



Microbiology Practical

Lecture _01



Microbiology Team 430

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Streptococcus pyogenes (Group A Strep)

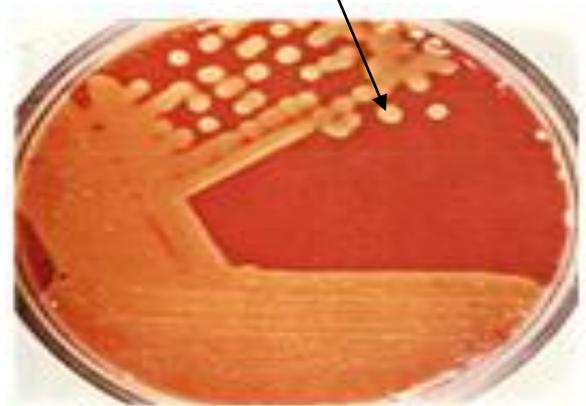
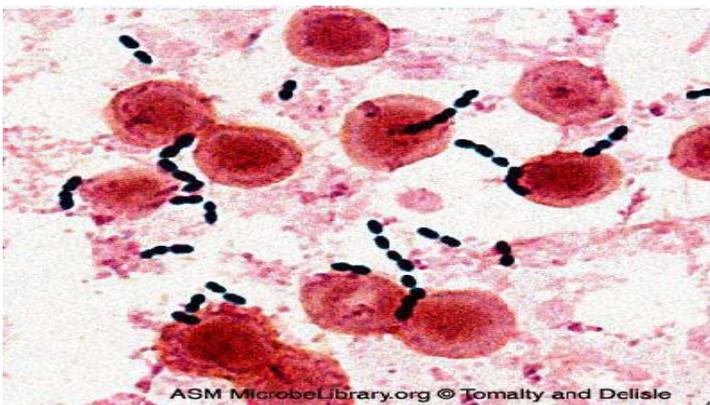


When we do gram stain, we are going to see

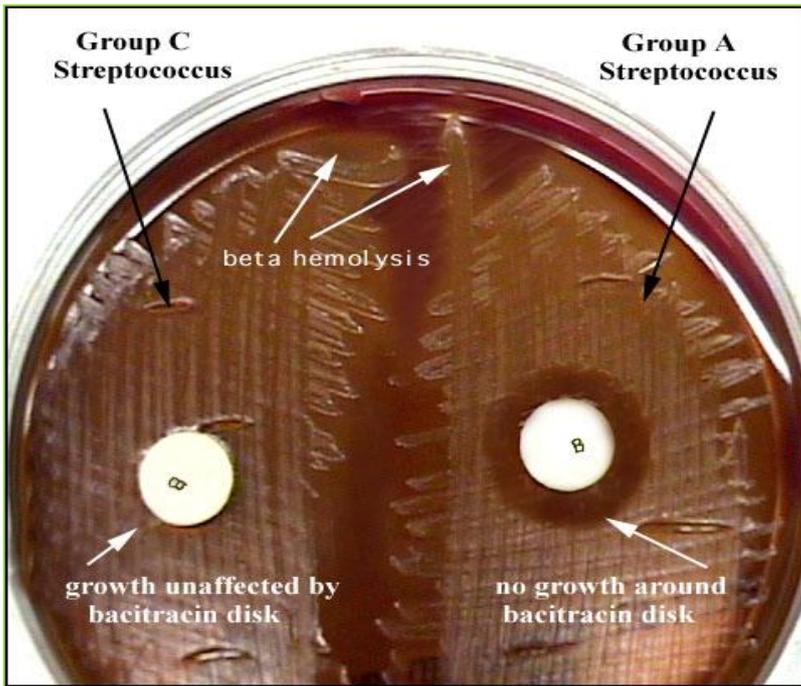
- Blue, cocci spherical & arranged either in pairs or chains.

- Carried by many people in throat or on skin
Often no symptoms
- Cause of :
 - Strep throat
 - Impetigo
 - Necrotizing fasciitis.

These are the colonies & the clearance around is the hemolysis.

