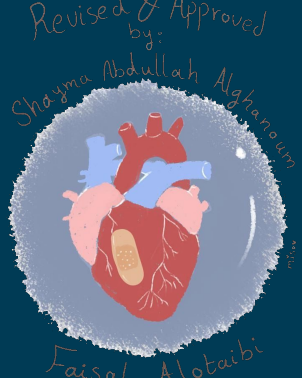
OSPE

Microbiology - Practical

TEAM 439
MICROBIOLOGY



VERSION 1

Objectives

- Recognize the signs & 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

These are the lectures in the OSPE (it is recommended to study them first)

1) **Bacteria causing URTI**
2) **Community acquired Pneumonia**
3) **Tuberculosis**

Colour index:

PURPLE BORDERS : GIRLS

GREEN BORDERS : BOYS

Any future corrections will be in the editing file, so please check it frequently.

Scan the code
Or click here

SCAN ME

Gram Stain

Gram +ve
(purple/blue)

- **Cocci**
- **Bacilli**

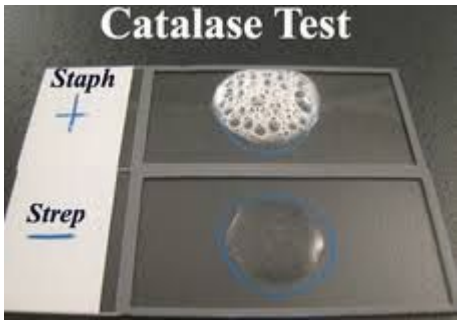
Gram -ve
(pink/red)

- **Cocci**
- **Bacilli**

Catalase Test

- **Staphylococcus**
- **Streptococcus**

Bubbles = Positive
Nothing = Negative



Hemolysis

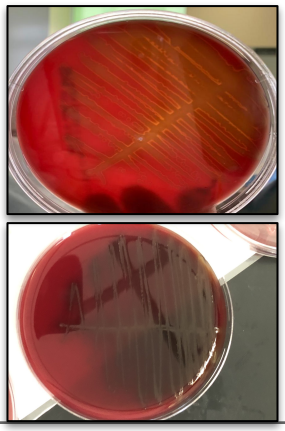
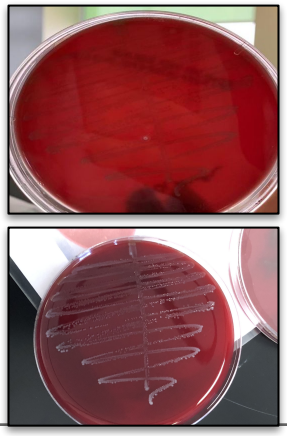
Alpha

Greenish zone around colonies

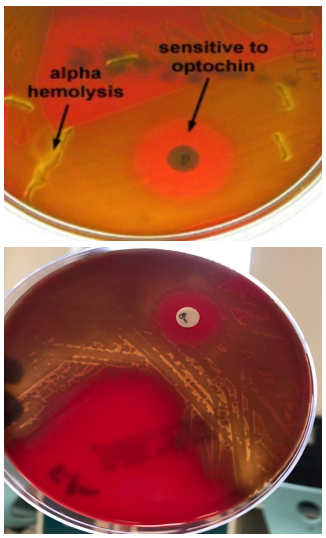
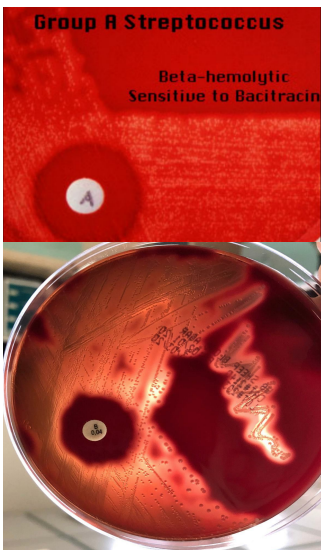
Beta

