

# THIRD CLASS

## *ASPERGILLUS SPP.*



# GENERAL CHARACTERISTIC FEATURES

- \*Second most common isolated fungus after Candida.
- \**A. fumigatus* is the species most commonly isolated
- \*pathogenic species are *A. flavus*, *A. niger* & *A. terreus*
- \*fast growing fungus.

*Aspergillus* species as examples of opportunistic fungi.

Unlike the primary pathogens, a large group of fungi are opportunistic pathogens that are capable of causing disease only in very special circumstances.

These fungi, which are monomorphic (growing as molds both in the patient and on culture media), usually cause disease in neutropenic patients or in patients whose PMN's do not function normally.

People with normal numbers of functional PMNs are resistant to infection.

The organisms are identified on the basis of morphology alone.

One very important member of this group is *Aspergillus fumigatus*.

This fungus has septate hyphae and a complex spore-forming apparatus consisting of a conidiophore that originates from the hyphae and supports the fruiting head (i.e., the spore-bearing structure). The mature colonies are usually gray-green in color.

Other species of *Aspergillus* differ in color and in the structure of the fruiting head.

# INFECTIONS

- *Aspergillus* species are ubiquitous saprophytes in nature
- In nature >300 species of *Aspergillus* exist, few are important as human pathogens

The *Aspergillus* species can cause a variety of clinical syndromes

## 1. Pulmonary aspergillosis

a) Allergic asthma

b) Bronchopulmonary aspergillosis

c) Aspergilloma

## 2. Invasive aspergillosis

## 3. Superficial infections



# *Aspergillus* spp.

## ➤ Colony morphology

- Surface at first white than any shade of yellow, green, brown or black depending on species
- Texture velvety and cottony
- Reverse is white, golden or brown

# *Aspergillus* spp.

## ➤ Microscopic morphology

- Septate hyphae(2.5-8.0  $\mu\text{m}$ )
- Unbranched conidiophore arises from foot cells
- The conidiophore is enlarged at the tip forming vesicle
- Vesicles are completely or partially covered with flask shaped phialides
- Phialides may develop directly on the vesicle(Uniseriate form) or be supported by metula (biseriate form)
- The phialides produce chain of round conidia(2-5  $\mu\text{m}$ )

# LABORATORY DIAGNOSIS

## Sampling:-

- \*For cerebrospinal infections, a lumbar puncture should be performed to collect CSF into sterile tube.**
- \*For Pleural and Peritoneal Effusions, a sample is collected by needle aspiration into sterile container.**
- \*Eye-corneal scrapings from the base and margins of the ulcer.**
- \*For the diagnosis of bronchopulmonary infection morning sputum or BAL (bronchioalveolar lavage) should be collected in a sterile container.**
- \*For systemic mycosis, pus swab from an ulcer or aspiration from unruptured abscess, or biopsy during surgical operation are collected by strict aseptic technique.**
- \*For urinary tract infection, mid-stream urine samples are collected into a wide mouth container.**

## Microscopic examination :-

### KOH mount

**The fungus appears as non-pigmented septate mycelium, 3-5 $\mu$ m in diameter with dichotomous branching and irregular outline.**

**Rarely the characteristic sporing heads of *Aspergillus Spp.* are present**

# Culture:-

\*pathogenic *Aspergillus Spp. generally* grows easily and relatively quickly on routine mycological and bacteriological media.

\*Clinical material inoculated on to SDA without cycloheximide at 25-37°C.

\* culture examined daily during first week, twice a week for further 4weeks of incubation before considering negative.

- ❑ Sub-culture of an isolate to **Czapek Dox** agar & 2% malt extractagar with incubation at 25c allows identification of most aspergilli using std monographs and taxonomic keys.
- ❑ **Potato dextrose agar** is particularly useful for induction of Sporulation.

➤Species are identified according to the morphology of their conidial structures

## *Aspergillus fumigatus*



SDA: Colonies are velvety/powdery at first, turning to smoky-green. Reverse is white to tan.

