

# Microbiology lab

## Respiratory system

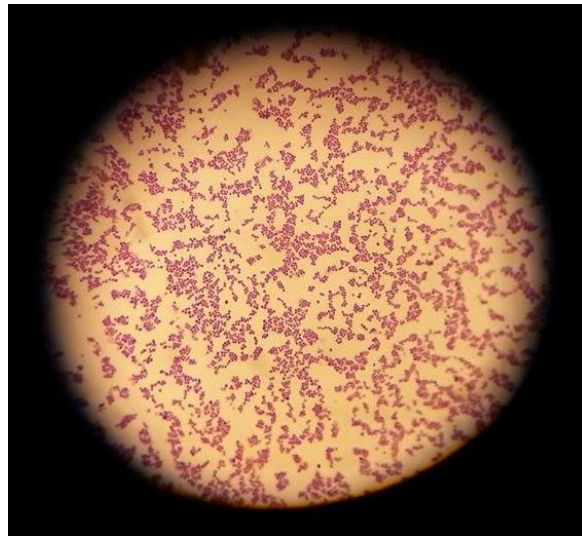
### Third medical year

#### Lab contents:

Gram positive bacteria (*Staphylococcus* and *Streptococcus spp*), two types of filamentous fungi (*Aspergillus* and *Penicillium spp*), and three types of *Candida* (*Candida albicans*, *Candida tropicalis*, and *Candida glabrata*).

## *Staphylococci*

- Gram stain: gram positive cocci arranged in clusters.



- Culture:
  - 1- We use blood agar for isolation of staphylococci.
  - 2- For coagulase test, **mannitol salt agar** is used (pink colored medium).



Here we are concerned about two types of *Staphylococcus* spp:

1- *Staphylococcus aureus*: is a mannitol fermentor, changes the color of the media into deep yellow. So it's coagulase positive.



2- *Staphylococcus albus*: is negative for mannitol fermentation and gives white colonies on mannitol salt agar. So it's coagulase negative.



- After complete identification, susceptibility test is performed for the *Staphylococcus aureus* (coagulase +) for suitable antibiotic profile.

## Streptococci

- Gram positive *Streptococcus* species are classified based on their hemolytic activity on blood agar into:
  - Alpha-hemolytic species cause partial hemolysis giving a greenish color on blood agar.
    - Streptococcus viridians*.
    - Streptococcus pneumonia*.
  - Beta-hemolytic species cause complete hemolysis and this appears as clear areas surrounding bacterial colonies.

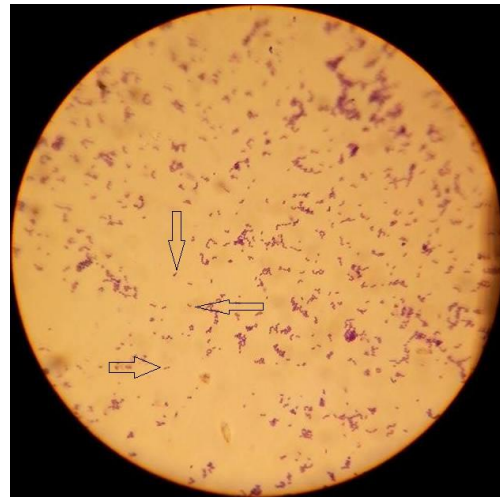
They are further classified according to sensitivity to bacitracin (antibiotic):

    - Group A (*Streptococcus pyogens*): bacitracin sensitive.
    - Group B (*Streptococcus agalactia*): bacitracin resistant.
  - Gamma-hemolytic species (*Enterococcus spp*): cause no hemolysis.