Clear zone around colonies

Hemolysis in Blood Agar

(Identification of streptococci) +ve cocci		
		
Beta hemolytic Streptococcus colonies	**Alpha hemolytic Streptococcus colonies**	**Gamma hemolytic Streptococcus colonies**
"complete hemolysis(clear zone) around the colonies "	"form greenish zone around the colonies "	"No change around the colonies"
St.pyogenes	**St.pneumoniae**	**Enterococcus faecalis**

Other Tests

	Optochin Test	Bacitracin Test
Sensitive If there is a clear space then it is sensitive	**S.pneumoniae** 	**Group A Strept (GAS)** 
Resistant	**Other, S.viridans**	**Other Beta hemolytic streptococci**

Case 1:

A 5 year old 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 38.5C, the tonsillar area and pharynx were obviously inflamed with some foci of pus.

What is the differential diagnosis? Write diseases with similar symptoms

Bacterial pharyngitis.

1-Mostly caused by Group A strept
2-Corynebacterium diphtheriae (Less likely since vaccines are up to date, DTP vaccine)

What investigations should be done?

Specimen: **Take a throat swab.** Then, two things can be done:
1- Culture it on blood agar and look for beta hemolysis.
2- Rapid antigen detection test RADT, such as Latex

After culture has grown:
1- Catalase test.
To differentiate between staphylococcus & streptococcus.
2- Gram stain.
Note that direct Gram stain from throat swab is not useful. It must be done from the culture.
3- Bacitracin susceptibility test (aka. Grouping).
To identify the group and differentiate between Group A strept and other groups of beta hemolytic streptococci.

Results (See pics next page)	
Culture on Blood agar	Beta hemolysis (colonies surrounded by clear zone of hemolysis
Gram stain from culture	Gram positive cocci in chains
Catalase test	No bubbles → (Negative)
Bacitracin susceptibility test	Bacitracin susceptible colonies

What is the likely identity of the organism?

Beta hemolytic (Group A streptococcus)

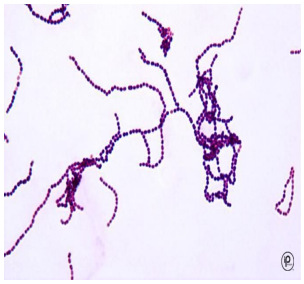
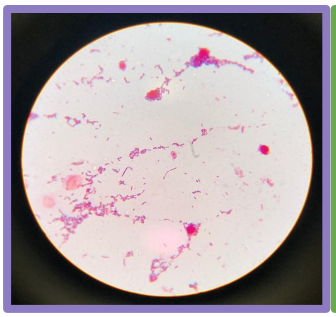
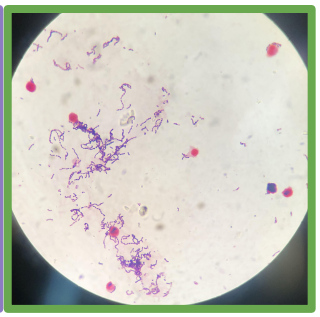
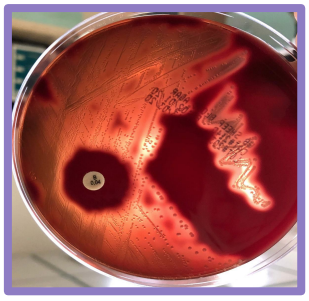
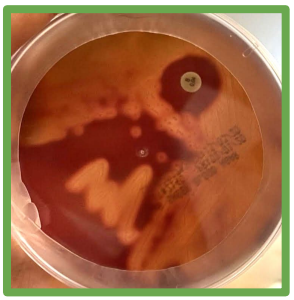
What is the best antibiotic therapy for this child?

Penicillin for 10 days

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

1- Rheumatic fever
2- Glomerulonephritis

Case 1, Continued..

Gram stain	   Gram positive cocci in chains
Blood agar	  Beta hemolytic colonies
Bacitracin susceptibility	   Beta hemolytic sensitive to bacitracin
Most likely organism	**Streptococcus pyogenes (Group A strept)**

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 38.8C and is found to have injected tympanic membranes.

What is the differential diagnosis?

