CNS Practical microbiology

THIRD YEAR, FACULTY OF MEDICINE, HASHEMITE UNIVERSITY

DR. JUMANA ABBADI

CNS specimens

- There are many specimens that can be taken from the central nervous system; cerebrospinal fluid (CSF), **brain abscess aspirates** or tissue biopsy samples. However, **CSF** is by far the most important, easily obtained sample by lumbar puncture.
- The role of **brain biopsy** in the diagnosis of encephalitis has declined since the advent of PCR testing in CSF. However, it still has a place for patients in whom diagnosis has not been obtained following extensive investigation, particularly if there are focal abnormalities on imaging (tumors).
- **Serum** samples can be obtained to help detect the cause of CNS infection.

Serum/blood samples

- A **serum serology sample** can help in the diagnosis of certain CNS invading microorganisms:
  - Mumps-specific IgM or IgG antibody titers from sera specimens.
  - Serologic testing for measles-specific IgM or IgG titers
  - Herpes simplex and Varicella-zoster (VZ) antibodies
  - Neurosyphilis (VDRL/RPR in serum, detection of antibodies by fluorescent treponemal antibody absorption (FTA-ABS) test)
- **Blood culture** can help aid the diagnosis, since some bacteria may circulate the blood during CNS infection (bacteremia). A blood culture can support the result of CSF culture.

CSF sampling

- Lumbar puncture
  1. Clean the puncture site with antiseptic solution and alcohol before needle insertion to prevent introduction of infection.
  2. Insert a needle at the L3-L4, L4-L5, or L5-S1 interspace.
  3. When the subarachnoid space is reached, the spinal fluid will appear in the needle hub.
  4. Slowly drain the CSF into the sterile leakproof tubes.
  5. Three tubes are generally required for microbiology, hematology, and chemistry testing.
  6. The second tube drawn will generally go to microbiology, and the last tube drawn will generally go to hematology.

- Contraindications of lumbar puncture:
  - Possible raised intracranial pressure (ICP) with risk for cerebral herniation
  - Thrombocytopenia or other bleeding diathesis, including ongoing anticoagulant therapy
  - Suspected spinal epidural abscess
- LP is a relatively safe procedure, but minor and major complications can occur.
  - Post-LP headache
  - Infection
  - Bleeding
  - Cerebral herniation
  - Minor neurologic symptoms such as radicular pain or numbness
  - Back pain

CSF macroscopic examination

- Normal CSF appearance is clear and colorless
- Macroscopic examination after LP:
  - Color (bloody due to hemorrhage or traumatic tap)
  - Clarity (turbidity is nonspecific but indicates the presence of an underlying pathology or purulent bacterial meningitis)
  - Viscosity (thick in severe purulent or cryptococcal meningitis)
  - Presence of clots (elevated proteins due to TB meningitis)

CSF sample analysis

- Chemistry tube:
  - To measure glucose and protein
  - Electrophoresis (to detect IgG band in multiple sclerosis)
- Hematology tube is for cell count and differential (RBCs, WBCs, etc)

CSF Analysis

TABLE S–19	Findings of Cerebrospinal Fluid Analysis: Normal versus Infection				
CLINICAL SITUATION	**LEUKOCYTES/ MM³**	**% POLYMORPHONUCLEARS**	**GLUCOSE (% OF BLOOD)**	**PROTEIN (MG/D)**	Appearance
Children and adults					
Normal	0-5	0	≥60	≤30	Clear
Viral infection	2-2000	≤50 mainly lymphocytes	≥60	30-80	Clear
Pyogenic bacterial infection	5-5000	≥60 mainly neutrophils	≤45[b]	>60	Cloudy
Tuberculosis and mycoses	5-2000	≤50 mainly lymphocytes	≤45	>60	Cloudy
Neonates					
Normal (term)	0-32	≤60	≥60	20-170 (90)	
Normal (preterm)	0-29	≤60	≥60	65-150 (115)	

[b]Usually very low.

- Microbiology tube:
  - Direct wet mount preparation (detects the presence of *Candida*, *Amoeba* and/or *Cryptococcus species*)
  - Gram stain (gram positive or negative, Cocci or bacilli).
  - Acid fast stains (for detection of *Mycobacterium tuberculosis*).
  - India ink stain (to detect the presence of *Cryptococcus neoformans*)
  - CSF culture and sensitivity for bacteria and fungi.
  - CSF culture for viruses.
  - CSF Serological tests
  - Rapid antigen detection tests
  - Molecular diagnosis (CSF PCR testing; to detect DNA/RNA)

CSF Culture and sensitivity

- CSF viral, bacterial (including mycobacterial) and fungal cultures remain the main stay in the diagnosis of infectious meningitis.
- Culture on routine media such as blood, chocolate and MacConkey agars.
- Culture on specific media such as Thayer-Martin agar, Lowenstein-Jensen medium for TB, SDA medium for fungal causes.
- Sensitivity tests done to identify the appropriate antimicrobial therapy and need up to 72 hours for results to be obtained.
- **CSF bacterial culture** requires up to 72 hours for final identification.
- **CSF fungal culture** needs up to 14 days for proper final identification.
- A recent study reported that **CSF mycobacterial culture** had a sensitivity of 22% and a specificity of 100% in diagnosis of tuberculosis meningitis.

- **CSF for viral culture:**
  - The virus for which culture remains most uniquely useful is HSV. Cell culture is also sometimes applied to the detection of CMV, VZV and the enteroviruses. It can also be used to detect measles, rubella, and mumps viruses. However, due to the long time and low sensitivity, CSF viral culture is often unable to provide the timely diagnosis required for optimum patient management and the rapid tests (PCR) are gradually replacing viral culture.
  - Because no one cell culture type can support the growth of all medically relevant viruses, virology laboratories must maintain several different cell culture types. The minimum requirements are a primary monkey kidney cell line, a human fibroblast line and a continuous human epithelial cell line such as HEp-2.

- Growth of viruses in cell culture is usually detected by visualizing morphological changes in the cells, known as cytopathic effect (CPE). The characteristics of the CPE are often sufficiently distinctive to allow the laboratory to be suspicious of which virus is responsible.
- Viral cultures usually take a long time to grow. The time required to detect CPE varies from 1–2 days after inoculation for herpes simplex virus (HSV) to 1–3 weeks for CMV. **Enteroviruses** are the easiest viruses to culture with 75% sensitivity and changes appear between 3 to 8 days.
- Newer culture methods have been developed that detect viruses faster than ordinary culture methods such as shell vial culture.

CSF serology

- Definitive serological diagnosis of CNS infections is established by detecting IgM antibodies or demonstrating at least a fourfold increase in neutralizing antibody titers between acute- and convalescent-phase CSF.
- In general, due to delay in antibody response after symptom onset, a negative antibody test cannot be used to rule out infections and retesting may be required.
- In most circumstances nucleic acid amplification tests have surpassed antibody-based detection as the test of choice.
- These assays still have a valuable role in:
  - CSF IgM is the most widely used test for West Nile virus (WNV) infections; antibody may appear as early as 3 days and persist for up to 3 months.
  - Tests used for screening and diagnosis of neurosyphilis. Neurosyphilis can be confirmed by a positive CSF venereal disease research laboratory (VDRL) test.
  - Detection of antibodies to varicella zoster virus (VZV) IgG in CSF.

Rapid antigen detection

- Among the antigen assays for CNS infections, *Cryptococcal* antigen is the most widely used. The test relies on detection of *Cryptococcus* capsular polysaccharide antigens in CSF.
- Detection of galactomannan (GM) antigen and (1,3)-β-D-glucan (BDG) in CSF can aid in the diagnosis of CNS aspergillosis or other invasive fungal infections
- For acute bacterial meningitis, a rapid antigen assay is available to detect *Hemophilus influenza*, pneumococcal and meningococcal capsular antigens.

Molecular diagnosis of CNS infections

- Because of higher sensitivity and specificity, nucleic acid in vitro amplification based molecular techniques are now widely used.
- Molecular methods have dramatically improved the ability to diagnose CNS infections in a reasonable and effective time frame.
- Divided into 2 types:
  - Monoplex assays.
  - Multiplex assays.

