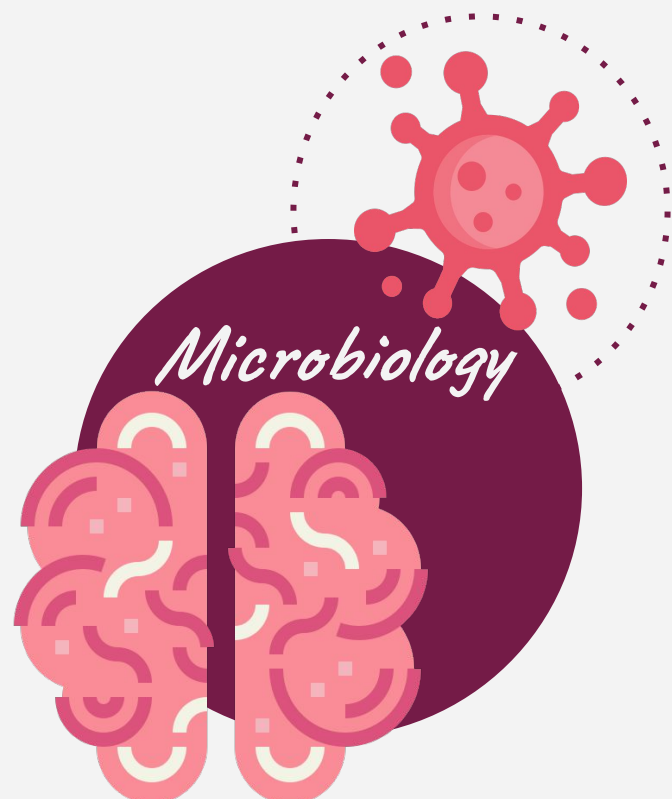


Examination of CSF

Objectives:

- Identify the functions of CSF.
- Recognize the normal and abnormal constituents of CSF.
- Understand the role of CSF in diagnosis of different diseases of CNS.
- Interpret the microbiological investigation results of CSF.
- Hands-on the procedure for estimation of total protein in CSF.



Color index

- | | | | |
|---|---------------|---|--------------|
| ● | Girls' slides | ● | Boys' slides |
| ● | Main content | ● | Extra |
| ● | Important | ● | Drs' notes |



Scenario



A 15-year-old healthy male visited emergency room presenting with fever, headache, vomiting and drowsiness.

Physical examination showed decreased level of consciousness, neck stiffness, skin rash and high temperature (38 °c). Cerebrospinal fluid (CSF) examination revealed opening pressure of 210 cm H₂O. The doctor in the emergency department takes a detailed history and conducts a clinical examination. Because of clinical findings, he decides to do a lumbar puncture.



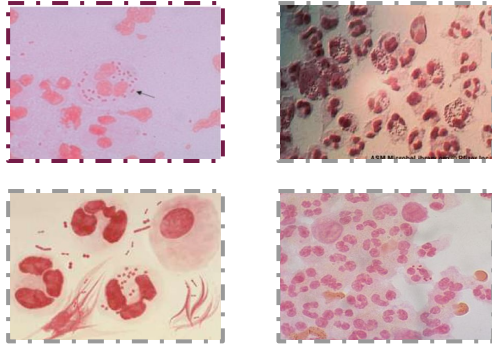
*The characteristic skin rash (purpura) of meningococcal septicemia, caused by *Neisseria meningitidis*

The results of the lumbar puncture are shown in the table below.

Lumbar Puncture Results		
CSF	Patient results	Normal range
Appearance	Turbid (cloudy) 	Clear 
WBCs (cells/mm ³) and differential	8,320 Mainly Polymorphonuclear leukocytes (84%)	Few (<5)
Protein	5.0	0.1-0.4 g/L
Glucose	1.3	3.0-4.5 mmol/L
Chloride	110	115-130 mmol/L

Microbiology lab results

Microscopic Appearance



Gram stained smear from CSF showing :
Gram negative intracellular diplococci + many pus cells

Culture



Growth on chocolate agar

Q1- What is the provisional clinical diagnosis?

Acute Pyogenic (bacterial) Meningitis

Q2- What is the most likely infection responsible

Bacterial infection (Neisseria Meningitidis)

Extra

Q3- What is your justification for your answer to question two?

