Lecture-16 & 17: Meningitis and other infections of the central nervous system (CNS)

Diagnosis of bacterial brain abscess and Anaerobic infections:

Brain abscess is a serious and deadly clinical body. Pyogenic infection of brain parenchyma begins with a localized area of inflammatory change referred to as cerebritis. This early stage of infection has characterized by increased blood vessel **permeability** without angiogenesis. When unrecognized, this process will progress to an immature capsular stage and then to brain abscess, a condition defined by an area of parenchymal infection containing pus encapsulated by a vascularized membrane.

Anaerobic and microaerophilic cocci, gram-negative and gram-positive anaerobic bacilli were the predominating bacterial isolates. **Many brain abscesses have mixed bacterial infections**. The predominant organisms include: ***Staphylococcus aureus***, aerobic and anaerobic streptococci (especially ***Streptococcus intermedius*****),** ***Bacteroides***, and ***Fusobacterium*** species, **Enterobacteriaceae**, ***Pseudomonas*** species, and other anaerobes. Less common organisms include; *Haemophillus influenzae*, ***Streptococcus pneumoniae*** and ***Neisseria meningitides***. Also bacterial abscess caused by *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella* spp., *Proteus* spp., *Enterobacter* spp., *Bacteroides* spp. And *Propionibacterium* spp.

Cerebrospinal fluid (CSF) is a watery fluid, continuously produced and absorbed, which flows in the ventricles (cavities) within the brain and around the surface of the brain and spinal cord.

Functions of CSF:

- Hydrolic shock absorber
- Regulation of intracranial pressure

- Impacts the hunger sensation and eating behaviors

Bacterial infection of CSF cause **meningitis**, which ranks high among medical emergencies, and early, rapid, and exact diagnosis, is more essential. Diagnosis of meningitis depends on maintaining a high index of thought, obtaining **adequate specimens properly, and examining the specimens quickly.**

The most urgent diagnostic issue is the differentiation of acute purulent bacterial meningitis from aseptic (sterile) and granulomatous meningitis. The immediate **decision usually based on the cell count, the glucose concentration in CSF and blood and protein content of cerebrospinal fluid,** the results of **microscopic examination for microorganisms.** In addition, the results of **culture, serologic tests, nucleic acid amplification tests, and other laboratory procedures.**

Common Causes of Meningitis:

- Coagulase negative Staphylococci (especially *Staph. epidermidis*), *Staph. aureus*.
- Aerobic gram-negative bacilli, *Propionibacterium acnes*.
- Serogroup B streptococci *(Strep. agalactiae)* cause infection to neonates to age 3 months of age.
- *Escherichia coli* infect mainly neonates.
- *Listeria monocytogenes* also infect neonates; elderly; immunocompromised children
- *Haemophilus influenzae* infect children 6 months to 5 years
- *Neisseria meningitidis* infect all ages
- *Streptococcus pneumoniae* infect all age groups; highest incidence in the young age.

Specimens

As soon as infection of the central nervous system has suspected, **blood samples** has taken for culture and **cerebrospinal fluid** (**CSF**) has obtained. **To obtain cerebrospinal fluid, perform lumbar puncture with strict aseptic technique (Figure 1).**

Cerebrospinal fluid is usually collected in three to four portions of 2–5 ml each, in sterile tubes.

If bacterial meningitis has suspected, **CSF is the best clinical specimen** to use for isolation, identification, and characterization of the etiological agents. Suspected agents should include ***N. meningitidis, Strep. pneumoniae*, and *H. influenzae* and other pathogens in some cases.**