- **Monoplex Assays:**
  - One of the first molecular assays used successfully for CNS infection diagnosis was for detection of HSV in cerebrospinal fluid (CSF). PCR quickly became the test of choice when studies demonstrated that CSF PCR was equivalent to culture of brain tissue for diagnosis of HSV encephalitis and meningitis.
- **Multiplex assays:**
  - Several multiplex PCR assays have been developed to identify bacterial pathogens in CSF targeting the most common causes of meningitis: *S. pneumoniae*, *N. meningitis*, *H. influenzae*, *L. monocytogenes*, *S. agalactiae*, *S. aureus*, *E. coli*, and *M. pneumoniae*.
  - Another multiplex PCR can simultaneously detect eight bacterial and viral pathogens in CSF including *N. meningitis*, *S. pneumoniae*, *E. coli*, *S. aureus*, *L. monocytogenes*, *S. agalactiae*, HSV-1/2, and VZV.
  - The BioFire FilmArray Meningitis/Encephalitis panel is currently the only FDA cleared multiplex assay for the detection of **six bacterial, seven viral** (cytomegalovirus, enterovirus, HSV-1, HSV-2, human herpesvirus 6 (HHV-6), human parechovirus and VZV), and **single fungal** (*Cryptococcus neoformans*) target in CSF

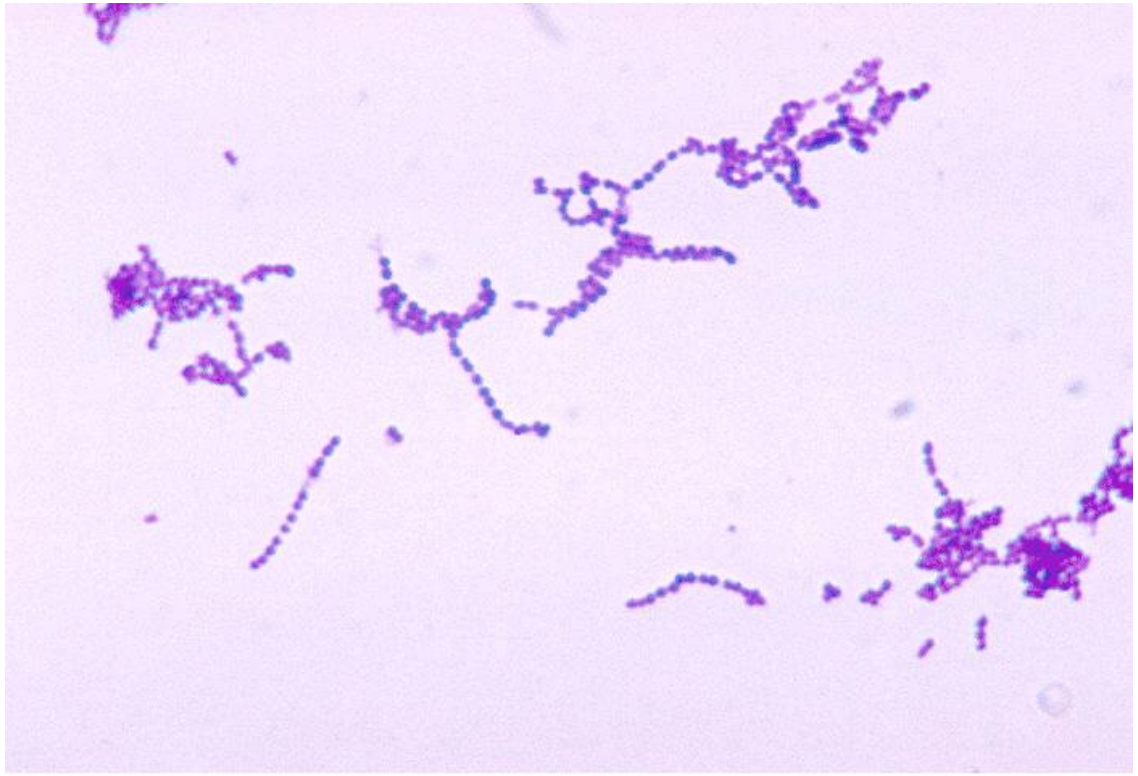
Major CNS pathogens

- Group B *Streptococcus*
- *Streptococcus pneumonia*
- *Neisseria meningitidis*
- *Hemophilus influenza*
- *Listeria monocytogenes*
- *Staphylococcus aureus*
- Gram negative rods (enterobacteriaceae; *E. coli*, *Klebsiella spp.*, etc.…)
- *Mycobacterium tuberculosis*
- Fungal pathogens

Group B *Streptococcus or S. agalactiae*

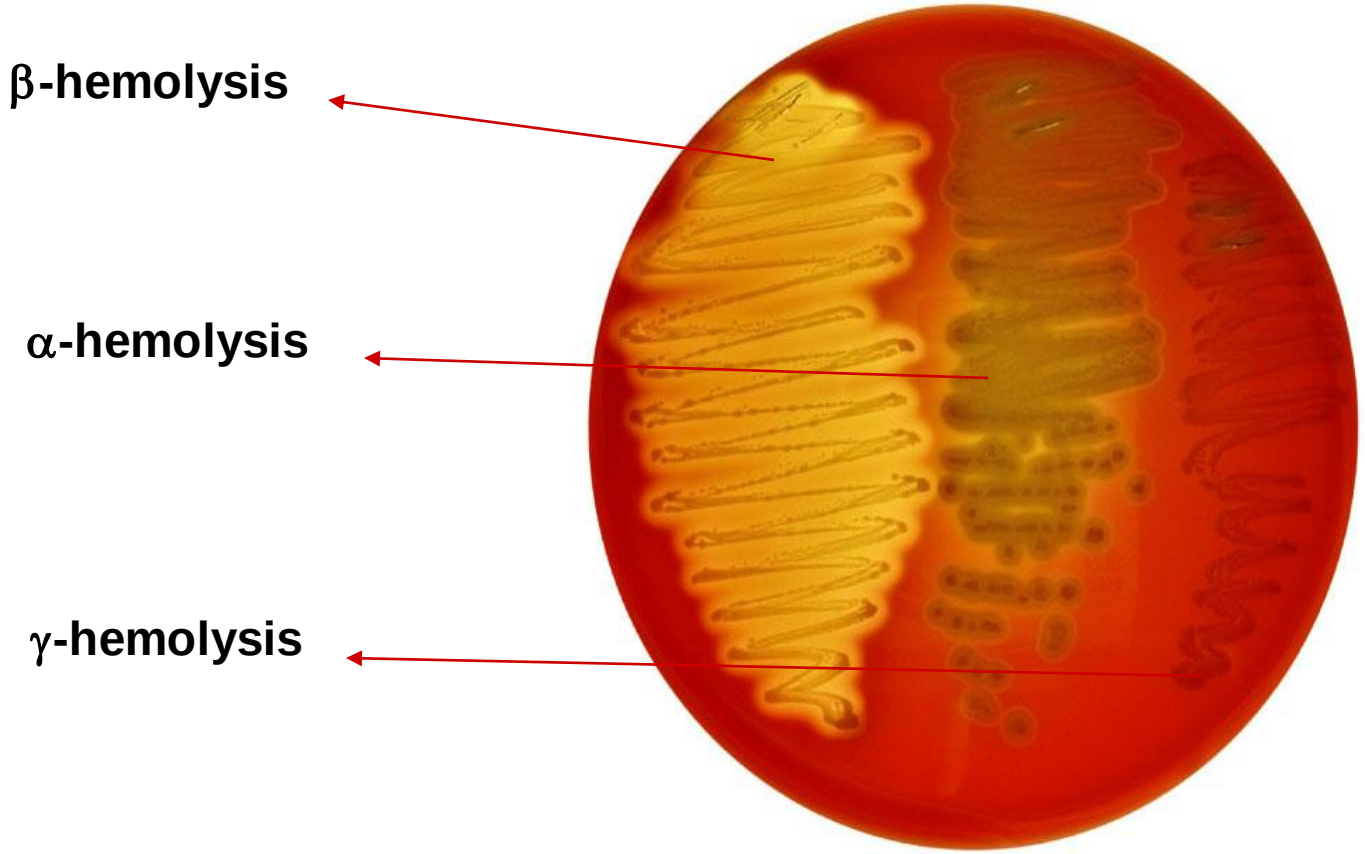
- *S. agalactiae* causes meningitis and septicemia among newborns. Neonates become infected during delivery through maternal genital tracts colonized by GBS.
- Characteristics:
  - Gram positive cocci in chains.
  - Catalase negative
  - Gray-white colonies on blood agar.
  - Produce a narrow zone of beta hemolysis on blood agar. Sometimes we must remove the colony aside to see the beta hemolytic zone below it.
  - Bacitracin resistant
  - CAMP test positive

S. agalactiae gram stain



Streptococcus sp., Gram stain. (Credit: CDC, PHIL.)