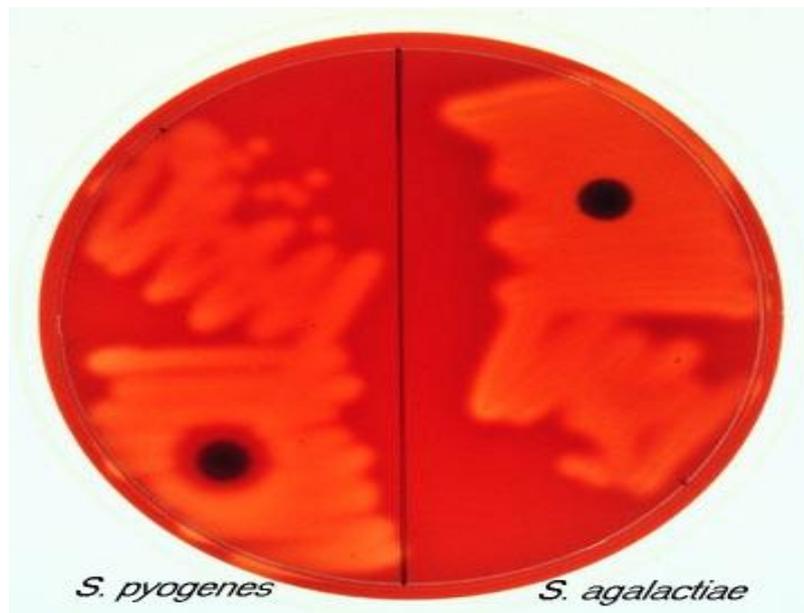


Streptococcus pyogenes. Left. Gram stain of *Streptococcus pyogenes* in a clinical specimen. Right. Colonies of *Streptococcus pyogenes* on blood agar exhibiting beta (clear) hemolytic.



- Principle:
 - Bacitracin test is used for presumptive identification of group A
 - To distinguish between *S. pyogenes* (susceptible to B) & non group A such as *S. agalactiae* (Resistant to B)
 - Bacitracin will inhibit the growth of gp A *Strep. Pyogenes* giving zone of inhibition around the disk.
- Procedure:
 - Inoculate BAP with heavy suspension of tested organism.
 - Bacitracin disk (0.04 U) is applied to inoculated BAP.
 - After incubation, any zone of inhibition around the disk is considered as susceptible.

Bacitracin sensitivity



Case 1:

A 5 year boy was brought to king Khalid University hospital, outpatient department complaining of fever and **sore throat**. He had regular vaccination history. On examination his temperature was 38.5° c, **the tonsil area and pharynx were obviously inflamed** with some foci of pus.

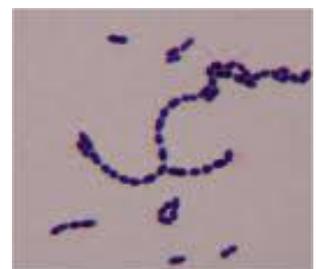
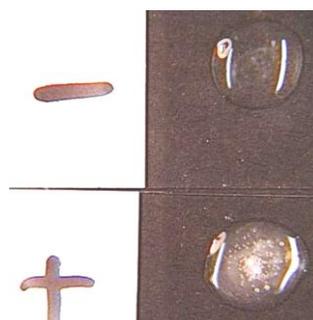
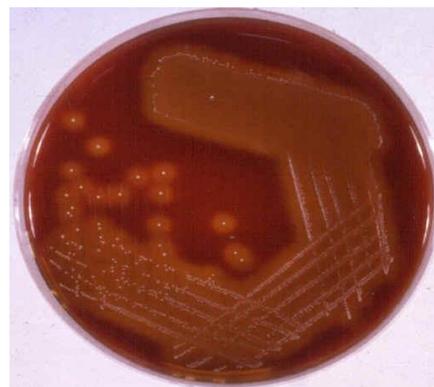
1. What is the differential diagnosis?

1- Diphtheria: elected buz diphtheria pt always has pseudomembran.

2. What investigation should be done?



- Lab tests:
 - The full blood count showed a total white cell count of 15000/ml. Throat swab culture showed **colonies with clear haemolysis** on blood agar. They were **catalase negative**. The **gram stain** of these colonies showed **gram positive cocci in chains**.



