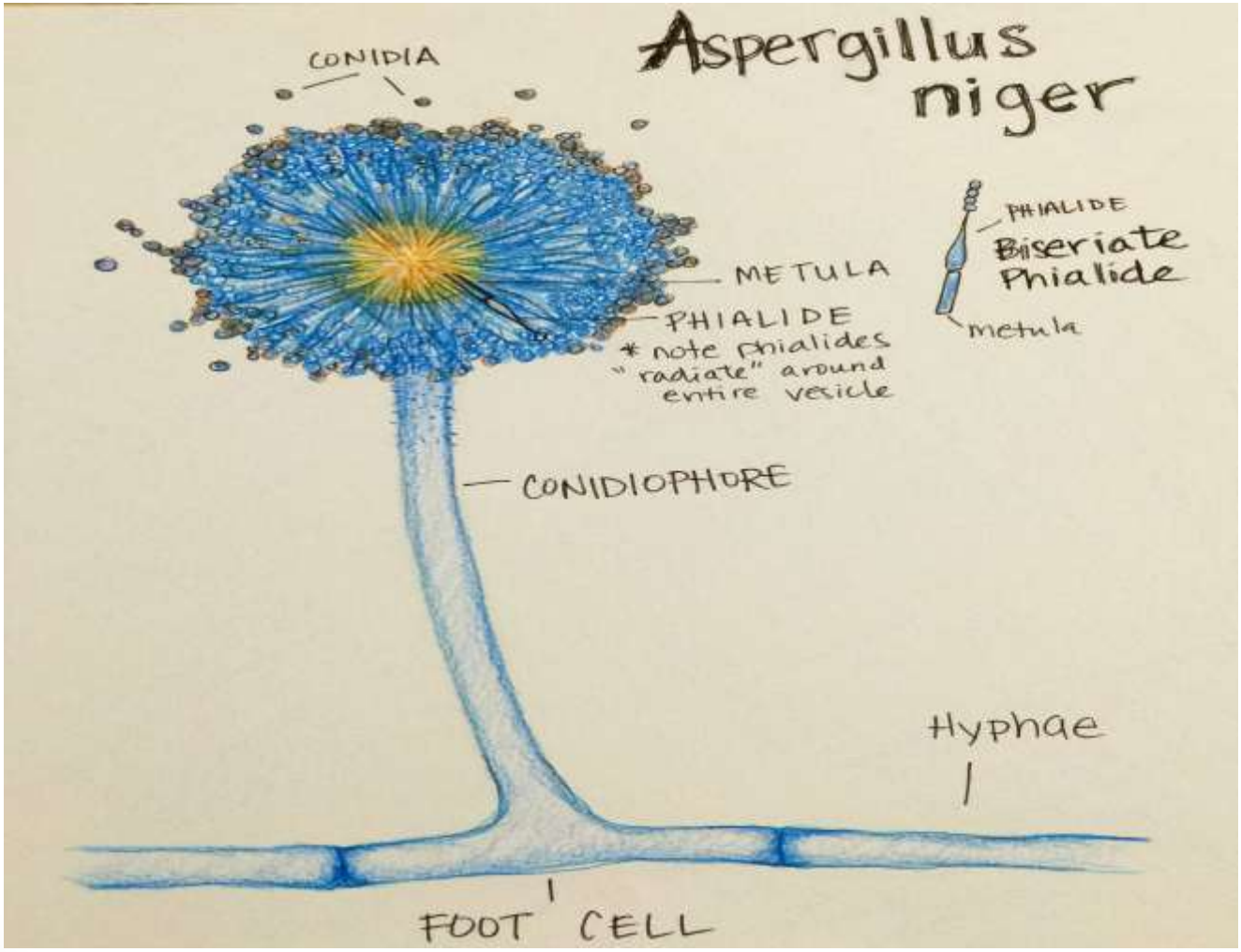
Aspergillus niger van Tieghem



Aspergillus niger (a) Culture and (b) conidial head morphology.
Note: Conidial heads are biseriate, large, globose, dark brown, becoming radiate with the phialides borne on metulae.