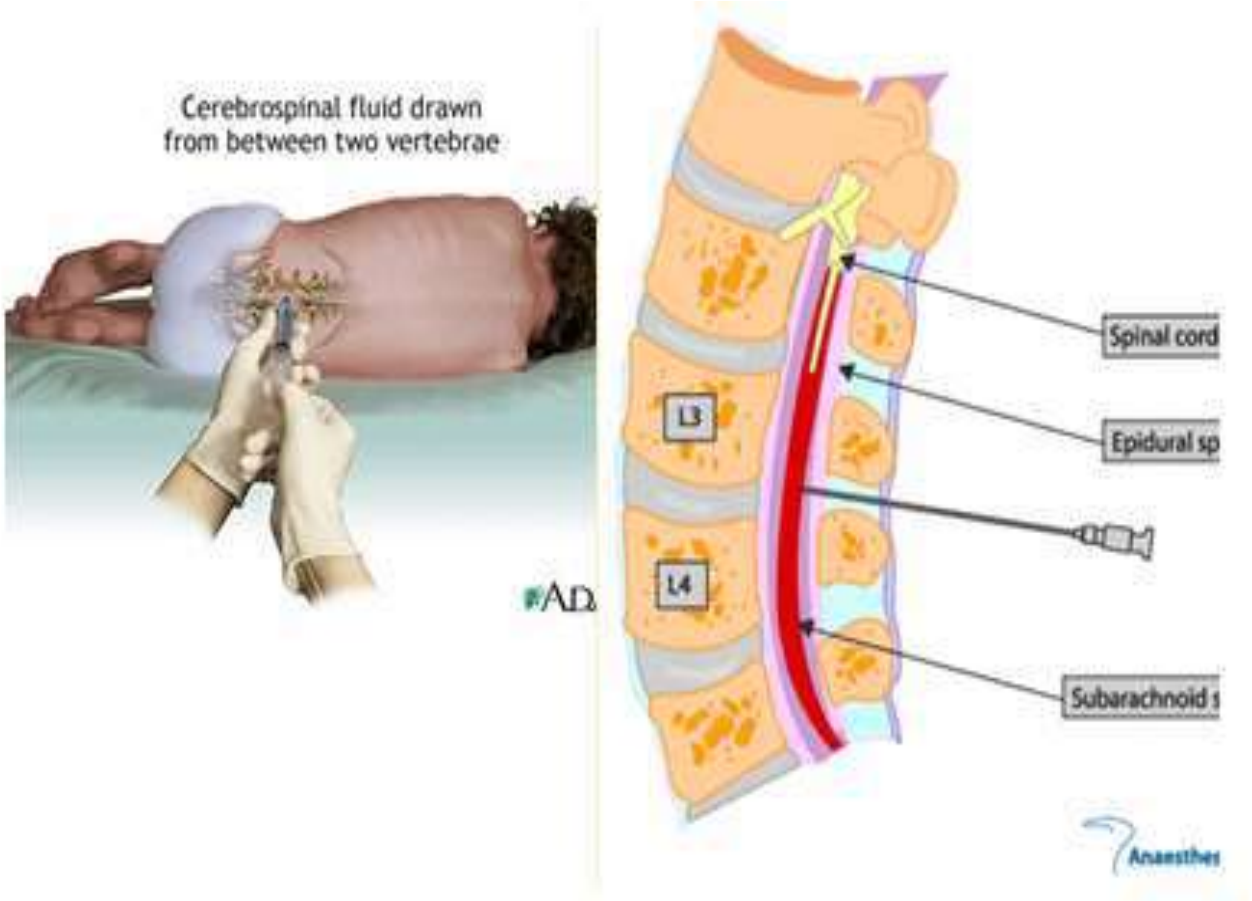


Figure (1): Collection of cerebrospinal fluid (CSF) by lumbar puncture.

Microscopic Examination

Smears have made from the sediment of centrifuged cerebrospinal fluid. Using a cytospin centrifuge to prepare the slides for staining has recommended because it concentrates cellular material and bacterial cells more effectively than standard centrifugation **(Figure 2).**

Smears have stained with Gram stain. Study of stained smears under the **oil immersion** objective may reveal **intracellular gram-negative diplococci (meningococci),**

extracellular lancet-shaped gram-positive diplococci (pneumococci), or small gram-negative rods (*Hemophilus influenzae* or enteric gram-negative rods).