CSF: ↑ WBCs (neutrophils) + ↑ Protein + ↓ Glucose + there is Polymorphs. Gram stain : Gram -ve diplococci

Extra

Q4- Describe the microorganism's appearance under the microscope?

Gram negative intracellular bean-shaped diplococci.

Extra

Q5- Name the media used for growing such organism?

Thayer-Martin agar or Chocolate agar

Q6- What further investigation would you like to do at this stage?

CSF culture and smear, Blood culture to check for septicemia, antibiotic sensitivity test, PCR (polymerase chain reaction) (DNA detection), Serology (Antigen detection). (antigen = capsule), glucose maltose utilizing , fermentation test

Q7- Mention 2 recommended empirical antibiotics that can be used in such a case?

Ceftriaxone with Vancomycin, For 10-14 days

The patient showed complete recovery after administration of ceftriaxone for 10 days




Epsilon-meter test (E test)

Scenario

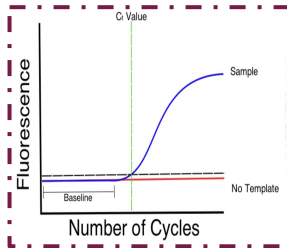
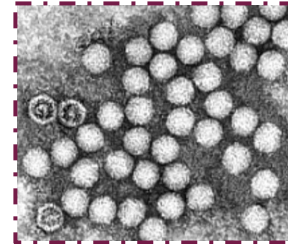
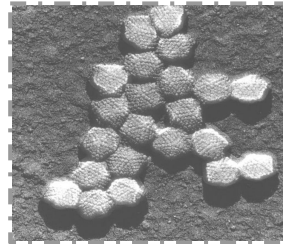
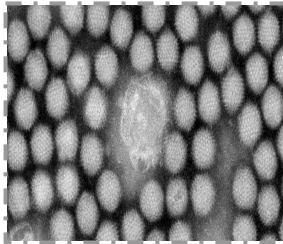
A 10-year-old boy is brought to the emergency department (A&E) at KKUH accompanied by his mother. He has **fever, headache, and vomiting** for the last 2 days. Clinical examination confirmed that he has meningeal irritation. The doctor decided to do a lumbar puncture.

The results of the lumbar puncture are shown in the table below:

Lumbar Puncture Results		
CSF	Patient results	Normal range
Appearance	Clear	
WBCs (cells/mm ³) and differential	100 mainly lymphocytes (80%)	Few (<5)
Protein (g/L)	0.5	0.1-0.4 g/L
Glucose (mmol/L)	3.7	3.0-4.5 mmol/L
Chloride (mmol/L)	100	115-130

Microbiological Finding

Electron Micrograph of Enterovirus



CSF Molecular testing is **positive** for Enterovirus

Extra

Q1- What is the diagnosis?

Aseptic meningitis

Q2-What is the most likely infection responsible?

Viral infection

Q3- What is your justification for your answer to question two?

↑WBCs (lymphocytes)+ moderate ↑ Protein + normal / or slightly decreased Glucose + high Lymphocytes

Q4- What further investigation would you like to do at this stage

CSF culture and smear, Blood culture, CBC, Protein and glucose levels,PCR (RT.PCR)

Scenario

A 38-year old male, not known to have any medical illness, in his usual state of health, he was visiting his family after which he felt tired and went to his house. He's brother was trying to call him several times and was found to be on the floor with LOC as he was not answering them, he was closing his eyes with his bilateral upper limbs contracted As per the brother. The patient complained of headache 2 days back

Physical Exam Vitals & Measurements:

T: 38.2°C (Axillary)

HR: 102

RR: 24

BP: 106/53

SpO2: 100%

WT: 90kg

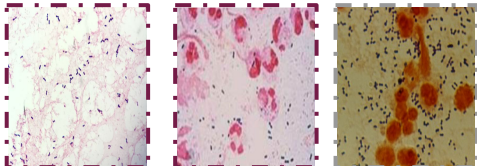
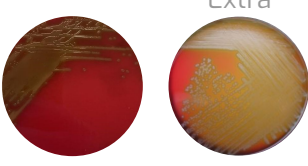
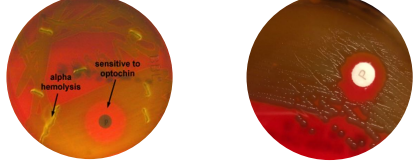
GCS: (E=1, V=2,M4).