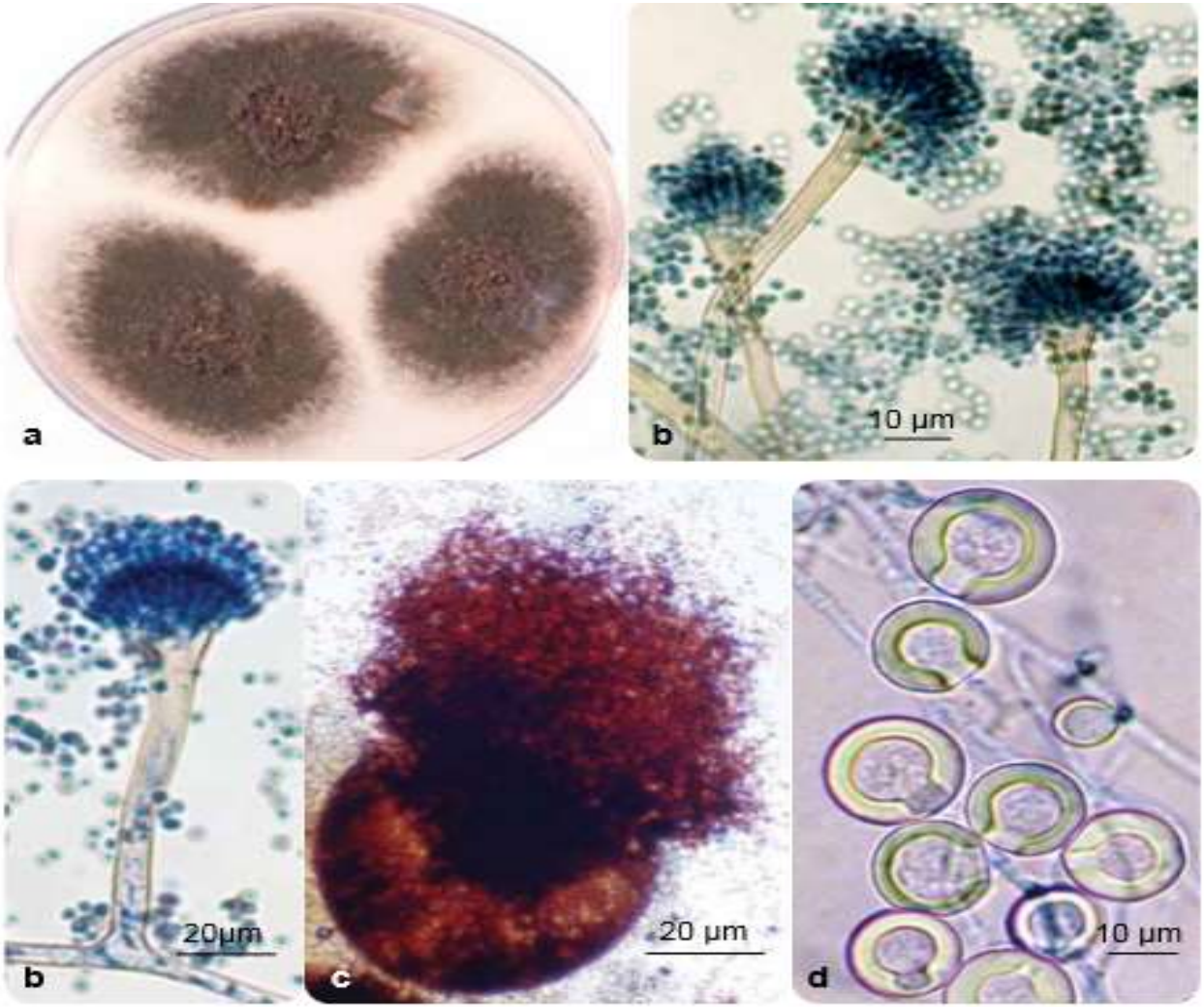
Aspergillus niger Complex



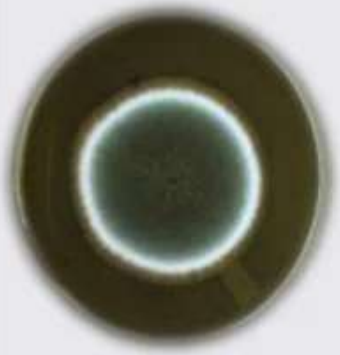
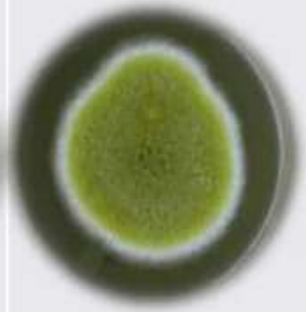

Aspergillus niger
CONIDIA
METULA
PHIALIDE
* note phialides "radiate" around entire vesicle
CONIDIOPHORE
Hyphae
FOOT CELL
PHIALIDE
Biseriate Phialide
metula

Aspergillus nidulans complex

- Aspergillus nidulans is a typical soil fungus with a worldwide distribution, it has also been reported to cause disease in human and animals.
- **Macroscopically:** colonies are typically plain green in colour with dark red-brown cleistothecia developing within and upon the conidial layer. Reverse may be olive or purple-brown.
- **Microscopically:** conidial heads are short, columnar (up to 70 x 30 µm in diameter) and biseriate. conidiophore stipes are usually short, brownish and smooth-walled. conidia are globose (3-3.5 µm in diameter) and rough-walled.
- **Key Features:** conidial heads are short, columnar and biseriate. Stipes are usually short, brownish and smooth-walled. conidia are globose and rough-walled.



Aspergillus nidulans (a) culture and (b) conidial head morphology, (c) cleistothecium of *Emericella nidulans* (anamorph *A. nidulans*) showing numerous reddish-brown ascospores and (d) thick-walled Hülle cells.

	A. fumigatus	*A. niger*	*A. flavus*	*A. terreus*
Macroscopic morphology	• Velvety or powdery • At first white than turning dark greenish to gray with a narrow white border. • Reverse white to tan	• Wooly • At first white to yellow than turning black • Reverse white to yellow	• Velvety • Yellow to green or brown • Reverse goldish to red brown	• Usually velvety • Cinnamon brown • Reverse white to brown