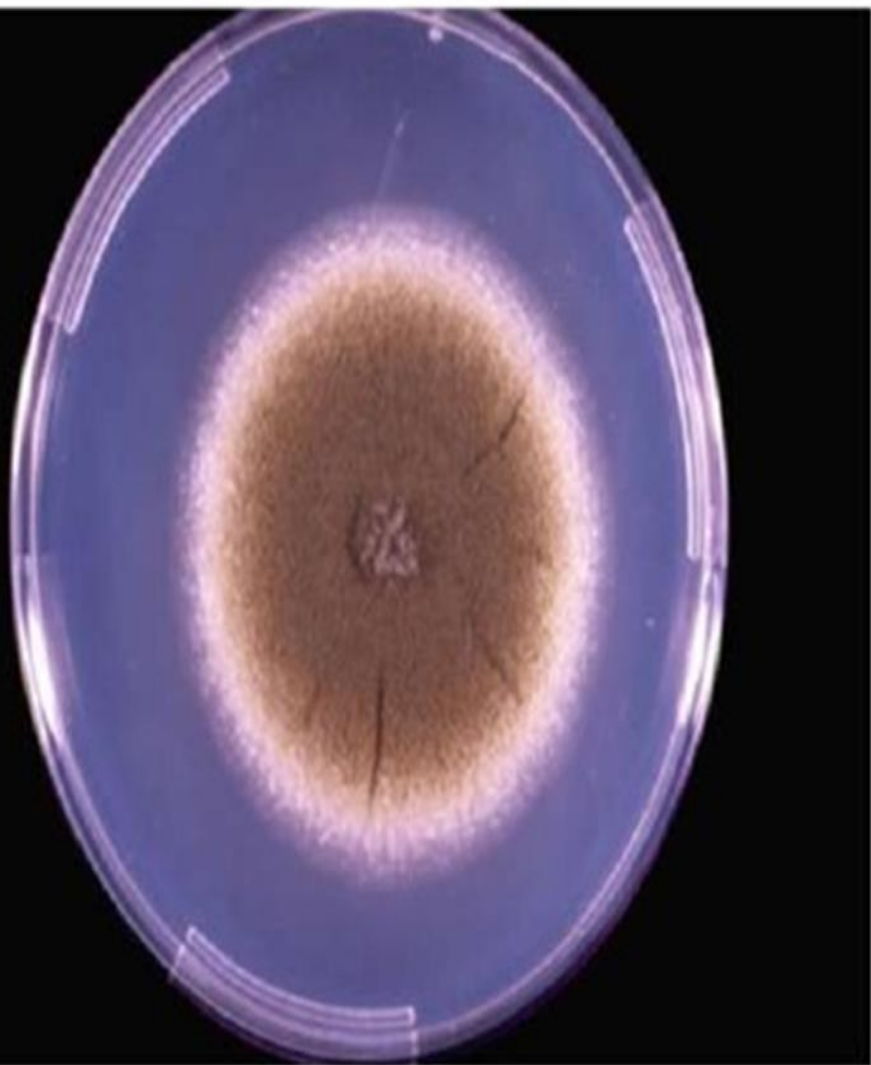




*Aspergillus niger* on Czapek dox agar. Colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads.



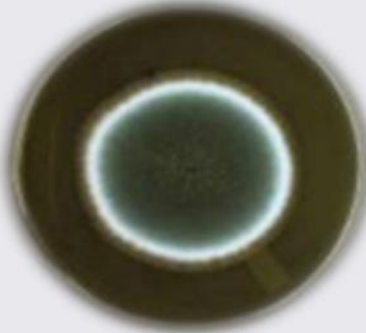
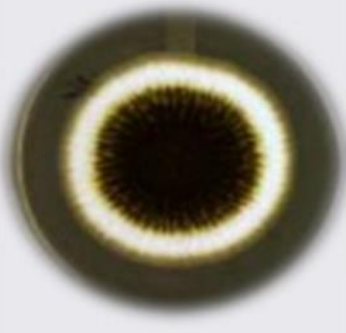
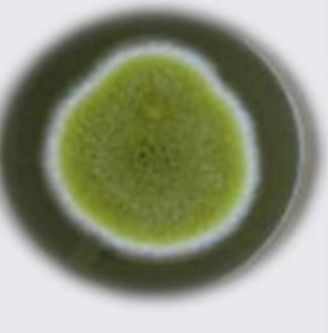

*Aspergillus terreus* on Czapek dox agar showing typical suede-like cinnamon-buff to sand brown colonies. Reverse yellow to deep dirty brown.







*Aspergillus flavus* on Czapek dox agar. Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age.

**SDA:** colonies are velvety, yellow to green or brown. Reverse is golden to red brown.

# SUMMERY OF IDENTIFY

<i>Aspergillus spp.</i>	<i>A. fumigatus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. terreus</i>
<b>Macroscopic morphology</b>	<ul style="list-style-type: none"> <li>• Velvety or powdery</li> <li>• At first white than turning dark greenish to gray with a narrow white border.</li> <li>• Reverse white to tan</li> </ul>	<ul style="list-style-type: none"> <li>• Woolly</li> <li>• At first white to yellow than turning black</li> <li>• Reverse white to yellow</li> </ul>	<ul style="list-style-type: none"> <li>• Velvety</li> <li>• Yellow to green or brown</li> <li>• Reverse goldish to red brown</li> </ul>	<ul style="list-style-type: none"> <li>• Usually velvety</li> <li>• Cinnamon brown</li> <li>• Reverse white to brown</li> </ul>
<b>Picture</b>				

<i>Aspergillus spp.</i>	<i>A. fumigatus</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>A. terreus</i>
<b>Microscopic morphology (conidiophores)</b>	Short smooth (<300µm)	Long smooth (400-3000 µm)	Variable length Rough, pitted, spiny	Short smooth (<250µm)
<b>Microscopic morphology (phialides)</b>	<ul style="list-style-type: none"> <li>• Uniseriate</li> <li>• Usually only on upper two-third of vesicle</li> <li>• Parallel to axis of conidiophore</li> </ul>	<ul style="list-style-type: none"> <li>• Biseriate</li> <li>• Cover entire vesicle</li> <li>• Form “radiate” head</li> </ul>	<ul style="list-style-type: none"> <li>• Uniseriate and biseriate</li> <li>• Cover entire vesicle</li> <li>• Point out in all direction</li> </ul>	<ul style="list-style-type: none"> <li>• Biseriate</li> <li>• Compactly columnar</li> </ul>
<b>Diagram</b>				



# IMMUNODIAGNOSIS

- **Detection of Antibody = Immunodiffusion**  
**, BALISA** (biotin-avidin amplification sys)
- **Detection of Antigen = Latex**  
**agglutination, RIA, ELISA, BALISA**
- **Molecular techniques = DNA**  
**sequencing, PCR (realtime PCR), DNA probe**