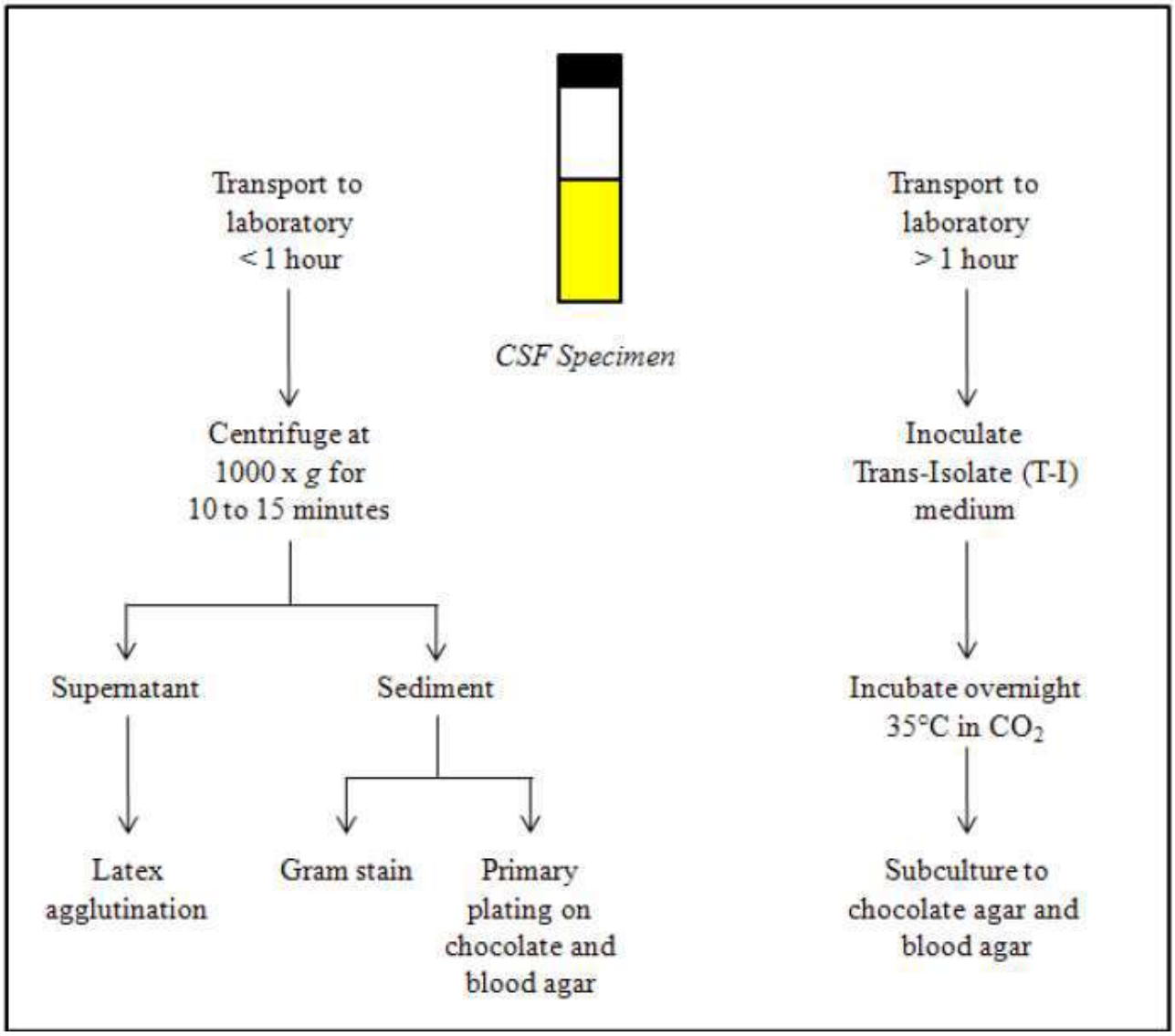


Figure (2): Cerebrospinal fluid (CSF) isolation and identification.

Culture

The culture methods used must help the growth of microorganisms most commonly encountered in meningitis. Sheep **blood and chocolate agar together** grow almost all bacteria that cause meningitis.

Follow-Up Examination of Cerebrospinal Fluid

The return of the cerebrospinal **fluid glucose level** and **cell count** toward normal is good evidence of adequate **diagnosis** and therapy.

Neisseria meningitids are; 1- **gram-negative. 2- coffee-bean shaped diplococci that may occur intracellularly or extracellularly in polymorphic nuclei (PMN) leukocytes. 3- (PMNs or neutrophils are often more than 1000 WBCs/cu mm). 4-** ***Neisseria meningitidis*** is a **fastidious organism, aerobic diplococci,** which **5-** grows best at 35-37°C with ~5% CO2 (or **in a candle-jar**). **6-** It can grow on both a blood agar plate (BAP) and chocolate agar plate (CAP). **7-** Colonies of *N. meningitidis* are grey and **unpigmented** on a BAP and appear round, smooth, moist, shiny, and convex, with a clearly defined edge. ***N. meningitidis*** appear as large, colorless to grey, opaque colonies on a CAP (Figure 3, 4).