Picture				

	A. fumigatus	*A. niger*	A. flavus	A. terreus
Microscopic morphology (conidiophores)	Short smooth (<300µm)	Long smooth (400-3000 µm)	Variable length Rough, pitted, spiny	Short smooth (<250µm)
Microscopic morphology (phialides)	• Uniseriate • Usually only on upper two-third of vesicle • Parallel to axis of conidiophore	• Biseriate • Cover entire vesicle • Form "radiate" head	• Uniseriate and biseriate • Cover entire vesicle • Poin out in all direction	Biseriate Compectly columnar
Diagram				

Pathogenesis

- Aspergillus infection is typically acquired through inhalation of conidia into the lungs, although other routes of exposure, such as oral or aerosol exposure to contaminated water, may also occur. Cutaneous exposure through surgical wounds, contaminated intravenous catheters, can lead to cutaneous infections .
- Invasive aspergillosis is uncommon in immunocompetent patients, although infection in apparently normal hosts does occur
- Hence, despite the ubiquitous nature of the organism and frequent exposures to Aspergillus conidia, normal host defenses do not readily permit invasive pulmonary aspergillosis to occur.
- Aspergillus species commonly produce toxins, including aflatoxins, ochratoxin A, fumagillin, and gliotoxin, that can contribute to clinical manifestations following exposure and may contribute to virulence in specific settings .

