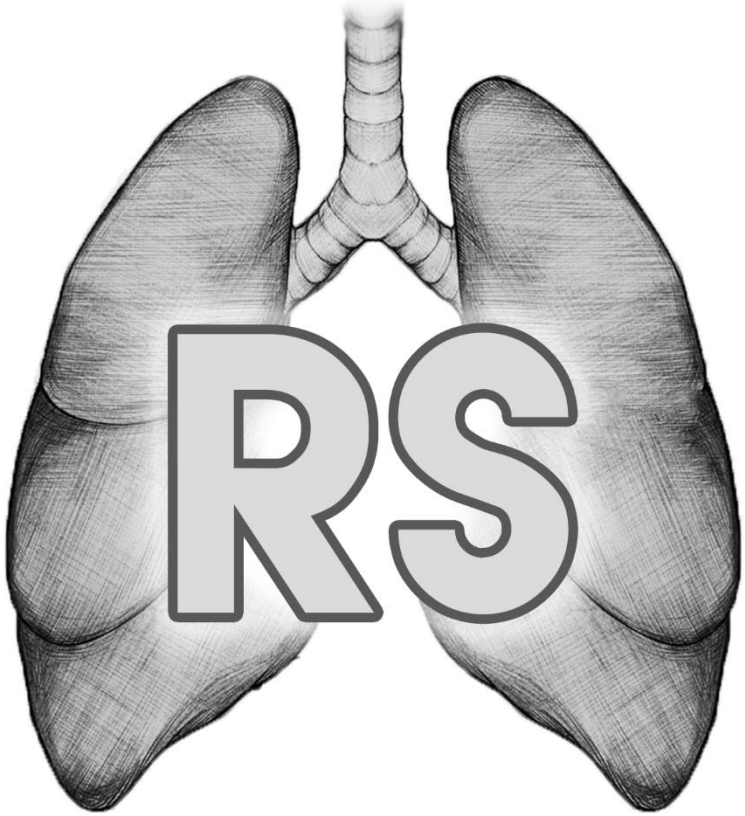


MICROBIOLOGY

☑ Sheet ○ Slide ○ Handout

Number

1

Subject

Micro Lab

Done By

Omar Saffar

Date:

Price:

Gram +ve

Staphylococcus & streptococcus

Main topics we will talk about in this lab:

-two major types of staph. & differences between them

-streptococcus groups (alpha,beta,ghama)
-major cultures media

Staphylococcus groups

"catalase +ve"

Two major types of staphylococcus are used in lab procedures due to their significance in culture media;staph.auerus(mostly pathogenic) & staph.albas(opportunistic) which are

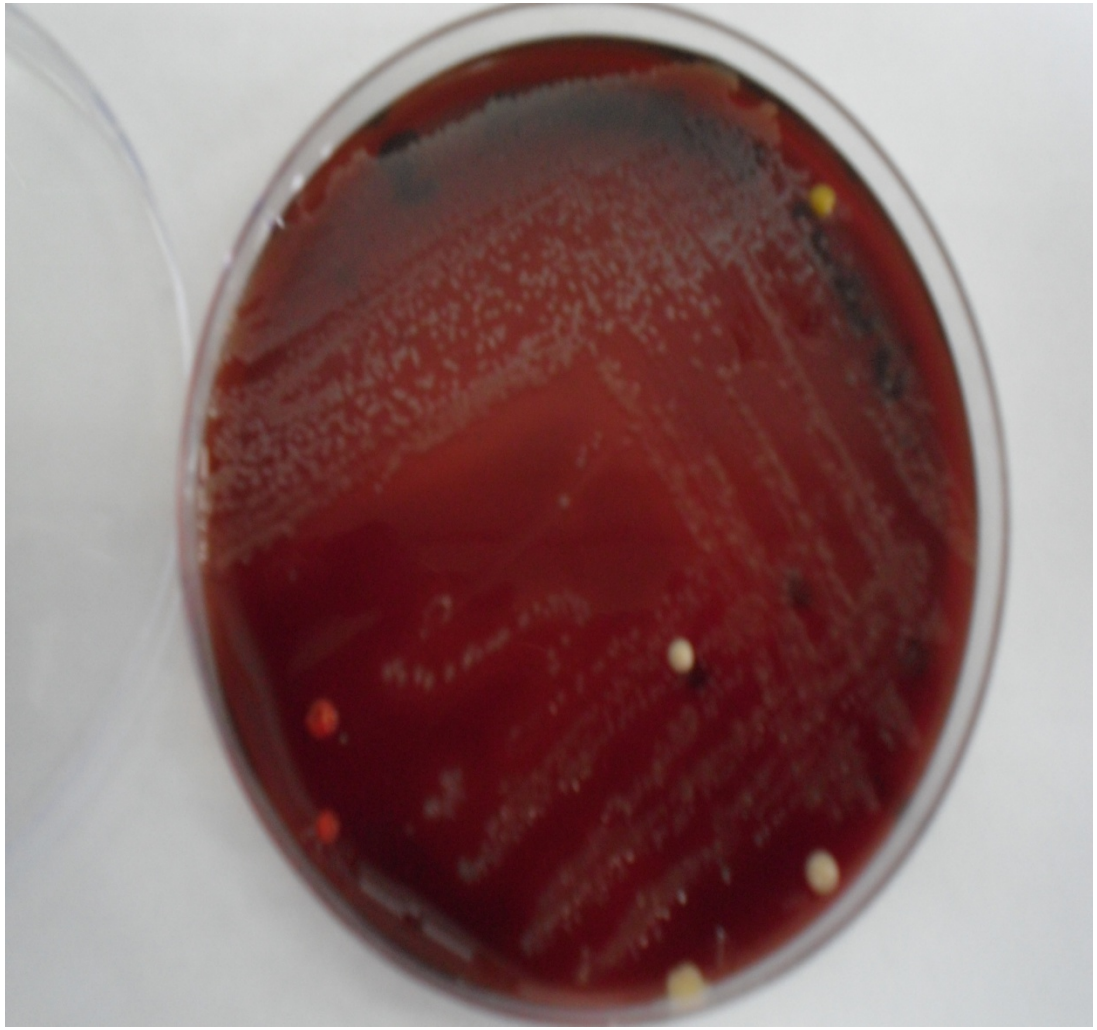
Most Common

Incubated mainly on two types of culture media:

-blood agar (general purpose medium) is a media that provides enough nutrients in which most any microorganism will utilize for growth. Allows for a wide variety of microorganisms "gram +ve,gram –ve,yeats"to grow. whether they are normal or pathogenic bacteria.

-~~CLED media (Cysteine Lactose Electrolyte Deficient agar), originally it has a green color~~

~~& its selective in this case to identify between staph.albas & staph.auerus~~



Staphylococcus in blood agar appear as a large white colonies

Throat swab culture:

T~~hroat swab is cultured on blood agar media & after 24 hours of incubation on 37 degree, mixed culture is formed as a mixture between microorganisms.~~

~~Staph. appear as a large white colony on blood agar so we take & culture it on cled media. This is Called" sub culture"~~

If the cled color turned into golden yellow then it's a staph.aeurus & is coagulase +ve,while in albas it stays the same.however,staph.albas sometimes appear as aeurus on cled that's why its placed by more specific media which is the " manitol salt agar"

That is pink in color so when it turned into;

-golden yellow: then it's a staph.aeurus(similar to cled)

-stays pink with white cells then it's a staph.albas

+others

Manitol Salt Agar allows only staphylococcus bacteria to grow on it

Staph.aeurus in cled agar appears golden yellow

If its coagulase +ve. they are pathogenic bacteria

Coagulase enzyme turns fibrinogen to fibrin !

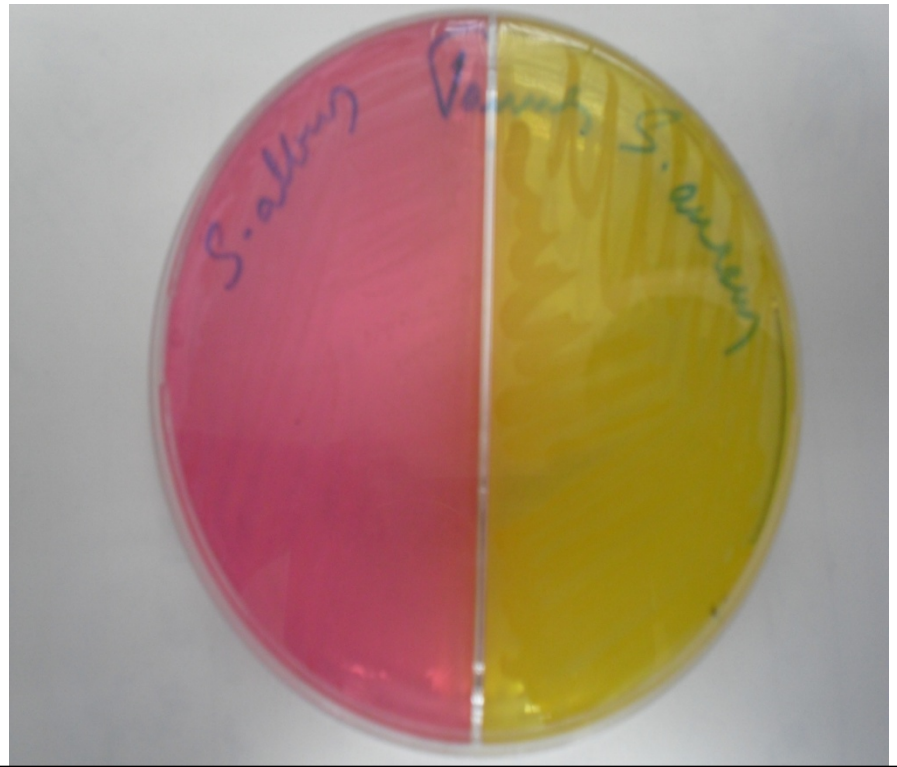
so if we put staph aureus into blood plasma it will induce clotting!!!

