MICROBIOLOGY PRACTICAL TEAMWORK 437

BACTERIA CAUSING RESPIRATORY TRACT INFECTIONS

Objectives:

Recognize signs and symptoms of different bacterial respiratory tract infections

Be able to come up with a short differential to relevant cases and identify the most likely causative organism

Discuss the diagnosis and treatment of different bacterial respiratory tract infections

Explain the laboratory work up of important respiratory pathogens and be able to interpret microbiological laboratory results

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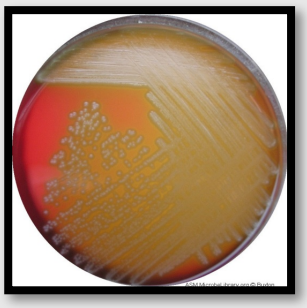
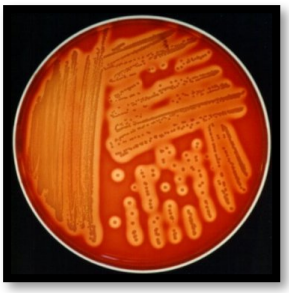
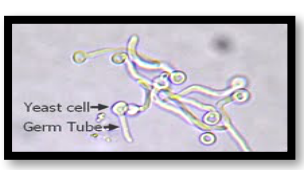
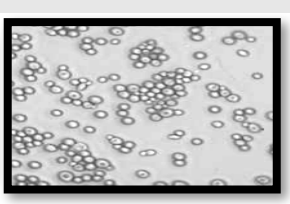
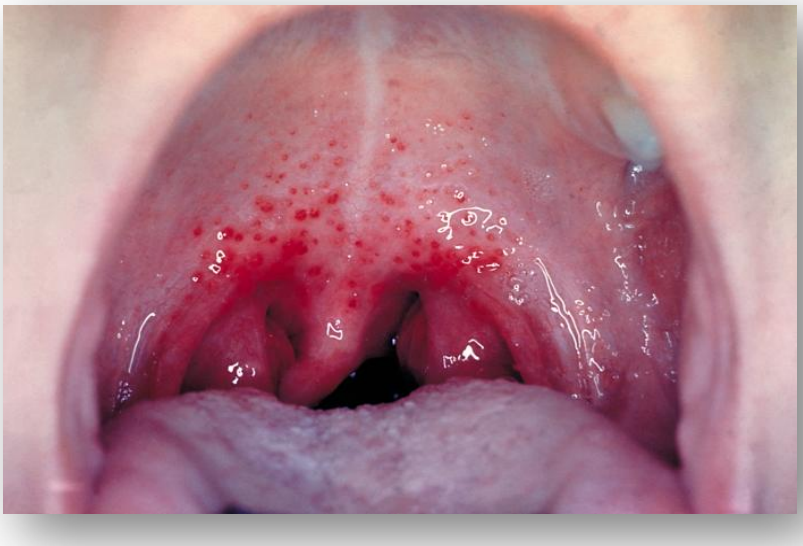
TYPES OF HAEMOLYSIS ON BLOOD AGAR

HAEMOLYSIS TYPE	DESCRIPTION	IMAGE
Alpha haemolysis	colonies surrounded by partial haemolysis with greenish color	
Beta haemolysis	colonies are surrounded by a clear zone	

DIFFERENT TESTS USED IN THE LAB

Test	Use	Positive	negative
CATALASE TEST	To differentiate between Staphylococcus & Streptococcus	 Staphylococcus	 Streptococcus
BACITRACIN SUSCEPTIBILITY	To differentiate between Streptococcus gp.A & any other group in beta haemolysis Streptococcus spec.		
OPTOCHIN SUSCEPTIBILITY (optochin disk)	To differentiate between Streptococcus pnumoniae & other alpha haemolysis Streptococcus spec.	 S. pnumoniae	
GERM TUBE TEST *for fungas	FOR IDENTIFICATION OF CANDIDA ALBICANS		

CASE 1



A 5 year boy was brought to KKUH, outpatient department complaining of fever and sore throat. His vaccination history was up to date. On examination his temp. was 38.5°C, the tonsillar area and pharynx were obviously inflamed with some foci of pus.

