Diagnosis by organ system Blood stream infections

Blood is a combination of plasma and cells that circulate through the entire body. It is a specialized bodily fluid that supplies essential substances around the body, such as sugars, oxygen, and hormones.

In healthy subjects, the blood is sterile

• There are various routes that organisms take to reach the blood.

1- **Pneumococcus** colonizing the upper airways could be aspirated into the lungs during sleep and go on to cause a lobar pneumonia; from here it can enter the blood

2-**The presence of bacteria in the blood requires identification of the likely source.**There is the obvious association of ***Escherichia coli*** in blood and an ascending urinary tract infection (UTI).

3-The patient with endocarditis caused by **a streptococcus of the mouth flora, such as Streptococcus sanguinis,** can have poor dentition (Poor oral health), and this needs to be addressed as part of the patient's management, usually involving the maxilla-facial surgical team and also called periodontal organisms of dental infections.

4- **More unusual situations** occur, and one is the identification of ***Streptococcus gallolyticus*** in blood culture. This organism is a minor member of the normal flora of the colon.

• **However, it is recognized that there is an association that can develop between it and a large bowel malignancy, likely due to a specific interaction between the organism and these malignant cells.**

• The ***Streptococcus*** gains a selective growth advantage, from where it accesses the blood. Once in the blood it has the potential to initiate infective **endocarditis.**

Blood is cultured to detect and identify bacteria or other cultivable microorganisms (yeasts, filamentous fungi). The presence of such organisms in the blood is called bacteraemia or fungaemia, and is usually pathological.

bacteraemia defines the presence of bacteria as detected by the culture of blood.

• **Septicemia** also defines the presence of bacteria in blood, but it signals a sense of urgency in the management of the patient.

• **The terms sepsis and septic shock** are also used and, with clinical parameters such as fever, hypotension, tachycardia, multiorgan failure and leucocytosis, alert the clinician to the severity of the situation, and the need for immediate action in the management of the patient.

Bacteremia types :

1.A transient bacteremia (a single episode lasting less than 30 minutes or so) can arise from **a pneumococcal pneumonia, or pyelonephritis caused by *Escherichia coli.***

2.An intermittent bacteremia manipulation (guidance) of **an extravascular** site, such as a ***Staphylococcus aureus*** **abscess,** where bacteria enter the lymphatics at irregular intervals, and from there, to the blood.

3.A continuous bacteremia an **intravascular** source, and endocarditis is the most important example.

• Once bacteria enter the blood, they have the potential to settle (become down) in other sites of the body, and set up another focus of infection.

• **The bacteria can cross the synovial membrane of a joint to initiate septic arthritis**.

Blood collection

blood should be taken **before antibiotics are administered**.It is recommended that two or preferably three blood cultures be obtained.

Blood Culture Media

Basic blood culture media contain a **nutrient broth and an anticoagulant**. Most blood culture bottles available commercially contain **tryptic soy broth, brain heart infusion broth, supplemented with peptone, or thioglycolate broth ,** Special media, such as **Middlebrook 7H9 broth with 0.05% SPS or BHI broth with 0.5% polysorbate 80**, enhances the recovery of *Mycobacterium spp*

• **Tryptic soy broth (TSB)** should be able to support growth of all clinically significant bacteria.

• the blood should be mixed with 10 times its volume of broth a (**1:10 ratio)** of blood to medium was required for successful bacterial growth (5 ml of blood in 50 ml of broth) to **dilute any antibiotic present and to reduce the bactericidal effect of human serum**. **Any medium showing turbidity should not be used**

• If strictly aerobic bacteria **(*Pseudomonas, Neisseria*)** the bottle should be **vented** as soon as it is received in the laboratory, by inserting a sterile cotton-wool-plugged needle through the previously disinfected diaphragm.**the use of a diphasic blood-culture bottle, with a broth phase and a solid-slant phase** on one of the flat surfaces of the bottle (Castaneda bottle), is recommended for the cultivation of Brucella spp.

• Blood-culture bottles should be incubated at 35–37 ◦C and routinely inspected twice a day (at least for the first 3 days) for signs of microbial growth.

• Whenever **visible growth appears**, the bottle should be opened aseptically, a small amount of broth removed with a sterile loop or Pasteur pipette, and a Gram-stained smear examined for the presence of microorganisms.

Table (3): Summary of bacterial blood infections.

Infection	Most Important Pathogens	Laboratory diagnosis
Endocarditis	*Streptococcus* spp. (60–80%) *Staphylococcus* spp. (20–35%) Gram-negative rods (2–13%) Numerous other bacterial spp. (5%) Fungi (2–4%) Culture negative (5–25%)	**Blood culture**, three sets from three different sites, within 1–2 h, before antimicrobials if possible. 10–20 ml venous blood into one aerobic and one anaerobic bottle, respectively.
Bacteria	***Staphylococcus aureus*** ***Streptococcus pneumoniae*** ***Enterobacteriaceae*** ***Mycobacterium tuberculosis***	Microscopy and culture from punctate DNA test from punctate if required
	Mycoplasma pneumoniae	Serology; culture from punctate
	Neisseria spp. Gram-negative anaerobes *Actinomyces* spp. *Nocardia* spp.	Microscopy and culture from punctate
	Rickettsia spp. *Chlamydia trachomatis*	Serology

Anticoagulation

1-Heparin 2-EDTA 3- citrate 4- Sodium polyanethol sulfonate(SPS, Liquoid) in concentrations of 0.025% to 0.03% is the best anticoagulant available for blood cultures

Specimen Volume

1-collection of two sets of cultures using **10 to 20 mL** of blood per culture is strongly recommended for **adults 2**- for **infants and small children**, only **1 to 5 mL** of blood should be drawn for culture.

Lec 2 -3

Bacterial Infections of the Gastrointestinal (GI) Tract (Gastroenteritis)

Diarrheal diseases are the second leading cause of death; about 48 million enteric infections occur each year. Most of these infections cause morbidity and death, particularly in elderly people and children younger than 5 years of age.

Pathogenesis:

Host factors: The human host has numerous defenses factors that prevent the disease produced by enteric pathogens such as the acidity of the stomach, the normal peristalsis the mucous layer coating the epithelium. Moreover, the normal flora prevents colonization by potential pathogens.