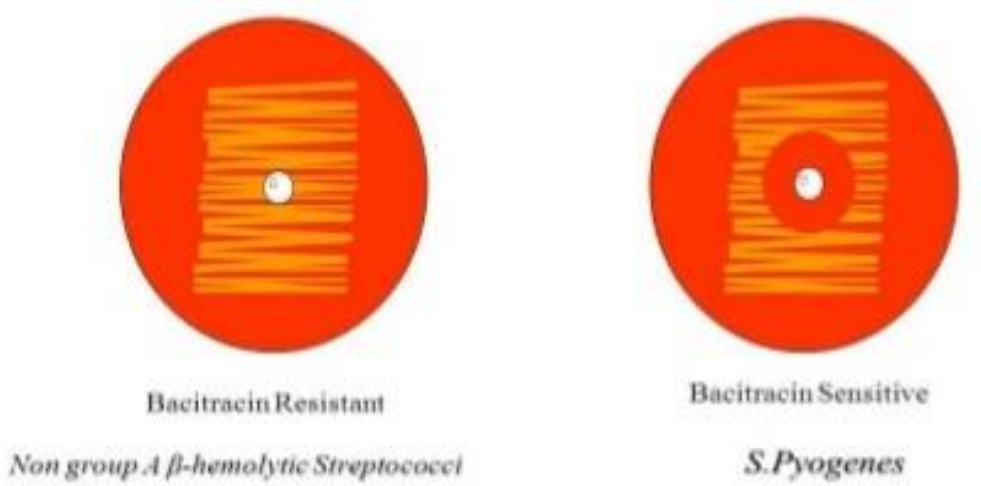
Types of hemolysis on blood agar



S. agalactiae on blood agar

Bacitracin test

- The traditional method of differentiating *S. agalactiae* from *S. pyogenes* is the use of **bacitracin sensitivity test**. A bacitracin (antibiotic) disk is used, and if no zone of inhibition is seen, the bacteria is considered resistant to Bacitracin, for example, *S. agalactiae*. If any zone of inhibition of growth is observed the test is considered positive; *S. pyogenes*.



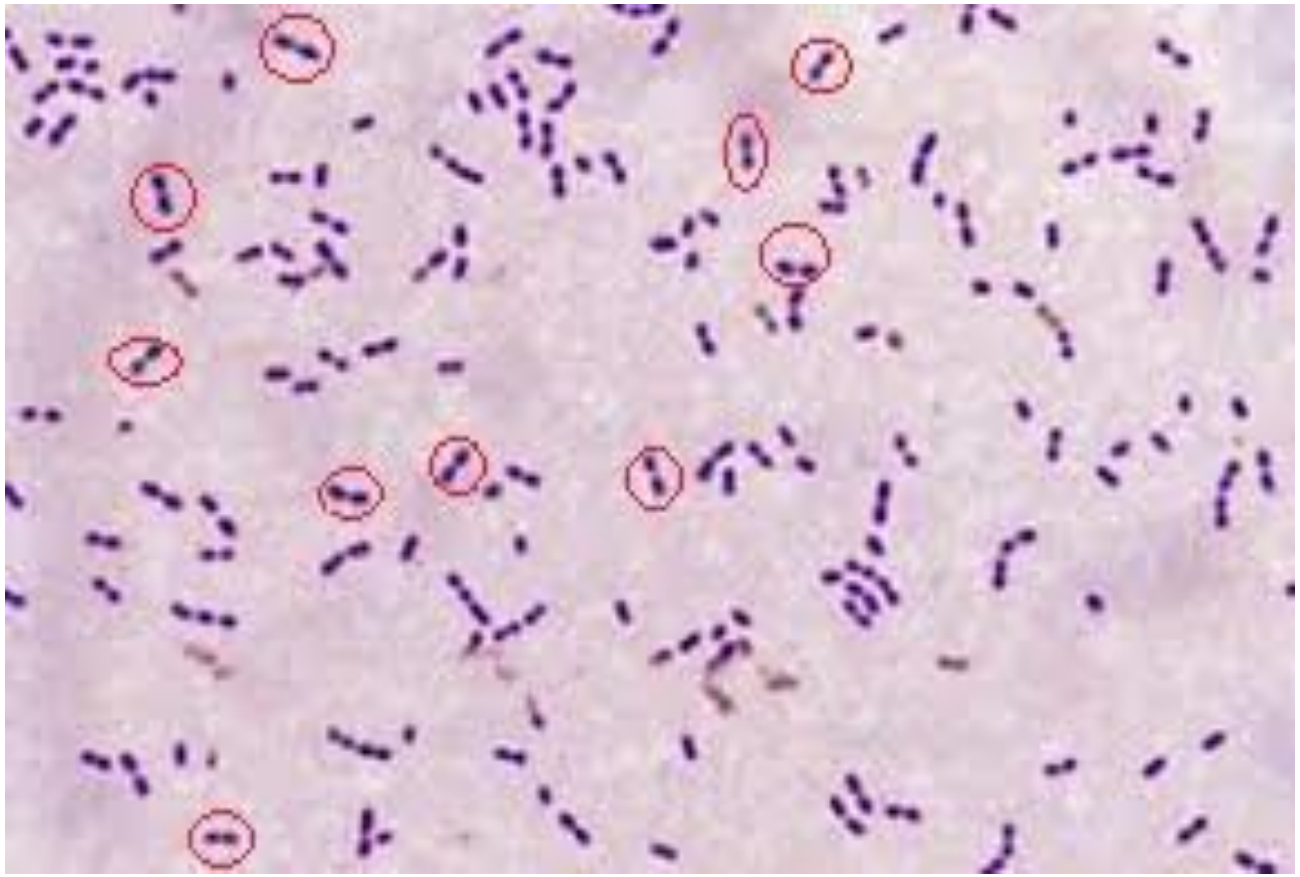
CAMP test

- The CAMP factor is a diffusible extracellular protein that acts synergistically with staphylococcal-lysin to cause **enhanced lysis** of red blood cells.
- A blood agar plate is inoculated by making perpendicular streaks of a lysin producing strain of Staphylococcus aureus and the organism to be tested.
- An **arrowhead-shaped zone** of enhanced hemolysis in the area into which both the lysin and the CAMP factor have diffused represents a positive test result. *S. agalactiae* gives a **positive** test result.

Streptococcus pneumonia

- *Streptococcus pneumonia* is also a major cause of meningitis and ear and sinus infections.
- Characteristics:
  - Gram-positive cocci arranged in pairs or diplococci
  - Catalase negative.
  - Colonies are usually transparent, slightly mucoid, or flattened.
  - Alpha-hemolytic on blood agar.
  - Optochin susceptible.