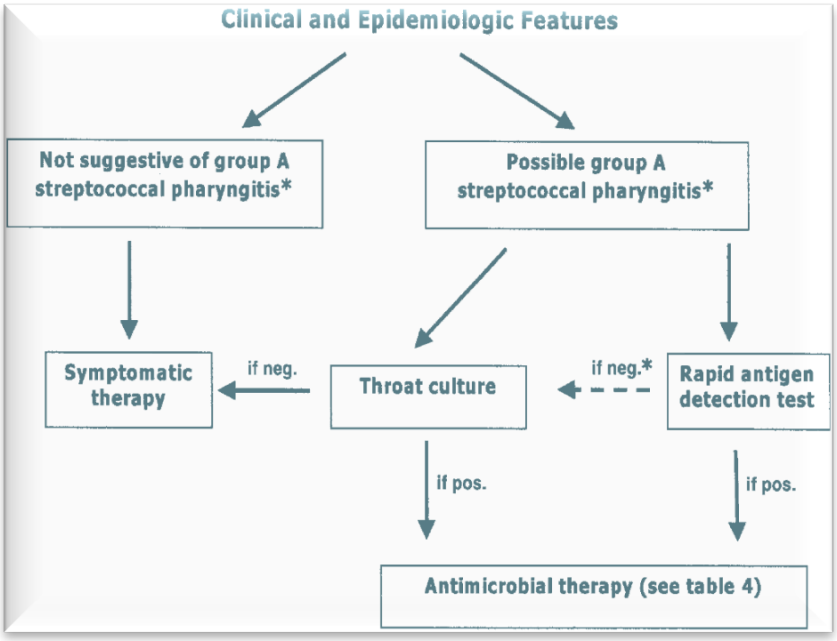
1. What investigations should be done?

- Specimen => throat swab
  1. (Rapid Antigen Detection Test) RADT
  2. CULTURE ON BLOOD AGAR
  - ❖ Direct gram stain from throat swabs is not useful (because it is contaminated with normal flora)

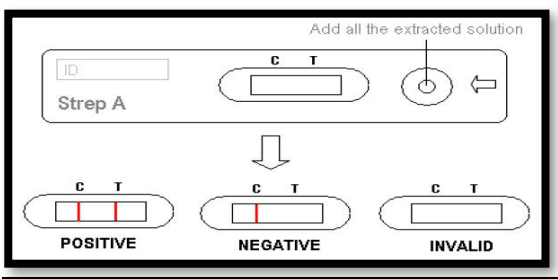
- Culture work up
  1. CATALASE TEST (To confirm it is Staph. Or Strept.)
  2. GRAM STAIN
  3. BACITRACIN SUSCEPTIBILITY TEST