Rigid neck

Pupils:2-3 mm with sluggish reaction to light.

- **Assessment/Plan:** 38 years old male, not known to have any medical illness, presented with decreased LOC along with documented high-grade fever in the ER. LP done by ER Team

Microbiology lab results

Microscopic Appearance	Blood agar	Optochin test
 <p>Direct gram stain of a CSF deposit shows gram-positive diplococci with lanceolate shape and polymorphonuclear leukocytes</p>	 <p>colonies appear as small, grey, moist, and produce a zone of alpha-hemolysis</p>	 <p>Gray white, alpha-hemolytic colonies recovered on sheep blood agar with increased CO₂ from spinal fluid sediment was Optochin sensitive</p>

All the questions are extra

Q1- What is the diagnosis?

Acute Pyogenic (bacterial) Meningitis

Q2- What is the most likely infection responsible

Bacterial infection (pneumococcal Meningitis)

Q3- Describe the microorganisms appearance under the microscope

Gram-positive diplococci with lanceolate shape and polymorphonuclear leukocyte (many pus cells)

Q4- Name the media used for growing such organism

Blood agar

Q5- Describe the microorganisms appearance on blood agar

Alpha hemolytic streptococci. Gray white, alpha-hemolytic colonies recovered on sheep blood agar with increased CO₂ from spinal fluid sediment was Optochin sensitive

Q6- Describe the microorganisms reactivity towards the optochin test

Optochin sensitive

Q7- What further investigation would you like to do at this stage

CSF culture and smear, Blood culture, CBC, Protein and glucose levels, PCR.

Q8- Mention two of the recommended antibiotics that can be used as empiric treatment in such a case

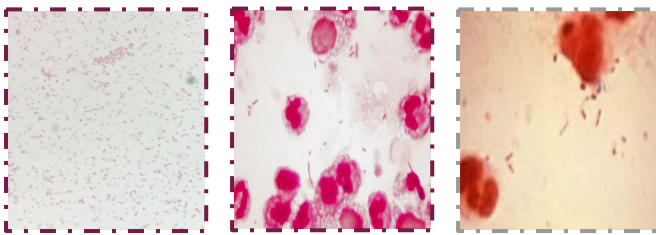
Vancomycin + ceftriaxone

Scenario

A 3-year old normal child became acutely ill, with temperature (40°C). She had neck stiffness and vomiting. There was no rash or bruising but the left ear drum was inflamed. The clinical diagnosis of meningitis was confirmed, and blood and cerebrospinal fluid (CSF) samples were taken immediately, and intravenous antibiotics started. The CSF showed increased numbers of neutrophil leukocytes and a few Gram-negative coccobacilli. Culture on chocolate agar is shown. The full blood count showed high neutrophil count and high C-reactive protein.

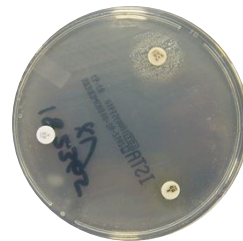
Microbiology lab results

Microscopic appearance



Direct gram stain of a CSF deposit shows Gram-Negative pleomorphic coccobacilli with many polymorphonuclear leukocyte.

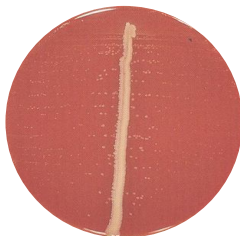
Culture on Nutrient agar for identification of H. Influenza



- Growth around XV factors (requires both factors XV),
- No growth around X or V alone

Blood agar

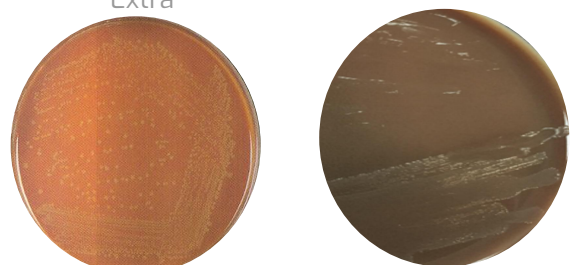
Extra



Growth on blood agar showing **satellitism** adjacent to a streak of S.aureus, S.aureus producing surplus factors increasing growth of adjacent H.influenzae

Chocolate agar

Extra



All the questions are extra

Q1- What is the diagnosis?