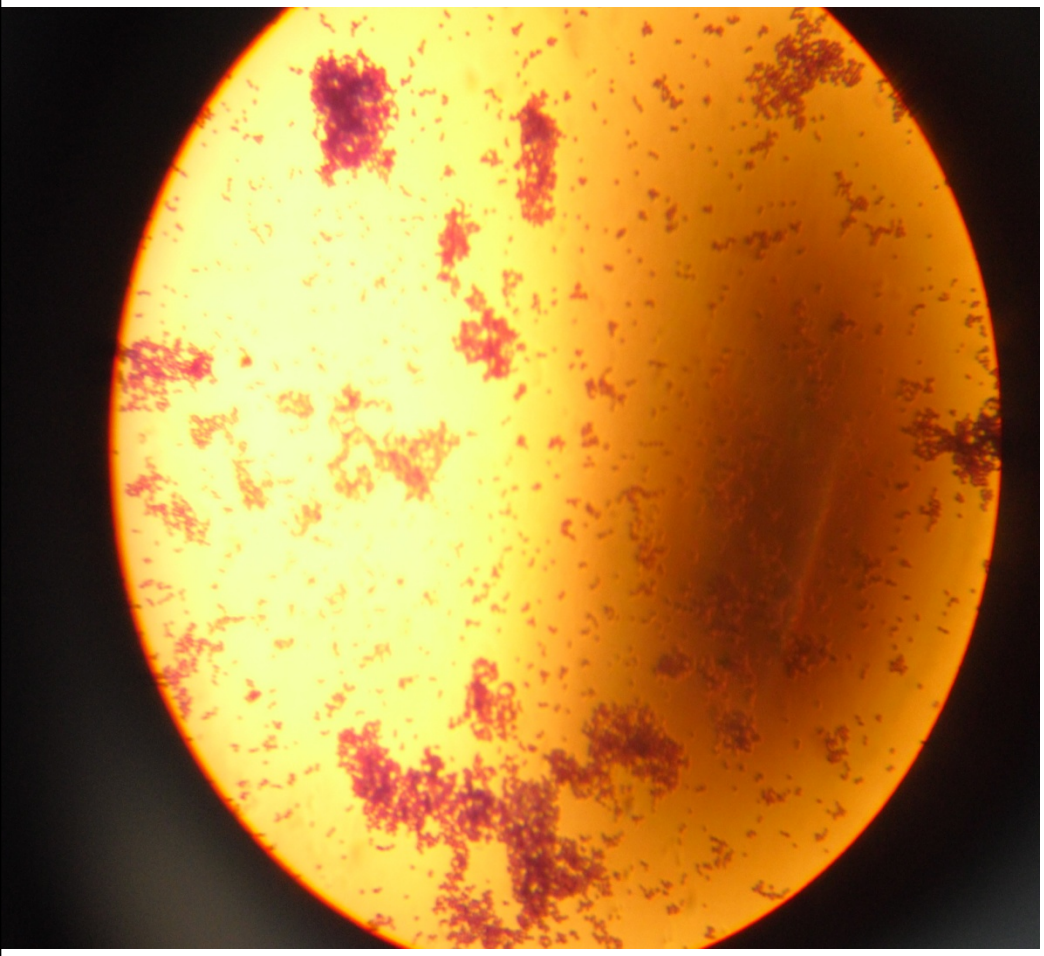
Manitol salt agar:

Staph.aeurus → yellow golden

~~Staph.albas~~ → pink

Other types of staph





Staph. Under microscope appear as gram +ve clusters

Note:

Under microscope we cant distinguish between staphylococcus groups;auerus & albas

To differentiate between staph. And strep.:

-In blood agar:

Staph.: larger colonies.

Strep.: pinpoint colonies.

- Catalase test:

Staph.: +ve reaction which means that it can convert H_2O_2 into H_2O and O_2 by catalase enzyme.where air bubbles are formed.

Strep.: -ve reaction.

"Streptococcus groups"

α β γ

Based on cell wall hemolytic activity on blood agar, are divided into:

α-Streptococcus: destroy RBC partially on blood agar → green

β-Streptococcus:all beta strains produce hemolysin enzyme which result in complete digestion of RBC on blood agar. Examples include Streptococcus haemolyticus. → Yellow

GAMMA- Streptococcus: no effect on blood agar

β-Streptococcus:

The most significant groups are: A & B

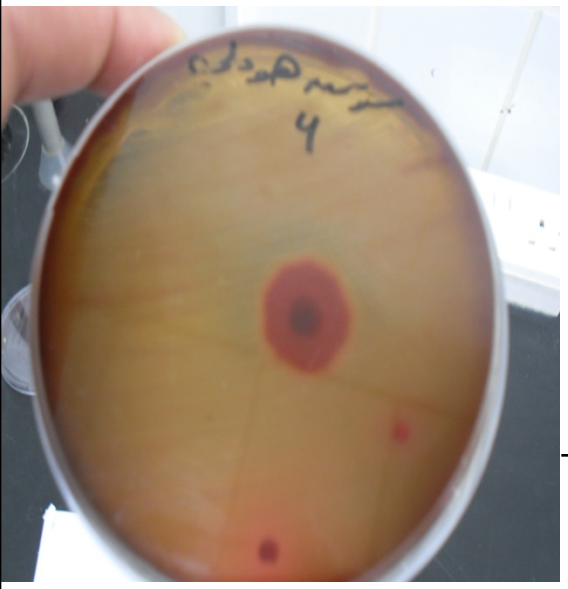
A: is the only strain that is susceptible to Bacitracin(BA) antibiotic disk

Where inhibition zone is formed around.