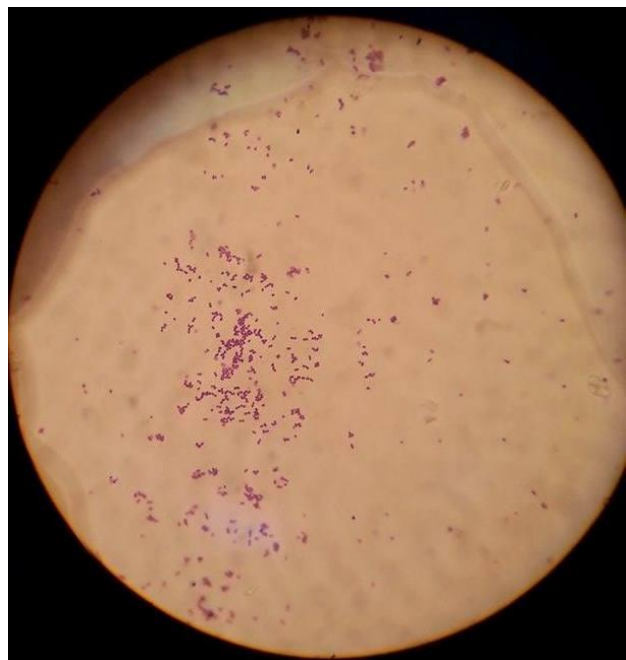
- Gram stain:**

1- *Streptococcus viridians*: gram positive short streps.

2- *Streptococcus pneumonia*: gram positive diplococci lancet shape, (where the arrows point in the figure.)



3- Beta hemolytic *Streptococci*: gram positive in long strep form, as shown in the figure.



- Culture:

**Blood agar** is used.

Alpha haemolytic *Streptococci* turn the colour of blood agar from red into greenish zone because of partial haemolytic activity.

- *Streptococcus pneumoniae*: susceptible to optochin disc
- *Streptococcus viridians*: resistant to optochin disc

Beta haemolytic *Streptococci* turns the colour of blood agar from red into colourless zones because of complete haemolytic activity, and divided into:

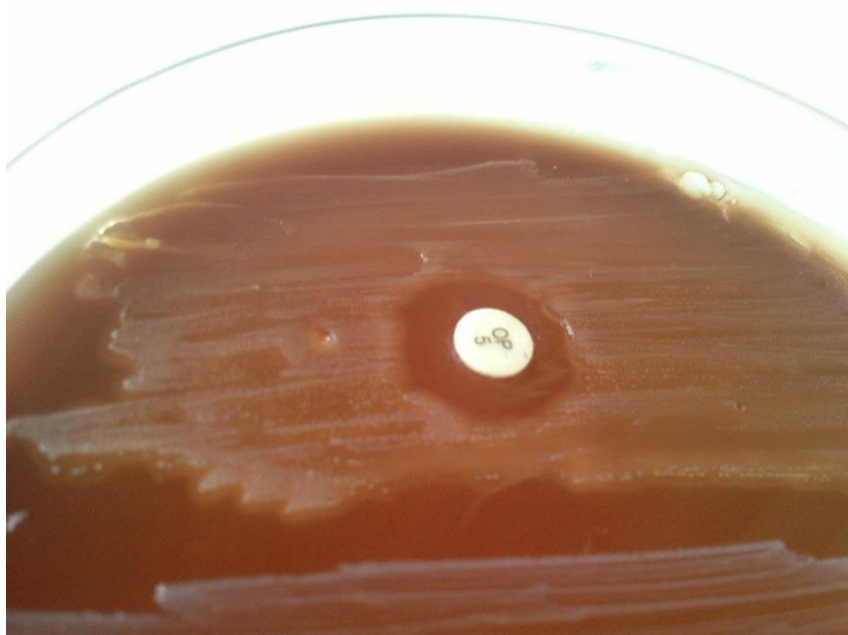
A- Bacitracin susceptible (Group A)

B- Bacitracin resistant (Group B)

The next two pictures are of Alpha haemolytic *Streptococci*;

*Streptococcus viridans*, above.

*Streptococcus pneumoniae*, below.