Acute Pyogenic (bacterial) Meningitis

Q2-What is the most likely infection responsible

Bacterial infection

Q3- What is the most probable Pathogen cause this infection?

Haemophilus Influenzae type B.

Q4- Describe the microorganism's appearance under the microscope?

Gram-Negative pleomorphic coccobacilli with many polymorphonuclear leukocyte

Q5- Name the media used for growing such organism?

Chocolate agar , Blood agar (only in case of satellitism) and Nutrient agar with X and V factors.

Q6- Describe the microorganism's appearance on Chocolate Agar?

Grey mucoid colonies of Haemophilus Influenzae due to the presence of X and V factors.

Q7- Describe the microorganisms morphology on Nutrient Agar?

H. influenzae:Growth around XV factors (requires both factors XV) no growth around X or V alone the optimum growth temperature is (35°C - 37°C in 5% CO₂).

Q8- Describe the microorganisms morphology on Blood Agar?

Growth on blood agar showing satellitism adjacent to a streak of S.aureus. S.aureus producing V factor (haemophilus require both x and v) increasing growth of adjacent H.influenzae.

Q9- Mention two recommended empirical antibiotics that can be used in such a case?

Ceftriaxone with Vancomycin.



All this page is extra

Scenario

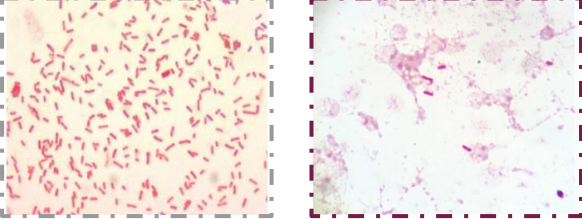
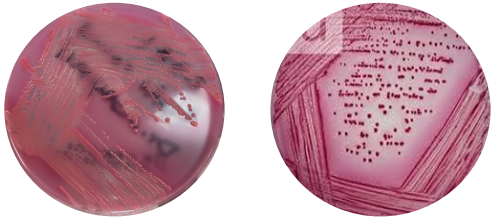
A 10 year old boy has been admitted to the ER with fever, headache, vomiting and confusion. Microscopic examination showed gram -ve bacilli, 300-2,000 Neutrophils, protein level is elevated, glucose level is decreased and oxidase -ve test.

The results of the lumbar puncture are shown in the table below:

Lumbar Puncture Results

CSF	Patient results	Normal range
Appearance	Turbid (cloudy) 	Clear 
WBCs (cells/mm ³)	(>5) mainly neutrophils	Few (<5)
Protein (g/L)	1.7 g/l	0.1-0.4 g/L
Glucose (mmol/L)	1.6 mmol/L	3.0-4.5 mmol/L

Microbiology lab results (Mentioned in the Dr's Slides)

Microscopic Appearance	Culture
 <p data-bbox="316 790 555 824">Gram-Negative Bacilli</p>	 <p data-bbox="1270 510 1337 539">Extra</p> <p data-bbox="948 772 1417 842">Culture on MacConkey agar E.coli appear pink as they ferment lactose</p>

All the questions are extra

Q1- What is the diagnosis?

Acute Pyogenic Meningitis.

Q2- What is the most likely infection responsible?

Bacterial infection

Q3- What is the most probable Pathogen isolated?

Escherichia Coli.

Q4- Describe the microorganism's appearance under microscope?

Gram negative bacilli (rods).

Q5- Name the media used for growing such organism?

MacConkey's agar.

Q6- Describe the microorganisms appearance on MacConkey Agar?

Lactose fermenter (appear pink colonies).

Q7- Mention two recommended empirical antibiotics that can be used in such a case?