E.X: strep.pyogens

B: its resistant to bacitracin antibiotic disk so has no inhibition zone

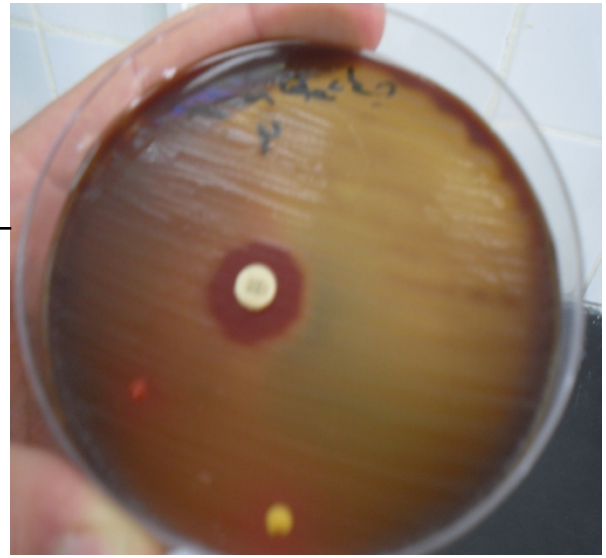
E.X: strep.agalactiae



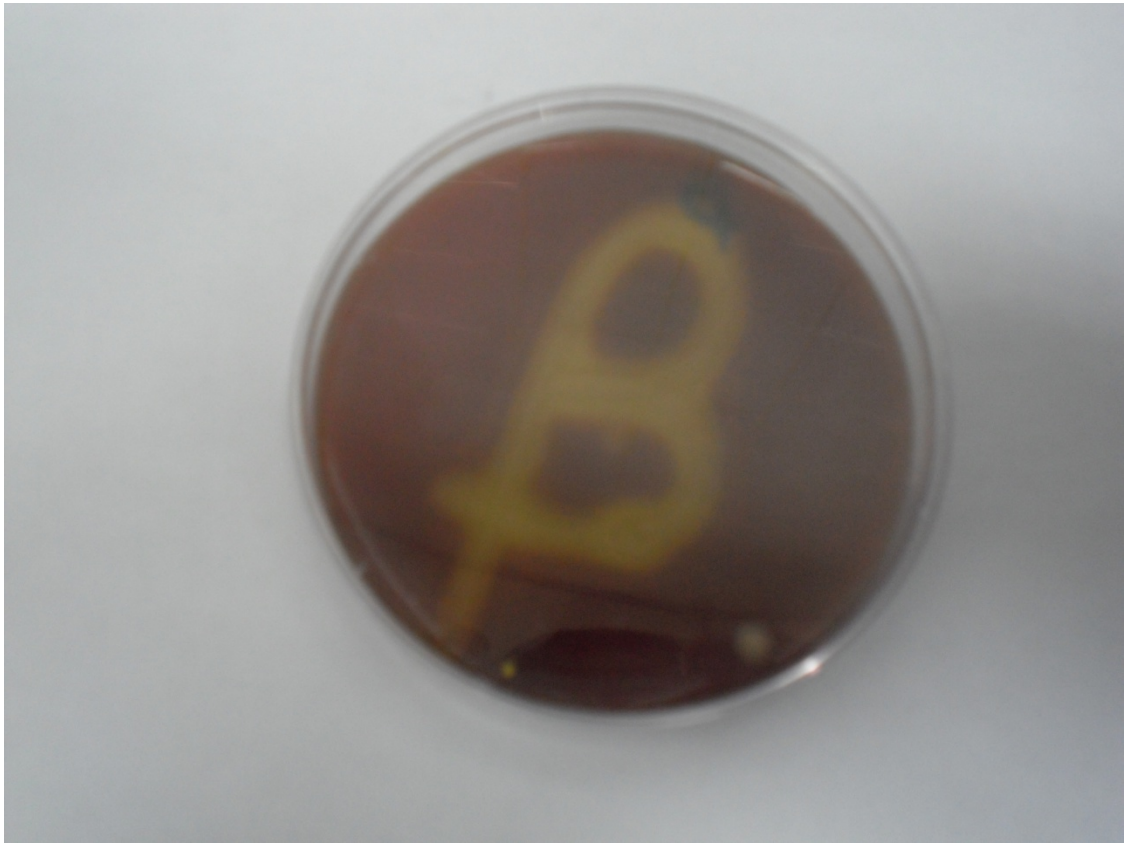
group ~~B~~ A

beta -> bacitracin

Group A



Here we can’t say whether its group A or B because there is no antibiotic disk so we just say its ***β-hemolytic strep.***



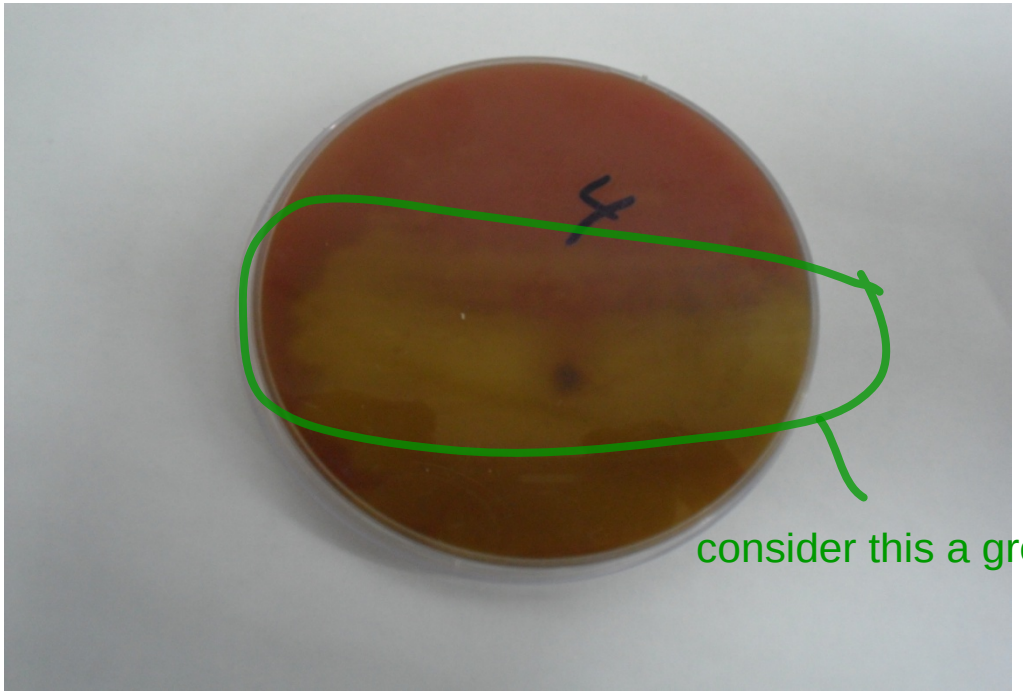
α- hemolytic streptococci :

It lyses blood partially in vitro, the place of bacterial growth is grayish green.