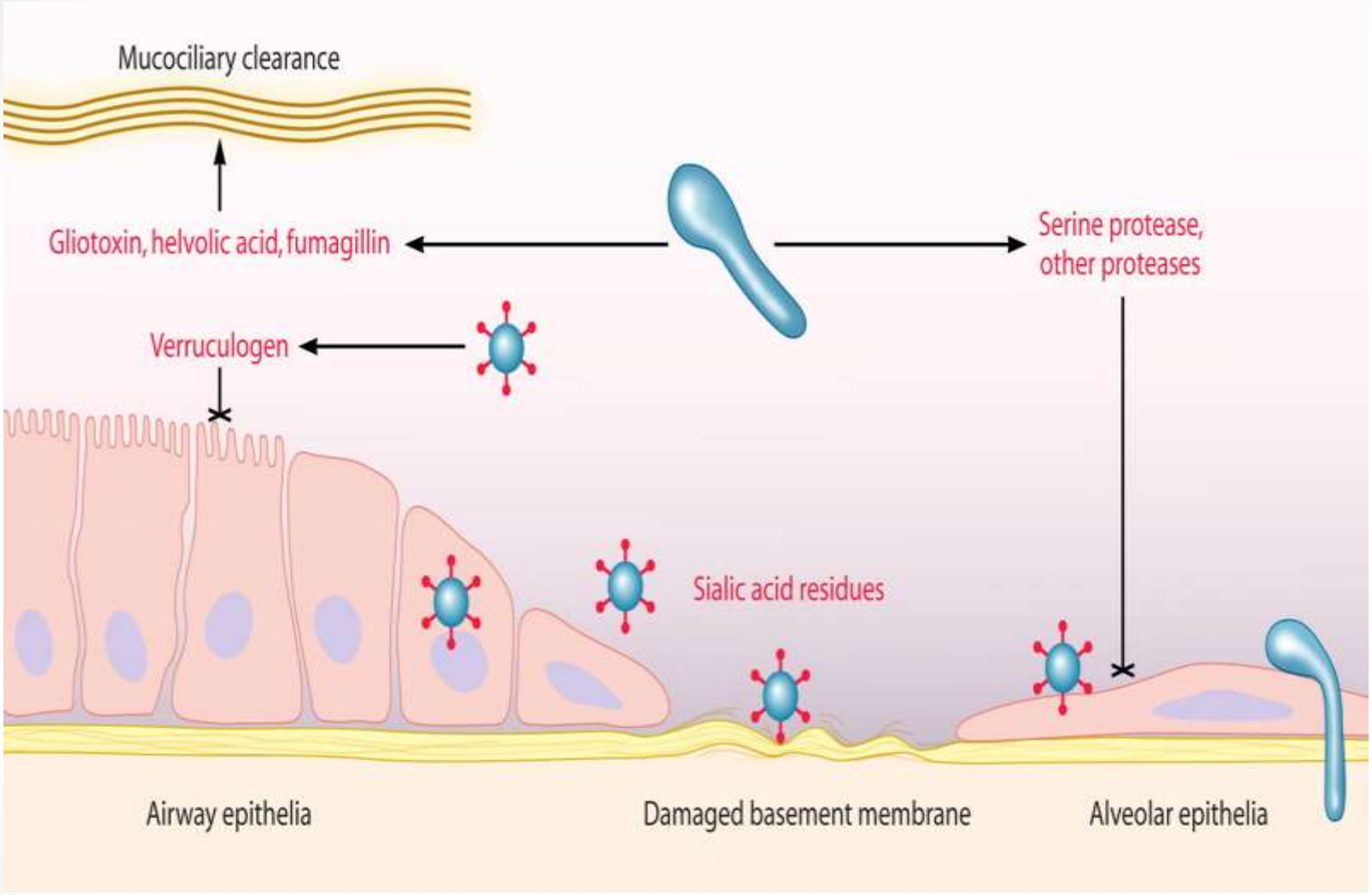
- For example, gliotoxin significantly impacts macrophage and neutrophil function,
- Other pathogenic factors include production of a variety of proteases and phospholipases, which are commonly produced by pathogenic strains.