Biochemical tests have recommended confirming the identity of cultures that morphologically appear to be ***N. meningitidis*** such as **8- oxidase test (+)** and **carbohydrate utilization (acid production from glucose, maltose).** If the oxidase test is positive, carbohydrate utilization testing should have performed. If the carbohydrate utilization test **indicates** that the isolate may be *N. meningitidis*, **9- serological tests** to identify the serogroup should performed. Additional methods for identification and characterization of ***N. meningitidis*** using molecular tools like **10-** PCR technique.

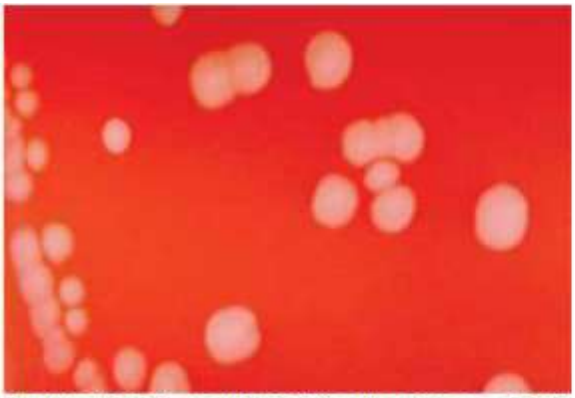


Figure (3): *N. meningitidis* colonies on a BAP

Figure (4): *N. meningitidis* colonies on a CAP

Streptococcus pneumoniae may occur **intracellularly** or **extracellularly** as gram positive diplococci, but can also occur as single cocci or in short chains of cocci. ***Strep. pneumoniae*** is a **fastidious** bacterium, **growing best at 35-37°C with ~5% CO2 (or in a candle-jar).** It is usually **culturing on media that contain blood**, but can **also grow on a chocolate agar plate (CAP).** On a blood agar plate (BAP), colonies of ***Strep.***

pneumoniae appear as **small, grey, moist** (sometimes **mucoid**), colonies and characteristically produce a zone of **alphahemolysis** (green) (**Figure 5**).

The alpha-**hemolytic property differentiates** this organism from many species, but not from the commensal **alpha-hemolytic (viridans)** streptococci. Differentiating pneumococci from viridans streptococci is **difficult** as young pneumococcal colonies appear raised, similar to viridans streptococci. However, once the pneumococcal **culture ages 24-48 hours, the colonies become flatten**, **and the central portion becomes depressed**, which **does not occur with viridans streptococci (Figure 6).**

For the identification and characterization procedures, it is essential to test alpha-hemolytic colonies that are less than a day old, typically grown overnight at 35-37°C with ~5% CO2 (or in a candle-jar).

The specialized tests have used to identify colonies on a BAP that resemble pneumococci (Figure 7). ***Strep. pneumoniae*** **can be identified using Gram stain, catalase (-), and optochin tests (see figure 8) (<14mm diameter) at the same time, with bile solubility (+) as a confirmatory test.** If these tests indicate that, the isolate is *Strep. pneumoniae*, then **serological tests used** to identify the serotype caught performed. This sequence of testing is an efficient way to save costly serotyping reagents and time. Additional methods for identification and characterization of ***Strep. pneumoniae*** using **molecular tools**.

Figure-5 : *Strep. pneumoniae* colonies with a surrounding green zone of alpha-hemolysis (black arrow) on a Blood Agar Plate.

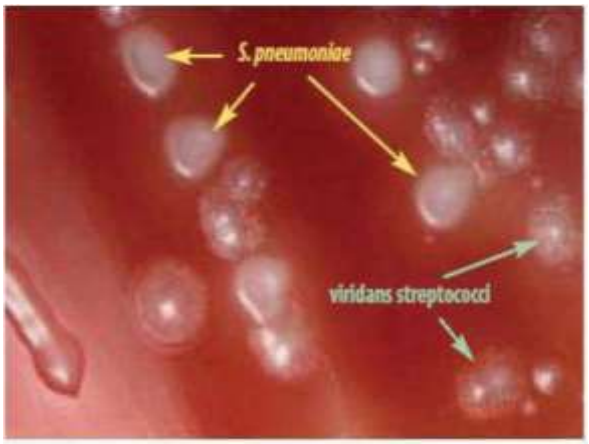


Figure-6 : *Strep. pneumoniae* colonies have a flattened and depressed center afte

24-48 hours of growth on BAP, whereas the viridans streptococci retain a raised center.