1. What is the likely identity of the organism?
Strep. Pyogenes.
2. What is the best antibiotic therapy for this child?
Penicillin.
3. If not treated what complication may this child have after 6 weeks period?
Rheumatic fever

In General

If pt came to the hospital & has the symptoms of RTI, the specimen that we will take is throat swap → once received in the lab, it should be gram stained → we will see this picture (the red one above). So, u can see

1. The organism either in chains or pairs + neutrophils.
2. The organism is blue.



Then we culture the specimen in blood agar → incubated under 37 c for 18 hr. → the organism growing forming colonies (Beta haemolysis) → Bacitracin test (to know if it is group A or B) → Biochemical test (which is catalyze test) (if we r confused if it is strep or staph)

Streptococci are large group. All these bacteria when they grown in the blood agar, they hemolyses the RBCs. This breaking down produces either Beta hemolytic (clear destruction) or alpha hemolytic.

So we have 3 tests:

- Gram stain: shows gram + cocci in chains.
- Culture in blood agar: produces Beta hemolysis.
- Biochemical test (catalyze): to differentiate between strep & staph.
 - Catalyze – is a strep
 - Catalyze + is a staph

- Bacitracin test (Bacitracin sensitivity test):

Is a filled of paper impregnated with antibiotics.

- What we do?

Spread the organism on the plate, then we add this disk, the antibiotics will diffuse & if it is group A, it will inhibit the growth of the organism but if it is group B, the organism will grow.

group A

•in Bacitracin if the result is Inhibition of the growth of the organism.

group B

•in Bacitracin if the result is continuation of growing of the organism .

Strep

•Biochemical catalyze is negative

Staph

•Biochemical catalyze is positive

B-
hemolysis

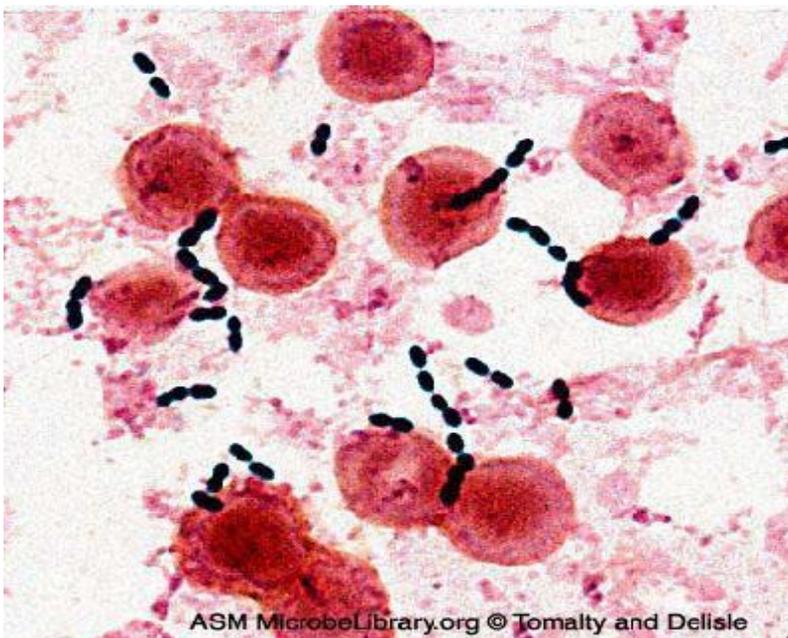
•Compleat destruction of RBCs

Alpha-
hemolysis

•Incomplet destruction of RBCs which leaves greenish discolorition

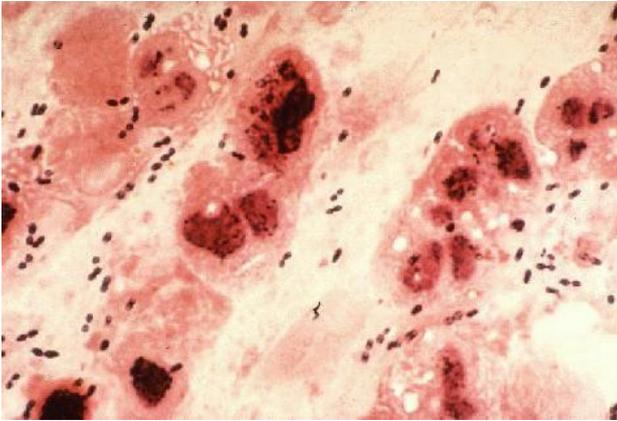
Q)

Explain what you see in this picture?



- Gram + cocci.
- Rounded.
- Polymorphic neutrophils (pus cells).
- Most properly it is strep. Pyogen.