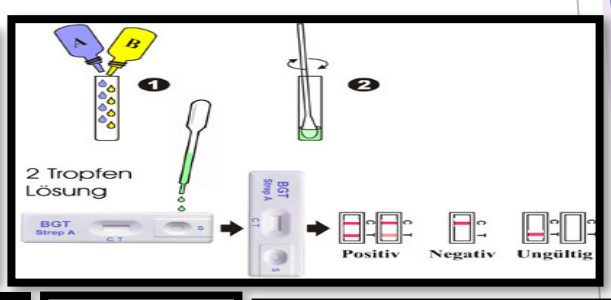
CLINICAL AND EPIDEMIOLOGIC FEATURES

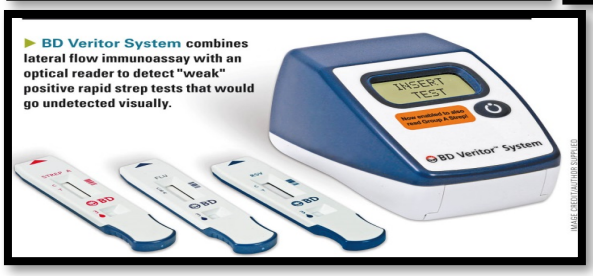
EXTRA page

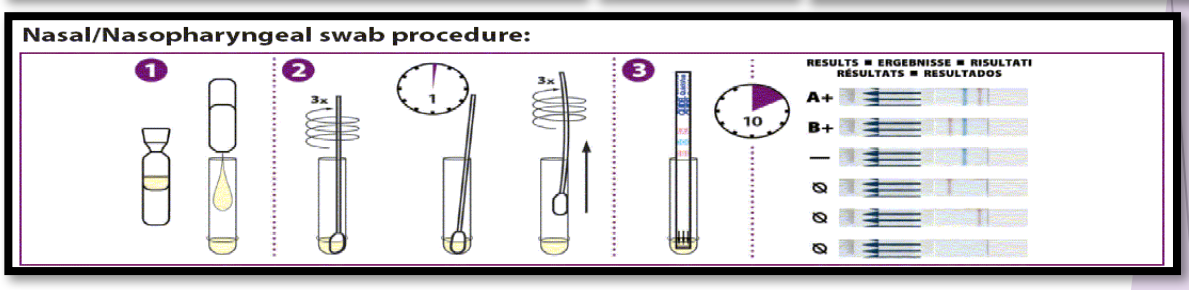


RADT









BACITRACIN SUSCEPTIBILITY

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 inhibits the growth of S. pyogenes giving zone of inhibition around the disk

Procedure:

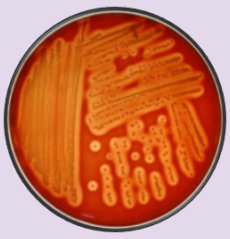
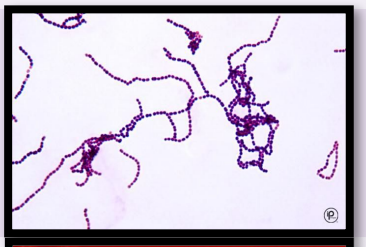
- Inoculate BAP with heavy suspension of tested organism

-Bacitracin disk (0.04 U) is applied to inoculated BAP

- After incubation, any zone of inhibition around the disk is considered as susceptible

LAB TEST RESULTS

Important

TEST	RESULT	IMAGE
CULTURE ON BLOOD AGAR	Beta haemolyis (colonies surrounded with clear zone of haemolysis)	
CATALASE TEST	No bubbles → catalase negative	
GRAM STAIN FROM CULTURE	Gram positive cocci in chains	
BACITRACIN SUSCEPTIBILITY TEST	Bacitracin Susceptible colonies	

Streptococcus pyogenes (Group A Streptococci)

1.What is the differential diagnosis?

Acute Pharyngitis or Tonsillitis

2.What is the likely identity of the organism?

Beta haemolytic Group A Streptococcus (streptococcus pyogenes)

3. What is the best antibiotic therapy for this child?

Penicillin for 10 days
If allergy: Clindamycin or Macrolide (e.g Erythromycin , Clarithromycin)

4. If not treated what complication may this child have after 6 weeks period?

1- Peritonsillar and Parapharyngeal spaces abscesses
2- Rheumatic Fever
3- Glomerulonphritis

CASE 2

A 3-year-old girl is brought to the emergency room by her mother because she has a fever and complains that her ear hurts. She has no significant medical history. Her temperature is 38.8°C and is found to have injected tympanic membranes.

1.What is the differential diagnosis?