Neonates: Ampicillin+Gentamicin+Cefotaxime



Children & Adults: Ceftriaxone (Cefotaxime) with Vancomycin

Elderly >50 years or at risk for Listeria: Ampicillin + Ceftriaxone (Cefotaxime) +Vancomycin

Scenario

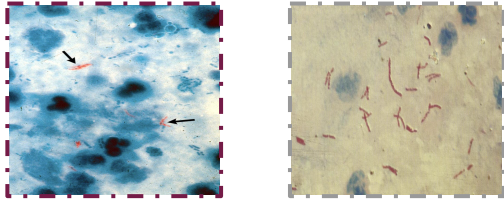
Scenario: A 65-year-old is referred from a general practitioner because of headache, fever, excessive sweating at night, and weight loss over the last 4-5 months. He has lost his appetite for food. On examination, there is neck rigidity. Laboratory tests including blood count, serum and electrolytes, blood urea, creatinine and blood culture are all normal. The doctor decided to do a lumbar puncture

The results of the lumbar puncture are shown in the table below:

Lumbar Puncture Results		
CSF	Patient results	Normal range
Appearance	Turbid (cloudy) 	Clear 
WBCs (cells/mm ³)	300 mainly Lymphocytes (80%)	Few (<5)
Protein	0.8	0.1-0.4 g/L
Glucose	2.0	3.0-4.5 mmol/L
Chloride	115	115-130 mmol/L

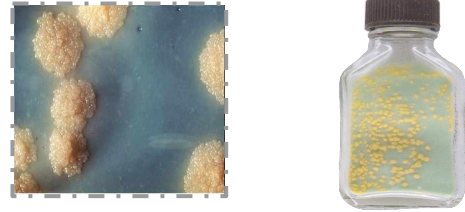
Lab investigation

Microscopic Appearance



Direct Ziehl – Neelsen Stained Smear of a CSF deposit shows Acid – Fast Bacilli (AFB)

Lowenstein-Jensen Medium



Colonies or growth is Rough, Tough and Buff

Extra

Q1- What is the diagnosis?

Chronic Bacterial Meningitis

Q2- What is the most likely infection responsible?

Mycobacterial infection

Q3- What is your justification for your answer to question two?

↑ WBCs + ↑ Protein + ↓ Glucose + present of Lymphocytes + ↓ chloride

Extra

Q4- What is the most probable Pathogen isolated?

Mycobacterium Tuberculosis

Extra

Q5- What is the stain used to identify such organism?

Ziehl-Neelsen (ZN) stain for Acid Fast Bacilli (AFB).

Extra

Q6- Describe the microorganism's appearance under microscope?

Acid Fast Bacilli (AFB) with a blue background.

Extra

Q7- Name the media used for growing such organism?

Lowenstein-Jensen (LJ) media.

Extra

Q8- Describe the culture on Lowenstein-Jensen?

Colonies or growth is rough, tough and buff.

Q9- What further investigation would like to do at this stage?

CSF culture (on LJ media for 2 to 3 weeks) , PCR, CBC, Tuberculin skin test, chest X-ray

Q10- Name the drug used to treat such infections?

For the first 2 months: Rifampicin + Isoniazid (INH) + Ethambutol + Pyrazinamide.
Then, for 4-6 months: Rifampicin + Isoniazid (INH).

Dr. Khalifa

- For most of the patients who are suspected to have meningitis, we do Gram stain, culture, and CSF analysis and other routine tests. However, additional tests such as TB culture are only required when there is a clinical suspicion or chronic symptoms.
- For TB meningitis, focus on the history of the case that is given.
- It is more important to rule out bacterial meningitis because it is a life threatening condition.
- Viral encephalitis with HSV (herpes simplex virus) is considered to be a medical emergency and a life threatening condition.
- Tests for fungal infections are requested for specific populations only (immunocompromised and HIV patients).
- Do not focus on the specific numbers of glucose and protein. You should just know when it is elevated or decreased. However, white blood cells count (WBC) is important to differentiate between bacterial/viral infections.
- **Make sure you know the different organisms and treatment regimens according to the age groups very well (check our theoretical lecture).**

Dr. Raed

- H. influenzae can be grown in a blood agar ***only if*** the agar plate was previously streaked with Group B strept. The liberation of V factor by hemolysis of Group B streptococci contributes to the growth of the H.influenzae around the streak line. This phenomenon or a property is known as satellitism.

Dr. Fawzia

- Neisseria meningitidis is the most important organism that causes skin rashes.
- Neisseria meningitidis is one of the rare organisms that are still sensitive to penicillin, and although penicillin is mainly used for Gram +ves, neisseria is an exception.
- Why do we use vancomycin and ceftriaxone in cases of neisseria meningitidis then? Because we need empiric treatment before the identification of the organism, however; after identification of neisseria we usually change the antibiotic to penicillin.