Streptococcus pneumonia (Pneumococci)



(Pneumococci): Q) describe what u see in this picture?

1. Founded in pairs (diplococci).
2. Gram + & pus cells
3. Irregular spherical, it is ovoid.
4. Causes lobar pneumonia (mainly) but sometimes causes otitis media.
5. Usually the specimen received in the lab is sputum (for culture or for gram stain)
6. It is catalyze negative



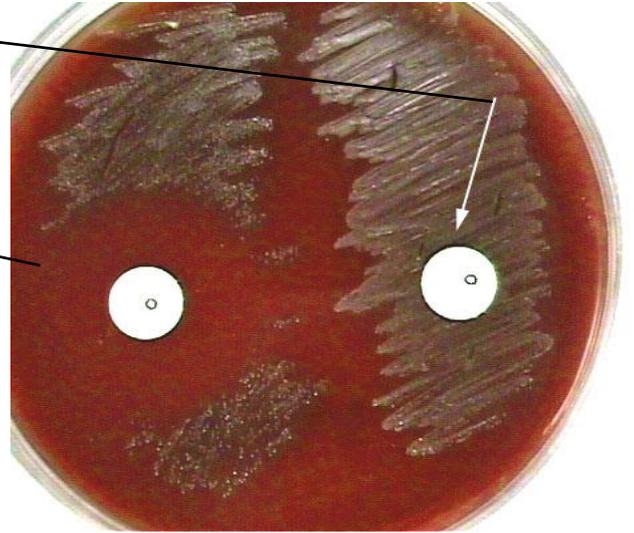
*Alpha hemolytic which produces greenish discoloration. (her it is not clear but under the microscope it s very clear.

- Alpha-hemolytic *Streptococcus* species "Viridans group" streptococci, including species such as the *Streptococcus mutans*, *mitis*, and *salivarius* groups display alpha hemolysis.
- There are 2 groups which produces Alpha hemolytic :
 1. Strep pneumonia (the pathogenic one).
 2. Strep. Viridans.
 In order to differentiate between them we do optochin susceptibility test

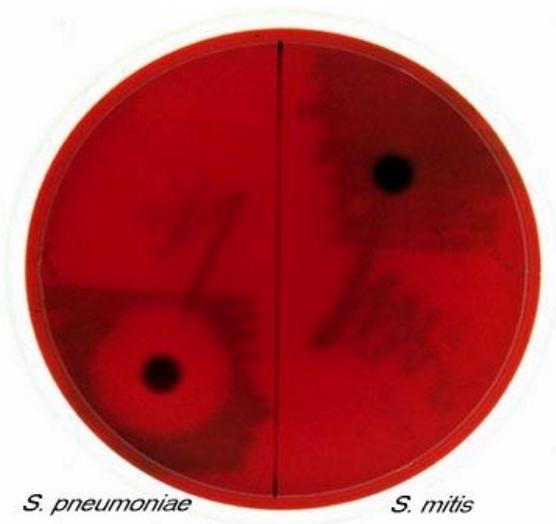
Optochin Susceptibility Test

Optochin resistant, *S. Viridans*

Optochin susceptible
S. pneumoniae



Optochin Sensitive



- If you have the specimen → gram + strep → dipole cocci → growing in blood agar, it gives Alpha hemolytic → optochin test (sensitive) → most properly strep pneumonia.

CASE 2

A 28 Year Old Female presented to the accident and emergency of KKUH with a sudden **onset of fever**, right sided **chest pain** and **productive cough** of **purulent sputum**. On examination her temperature was 39 °C. There were Rhonci and **dullness** on the right side of the chest. X-ray showed massive **consolidation** on the right side of the chest.

History showing acute infection → sudden onset of fever.

N.B: In acute infection we are suppose to find high fever, but in chronic it lasts for long time.

1. What is the most likely diagnosis?

Pneumonia → “key words: **dullness and consolidation**”

2. What investigation should be done?

*CBC → shows leukocytosis (lots of neutrophils with pus cell).

*Sputum culture

*NP swab → for respiratory viruses.

*Urine antigen → for Legionella.

LAB TESTS

- The blood counts showed a total **white cell count 45,000/ ml** 90% of the cells were **neutrophils**. The **sputum culture** showed **alpha hemolytic colonies** on blood agar. The gram stain showed **gram positive diplococci**. which were **catalase negative** , this organism was confirmed to be **optician susceptible**.