Microorganisms That Cause GI Infection for Each Primary Pathogenic Mechanism

Mechanism	Examples of Microorganisms
Toxin Production	***Vibrio cholera***
Enterotoxin	**Noncholera vibrios**
	***Shigella dysenteriae* type 1**
	Enterotoxigenic *Escherichia coli* (ETEC)
	***Salmonella* spp.**
	***Clostridium difficile* (toxin A)**
	Aeromonas
	Campylobacter jejuni
Cytotoxin	***Shigella* spp.**
	***Clostridium difficile* (toxin B)**
	Enterohemorrhagic *Escherichia coli*
Neurotoxin	***Clostridium botulinum***
	Staphylococcus aureus

	Bacillus cereus
Attachment/	**Enteropathogenic *Escherichia coli* (EPEC)**
Adherence	**Enterohemorrhagic *Escherichia coli* (EHEC)**
Invasion	***Shigella* spp.**
	Enteroinvasive *Escherichia coli* (EIEC)
	Campylobacter jejuni
	Yersinia enterocolitica

Laboratory Diagnosis of Gastrointestinal Tract Infections

Specimen Collection

Stool Specimens for Bacterial Culture

Feces and rectal swabs are the most readily available specimens. The presence of blood, mucus must be noted.

Direct detection of agents

Wet Mounts: A direct wet mount of fecal material is the fastest method for detecting motile trophozoites of intestinal parasites.

Stains

Feces may be Gram stained for detection of certain etiologic agents.

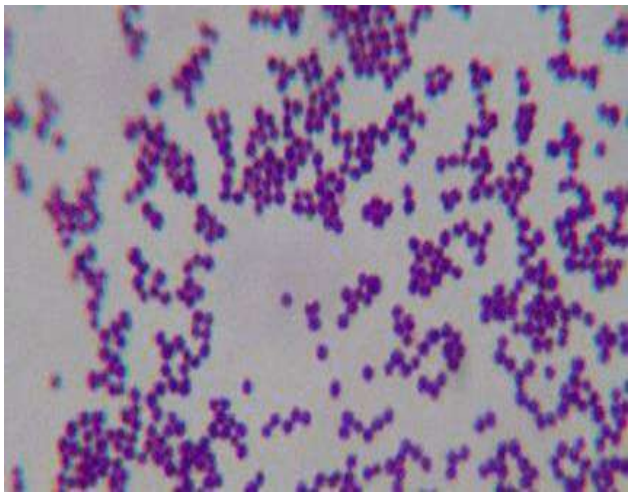
Culture for isolation of bacteria.

Stools should be suspended in broth and cultured on ordinary media as well as on selective/differential media **(MacConkey agar, EMB agar).**

If **Salmonella** infection has suspected, the specimen is inoculated in **Selenite broth** for 18 hours before it has placed in differential media (**Hektoen enteric or S-S agar**.

For ***Vibrio cholerae*** suspected infection, **TCBS agar** is used.

<u>Staphylococcal Food Poisoning:</u> When ***Staphylococcus aureus*** grows in food, it may produce enterotoxins that, cause symptoms such as nausea, diarrhea, cramping, and vomiting within one to six hours. The enterotoxins are **proteins** that are resistant to **low pH**, allowing them to pass through the stomach. They are **heat stable** and are not destroyed by boiling at 100 °C. Even though the bacterium itself may be killed, the enterotoxins alone can cause vomiting and diarrhea. *Staph. aureus* is diagnosed by staining and culturing on Mannitol agar (mannitol fermenting) **and confirmed by identifying the toxin in**



***Staph. aureus*- gram stain**

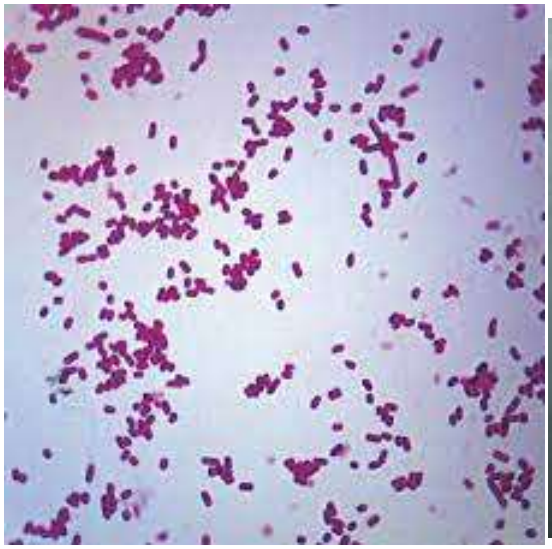
a food sample or in biological specimens (feces or vomitus) from the patient. Serological techniques, including ELISA, can also be used to identify the toxin in food samples.

<u>Shigellosis (Bacillary Dysentery).</u>

When gastrointestinal illness is associated with Shigella, it is called **bacillary dysentery, or shigellosis.** Infections can be caused by *S. dysenteriae*, *S. flexneri*, *S. boydii*, and/or *S. sonnei* that colonize the GI tract. Shigella is gram negative non-motile rod shaped. It invades intestinal epithelial cells. Shigella can escape from the immune system and then live within the cytoplasm of the cell or move to adjacent cells. More severe cases may result in ulceration of the mucosa, dehydration, and rectal bleeding. Patients may develop hemolytic uremic syndrome (HUS), a serious condition which may cause kidney failure. *S. dysenteriae* is able to produce Shiga

toxin, which targets the endothelial cells of small blood vessels in the small and large intestine.

Stool samples are analyzed using serological or molecular techniques. The presence of WBCs and blood in fecal samples occurs in about 70% of patients.



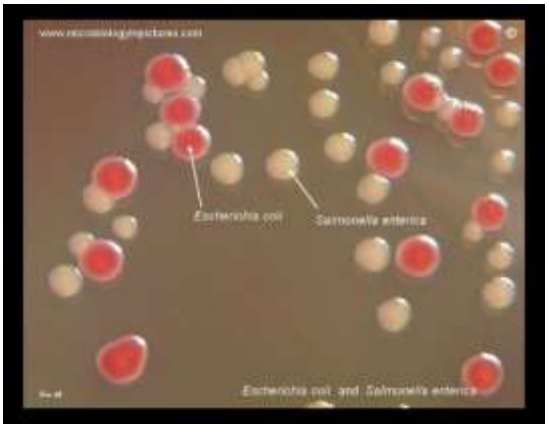
Shigella- gram stain

Shigella-on MacConkey agar

<u>Salmonellosis</u>.