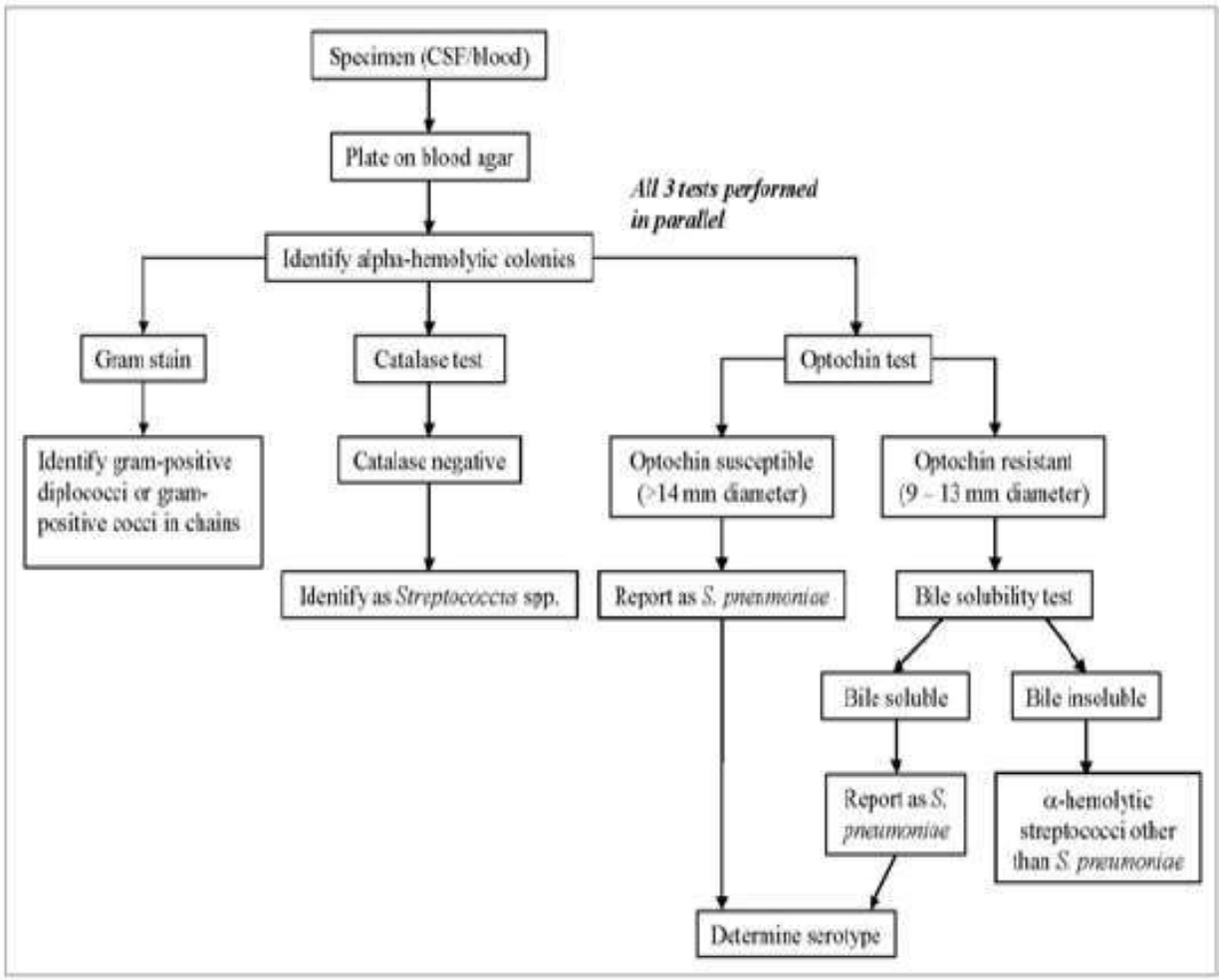


Figure (7): Flow chart for identification and characterization of a *Strep. pneumo* isolate.

Haemophilus Influenzae are small, pleomorphic, **gram-negative bacilli** or coccobacilli with random arrangements. ***H. influenzae*** is a fastidious organism, which grows best at 35-37°C with ~5% CO_2 (or in a candle-jar) and requires **hemin** (X factor) and **nicotinamide-adenine-dinucleotide** (NAD, also known as V factor) for growth.