From the description blood agar indicates:

1- **Alpha hemolytic diplococci colonies, catalase negative, optician susceptible** → “these three are characteristics of *S.pneumoniae*”.

3. What should have been the empirical therapy for this case and why?

- MRSA = Vancomycin , Linezolid, if the patient has kidney failure
- Chlamydophila psittaci = Macrolide or tetracycline
- Sterp.pneumonia= tetracycline.

CASE 3

Abdul Karim is a 45 year old Saudi man who was admitted to King Khalid University Hospital because of 2-3 month history of **loss of appetite, weight loss, and on and off fever**

with attacks of **cough**. Two days before admission .he **coughed blood (haemoptysis)** Abdul karim is **diabetic** for the last 5 years. His father died of **tuberculosis** at the age of 45 yrs.

Key words: low grade of fever, loss of weight & cough up with blood.

These are usually the symptoms of T.B

On examination Abdul Karim looked weak with a temperature 38.6 °C, CVS and Respiratory system examination was unremarkable.

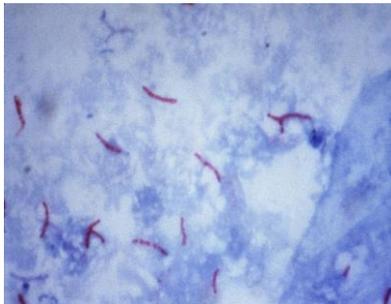
- The **chest X- ray** done showed **multiple opacities and cavities**
- **The ESR was increased** (85 m /hour)

Investigation: x-ray & sputum

1. What further tests should be done?

- Other test is PCR for the organism
- Tuberculin test, measurement of interferon gamma (IF-gamma), biochemical test and sensitivity tests

- **Sputum Microscopy** → Mycobacterium tuberculosis: **Ziehl-Neelsen stain**



stain

– Why?! Mycobacterium tuberculosis is supposed to be gram +ve streptococci **but** it cannot be stained by gram stain “because of the waxy wall, which does not allow the gram stain to penetrate inside the cell that's why it stained by **Z-N stain**”

– **Result:** organism will be red and background will be blue.



Growth on

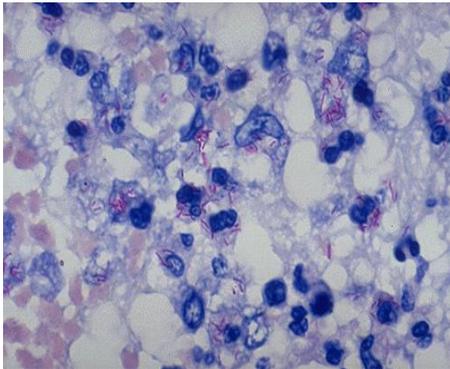
LJ medium (selective for Mycobacteria)

- Sputum is cultured in LJ media “special media for M.tuberculosis”
- **This media contain** : solidifying agent which is the egg & inhibitory substance: malachite green “type of type”

- **Result** : colonies are enlarged

Other investigation:

- **Sputum smear showed AFB**



2-What is the probable diagnosis?

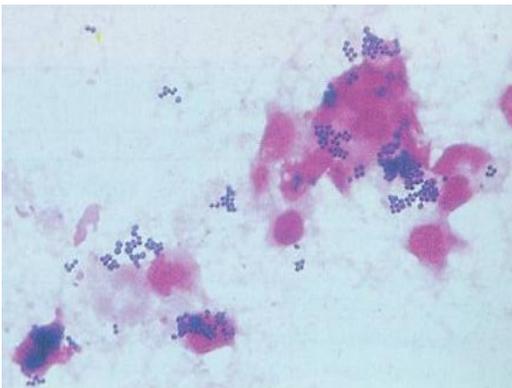
Key word: **weight loss, coughed blood (haemoptysis) and fever.**

Diagnose is: **tuberculosis**

3-How can the diagnosis be confirmed?