CASE1: A previously healthy 8 years old boy was admitted to the hospital with a reduced level of consciousness,, headache and vomiting for a day and a half. At admission, CSF analysis revealed WBCs to be (6,484 cells/mm³ mainly neutrophils (98%) Normal is <5) Protein is 5 (normal 0.1-0.4 g/L) Glucose 1.3 (normal 3.0-4.5 mmol/L) Gram stain is shown in the pictures.

Q1: What is the most likely diagnosis?

Acute Pyogenic Meningitis

Q2: Describe the microorganism's appearance under the microscope?

Gram-Negative pleomorphic coccobacilli with many polymorphonuclear leukocyte (pus cells)

Q3: What is the most likely causative agent?

Haemophilus Influenzae type B.

Q4: Name the media used for growing such organism?

Chocolate agar, nutrient agar with X and V factors, Blood agar (only in case of satellitism).

Q5: How would you confirm the causative organism?

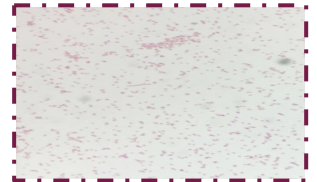
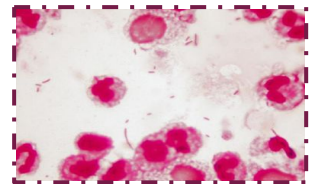
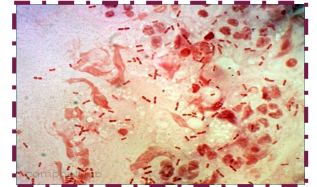
Growth in the presence of **BOTH** X and V factors

Q6: What is the appropriate treatment for this patient and for how long?

Ceftriaxone with Vancomycin for 10-14 days

Q7: What are the virulence factor that this organism possesses?? (theoretical)

It has a capsule made of a polymer of PRP (Polyribosylribitol Phosphate)



CASE2: A 72 years old man was admitted to the emergency department with a reduced level of consciousness after a day of flu-like symptoms, headache and vomiting. After arrival to the hospital, he appeared somnolent with a Glasgow Coma Score (GCS) of 9. Physical examination revealed nuchal rigidity with no focal neurological deficits. At admission, biochemical analyses revealed a neutrophil leucocytosis, elevated C reactive protein and procalcitonin levels, and arterial blood gas analysis showed a slight metabolic acidosis. Lumbar puncture revealed a cloudy cerebrospinal fluid (CSF) with elevated white blood cell count and protein levels, and a decreased CSF-serum glucose ratio. Gram stain is shown in the pictures.

Q1: What is the most likely diagnosis?

Acute Pyogenic Meningitis

Q2: Describe the microorganism's appearance under the microscope?

Gram-positive diplococci with lanceolate shape and polymorphonuclear leukocyte (with many pus cells)

Q3: What is the most likely causative agent?

S. pneumoniae (pneumococcal infection)

Q4: Name the media used for growing such organism?

Blood agar

Q5: Describe its appearance on the media?

Alpha hemolytic streptococci

Q6: How would you confirm the causative organism?

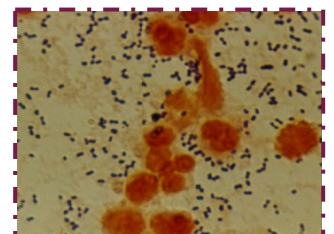
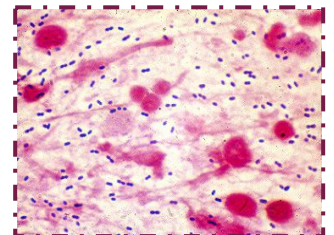
Optochin test (sensitive)

Q7: What is the appropriate treatment for this patient and for how long?

Vancomycin + ceftriaxone + ampicillin (as the patient is above 50 years).

Q8: What are the virulence factor that this organism possesses? (theoretical)

Capsule + Pneumolysin



CASE3: A 35-year-old firemen came to our hospital with complaints of on and off fever, night sweating, loss of appetite of 1-month duration. Patient recently became irritable and was in altered sensorium since 4 days. There was no history suggestive of respiratory, cardiac, or urinary abnormalities. Evaluation for sexually transmitted disease was negative. On examination patient was pale, febrile with toxic look, disoriented with Glasgow coma scale of 12/15. Patient had signs of meningeal irritation along with right sixth nerve palsy, resting tremors in both hands, generalized rigidity of extra pyramidal type (lead pipe and cogwheel) but he did not had any weakness. Patient had cognitive defects like apathy, psychomotor retardation, and impaired memory.