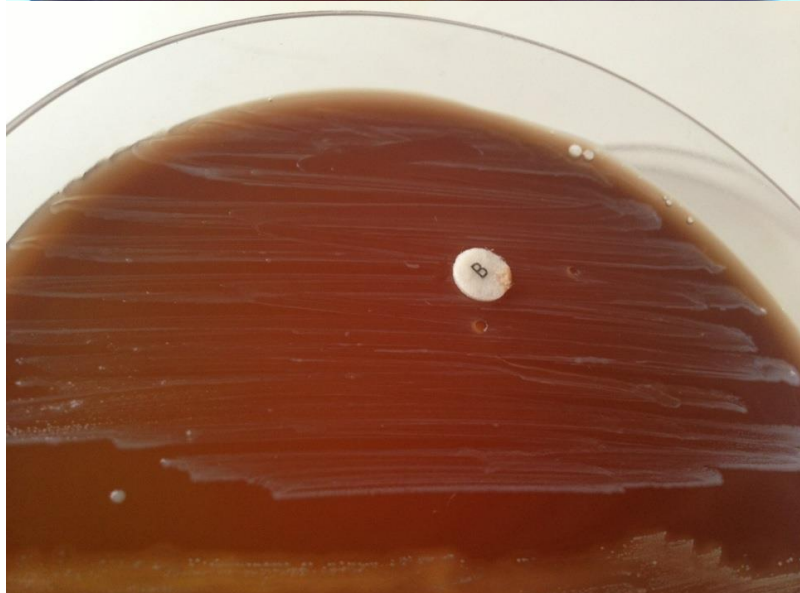


The next three pictures are of Beta haemolytic *Streptococci*;

This one is Group A.



This one is group B.



Here we see clearly the difference in sensitivity to bacitracin between group A and B:



## *Aspergillus*

- This filamentous fungus contains a lot of species like *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus parasiticus*, and others.

- ❖ *Aspergillus niger*:

This is a non-pathogenic filamentous fungi, this is an example on molds that contaminate pickles especially on pickled olives and fruits in addition to Aspergillosis disease caused by other species of this fungi.

- Culture:

**Sabaroud dextrose agar** is used.

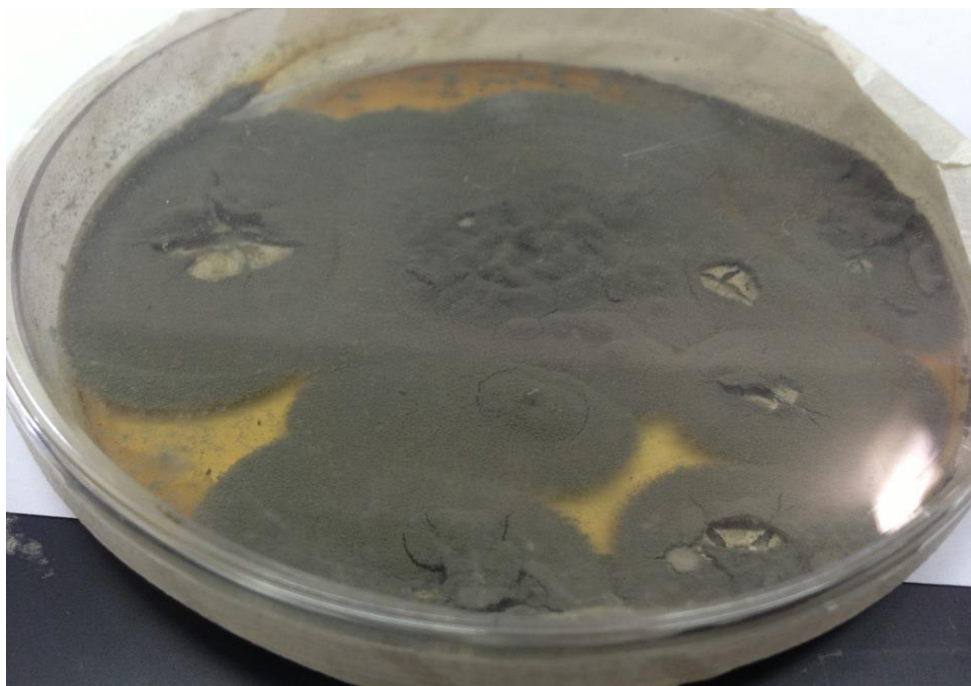
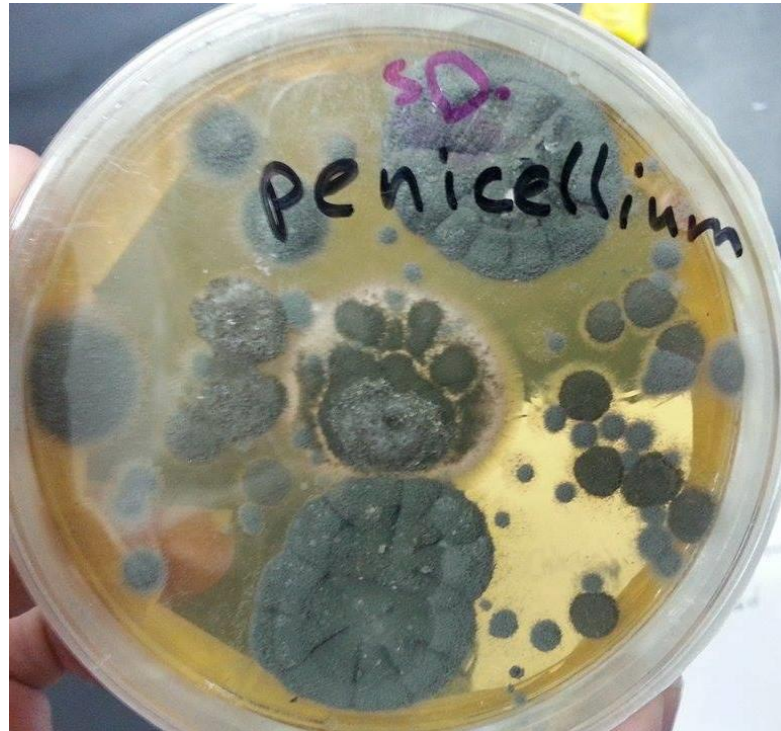
*Aspergillus niger* colony develop on the surface of media within seven to ten days of incubation at 25-30 degrees Celsius after the development of hyphae and black spores.





## *Penicillium* spp

- These filamentous fungi include a lot of species; one example is *Penicillium notatum* which produces the penicillin.
- Culture:  
**Sabaroud dextrose agar** is used as well. Each of the many species of *Penicillium* fungi produces a distinct color of colony on it like black, green, blue, and others. Here is shown a *Penicillium* species that produces an olive-green-coloured colony, It is powdery looking because of the very small spores.



## *Candida*

Yeast is a big family which includes both non-pathogenic members such as *Saccharomyces cerevisiae* (baking yeast), and pathogenic members like *Candida albicans*, *Candida tropicalis*, and *Candida glabrata*.

### ❖ *Candida albicans*

- It causes many infections such as those in immune-compromised patients, in children as thrush on their tongues, and in vaginitis cases isolated from vaginal swabs.

- Culture:

**Sabaroud dextrose agar** is used as well. The colony produced by *Candida albicans* is creamy colored with yeast smell.



- Gram stain:

Gram positive oval big cells.

- Identification of Candida albicans by Germ tube test.

Few colonies are suspended with serum in tube and incubated for four hours then wet mount preparation is stained by crystal violet (gram stain) and observed under the microscope, pseudohyphae are the character of *Candida albicans*.





❖ *Other candida spp*

Identification by: **Chrome agar medium.** It's a transparent agar medium and it contains chromogenic mixture. It can differentiate between a lot of types of *Candida spp.*

Suspected *Candida* colonies are subcultured into chrome agar medium and incubated for 48 hours at 37 degrees Celsius.

Results:

*Candida albicans* produces green colonies.

*Candida tropicalis* produces blue colonies.

*Candida glabrata* produces pink colonies.



**Supervised by:  
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