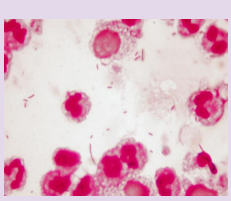
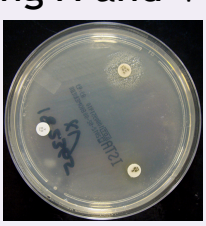
Haemophilus influenzae, S. aureus, Streptococcus Pneumoniae (all cause otitis media)

2. What investigations could be done?

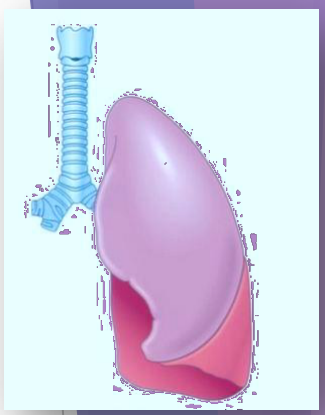
LAB. TESTS:

- Gram stain From ear discharge.
- Culture of the specimen on blood, chocolate and MacConkey agar.
- Biochemical tests.
- Antibiotic susceptibility test.

LAB TEST RESULTS

test	result
Gram stain From ear discharge	Gram negative coccobacilli 
Nutrient agar with X and V factors هذا اغار فاضي بس فيه ٢ ديسك فيهم X AND V factors)	Haemophilus influenzae grow around the disc containing X and V factors 
Culture on chocolate agar الاغار الوحيد اللي تنمو فيه Haemophilus influenzae)	

CASE 3



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

1. What is the differential diagnosis?

Streptococcus Pneumoniae, staphylococcus aureus, Haemophilus influenzae …. all causes chest infection (lobar pneumonia)

2. What investigations should be done?

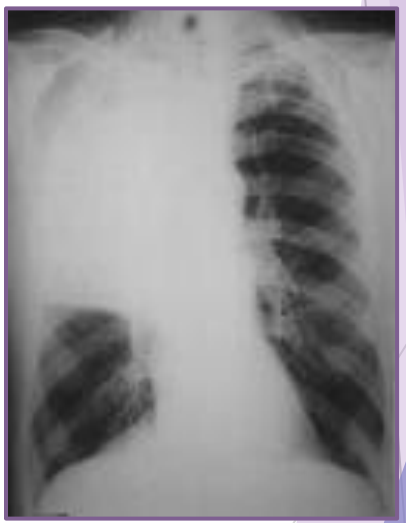
LAB.TESTS:

- ❖ Blood work: CBC
- ❖ Sputum specimen:

1. Gram stain for sputum
2. Culture on blood, chocolate and McConkey agar

- ❖ Culture work up:

1. Catalase test
2. Optochin susceptibility test
3. Antibiotic susceptibility test



The chest X- ray showed massive consolidation on the right side of the chest.

3. What is the most likely organism?

Streptococcus pneumoniae

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

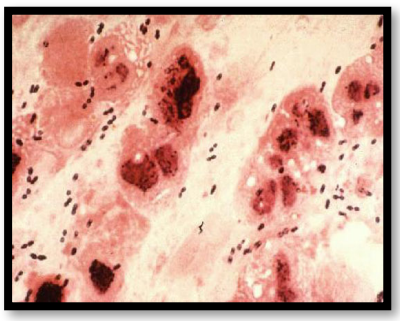
Ceftriaxone + vancomycin

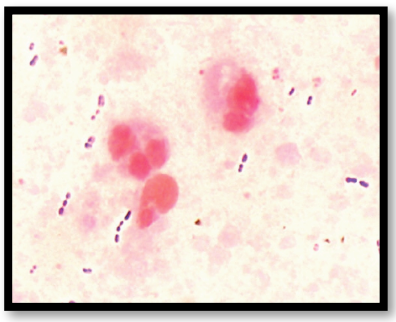
Because the organism may be Pencillin resistant.

Microscopic appearance:

❖ **Gram stain from sputum showed:**

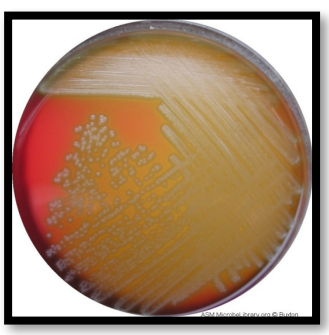
Gram positive diplococci (arranged in pairs):





Negative stains showing capsule:

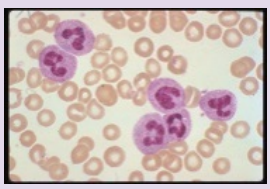
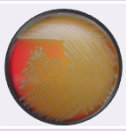
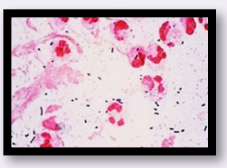
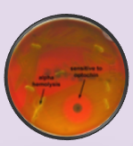
Culture:



❖ **Sputum culture showed:**

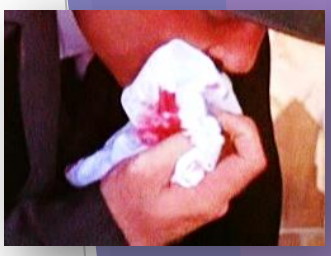
Alpha haemolysis on blood agar (colonies surrounded by a partial haemolysis with greenish color)

LAB. TEST RESULTS (summary) :

test	result	
CBC	45,000/ml 90% of the cells were neutrophils	
CULTURE ON BLOOD AGAR	Alpha haemolysis	
CATALASE TEST	No bubbles → catalase negative	
GRAM STAIN	Gram positive diplococci in pairs	
OPTOCHIN SUSCEPTIBILITY TEST	Optochin susceptible colonies	

Streptococcus pneumoniae (Pneumococcus)

CASE 4



Abdul Karim is a 45 year old Saudi man who was admitted to KKUH because of 2-3 month history of loss of appetite, weight loss, and on and off fever with attacks of cough. On examination Abdul Karim looked weak with a temperature 38.6 °C, CVS and Respiratory system examination was unremarkable. 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 years.

1.What is the differential diagnosis?

Chronic Pulmonary infection (TB, virus, fungal)

2. What investigations-tests could be done?

1- X-ray
2- Sputum Microscopy: Ziehl-Neelsen stain (Shows Acid Fast Bacilli)
3- Culture: Growth on L.J medium (Selective for Mycobacteria)

X-ray

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

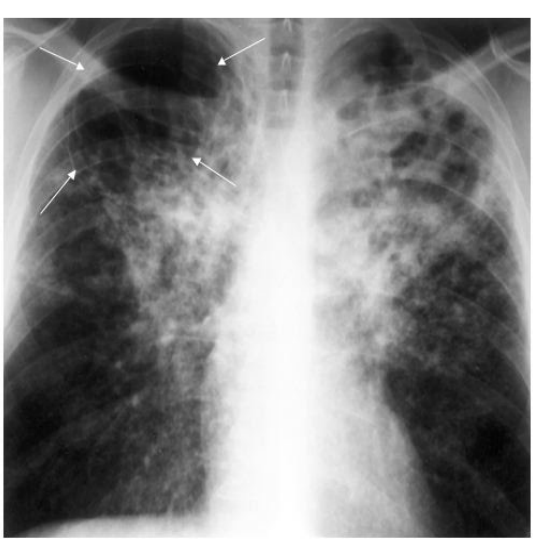
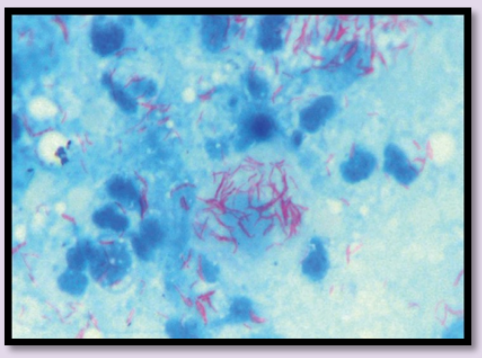
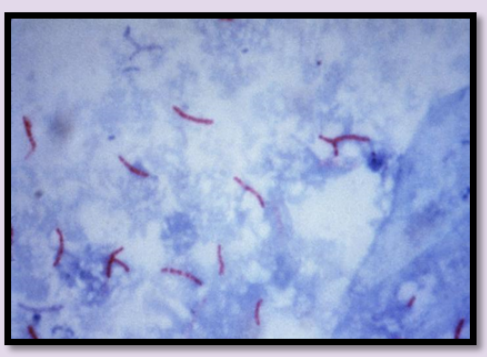


Figure 8. Chest x-ray with bilateral upper lobe opacities (white areas) with multiple cavities including a very large cavity in the right upper lobe (arrows).