Salmonella gastroenteritis (salmonellosis), is caused by the rod-shaped, motile, gram-negative bacterium Salmonella. Infection is caused by ingestion of contaminated food, raw eggs and raw poultry. Salmonella can cross the epithelial cell membrane and enter the bloodstream and lymphatic system. Infected individuals develop fever, nausea, abdominal cramps, vomiting, headache, and diarrhea. These signs and symptoms generally last a few days to a week.

Colony of *Escherichia coli* and *Salmonella enterica*. Growth on MacConkey agar. Red, lactose positive colonies of *E. coli* and colorless, lactose negative colonies of *S. enterica.*



Typhoid Fever.

S. typhi and *S. Paratyphi*, cause severe type of salmonellosis called typhoid fever. ***S. typhi*** penetrate the intestinal mucosa, grow within the macrophages, and are transported through the body, most notably to the liver and gallbladder. The macrophages lyse, releasing *S. typhi* into the bloodstream and lymphatic system.

The bacteria can be cultured from feces, urine, blood, or bone marrow. Serology, including ELISA, is used to identify Salmonella but confirmation with PCR test.

E. coli Infections.

There are five pathogenic groups of *E. coli,* but we will **focus here on four**, the most commonly transmitted through food and water.

1- Enterotoxigenic *E. coli* (ETEC), also known as **traveler's diarrhea**, causes diarrheal illness. The patients develop a **watery diarrhea, abdominal cramps, malaise (a feeling of being unwell), and a low fever**. **ETEC produces a heat-stable enterotoxin and adhesins called colonization factors that help the bacteria to attach to the intestinal wall**. **Diagnosis involves staining, culturing and PCR.**

2- Enteroinvasive *E. coli* (EIEC) it carries a large plasmid that is involved in epithelial cell penetration. The signs and symptoms include **watery diarrhea, chills, cramps, malaise, fever, and dysentery.**

3- Enteropathogenic *E. coli* (**EPEC**) can cause a potentially fatal diarrhea, especially in infants. Fever, vomiting, and diarrhea can lead to severe dehydration. **This *E. coli*** produces a protein (Tir) that attaches to the surface of the intestinal epithelial cells. As with ETEC.

Diagnosis involves staining, culturing and PCR.

4- Enterohemorrhagic *E. coli* (**EHEC**), the strains capable of causing epidemics. EHEC can cause disease ranging from relatively mild to life-threatening. **Symptoms include bloody diarrhea with severe cramping, but no fever.**

Diagnosis involves culture, often using MacConkey.

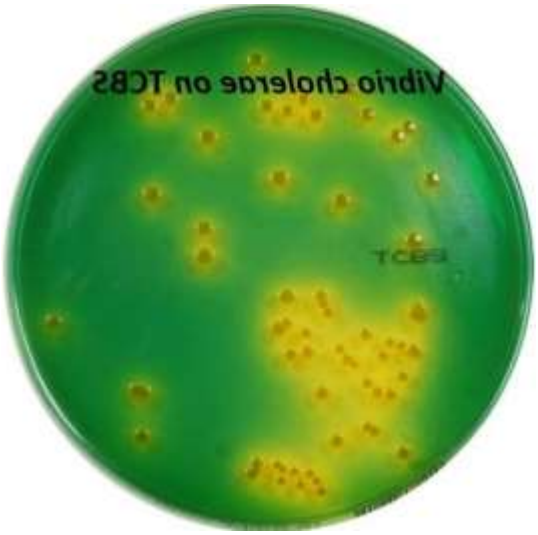
The Primary Groups of *E. coli* That Cause Diarrhea in Humans

Type	Primary Mode of Pathogenesis	Other Comments
Enterotoxigenic (ETEC)	Produces heat-labile (LT) or heat stable (ST) enterotoxins; genes of both toxins reside on a plasmid; LTs are closely related in structure and function to cholera toxin; STs result in net intestinal fluid secretion by stimulating guanylate cyclase	Common cause of traveler's diarrhea; infects all ages
Enteroaggregative (EAEC)	Binds to small intestine cells via fimbriae encoded by a large molecular weight plasmid, forming small clumps of bacteria on the cell surface; other plasmid-borne virulence factors include structured pilin, a heat-stable enterotoxin, novel anti-aggregative protein, and a heat-labile enterotoxin, all believed to be the cause of the associated diarrhea	Infects primarily young children
Enteroinvasive (EIEC)	Pathogenesis has yet to be totally elucidated; studies suggest that mechanisms by which diarrhea results are virtually identical to those of *Shigella* spp.	Very difficult to distinguish from *Shigella* spp. and other *E. coli* strains
Enteropathogenic (EPEC)	Initially attaches in the colon and small intestine and then becomes intimately adhered to intestinal epithelial cells, subsequently causing the loss of enterocyte microvilli (effacement); genes for attachment/effacement reside in a cluster on the bacterial chromosome (i.e., pathogenicity island)	Diarrhea in infants, particularly in large urban hospitals
Enterohemorrhagic (EHEC) OR	Attaches to and effaces gut epithelial cells in a similar manner as EPEC; in addition, EHEC elaborates shiga toxins	Although many outbreaks are caused by *E. coli* 0157:H7, other serotypes have been implicated in outbreaks and sporadic cases Gene recombination among strains makes classification difficult

****

Cholera

Cholera is a serious infection often associated with **poor sanitation**, It is caused by ***Vibrio cholerae* serotype O1, a gram-negative, motile (darting movement) by single polar flagellum, curved rod (comma shaped**. Because *V. cholerae* is killed by stomach acid, therefore, relatively large doses are needed to reach the intestines and cause infection. They attach to epithelial cells and release cholera enterotoxin. Within the intestinal cell, cyclic AMP (cAMP) levels increase, which activates a chloride channel and results in the release of ions into the intestinal lumen. It causes rapid dehydration and electrolyte imbalance. Diarrhea is so profuse that it is called "rice water stool,". Cholera is diagnosed by taking a stool sample and culturing for *Vibrio*. **The bacteria are oxidase positive and non-lactose fermentation on MacConkey agar**. ***V. cholerae* may also be cultured on thiosulfate citrate bile salts sucrose (TCBS) agar,** which is **th selective and differential medium for *Vibrio* spp., which produce a distinct yellow colony**.