S. pneumonia gram stain

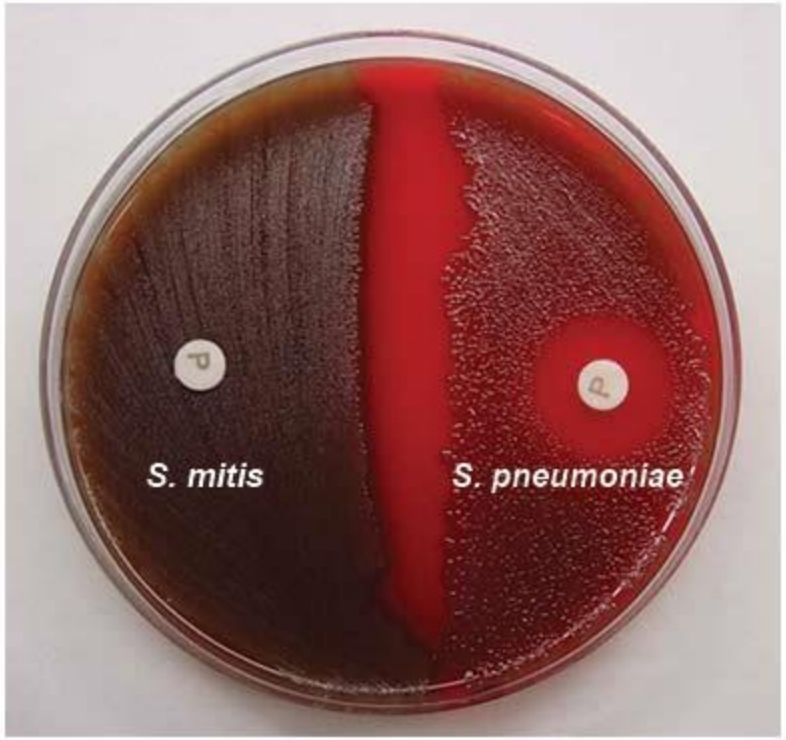


S. pneumonia on blood agar

Optochin sensitivity test

- Optochin is a chemical that kills *S. pneumonia* but does not kill other alpha-hemolytic streptococci. In this test, an Optochin impregnated paper disk is placed onto the surface of a blood agar plate that has previously been inoculated with the isolate.

- If the zone of no growth is **more than or equal to 16 mm** then the organism can be identified as *S. pneumonia.* If the isolate produces a smaller zone of no growth, then the isolate is not *S. pneumonia*.



Left Side

S. mitis

Resistant to optochin

Right Side

S. pneumoniae

Susceptible to optochin

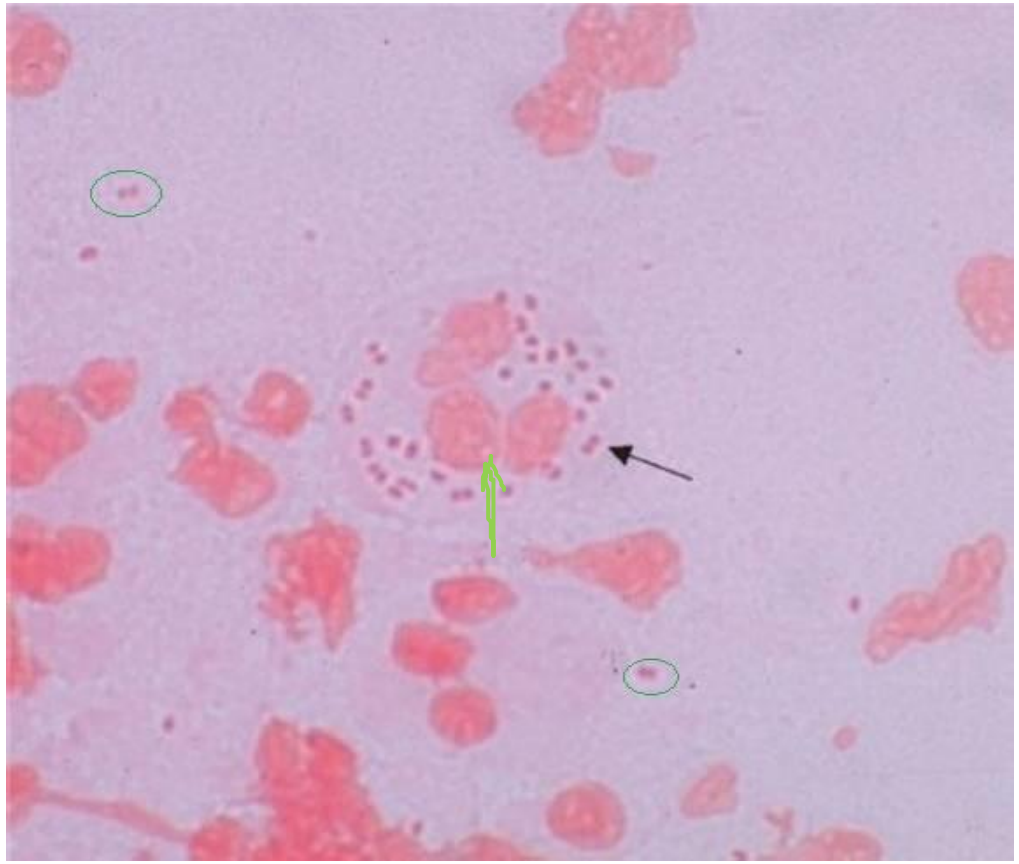
Neisseria meningitidis

- The usual manifestations of N. meningitidis disease are meningococcemia (*N. meningitidis* in the bloodstream), petechiae (tiny red spots on the skin), and meningitis.
- Characteristics:
  - A rapid presumptive diagnosis of meningococcal meningitis can be made when **leukocytes (WBCs)** and **Gram-negative diplococci** are observed in Gram-stained smears obtained from CSF specimens.
  - *N. meningitidis* colonies are gray, convex, and glistening, with a smooth, moist entire edge.
  - *N. meningitidis* will oxidize glucose and maltose.
  - *N. meningitidis* is oxidase positive.
  - *N. meningitidis* is catalase positive.

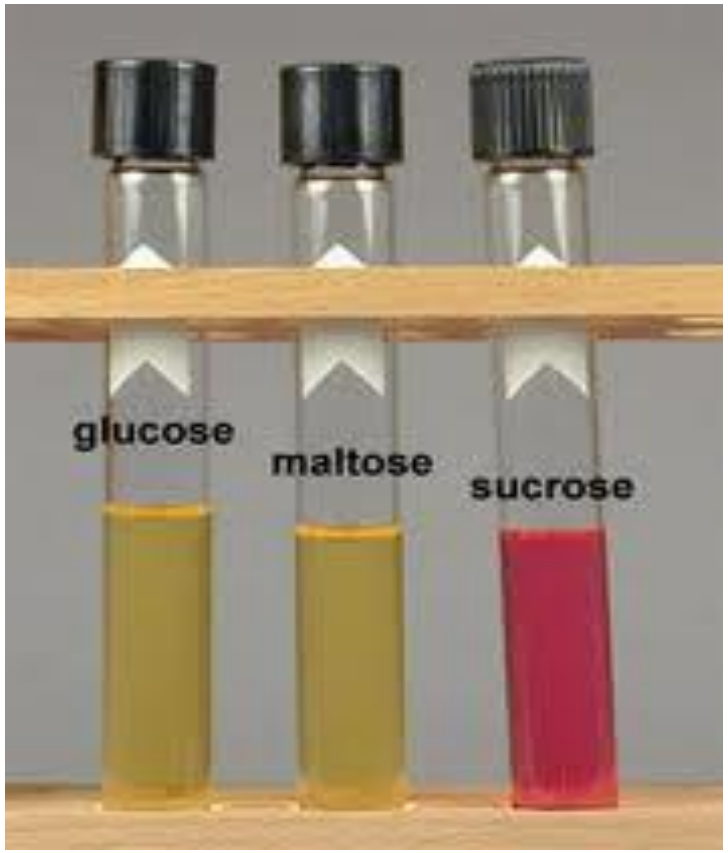
- Neisseria species are **capnophilic** (need a moist 5-7% CO_2 atmosphere) at 37 degrees Celsius.

- *Neisseria meningitidis* can grow on:
  - Blood agar
  - Chocolate agar
  - Selective media such as Thayer Martin agar, Martin Lewis agar, etc.