- The first line of host defense against inhalation of Aspergillus conidia is ciliary clearance of the organism from the airways, limiting access to deep lung structures for larger, less pathogenic conidia.
- In the pulmonary tissues, the alveolar macrophage is a potent defense, capable of ingesting and killing inhaled Aspergillus conidia
- The hydrophobic layer on the conidial cell surface immunologically protects Aspergillus against activation of the host innate immune cell response.
- After germination, the major line of defense against both swollen conidia and hyphae is the polymorphonuclear leukocyte.
- Neutrophil influx in preventing conidial germination and limiting hyphal invasion .
- Hyphae are too large to be effectively ingested, and hyphal damage occurs extracellularly.

- Swollen conidia and hyphae are both able to fix complement, which is important in phagocytic killing of the organism. Notably, ***A. fumigatus*** produces a complement inhibitor, which may play a role in its pathogenicity
- Host defenses against Aspergillus may be enhanced by opsonization of conidia with complement or other molecules such as mannose-binding protein or surfactant proteins .
- A deficiency in mannose-blinding lectin has been associated with increased risk for invasive pulmonary aspergillosis.
- Antibody responses due to prior exposures to Aspergillus are common, but antibodies are not protective against invasive infection nor are they useful for diagnosis of infection.

- The NADPH oxidase in phagocytes is essential in host defenses against Aspergillus species, as demonstrated by the increased susceptibility of patients with chronic granulomatous disease, an inherited disorder of NADPH oxidase, to Aspergillus infections
- In contrast to the deficient host defense responses in invasive infection, in noninvasive allergic forms of aspergillosis such as allergic bronchopulmonary aspergillosis (ABPA) or allergic sinusitis, the pathogenesis often relates to exuberant inflammatory host responses to the. ABPA begins with an allergic inflammatory response that follows after inhalation of Aspergillus conidia into the bronchi, where they germinate and form hyphae
- Colonizing hyphae then release allergens that are processed by HLA-DR2 or HLA-DR5 antigen-presenting cells. The resultant Th2 inflammatory response in bronchial tissue leads to excessive mucin production, recruitment of eosinophils, intermittent bronchial obstruction, and eventually bronchiectasis in some patients.

- Similarly, the pathogenesis of aspergilloma or fungus ball due to Aspergillus is not well defined but also seems to be associated with host responses to chronic colonization. In aspergilloma, the organism does not usually invade the tissues, but it colonizes a pulmonary cavity. Although tissue invasion resulting in a chronic necrotizing form of aspergillosis can occur, the pathogenic features leading from colonization to invasive disease are not clearly understood


Mucociliary clearance
Gliotoxin, helvolic acid, fumagillin
Serine protease, other proteases
Verruculogen
Sialic acid residues
Airway epithelia
Damaged basement membrane
Alveolar epithelia

Clinical forms of Aspergillosis

The clinical presentation of diseases produced by Aspergillus species is diverse and generally reflect the underlying immune status of the host and the host's response to the organism.

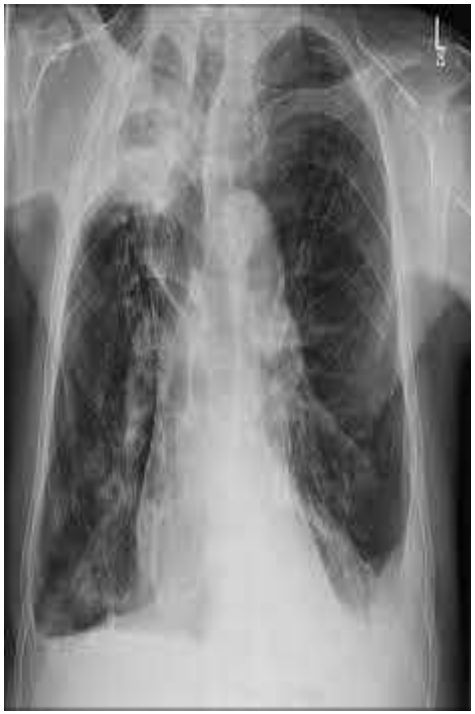
The syndromes of aspergillosis range from :