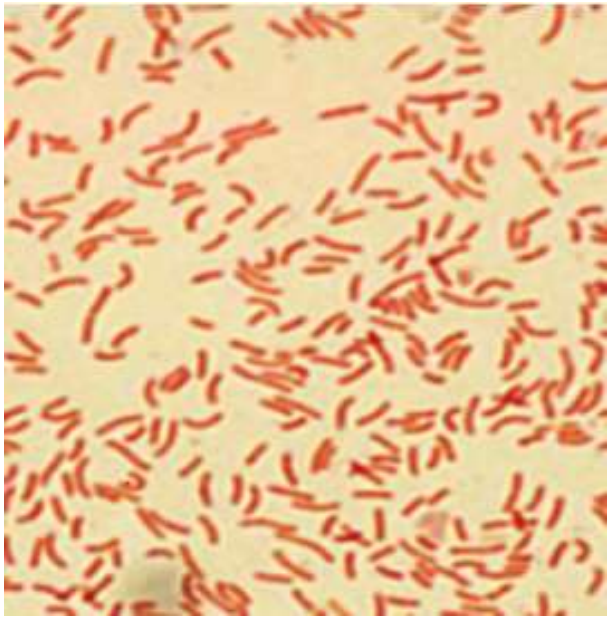


Helicobacter pylori and "Peptic Ulcers"

H. pylori is gram negative, motile, spiral (helical) shaped. It is able to tolerate the acidic environment of the human stomach and has been shown to be a major cause of **peptic ulcers**, which are ulcers of the stomach or duodenum. The bacterium is also associated with increased risk of stomach cancer.

H. pylori colonizes epithelial cells in the stomach using pili for adhesion. These bacteria produce **urease**, which stimulates an immune response and creates ammonia that neutralizes stomach acids. The infection damages the cells of the stomach lining. As a result, inflammation (gastritis) occurs. **Signs and symptoms include nausea, lack of appetite, bloating, burping, and weight loss. Bleeding ulcers may produce dark stools.** If no treatment is provided, the ulcers can become deeper, more tissues can be involved.

To diagnose *H. pylori* infection, multiple methods are available. In a **breath test, the patient swallows radiolabeled urea. If *H. pylori* is present, the bacteria will produce urease to break down the urea. This reaction produces radiolabeled carbon dioxide that can be detected in the patient's breath.** Blood testing can also be used to detect antibodies to *H. pylori*. **The bacteria themselves can be detected using either a stool test or a stomach wall biopsy.**



***H. pylori*-Gram stain**

Clostridia

Clostridia are the **anaerobic gram-positive rods** of greatest clinical importance. **Clinically** significant species of Clostridium include:

1. ***Clostridium perfringens***, which causes histotoxic (**tissue destructive**) infections **myonecrosis gas (gangrene**) and **food poisoning**.

2. ***Clostridium difficile***, which causes **pseudomembranous colitis** associated with antibiotic use.

3. ***Clostridium tetani***, which causes **tetanus (lockjaw‖).**

4**. *Clostridium botulinum*, which causes botulism.**

C. perfringens can cause anaerobic cellulitis and myonecrosis (gas gangrene). Some strains of *C. perfringens* also cause a common form of food poisoning. Pathogenesis of *C. perfringens* secretes a variety of **exotoxins**, **enterotoxins**, and **hydrolytic enzymes** that facilitate the disease process.

Exotoxins: *C. perfringens* elaborates at least **12 exotoxins**. The most important of these, and the one that seems to be required for virulence in tissue, is **α toxin**. α Toxin is a **lecithinase (Phospholipase-C)** that degrades lecithin in mammalian cell membranes, **causing lysis of endothelial cells as well as erythrocytes**, **leukocytes**, and **platelets**. **Perfringolysin** O, or **theta (θ) toxin**, is a cholesterol-dependent hemolysin and an important virulence factor. *C. perfringens* strains are grouped **A through E** on the basis of their spectrum of **exotoxins**.

Enterotoxin: *C. perfringens* enterotoxin, a small, heat-labile protein, acts in the lower portion of the small intestine.

Clinical significance: The disease processes initiated by *C. perfringens* result from a combination of infection and the production of exotoxins and/or enterotoxins and degradative enzymes.

Foodborne infection: *C. perfringens* is a common cause of foodborne infection in many situations. Typically, the onset of nausea, abdominal cramps, and diarrhea occurs 8 to 18 hours after eating contaminated food. Fever is absent and vomiting rare. The attack is usually self-limited, **with recovery within 1 to 2 days**. The occurrence of clinical

symptoms requires a large inoculum of 10^8 organisms or greater. Vegetative cells are consumed in the contaminated product, and *C. perfringens* then reproduces following ingestion (food infection) and produces toxin in vivo. Meats, meat products, are the most commonly implicated foods in *C. perfringens* foodborne illness.

Laboratory identification: Stool or diseased tissue specimens cultured anaerobically on blood agar, *C. perfringens* grows rapidly, producing colonies with a unique **double zone of hemolysis due to production of α toxin (partial hemolysis) and perfringolysin O (complete hemolysis)** as shown in the Figure. *C. perfringens* on blood agar exhibits double zone hemolysis due to two toxins: an inner zone of complete hemolysis (beta-hemolysis) from theta-toxin and a wider outer zone of incomplete hemolysis (alpha-hemolysis) from alpha-toxin. The inner zone is a clear, transparent ring around the colony, while the outer zone appears greenish due to the partial lysis of red blood cells.

Clostridium botulinum: ***C. botulinum*** causes **botulism**, which occurs in several clinical forms. Botulism is caused by the action of a **neurotoxin that is one of the most potent poisons known and causes a limp paralysis**. Contact with the organism itself is not required, and the disease can be specially due to ingestion of toxin contaminated food. Epidemiology **of *C. botulinum* is found worldwide in soil and aquatic sediments, and the spores frequently contaminate vegetables and meat or fish.** Under appropriate conditions, including a strictly anaerobic environment at neutral or alkaline pH, the organism germinates, and toxin is produced during vegetative growth. Because the toxin is often elaborated in food, outbreaks frequently occur in families or other eating groups. **Pathogenesis**: **There are several types of botulinum toxin, but human disease is almost always caused by types A, B, or E toxins**.