Otitis media

Haemophilus influenzae, S.aureus, Streptococcus Pneumoniae, or Moraxella catarrhalis.

What investigations should be done?

Specimen: **Middle ear fluid.** Then, two things can be done:

1- Gram stain (it is okay to do it directly without culture, as there is no normal flora in middle ear)
2-Culture of the specimen on blood, chocolate, and MacConkey agar.
Chocolate agar because we are suspecting H. influenza. MacConkey agar because we are suspecting Gram -ve.

After culture has grown:

1- Biochemical Tests.
2- Antibiotics susceptibility tests.

Results	
Gram stain from ear discharge	Gram -ve coccobacilli
Culture	Has grown on chocolate agar. No growth on Blood agar
Nutrients agar with X & V factors	Has grown around the disc containing both X & V factors

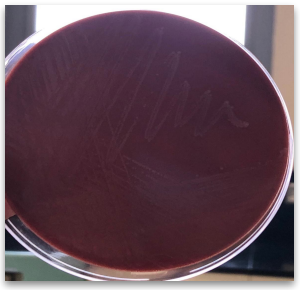
What is the likely identity of the organism?

Haemophilus influenzae

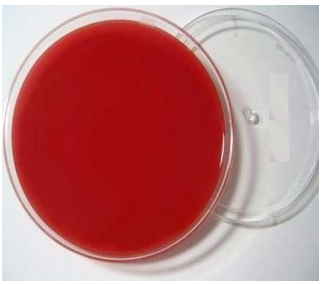
Case 2, Continued..

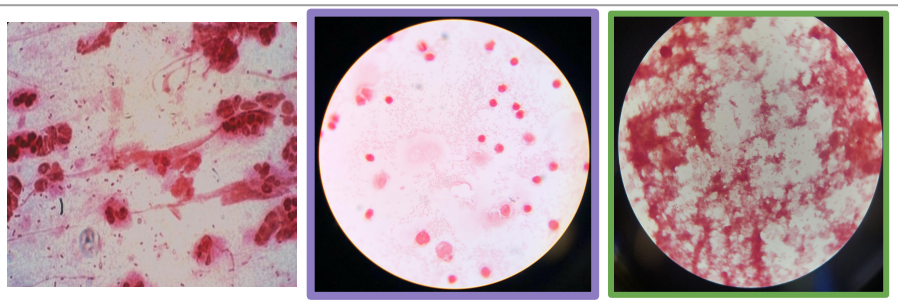
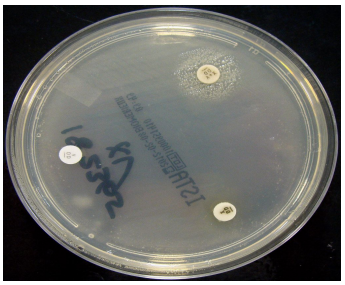
Ear Discharge Culture

Chocolate agar
Showing growth



Blood agar
Showing NO growth



Gram stain	
	Gram negative coccobacilli
Nutrient agar with X and V factors	
	Haemophilus influenzae grow around the disc contain X and V factors
Most likely organism	**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.

What is the differential diagnosis?

Typical lobar pneumonia

Mostly caused by **Streptococcus pneumoniae**

What investigations should be done?

1- Blood work, CBC
2- X-ray
3- Specimen: **Take a Sputum.** Then, two things can be done:

- Gram stain
- Culture of the specimen on blood, chocolate, and MacConkey agar.

After culture has grown:
1- Catalase test.
To differentiate between staphylococcus & streptococcus.
2- Optochin susceptibility test
To differentiate between strept. pneumoniae & strept, viridans.
3- Antibiotic susceptibility test.