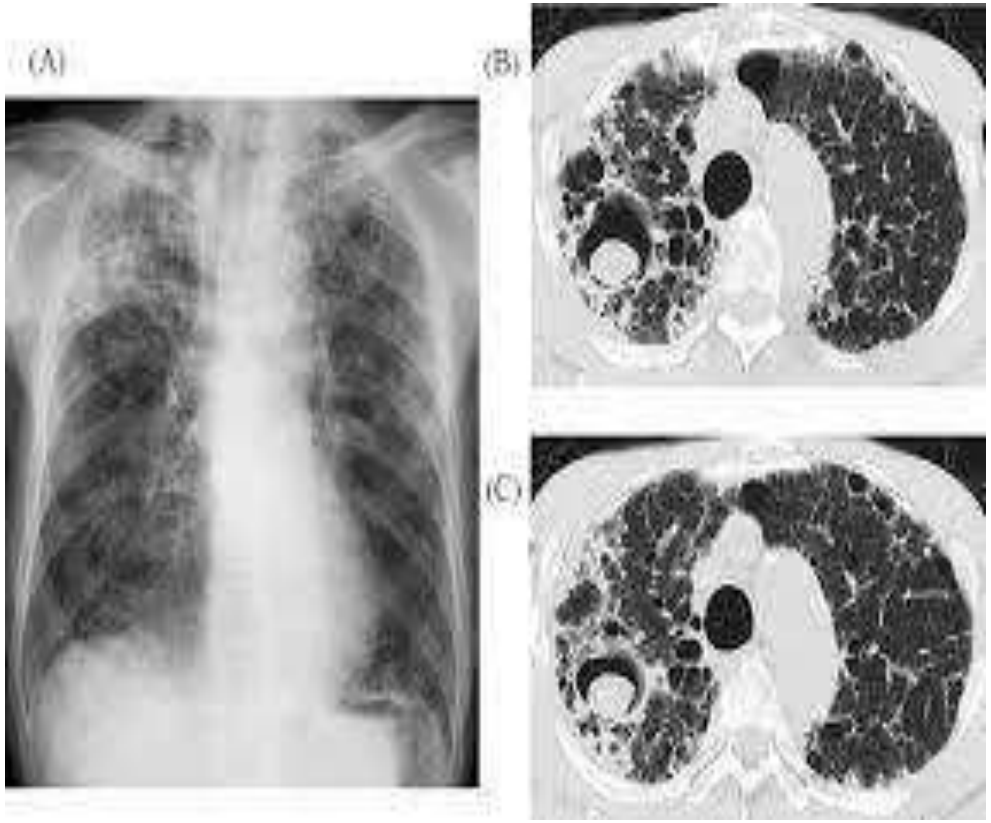
1. Asymptomatic colonization.
2. Superficial or saprophytic infection which include pulmonary aspergilloma and chronic pulmonary aspergiloosis
3. Allergic responses to the organism (ABPA)
4. Acute or subacute invasive disease.

Pulmonary Aspergilloma

- A pulmonary fungus ball due to Aspergillus (aspergilloma) is characterized by chronic, extensive colonization of Aspergillus species in a pulmonary cavity.
- Fungus balls may also develop in other sites, such as the maxillary or ethmoid sinus, or even in the upper jaw
- Typically Aspergillus fungus balls in the lung develop in cavities as a result of pre-existing infections or diseases, such as tuberculosis, histoplasmosis, sarcoidosis, rarely, Pneumocystis jiroveci pneumonia. The diagnosis of a pulmonary fungus ball, which can also be due to other molds, is usually made radiographically with the appearance of a solid round mass inside a cavity. The detection of Aspergillus antibodies are further evidence that the radiographic findings are consistent with a diagnosis of fungus ball due to Aspergillus; biopsy is not usually undertaken.