The botulinum and tetanus toxins constitute a homologous set of proteins whose **neurotoxicity**, **causing subsequent failure of neurotransmissio**n. In contrast to **tetanus toxin, which causes constant contraction or spasms. botulinum toxins affect peripheral cholinergic synapses by blocking the neuromuscular junction and inhibiting release of the neurotransmitter acetylcholine, preventing contraction and causing flaccid paralysis.**

Clinical significance: Classic botulism at food poisoning in which a patient first begins to experience difficulties in focusing vision, swallowing, and other cranial nerve functions, 12 to 36 hours after ingesting toxin-containing food but not essentially viable organisms. There is no fever or sign of sepsis. A progressive paralysis of striated muscle groups develops, and mortality rate is about 15 %, with the patient usually yielding to respiratory paralysis.

Laboratory identification: The organism can be cultured and identified by standard **anaerobic methods** (Isolation of a bacterium is usually performed on solid medium. Liquid medium is used to grow larger quantities of a culture of bacteria that have already been isolated as a pure culture on Enriched media **(blood agar, yeast extracts, or brain or heart infusions are useful in growing this fastidious organisms).** Toxin is also identifiable in serum, stool, and food.

BACILLUS SPECIES: Species of the genus Bacillus are **gram-positive**, form **endospores**, and are **strict aerobes**. Most of the species of Bacillus are found in soil and water and are usually essential in the medical laboratory as airborne contaminants**.**

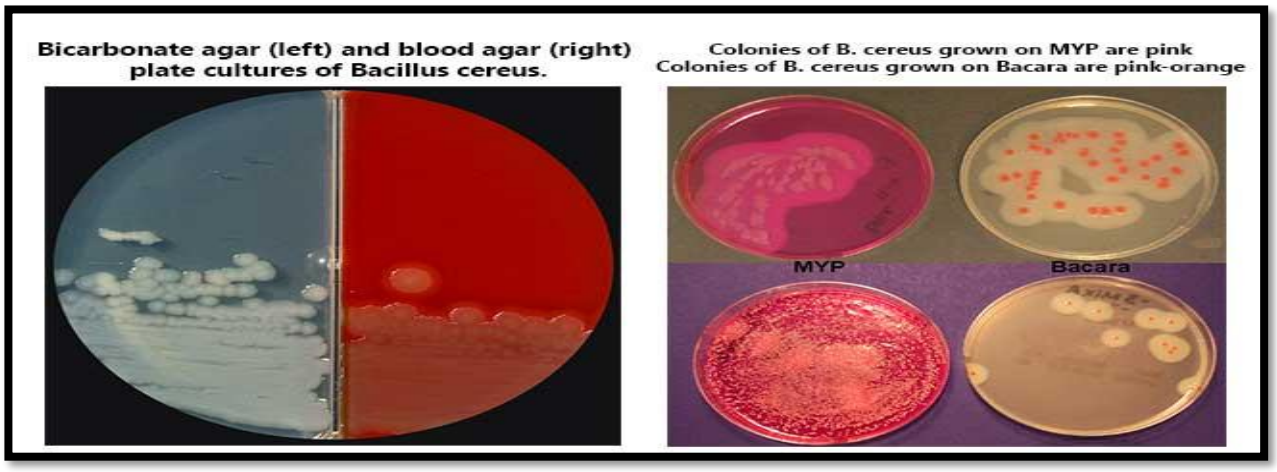
B. anthracis, the cause of the disease **anthrax**, is clinically the most important member of this genus.

Bacillus cereus*:** It is a soil organism which commonly contaminates rice and produce a tissue-destructive exotoxin. When large amounts of rice are cooked and allowed to cool slowly, the ***Bacillus cereus spores germinate, and the vegetative cells produce the toxin during log-phase growth or during sporulation. Food poisoning caused by *Bacillus cereus* **has two separate forms**;

1-**the emetic type, which is associated with cooked rice**, and

2-**the diarrheal type, which is associated with meat dishes and sauces**.

The emetic form is manifested by **nausea**, **vomiting**, **abdominal cramps**, and occasionally **diarrhea** and is **self-limiting, with recovery occurring within24 hours**. The diarrheal form has an incubation period of 1–24 hours and is manifested by **abundant diarrhea** with **abdominal pain** and **cramps**; **fever and vomiting are uncommon**.



Bacillus cereus is a gram-positive, rod-shaped, aerobic, motile, beta hemolytic bacterium commonly found in soil (on vegetables) and food (raw and processed). ***B. cereus*** bacterium is facultative anaerobes, and like other members of the genus Bacillus, can produce protective endospores. Its virulence factors include cereolysin and phospholipase C. The presence of Bacillus cereus in a patient's stool is not sufficient to make a diagnosis of Bacillus cereus disease because the bacteria may be present in normal stool specimens; a concentration of 105 bacteria or more per gram of food is considered diagnostic. Some strains of ***B. cereus*** produce ***cereins, bacteriocins*** active against different *B. cereus* strains or other Gram-positive bacteria.

Clostridium difficile

Clostridium difficile is a gram-positive rod that can be a commensal bacterium as part of the normal microbiota of healthy individuals. When the normal microbiota is disrupted by long-term antibiotic use, it can allow the overgrowth of this bacterium, resulting in antibiotic-associated diarrhea caused by *C. difficile*.

Patients at the greatest risk of *C. difficile* infection are those who are immunocompromised, have been in health-care settings for extended periods. Because this species can form **endospores**, it can survive for extended periods of time in the environment under harsh conditions. **This bacterium produces two toxins, *Clostridium difficile* toxin A (TcdA) and *Clostridium difficile* toxin B (TcdB)**. These toxins inactivate small GTP-binding proteins, resulting in cell death. Infections begin with focal necrosis, then ulceration with exudate, and can progress to **pseudomembranous colitis**, which involves **inflammation of the colon** and the development of a **pseudomembrane of fibrin containing dead epithelial cells and leukocytes.** The disease is characterized by **watery diarrhea, dehydration, fever, loss of appetite, and abdominal pain can result. Perforation of the colon can occur, leading to septicemia, shock, and death.**

<u>Diagnosis</u> of *C. difficile* infection:

Stool Test: The simplest way to detect *C. difficile* is through a stool test.