****A subtype of α-hemolytic strep. Is streptococcus viridans which is part of normal flora***

****A dangerous &pathogenic subtype is strep.pneumoina which appears in gram stain as gram +ve,diplococci(pairs),lancet shape(like num 8 or glasses shape) & its not completely round***

α- hemolytic streptococci ←



Strep. pneumonia is susceptible to an "optician disk" an antibiotic

Which is ~~18 mm in diameter~~ and is highly specific

For bacteria, when do purification((pure colony)) & apply a new

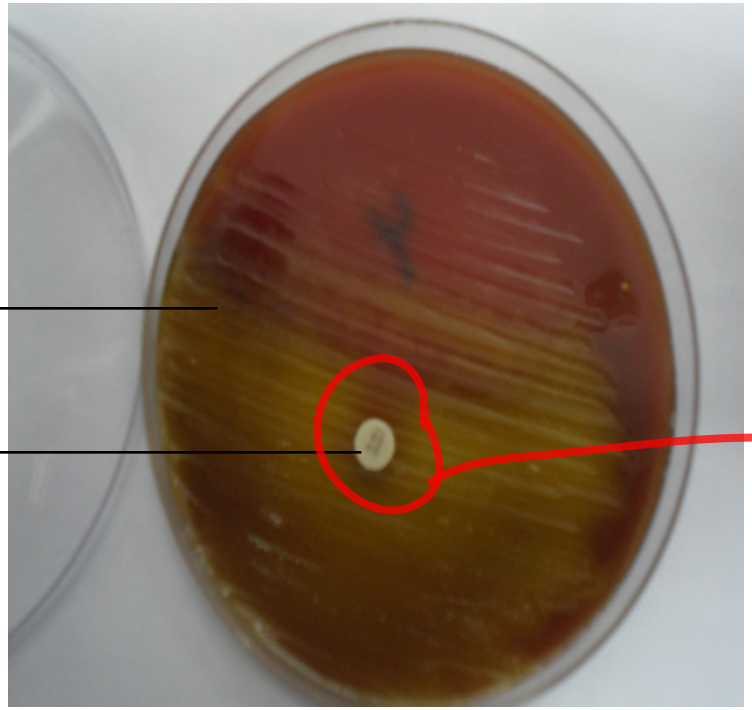
Bacteria to the disk & the zone was larger than 18mm

Then it's a streptococcus pneumonia((the greenish zone))

Viridans is resistant !

Strep. pneumonia

Optician disk



inhibition zone

Alpha -> optician

Y Non hemolytic streptococcus

***Enterococcus** is an example specially ((enterococcus faecalis)). We do **bile esculin** test for it cz it has no effect on blood agar. appears in gram stain*

~~***As gram +ve short chains((each consist from 4 or 5 cells)),While β-streptococcus shows long chains((like the necklace))*** .~~

GAMA hemolytic strep.

We use bile esculin test which is green in color, after incubation; in the second day it turned into black which is called now" esculetin" due to the presence of Iron & to the gamma strep D or Enterococcus

non-hemolytic groups
Group D & enterococcus -> bile esculin +ve
others -> "non enterococcus" bile esculin -ve

+ve

These pics are from the net ☺

−ve

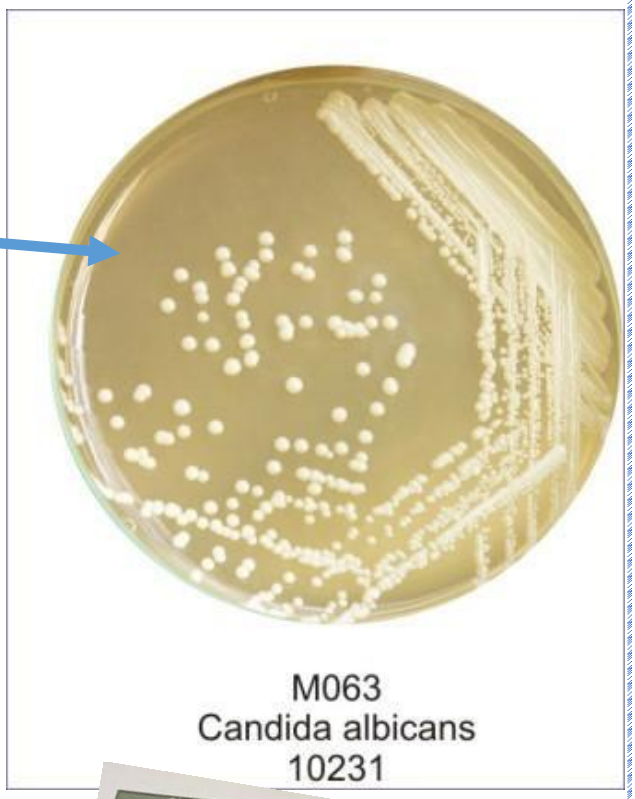
Enterococcus faecalis* & group D *strep. **hydrolyzes esculin in the presence of bile and turns more than half the medium dark brown. This is a positive result.**	***Streptococcus pyogenes*** **does not hydrolyze esculin in the presence of bile. No dark brown complex is formed. This is a negative result.**

Now were gonna talk about

Candida, Fungi & TB

Sabouraud dextrose agar for both

it's **Paige** in color



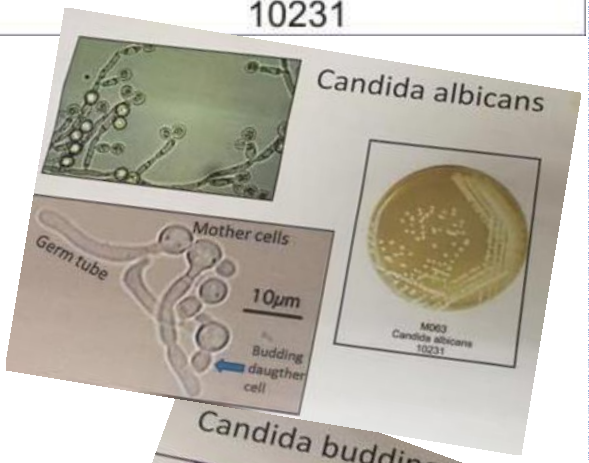
Candida:

Incubation period for candida or fungi is around 48 hours

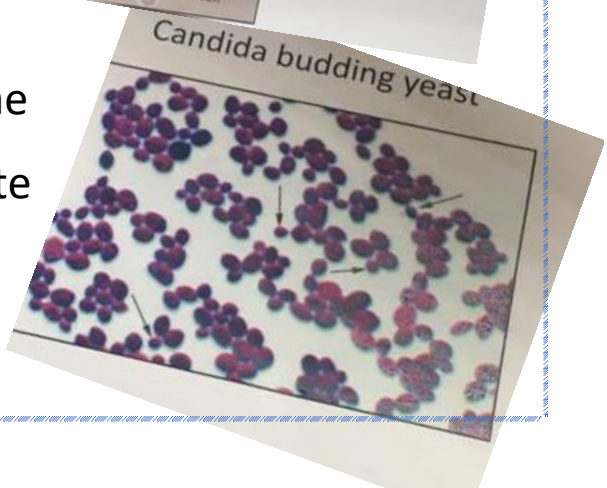
Candida Albicans is the most common type of yeast infection, called candida thrush!

"So it is a Yeast!" خميرة

Milky white color on the SD agar,

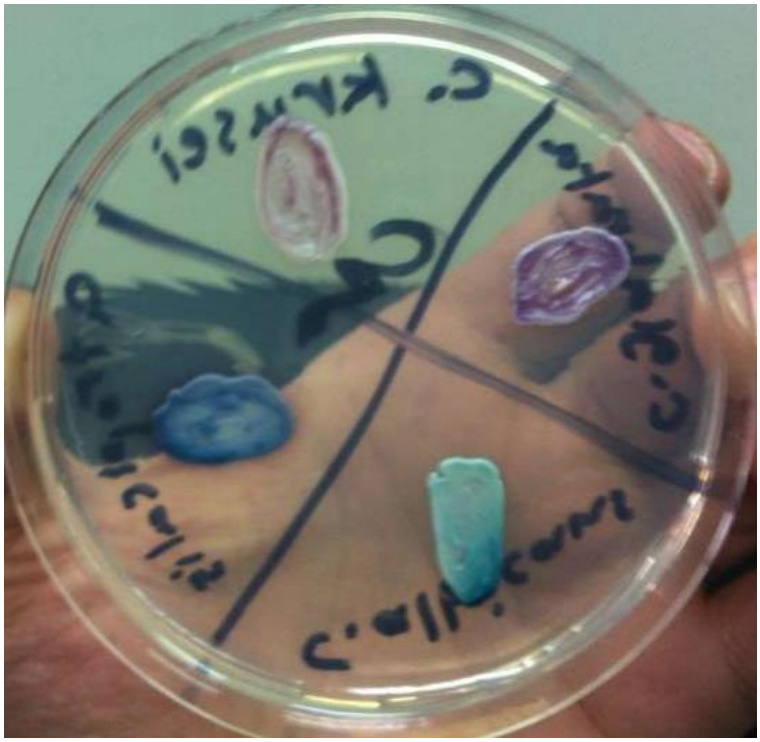


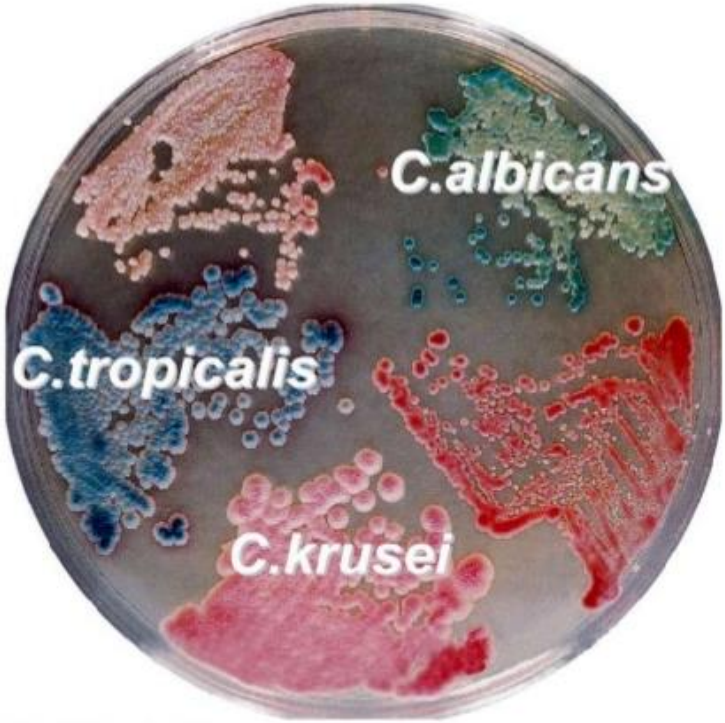
All Candidas grow by budding, and **only candida albicans** make pseudo-hyphae "germ tube", that's one way to differentiate between Candidas!