N. meningitidis gram stain



N. meningitidis colonies


glucose
maltose
sucrose


oxidase test
+

Hemophilus influenzae

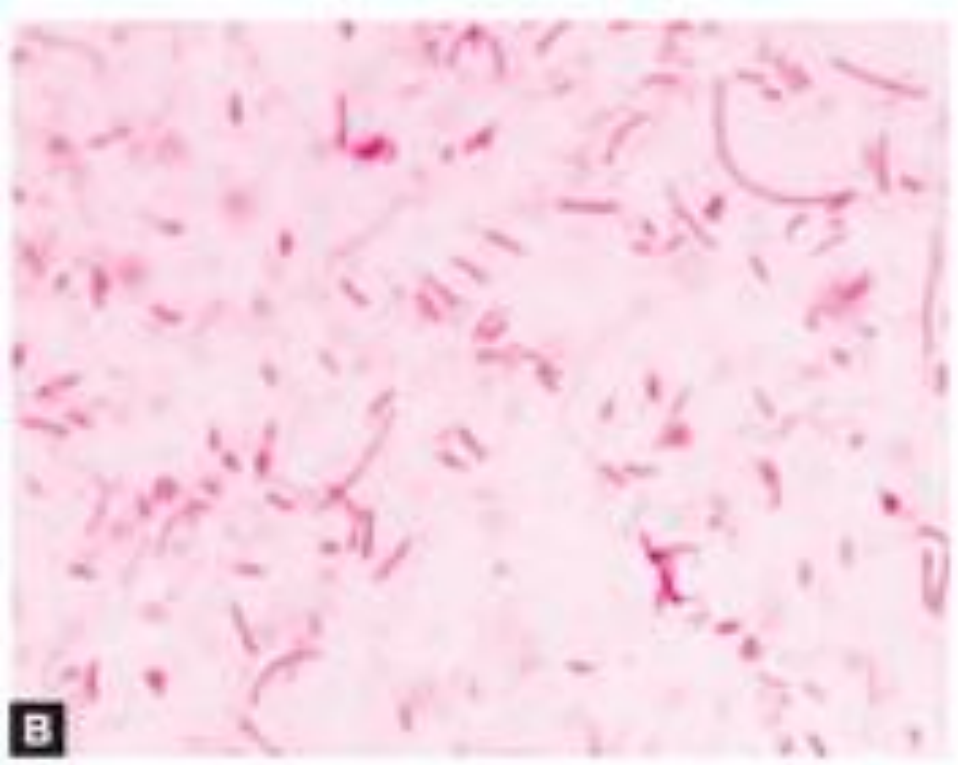
- *H. influenzae* is one of the three major causes of bacterial **meningitis** (*S. pneumonia and N. meningitides*).
- Characteristics:
  - Gram negative coccobacilli
  - **On chocolate agar:** *H. influenzae* colonies are grayish, semiopaque, smooth, and flat.
  - Oxidase and catalase positive

- In vitro growth of *H. influenzae* requires the culture medium to contain both:
  1. **Hemin**, referred to as X factor
  2. **Nicotinamide adenine dinucleotide** or NAD referred to as V factor.

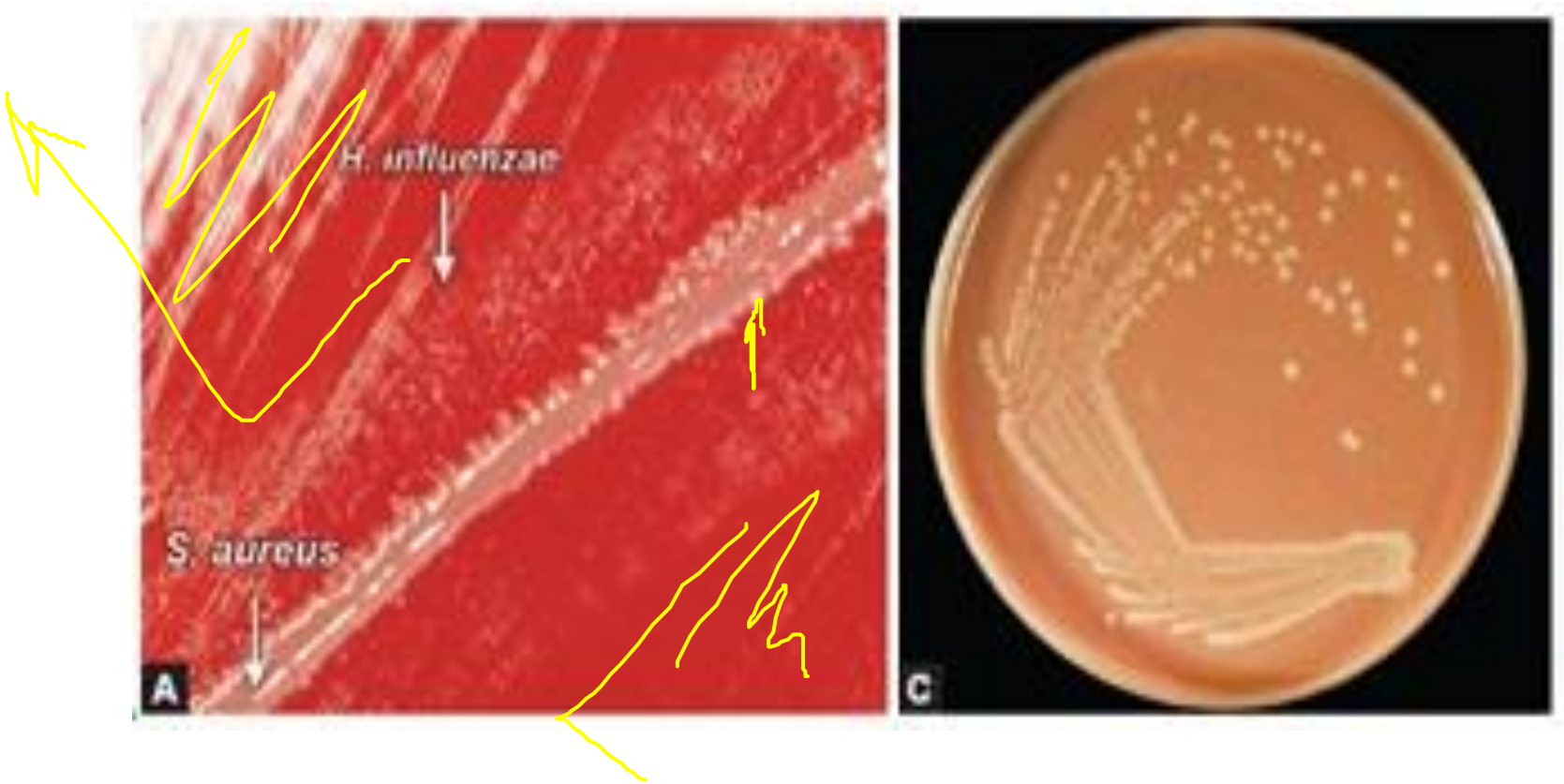
- Both X and V factors are present **inside** sheep red blood cells but *H. influenzae* will not grow on normal sheep blood agar. However, it will grow on **horse and rabbit blood agar** and **on chocolate agar;** because X and V factors are both present in this media.

- Sheep blood agar can be used to grow *H. influenzae* by the following method:
  - The inoculated sheep blood agar plate can be cross-streaked with a ***Staphylococcus*** **strain**. After lysing the sheep RBCs, V and X factors will be released into the medium.
  - *H. influenzae* will grow in the immediate vicinity of the *Staphylococcus* strain, a phenomenon called **satelliting**.