Blood Test: A blood test can reveal high levels of white blood cells, a sign of infection. Very high levels can signify a more severe *C. difficile* infection, in which a person may have watery diarrhea, intense stomach cramps, and dehydration.

Colonoscopy: A colonoscopy enables a doctor to examine the entire colon and rectum. The test can indicate whether inflammation is present, indicating a *C. difficile* infection.

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Diagnosis of bacterial respiratory tract infections

Bacterial infections of respiratory tract

Respiratory system has divided into two major parts:

- ✓ Upper respiratory tract includes (**nose and pharynx**)
- ✓ Lower respiratory tract includes (**larynx, trachea, bronchial tube and alveoli**).

Each part or organ of this system has **own resident microflora**. Many factors play a vital role in challenging and limitation of **number and type of microflora colonizing**. Also each parts of respiratory tract **having physical factors** such as **hair, mucus membrane** lining the tract, **cilia** movement, **sneezing**, **coughing** besides **oxygen tension** in lung, which act all collectively as **unbreakable defense** line.

In addition, **innate immunity** and **circulating antibodies stabilize natural balance**, which represents equilibrium state between **host immunity** and **action of pathogens**. Ear, eye and nose are all share common canal, so any infection of one of these parts may cause infection to others. **Nasal cavity** for example consider as a reservoir for genus **Staphylococcus** along with other **gram-positive bacteria**. Nasal cavity is the pathway for deeper parts of respiratory tract for example resident bacteria of **nasal cavity** may and **will find its way** to the system causing problems here location and **to nervous system** such as **meningitis**. **Ear infection**, on other hand may be the way for **enteric bacteria** to **reach to un-limited area in respiratory or nervous systems.** ***E. coli*** **meningitis** is one example among many of such cases. **Tonsils** are the major front line of defense, yet, it is frequently had infected with so many species of bacteria, **Gram-negative** as well as **Gram-positive** bacteria.

Infection of respiratory tract sometimes classified as adult or childhood infections in this regard, ***Bordetella Pertussis*** **the causative agents of whooping cough is the example of childhood infections**. **Respiratory infections may have classified as accidental or seasonal infections. The latter has associated with possible changes in the weather, from winter to summer and vice versa, bacterial infection may come second to viral infection in this aspect. Accidental infection is the infection that man acquired during daily life.**

No limitation for the types of bacteria that may **cause** infection to **respiratory system** regardless the way that bacteria inter the system. Most **of normal flora of upper respiratory tract** play an important role in **causing <u>opportunistic disease</u>. Staphylococus, Streptococcus, Haemophilus, Corynebacterium, Neisseria, Bacteroides, Fusobacterium, and Actinomyces,** are typical examples for these bacteria.

Nearly any type of **gram-positive** or **negative** bacteria **Pneumonia**, **Mycoplasma** and **Chlamydia spp**., can cause respiratory infection. On other hand, may **cause non-specific pneumonia**, while **Tuberculosis** caused by **Mycobacterium tuberculosis complex**, both of these diseases involved **lower** respiratory tract.

<u>Sore throat</u> is a common infection of upper respiratory tract caused specially by **hemolytic Streptococci**, besides other **gram-positive cocci** or **gram-negative bacilli (*Haemophilus spp*.)**.

The middle and inner ear are normally sterile, while outer ear and auditory canal contain the **normal flora of mouth and nose**. When a person coughs, sneezes or blow the nose these microorganisms may reach middle or inner ear and causing infection. **Tears in eyes decreases the number of microorganisms** that may find its way to eye because it's content of **lysozyme that destroys bacterial cells.** (fig.1)

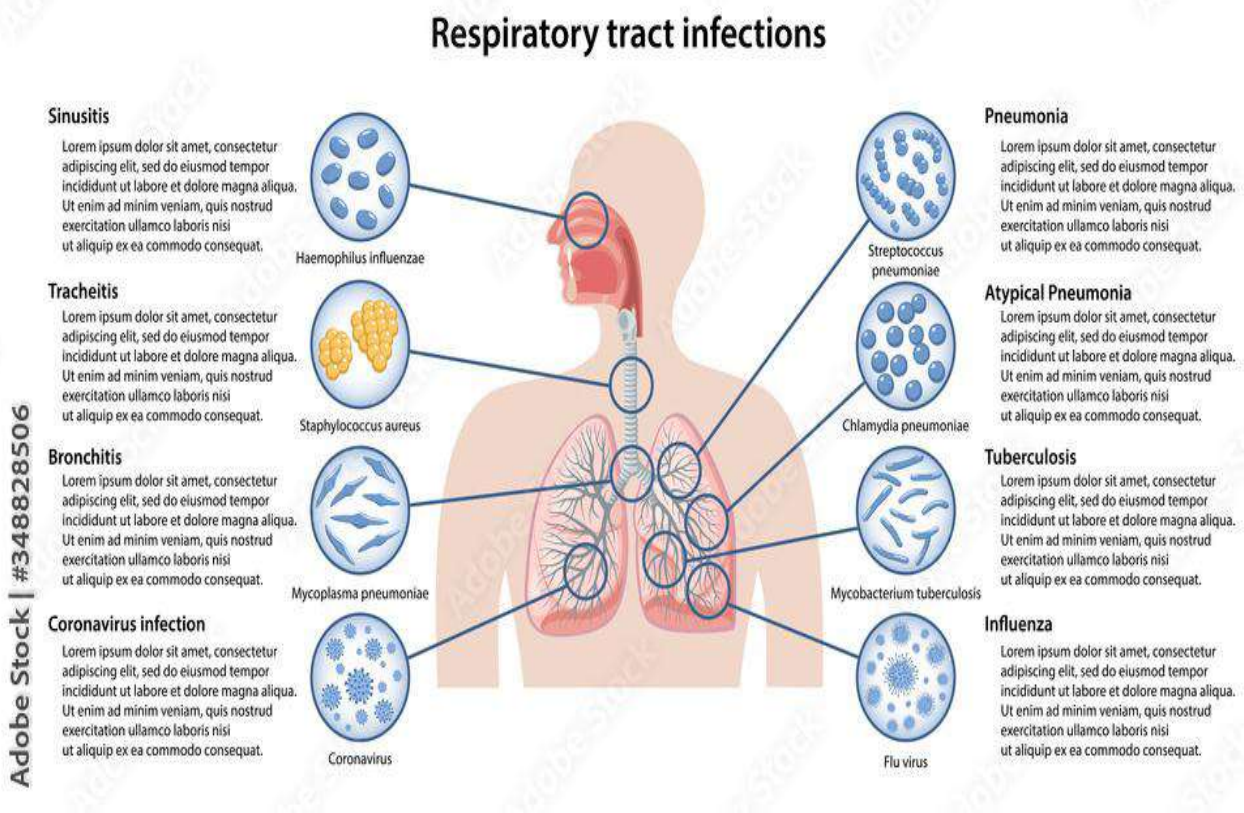


Fig. (1): Summary of bacterial respiratory tract infection

Bronchitis

1. Acute bronchitis: It is an acute inflammation of the tracheobronchial tree generally self-limited and with eventual (final) complete healing and return of function.