Microscopic Appearance	Culture
Ziel – Neelsen Stained Smear From Sputum Showing: Acid – Fast Bacilli (AFB)  	Sputum culture on Lowenstein-Jensen medium (selective for mycobacteria) showed: showing growth of Rough, Tough and Buff colonies   

3. What is the probable diagnosis?

Pulmonary TB

4- How can the diagnosis be confirm?

1- Measurement of Interferon-Gamma (IFNγ).

2- If the morphology on LJ media showed buff rough and tough colonies.

3- If the growth occurred at 37°C and produced 5-10% CO_2 .

Mycobacterium tuberculosis

CASE 5

A 5 year-old boy was brought to the emergency department complaining of sore throat , fever (38.5°C), and was found to have pharyngeal pseudomembranes

What is the differential diagnosis?

Diphtheria .... + اي مثال اخر

What investigation should be done?

1. Gram stain From culture.
2. Throat swab culture on blood tellurite.
3. ELEK's test

What is the likely identity of the organism?

Corynebacterium diphtheriae

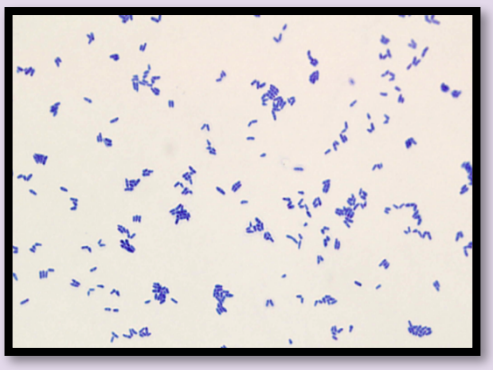
What is the best antibiotic therapy for this child?

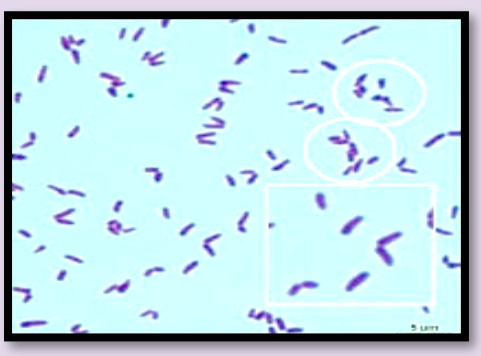
1. Anti-toxin
2. Penicillin , If allergic, use Erythromycin.

What complication may this child develop?

1. Local complication (descent of pseudomembrane)
2. Cardiac failure
3. adrenal infraction

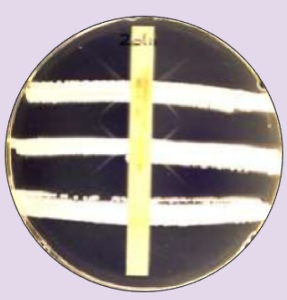
Microscopic Appearance	Culture
Gram stain From culture showed : Gram positive bacilli (chains' litter appearance)	Throat swab culture on blood tellurite showed: Black color colonies

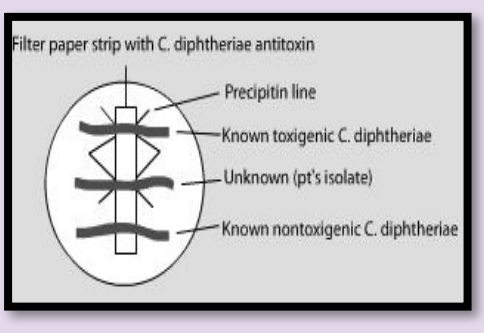




ELEK TEST

Toxin from culture of *C. diphtheriae* diffused and react with the diphtheria antitoxin defused from strip and produce precipitation lines → which demonstrate positive test (Diphtheria exotoxin production)





Corynebacterium diphtheriae