Q1: What is the most likely diagnosis?

Chronic Meningitis

Q2: Describe the microorganism's appearance under the microscope?

Positive AFB smears

Q3: What is the most likely causative agent?

Mycobacterium Tuberculosis

Q4: Name the media used for growing such organism?

Lowenstein-Jensen Medium (LJ)

Q5: Describe its appearance on the media?

Colonies or growth is rough, tough and buff

Q6: How would you confirm the causative organism?

- (1) Cell count → it will show lymphocytosis
- (2) Protein → it will show slight elevation
- (3) Glucose → it will show slight reduction
- (4) Gram stain (Negative) & Z-N stains (Positive for AFB)
- (5) Culture: LJ culture (for TB)
- (6) PCR

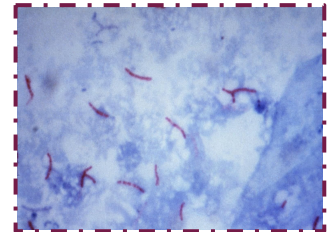
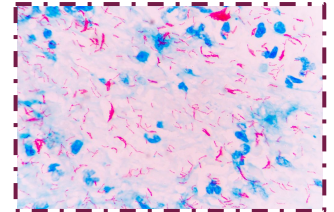
Q7: What is the appropriate treatment for this patient and for how long?

For the first 2 months: Rifampicin + Isoniazid (INH) + Ethambutol + Pyrazinamide

For the next 4-6 months: Rifampicin + INH

Q8: What are some expected complications of this condition? (theoretical)

Hydrocephalus and cranial nerve deficits



CASE4: A 1 week old boy was brought to the emergency department. His mom is complaining that he has fever, and that he was very irritable and he kept vomiting for the past three days. (oral temperature of 39°C) and other vital signs were as follows: systolic blood pressure of 160 mmHg, diastolic blood pressure of 90 mmHg, heart rate of 110 beats per minute, and respiratory rate of 16 per minute. CSF analysis revealed WBCs to be (7,837 cells/mm³ mainly polymorphonuclear leukocytes (84%) Normal is <5) Protein is 4.1 (normal 0.1-0.4 g/L) Glucose 1.5 (normal 3.0-4.5 mmol/L) Gram stain is shown in the pictures.

Q1: What is the most likely diagnosis?

Acute Pyogenic Meningitis

Q2: Describe the microorganism's appearance under the microscope?

Gram negative intracellular bean-shaped diplococci WITH pus cells

Q3: What is the most likely causative agent?

Neisseria Meningitidis

Q4: Name the media used for growing such organism?

Thayer-Martin agar or Chocolate agar

Q6: How would you confirm the causative organism?

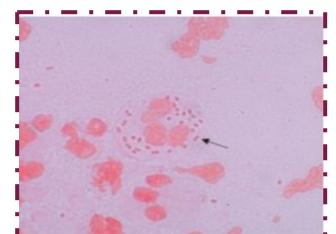
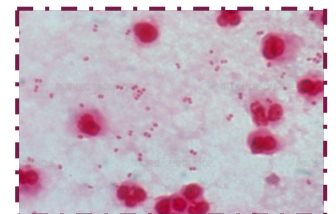
Maltose and glucose utilization/fermentation test

Q6: What is the appropriate treatment for this patient and for how long?

Ampicillin + Gentamicin + Cefotaxime for 10-14 days (neonate)

Q7: What is the stereotype of this organism that has a pandemic potential? (theoretical)

Type A (highly prevalent in african countries of meningitis belt)



Members Board

Team Leaders






Muneerah Alsadhan



Abdurahman Addweesh

Team Members

- Abdulaziz Alderaywsh
- Abdulrahman Alswat
- Albandari Alanazi
- Faisal Alotaibi
-  Ibraheem Altamimi
- Leen Almadhyani
- Mayasem Alhazmi
- Meshal Alhamed
- Meshal Althunian

- Mohammed Beyari
- Mona Alomiriny
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