H. influenza gram stain



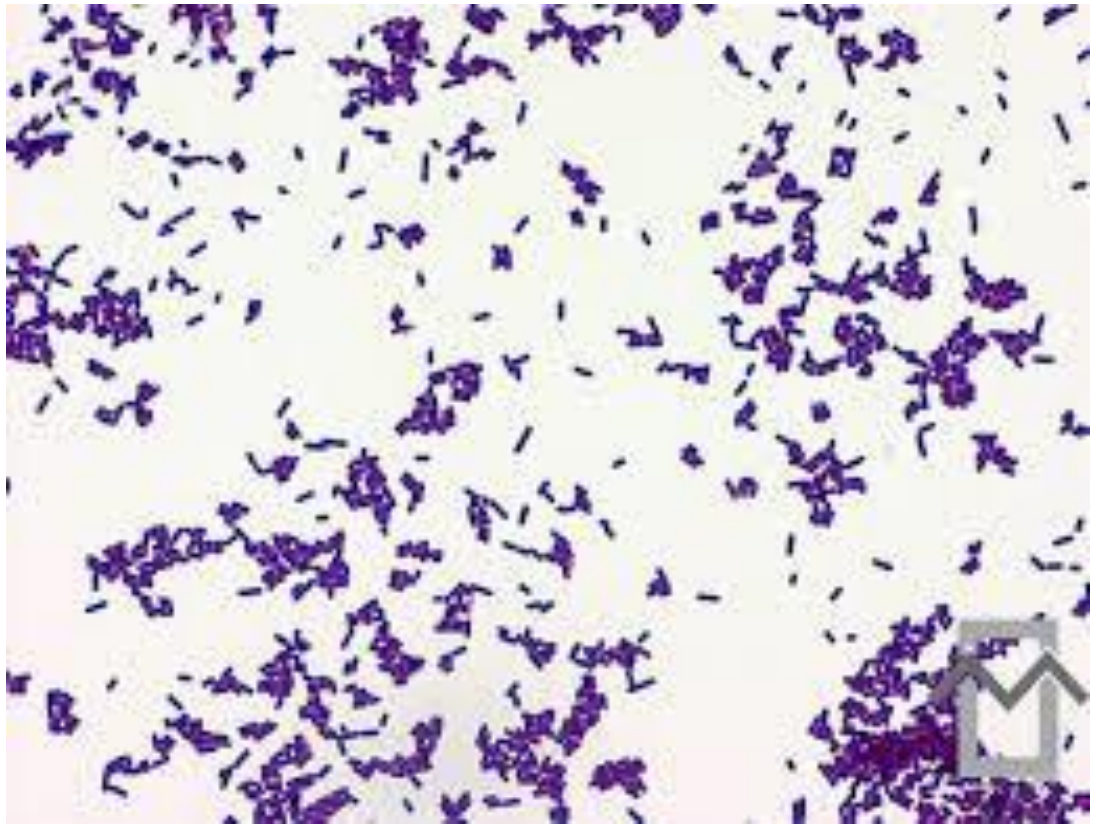
H. influenza colony morphology



Listeria monocytogenes

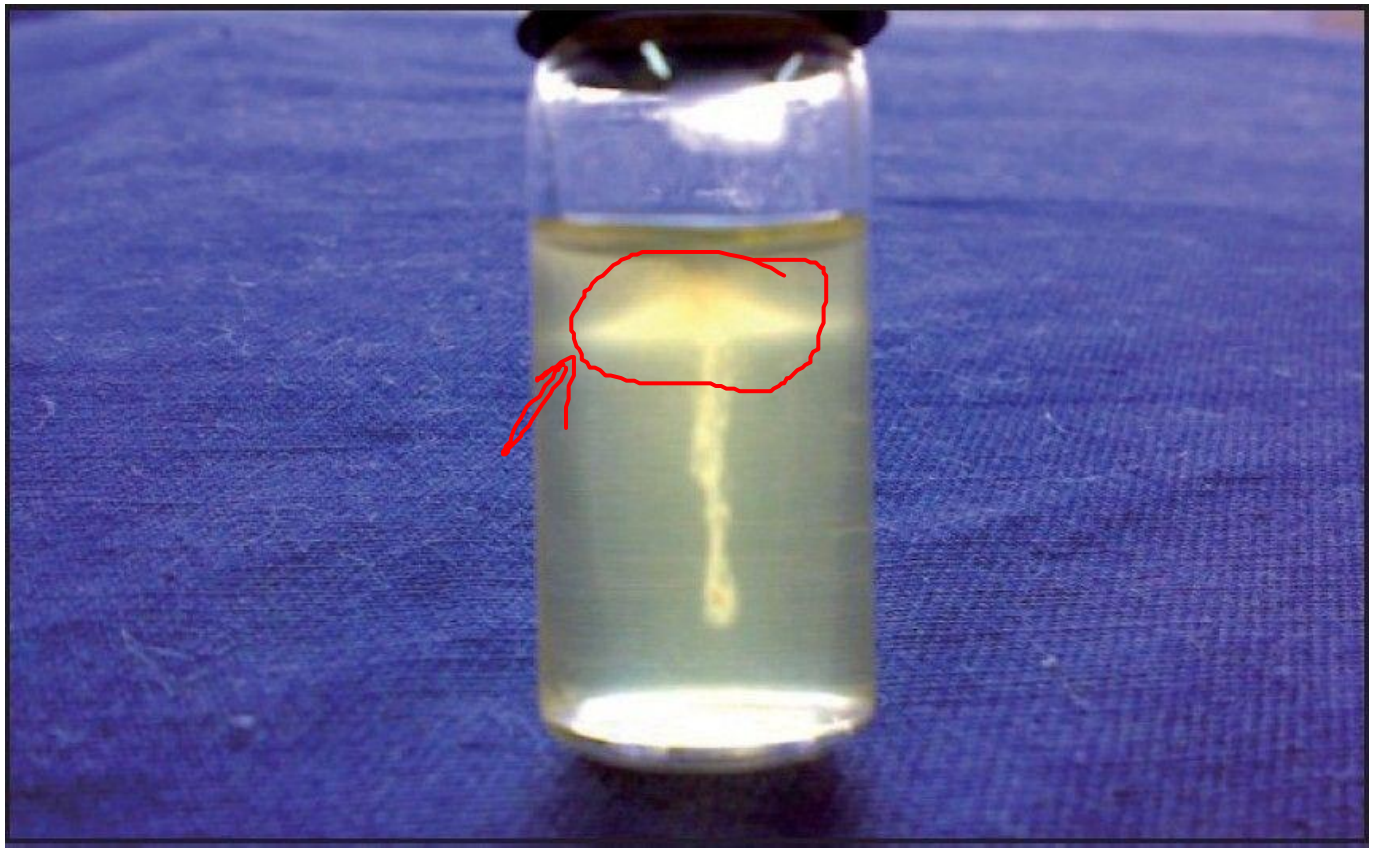
- *L. monocytogenes* is one of the major causes of neonatal meningitis.
- Characteristics:
  - Short, nonbranching Gram-positive bacilli on gram stain
  - Observation of tumbling motility in a wet mount (https://www.youtube.com/watch?v=bV_Wd7JCo6A)
  - A narrow zone of beta-hemolysis on blood agar, resembles growth of *Streptococci*.
  - Umbrella-shaped growth in semisolid agar medium.
  - Catalase positive.
  - Optimum growth at 30-37degrees but can grow at **4 degrees.**