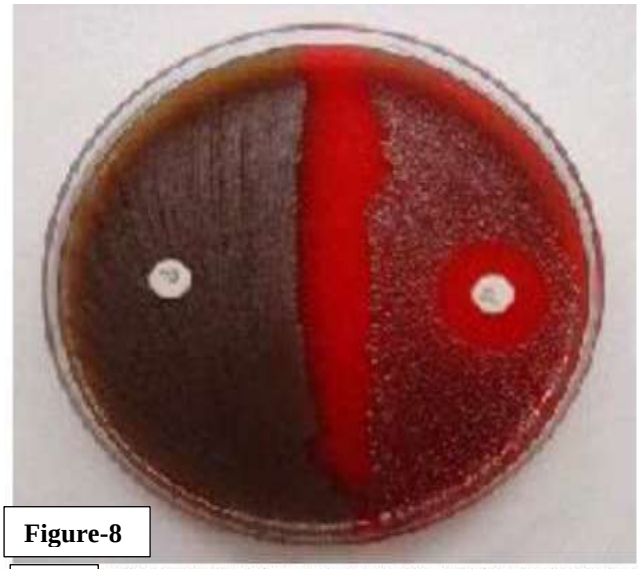
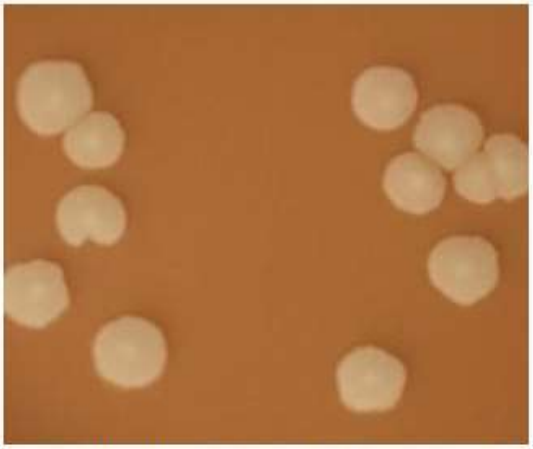


Figure-8

Optochin test for *Strep. pneumoniae* using optochin disks. The strain on the left is resistant to optochin with no zone of inhibition, and therefore is not a pneumococcus. The strain on the right is susceptible to optochin and is *Strep. pneumoniae*.

The standard medium used for growth of ***H. influenzae*** is a **chocolate agar plate (CAP),** which can be prepared with heat-lysed horse blood, a good source of both hemin and NAD, although sheep blood can also be used. Growth occurs on a CAP because NAD has released from the blood during the heating process of chocolate agar preparation and hemin is available from nonhemolyzed as well as hemolyzed blood cells. ***H. influenzae*** appear as **large, round, smooth, convex, colorless-to-grey, cloudy colonies on a CAP (Figure 9).** ***H. influenzae*** **produce a sharp indol smell,** plates should not be opened in order to smell the cultures. ***H. influenzae*** cannot grow on an unsupplemented Blood Agar Plate. **(Figure 10). Biochemical tests** have recommended confirming the identity of cultures that morphologically appear to be ***H. influenzae***. ***H. influenzae*** caught identified using **Kovac's oxidase** test and determining the necessity of hemin and **NAD as growth** requirements. If the **oxidase test is positive**, hemin and **NAD growth factor** requirement testing should **have performed.** If the growth factor requirement test indicates that the isolate may be ***H. influenzae*****, serological tests** to identify the serotype should have performed. This sequence of testing is an efficient way to save costly antisera and time. **Additional methods** for identification and characterization of ***H. influenzae*** using molecular tools like PCR technique. Some of most common bacterial causes summarized at table (1).

Figure (9): *H. influenzae* colonies on a CAP



. Figure (10): *H. influenzae* colonies on a CAP

Table (1): Examples of bacterial nervous system infections.

Pathogen	Risk Factor	Incidence
Streptococcus pneumoniae	Day care, HIV infection	Most common
Neisseria meningitidis	Crowded conditions	Outbreaks
Haemophilus influenzae		Significantly less common after vaccination
Listeria monocytogenes	Immune compromise, elderly	Less common
Group B streptococcus	Neonates	Decreased with antenatal detection of group B streptococcus
Escherichia coli	Neonates	Less common
Mycobacterium tuberculosis	Exposure, older age, immune compromise	Rare

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