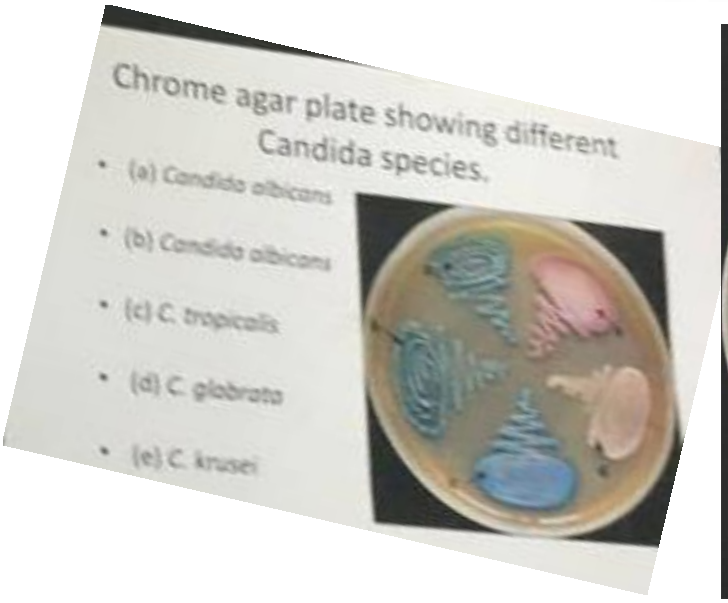


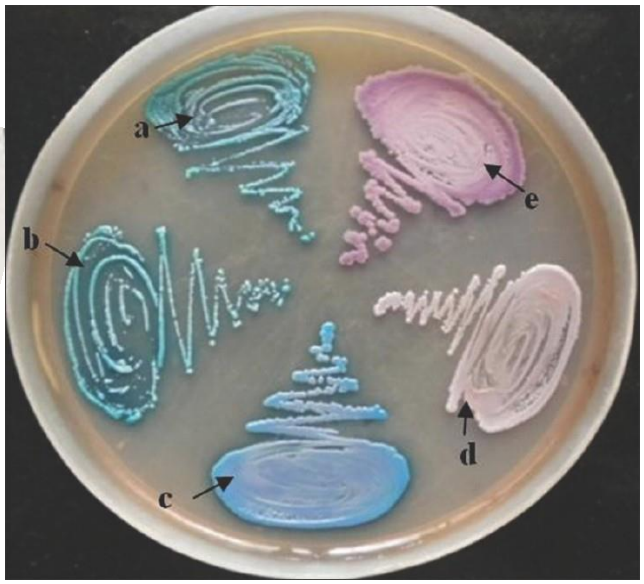
Another way which is by an expensive test, “Chromagar” a special agar for candidas and each candida can produce a different color on it

1. Candida albicans → green colonies
2. Candida tropicalis → Blue Colonies
3. Candida glabrata → pink glossy colonies
4. Candida Krusei → light pink, dry colonies





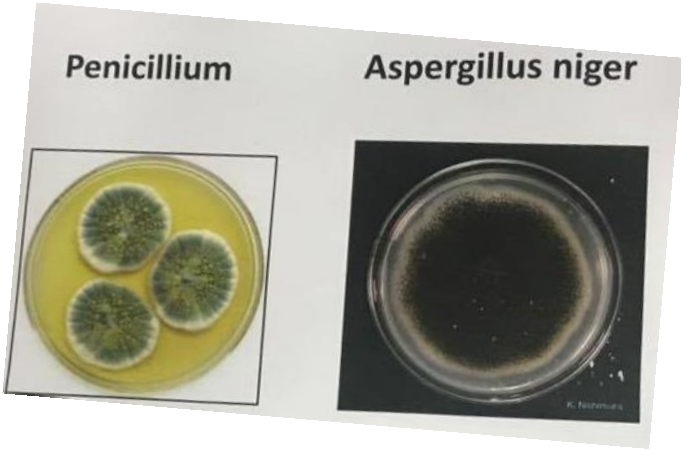




Fungi

I. **Aspergillus niger العفن "black"**

II. **Penicillium "green to blue, mostly green!"**



Same SD agar is used!!