L. monocytogenes gram stain



L. monocytogenes on blood agar

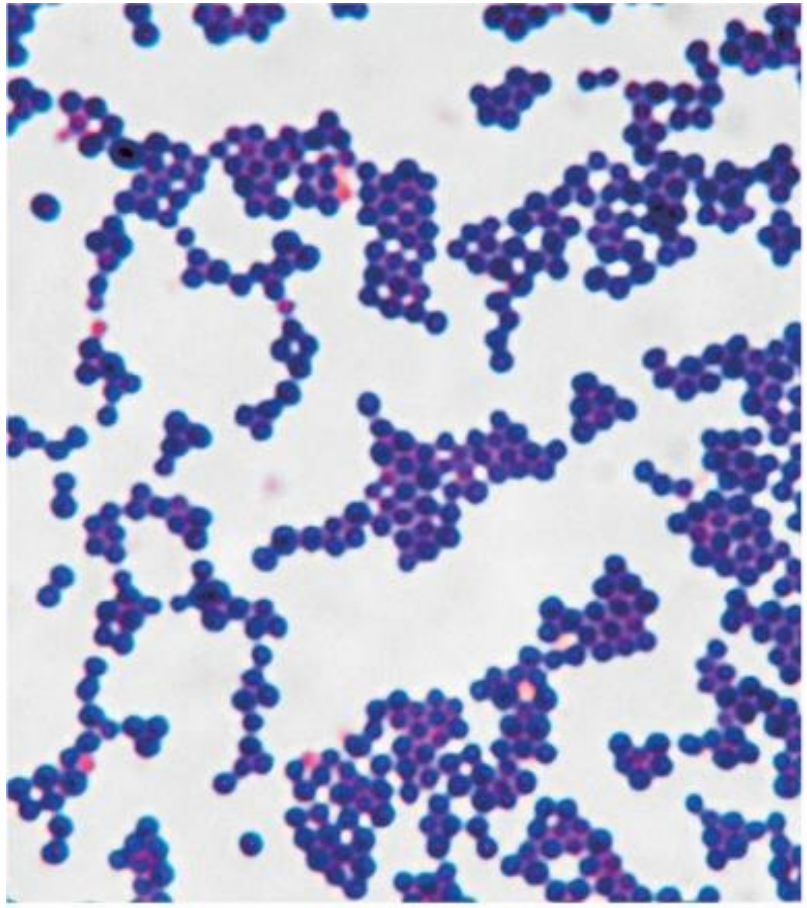
Umbrella shaped motility



Staphylococcus aureus

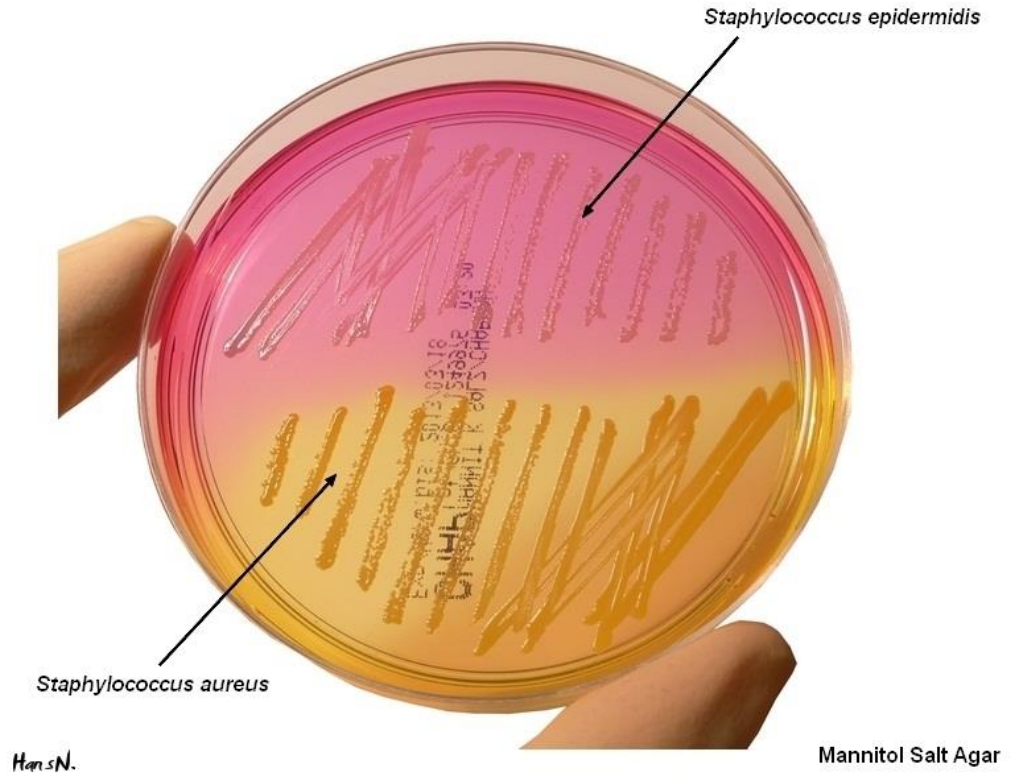
- It is also capable of causing meningitis.
- Characteristics:
  - Gram positive cocci in clusters
  - Colonies are large smooth, slightly raised, translucent, and pigmented. They may be off-white, gray, or yellow.
  - Colonies are often surrounded by a wide zone of beta-hemolysis.
  - Catalase positive
  - Coagulase positive
  - Mannitol fermenters

S. aureus gram stain



S. aureus colony morphology

S. aureus mannitol salt agar

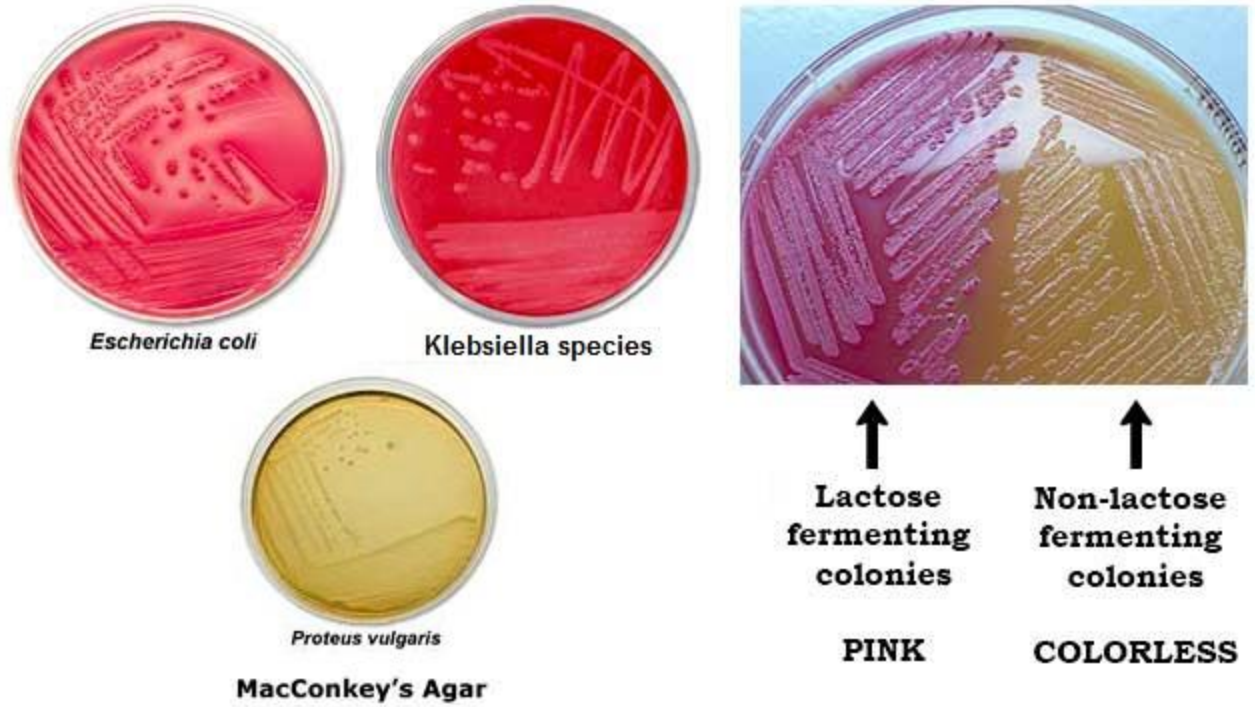


Enterobacteriaceae

- Some of the enterobacteriaceae can cause meningitis. Example: E. coli, Klebsiella pneumonia and *Proteus* species.
- Characteristics:
  - Gram negative bacilli on gram stain
  - Can grow on blood, chocolate and MacConkey agars each with a specific colony morphology
  - Catalase positive
  - Oxidase negative
  - *E. coli* and *Klebsiella species* are lactose fermenters that turn MacConkey agar into dark pink color.
  - All are motile except for *Klebsiella species.*

Enterobacteriaceae gram stain

MacConkey Agar



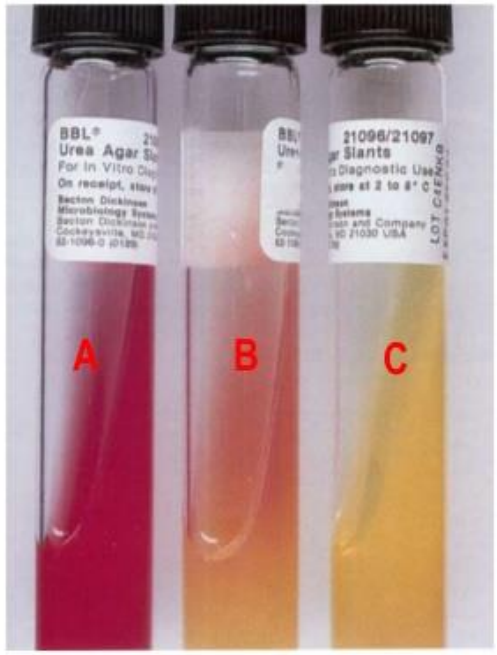
Biochemical tests

- Multiple biochemical tests must be done to differentiate the exact organism causing meningitis.