Aspergilloma





Chronic Forms of Pulmonary Aspergillosis

- Chronic necrotizing pulmonary aspergillosis is the descriptive term applied to cavitary lung disease, chronic respiratory symptoms, and the presence of serum precipitating antibodies to Aspergillus. Direct invasion of Aspergillus into the lung parenchyma without angioinvasion occurs, and this form of infection is described as a subacute or non-angioinvasive form of disease

- Aspergillus sinusitis is associated with fungal balls of the sinuses without tissue invasion .The maxillary sinus is the site most commonly involved. Clinical presentation is similar to that for any chronic sinusitis with chronic nasal discharge, sinus congestion, and pain. The diagnosis of a fungal ball is suggested on CT scan of the sinuses; positive cultures for Aspergillus, usually are obtained by aspiration of material from the sinuses. Management is usually directed at surgical removal of the lesion and confirmation that the fungal ball has not caused bony erosion

- Otomycosis is a condition of superficial colonization by Aspergillus, most typically A. niger. The usual clinical presentation is that of an external otitis media with ear pain and drainage. Examination of the ear canal may reveal the black conidiophores of A. niger. Treatment involves cleaning debris from the ear canal and the topical administration of a variety of agents, including cleansing solutions and topical antifungal agents.
- Other superficial or colonizing conditions due to Aspergillus include onychomycosis, which can be a chronic condition not responsive to antifungal agents directed at yeasts. Culture confirmation of Aspergillus as the etiologic agent may be useful in this setting.

ABPA

- ABPA is a chronic allergic response to colonization with Aspergillus. Classic criteria for establishing a diagnosis include (1) episodic bronchial obstruction (asthma); (2) peripheral eosinophilia; (3) immediate skin test reactivity to Aspergillus antigen; (4) precipitating Aspergillus antibodies; (5) elevated serum immunoglobulin E (IgE); (6) history of or presence of pulmonary infiltrates; and (7) central bronchiectasis .
- The detection of the first six criteria establishes a likely diagnosis, while the presence of all seven confirms the condition. Other secondary features that may be present include sputum cultures yielding Aspergillus, brown mucus plugs in expectorated sputum, elevated specific IgE

- In ABPA, typically the initiating event is for an asthmatic patient to develop an allergic reaction to inhaled Aspergillus. Following that reaction, mucus plugs develop in the bronchi and can be detected by the presence of hyphae in sputum.

Invasive Aspergillosis Following inhalation and germination of the conidia, Hyphae invade the lumens and walls of blood vessels, causing thrombosis, infarction, and necrosis. Symptoms include fever, cough, dyspnea, and hemoptysis. With or without dissemination

Diagnosis

- Sputum, other respiratory tract specimens, and lung biopsy tissue provide good specimens. Blood samples are rarely positive.

1. Microscopic examination

- On direct examination of sputum with KOH or calcofluor white or in histologic sections, the hyphae of Aspergillus species are hyaline, septate, and branch. The hyphae bear characteristic conidial structures: long conidiophores with terminal vesicles on which phialides produce chains of conidia . The shape of condophore , phalids and conidia differe with different SPP

2. Culture

- Aspergillus species grow within a few days on most media at room temperature range of Temp 10 to 50 c.

3. Serology

- The intradermal test for precipitins to *A fumigatus* is positive in over 80% of patients with aspergilloma or allergic forms of aspergillosis, but antibody tests are not helpful in the diagnosis of invasive aspergillosis. However, a serologic test for circulating cell wall galactomannan is diagnostic.

4. **Histopathological examination**

5. Radiographic

Treatment

1. Aspergilloma is treated with itraconazole or amphotericinB and surgery.
2. Invasive aspergillosis requires rapid administration of either of amphotericin B or voriconazole, often supplemented with cytokine immunotherapy.
3. Allergic forms of aspergillosis are treated with corticosteroids