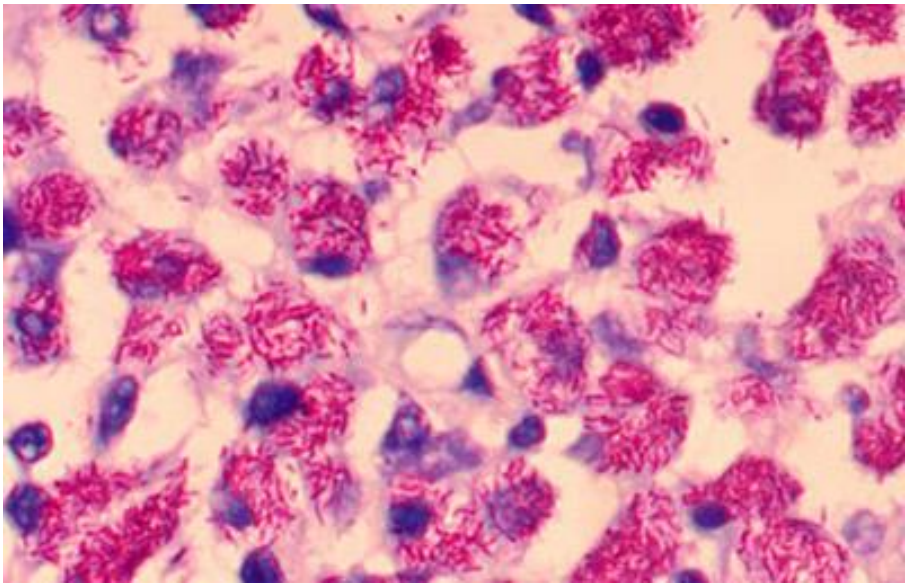
Aspergillus Niger

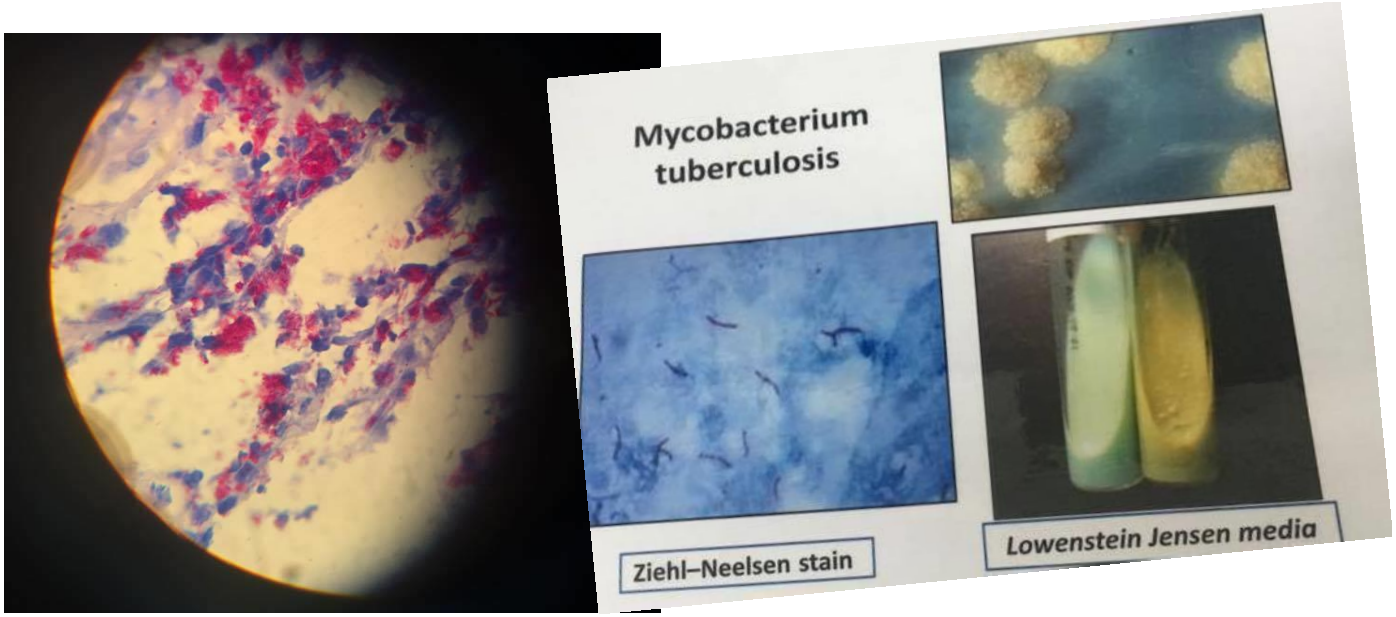
Penicillium

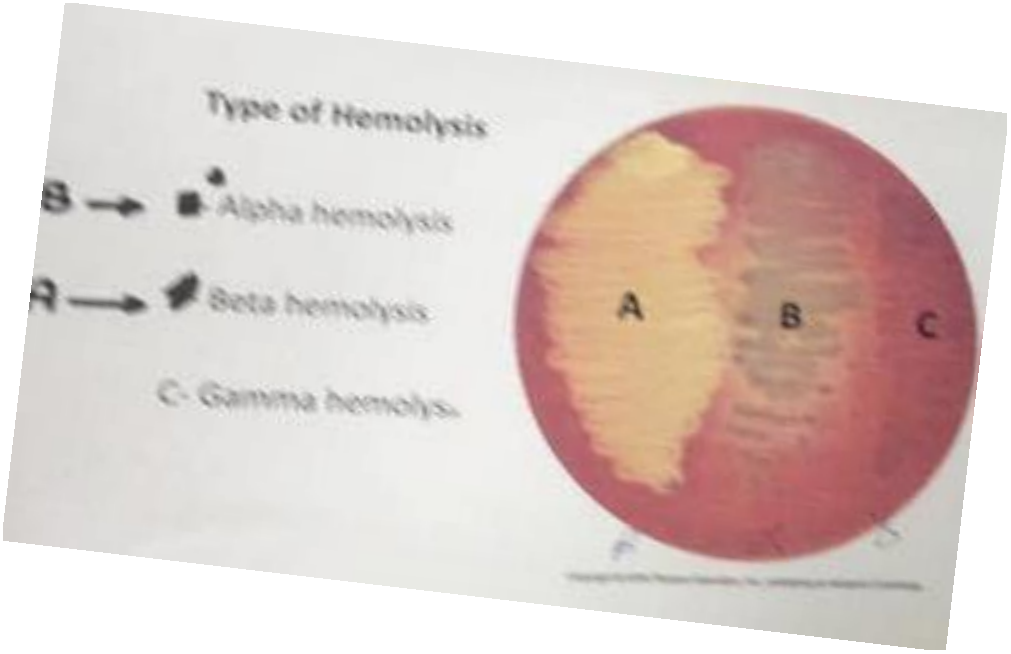
Mycobacterium Tuberculosis

"TB"

- Special media used for it called:
  - **Löwenstein–Jensen medium**
- Contains malachite green stain
- It's put in a veil (اناء) not a dish because of the long incubation period (4 weeks), to prevent it from getting dry.
- To inspect this bacteria under the microscope a special stain is used called **acid fast stain "Ziehl–Neelsen stain"**
- The outer wall of this bacteria is coated with wax, which makes it hard to be stained, so during preparation the temperature should be increased to melt this wax then the stain is placed to get through it and give color to the bacteria.
- The bacteria will get a **rosy** color under the microscope




Mycobacterium tuberculosis
Ziehl–Neelsen stain
Lowenstein Jensen media


Type of Hemolysis
S → Alpha hemolysis
R → Beta hemolysis
C- Gamma hemolysis
A
B
C