Gram reaction	Urease	Oxidase	Indole	Citrate	Lactose	Motility	Isolates
GNB	+	-	-	+	+	-	*Klebsiella* spp
GNB	+	-	V	+	-	+	*Proteus*
GNB	-	-	+	-	+	+	*Escherichia coli*

GNB= Gram negative bacilli; + = positive; - = negative

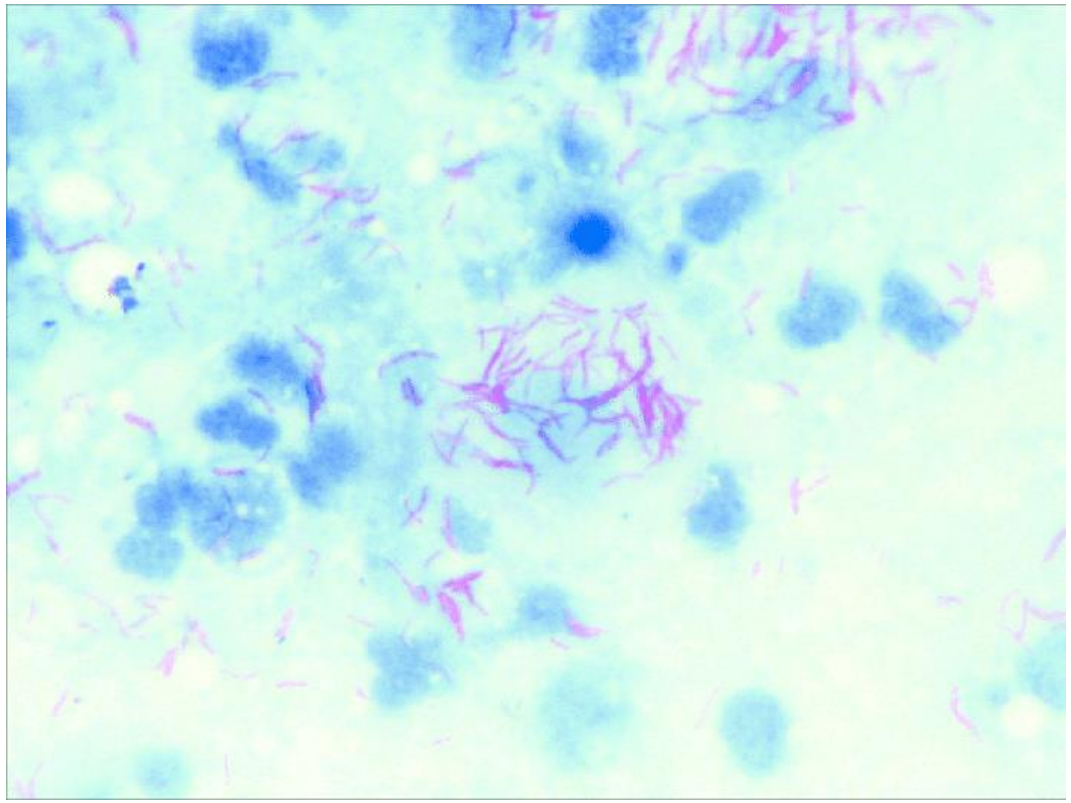
A. Positive: Proteus spp.
B. Positive: Klebsiella spp.
C. Negative: Escherichia coli



Urease test
(Christensens Urea agar)

Tuberculous meningitis

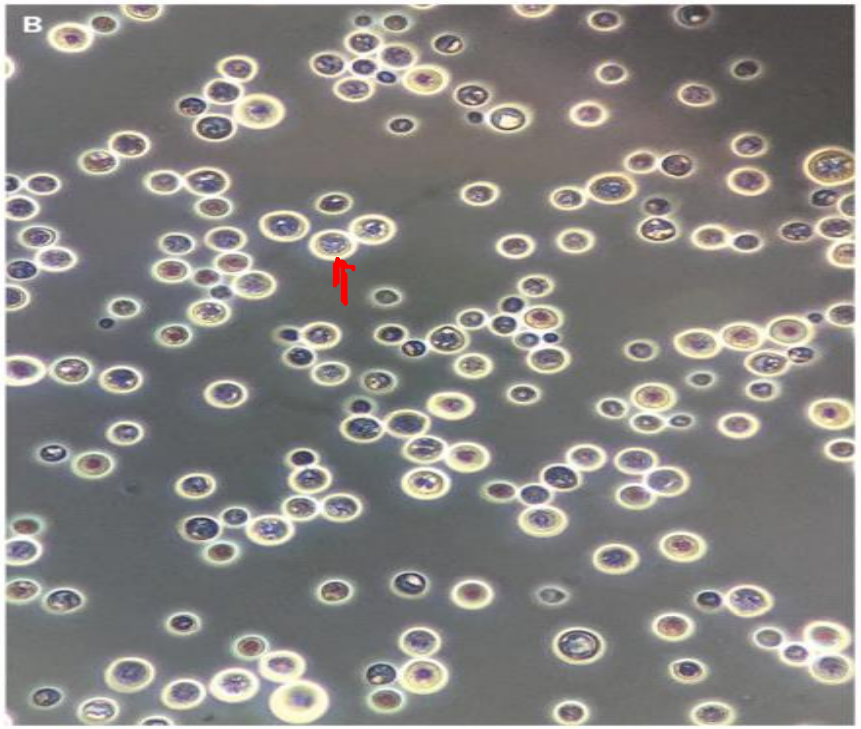
- Acid fast stain (positive for TB)

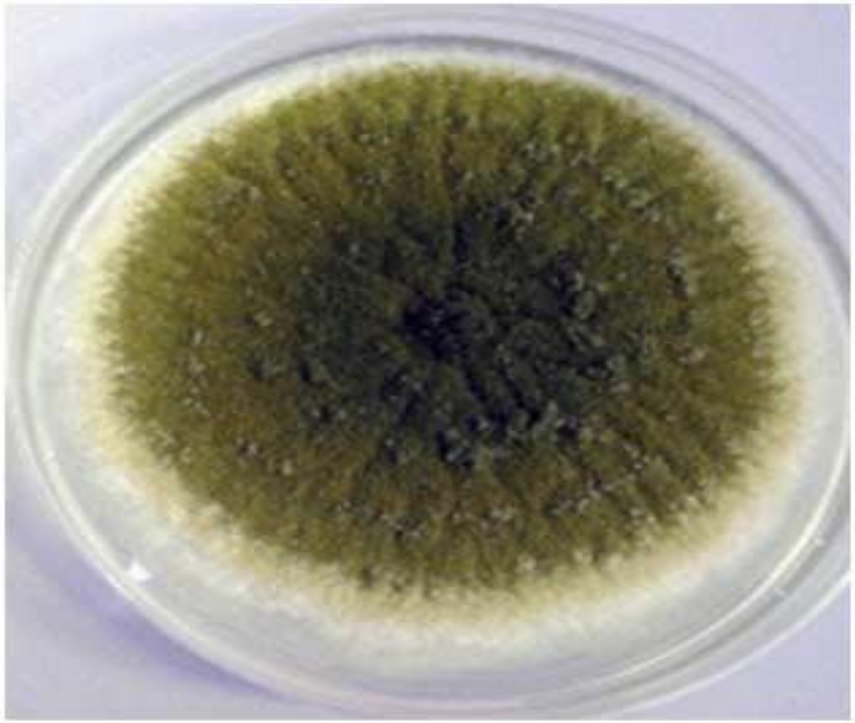


Lowenstein-Jensen medium for TB culture

Fungal tests

- Fungal cultures are done specific media for example Sabouraud Dextrose agar (SDA).
- India ink test for detection of *C. neoformans*.





Thank you ☺