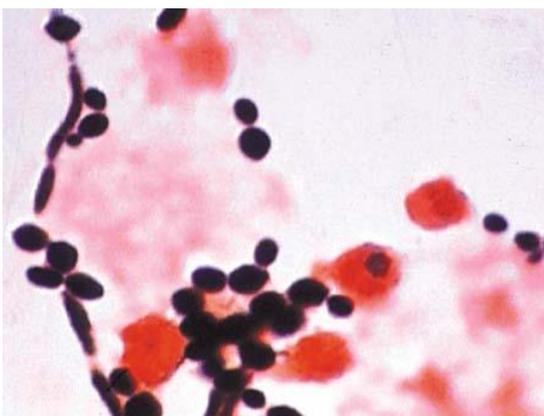
*Sputum culture & PCR.

Staphylococci → there is a Pus in the stain (which is in the red color)



Staphylococci: Are gram +ve cocci, usually arranged in clusters "spherical in groups"

Describe: Gram +ve cocci in clusters + neutrophills



Gram Stain of a Clinical Specimen of *Candida albicans*

Fungi → either mold or yeast

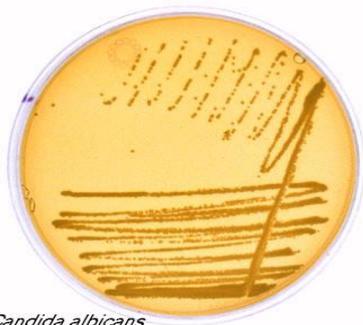
Candida albicans: oval with budding cell

Candida albicans are effect URT causing oral thrush especially in children

Gram stain: large budding cells generally oval budding yeast cell

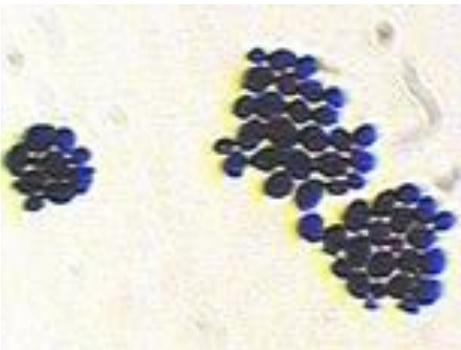
Description: budding yeast cell

Growth on Sabouraud's Dextrose Media



Candida albicans

Fungi usually growing in SDA "Sabouraud's Dextrose Agar"

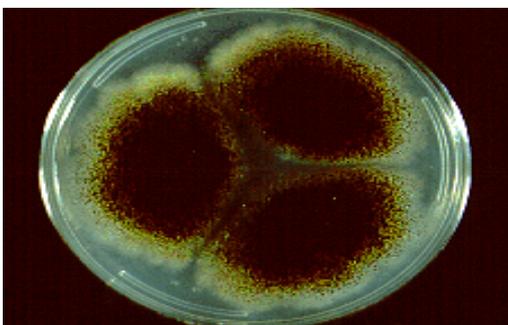


Gram stain of *Candida albicans* showing budding yeast celols

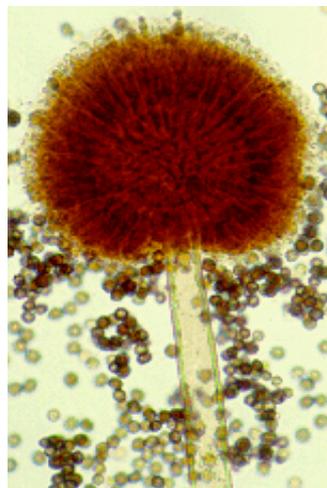
Without pus cells

Other mold fungi is *Aspergillus* causing URTI

Aspergillus Niger



*Culture of *Aspergillus Niger*.

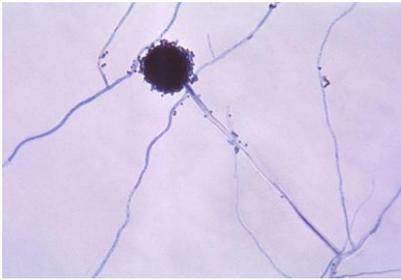


Growing in SDA looking cottony colonies.

Stained with Gemsa

Conidial head of *A. Niger*

Under the microscopy Looking like this



Aspergillus Niger

What U Should Know?

- The exam will be N sha'Alla 3 to 4 cases each case has 2 to 3 Qs.
- U should reach the diagnosis according to the history & the picture of the organism.
- U should know the treatment & complication.
- If u forgets the treatment write Penicillin (it doesn't mean that it is correct but 90% of the cases it will be N sha'Alla, so if u remember the answer write it, if u don't, write penicillin).