Results	
x-ray	Showed massive consolidation on the right side of the chest.
Gram stain from sputum	Gram +ve diplococci (pairs)
Culture	Alpha hemolytic on blood agar
Catalase	No bubbles (Negative)
Optochin Susceptibility	Susceptible
CBC	45,000/ml 90% of the cells were neutrophils

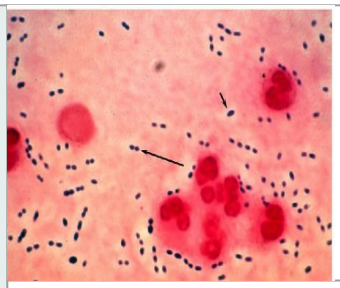
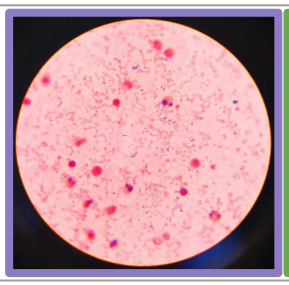
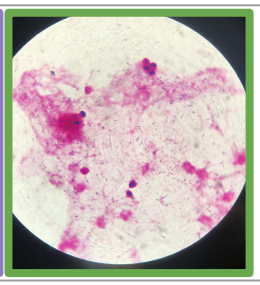
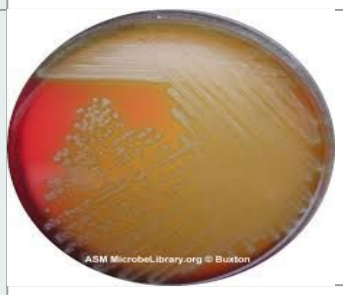
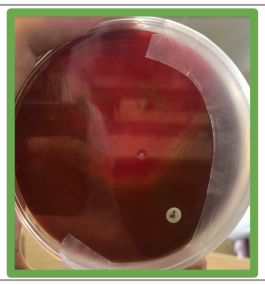
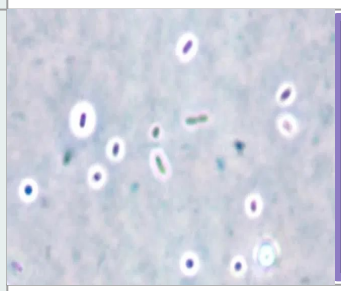
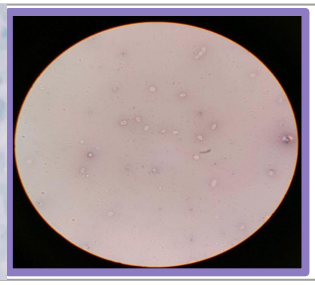
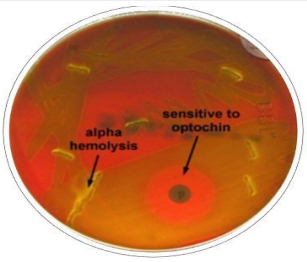
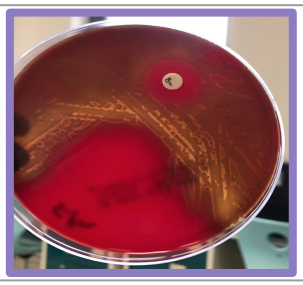
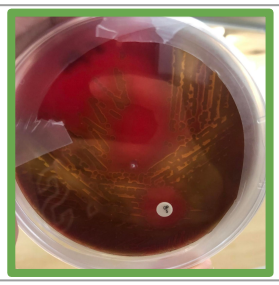
What is the likely identity of the organism?

Streptococcus pneumoniae (Pneumococcus)

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

Ceftriaxone or **macrolides**, because the organism may be Penicillin resistant.

Case 3, Continued..

Gram Stain	   Gram positive diplococci (arranged in pairs)
Blood agar	   Alpha hemolytic colonies
Negative stains	  Capsule
Optochin Test	   Optochin susceptible colonies
Most likely organism	**Streptococcus pneumoniae (pneumococcus)**

Case 4:

Abdulkarim is a 65 year old Saudi man who was admitted to KKUH with a 2-3 month history of loss of appetite, weight loss, and on and off fever with attacks of cough. On examination Abdulkarim looked weak with a temperature of 38.6 °C. CVS and Respiratory system examination was unremarkable. Two days before admission he coughed blood (haemoptysis). Abdulkarim is diabetic (for the last 5 years). His father died of tuberculosis at the age of 45 yrs.