Causative agent: ***Mycobacterium pneumoniae; Bordetella pertussis***

Laboratory diagnosis:

Specimen: **Sputum**

Procedure: **Gram staining, culture, biochemical and serological test** for microbe identification.

2. Chronic bronchitis

It has defined as chronic productive cough for at least three months in each of two successive years.

Causative factors: **Cigarette smoking; Air pollution;** Exposure to harmful stimuli

Bacteria that improve chronic bronchitis are: ***Streptococcus pneumonia; Haempphilus influenza; Mycoplasma pneumoniae Branhamella catarrhalis.***

Laboratory diagnosis:

Specimen: **Sputum**

Procedure: **Gram staining, culture, biochemical and serological test** for microbe identification.

Pneumonia: It is infection of the lung parenchyma.

Causative agents: ***Strep. pneumoniae, Staph. aureus, Hemophillus influenzae*** **and** ***Mycoplasma pneumoniae.***

Route of entry of microbes to the lung:

- ❖ Aspiration of oral and gastric secretion
- ❖ Haematogenous spread from distant foci
- ❖ Direct inoculation and local spread from surrounding tissue
- ❖ Inhalation

Laboratory diagnosis:

Specimen: **Lower respiratory secretion** which indicated by **greater than 25 Neutrophils and less than 10 squamous epithelial cells per high power field.**

Procedure: **Gram staining, culture, biochemical and serological test for microbe identification.**

Bacterial Diagnosis of TB infection

Tuberculosis: It is a disease caused by group of ***Mycobacterium spp***., namely Mycobacterium tuberculosis complex. ***M. tuberculosis*** is of human origin, ***M. bovis*** is of cattle origin, ***M. avium*** is of bird origin.

The main problem of these bacteria is:

1. Their high resistance to environmental stress such as dryness.
2. Survive in dry sputum for months.

3. Members of genus mycobacterium are very resistant to chemical and antibiotic treatment.

All these features are because of their highly **contents of cell wall of lipids**. Cell wall lipid content makes these bacteria **difficult to stain** with ordinary stains. Therefore, special stain is required (Acid Fast Stain: AFS). **AFS** depends on **penetration of Carbol-fuchsin dye to cell wall with aid of heat**, once it is in there, a complex of stain and lipid of cell wall is formed, this complex is **not removed** by normal **decolorizing agent (alcohol**), it **resists even the decolorizing** with acid-alcohol from which it takes its name (Acid Fast Bacteria).

Air born **droplets**, **milk**, or even **prolonged contact** with sick peoples consist collectively the major pathways for **transmission of disease**, yet, **air born rout** is the **important rout of entry,** fine particles containing one or two TB. **Cells travels** from patient for a distance of one meter **to another person** (air born) will enough to cause a disease in susceptible individual; normally these bacteria are overcoming by **host defense**. If bacteria succeeded to penetrate host defense, then **alveoli** will the **battlefield (area)** of the disease.

Bacilli are **multiply in macrophages protect themselves against killing process,** in a self-protection process host try to limit the drastic (severe) effect of the pathogen by forming a **tubercle**, which is a **matrix tissues**, **exudates**, **WBC**s, and other materials. ***M. tuberculosis*** tend to arrange in cord formation, which increase the immune response of host resulting in what is called hypersensitivity reaction which lead ultimately to tissue damage.

Lab. diagnosis:

Mycobacterium may come from a wide range of samples, these include; **sputum, lung wash, urine, wound, CSF, lymph secretion, bone, gastro-intestinal material.** The prime diagnostic parameter is **culturing of materials** (regardless the origin of it) on suitable culture medium, the medium commonly used is (**L-J M**), enriched media with **high contents** of **nutrition** to aid the **long period of incubation**. TB bacilli

appear as **hydrophobic colonies with wrinkled (crumpled) surface**. Because of long time of incubation,

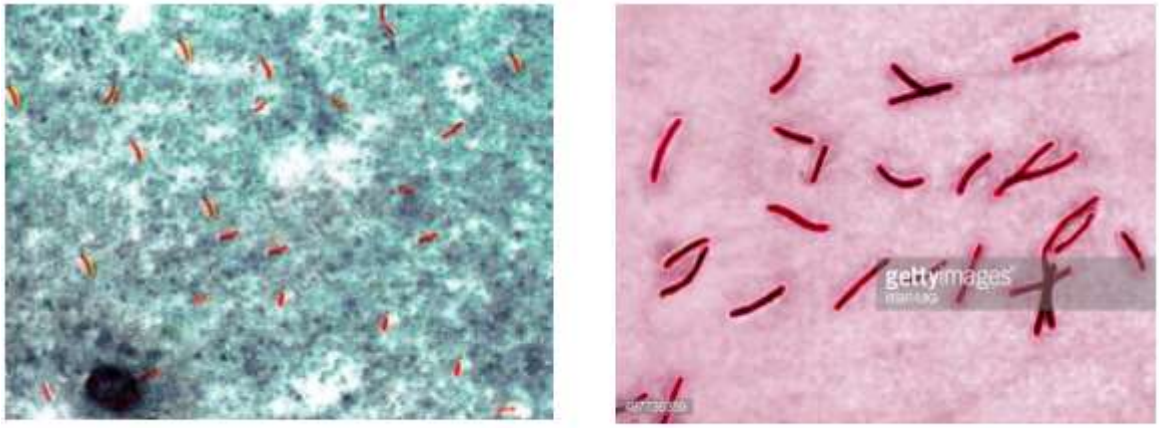


Fig.2 :*Mycobacterium tuberculosis* in Ziehl-Neelsen stained smear

An alternative diagnostic methods have employed such as **PCR** or other methods. **Blood film** might of little help in diagnosis of TB. Since **WBCs**, count may **still normal** with marked **elevation in number of monocytes**. **ESR** on the other hand might more evident in this regard, **ESR** is **shooting up reaching levels of 100 mm/h** or higher. Commercial kits for diagnosis of **IgM and IgG for TB**. Are available now in local markets.