What is the differential diagnosis?

Chronic pulmonary infection, **Tuberculosis** or Fungal infection.

Mostly caused by **Mycobacterium Tuberculosis**

What investigations should be done?

1- X-ray
2- ESR
3- Specimen: **Take a Sputum.** Then, two things can be done:
- Z-N stain
- Culture oN LJ media (selective for mycobacteria)

Results	
x-ray	Showed multiple opacities and cavities
ESR	Increased (85m/hour)
Z-N Stain	Acid fast bacilli
Culture	Growth of rough, tough, and buff colonies

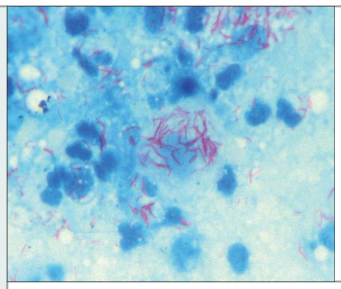
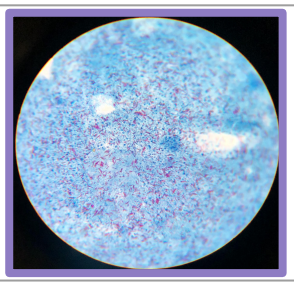
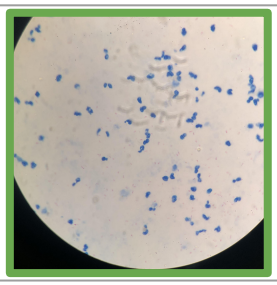
What is the the most probable diagnosis?

Pulmonary Tuberculosis

How can the diagnosis be confirmed?

1- X-ray
2- ESR
3- Specimen: **Take a Sputum.** Then, two things can be done:
- Z-N stain
- Culture oN LJ media (selective for mycobacteria)

Case 4, Continued..

Ziehl-neelsen stained smear from sputum	   Acid-Fast bacilli
Sputum culture on Lowenstein-Jensen Medium (Selective media used for mycobacteria)	   Growth of Rough, Tough and buff colonies
Most likely organism	**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 pharyngitis

What investigations should be done?

1- Specimen: **Take a Throat swab.** Then, what can be done is:

- Culture on blood tellurite.

After culture has grown:
1- Gram stain from culture, as it cannot be done directly with the throat swab.
2- Elek test to confirm toxin production

Results	
Gram stain from culture	Gram +ve bacilli (chinese letter appearance)
Throat Swab Culture on Tellurite	Black colonies

What is the likely identity of the organism?

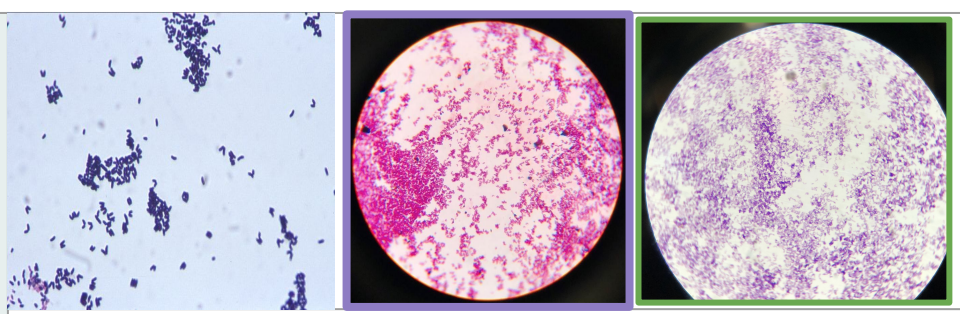
Corynebacterium diphtheriae

What is the best antibiotic therapy?

1. Anti-toxin
2. Penicillin, In case of allergy, Erythromycin

What complications may this child develop?

Myocarditis & Neuritis

Gram stain	 Gram positive bacilli (Chinese letter appearance)
Most likely organism	**Corynebacterium diphtheriae**

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- Abdulaziz Alsuhaim
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