AFB serves as a **screening test** in diagnosis of TB., the existence of **even a single** bacilli/ many microscopic fields is **enough to consider it " AFB positive**", yet the **absence** of AFB from the investigated sample **does not mean that " patient has no TB.** And vice-versa the existence of AFB does not mean that patient is a TB. Patient. Since may other bacteria such as **Nocardia** may show a similar appearance of TB.

Diphtheria disease: **the causative agent of this disease** is ***Corynebacterium diphtheria*e**

Diphtheria is most commonly an infection of the upper respiratory tract and causes fever, sore throat, **hypoxia** due to airway obstruction by the **pseudomembrane**.and malaise. The **pseudomembrane** is a thick, gray-green fibrin membrane, forms over the site(s) of infection as a result of the combined effects of bacterial growth, toxin production, necrosis of underlying tissue, and the host immune response.

The involvement of **cervical lymph nodes may cause profound swelling of the neck** (**bull neck diphtheria**) as shown in **figure-2**, causing Life-threatening systemic complications as a result of the action of **diphtheria toxin**.

Figure-2

Corynebacterium diphtheriae is a **Gram-positive, nonmotile, club-shaped bacilli.** Older cultures often contain **metachromatic granules** (**polymetaphosphate**) which stain **bluish-purple** with methylene blue.

Diagnosis

Culture media: **Loeffler agar** or **Mueller-Miller tellurite agar**.

Specimen: **Pharyngeal tonsils** swab. The most common assay for **toxigenicity** is the **Elek immunodiffusion test (Figure-3)**

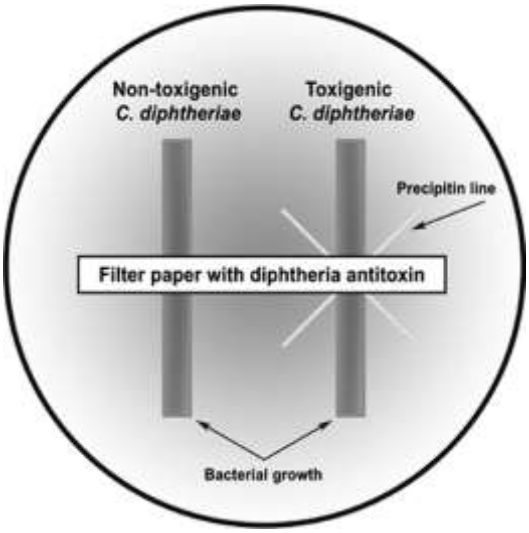


Figure-3: Procedure of Elek immunodiffusion test. A Sterile filter paper impregnated with diphtheria antitoxin is imbedded in agar culture medium. Isolates of *C. diphtheriae* are then streaked across the plate at an angle of 90° to the antitoxin strip. Toxigenic *C diphtheria*.

This test is based on the **double diffusion of diphtheria toxin and antitoxin in an agar medium.** A sterile, antitoxin-saturated filter paper strip is embedded in the culture medium, and ***C. diphtheriae*** isolates are streak-inoculated at a 90° angle to

the filter paper. The production of diphtheria toxin can be detected within 18 to 48 hours by the formation of a toxin-antitoxin precipitin band in the agar.

Whooping cough disease: the causative agent of this disease is *Bordetella pertussis*.

***B. pertussis* is a small Gram-negative rod-shaped, encapsulated, non-motile, obligate aerobes, catalase and oxidase positive**. Numerous antigens and virulence factors are produced by *B. pertussis.*

Symptoms and signs whooping cough:

- blocked or runny nose.
- sneezing.
- raised temperature.
- uncontrolled bouts of coughing that sounds like a 'whoop' or are followed by a 'whooping' noise.
- vomiting after coughing.

Diagnosis:

Specimen: nasopharyngeal secretions nasopharyngeal swabs.

These specimens should be immediately plated onto **Regan-Lowe medium** or **Bordet-Gengou agar** which is the most widely used.

***B. pertussis* on Bordet-Gengou Agar with blood**

Bordetella pertussis usually grows after 3 to 4 days of incubation at 37° C. (Also, it can be identified by API-NE, PCR and ELISA).

Infection of the urinary tract

The urinary tract consists of the kidneys, ureters, bladder, and urethra. Urine is normally a sterile fluid.

Urinary tract infections (UTIs) are characterized as being either upper (U-UTI encompasses the ureters and kidneys) or lower (L-UTI encompasses the bladder and urethra) based primarily on the anatomic location of the infection.

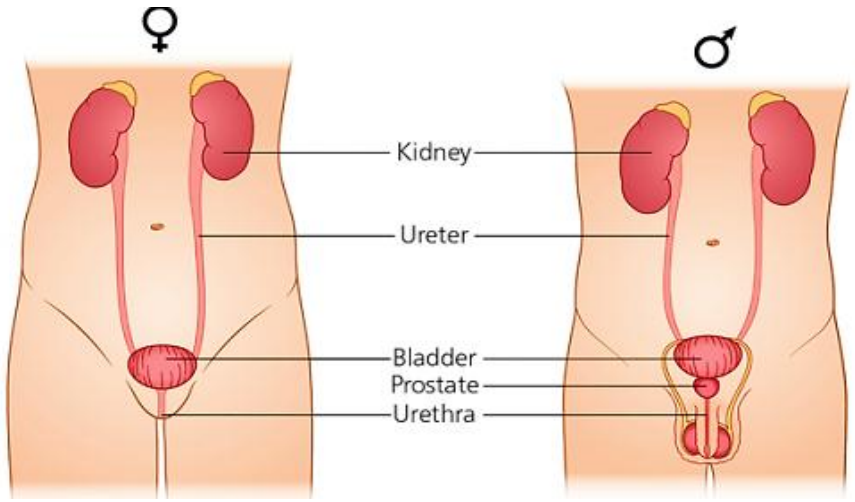


Figure (1): Male and female urinary tract system

A proper classification has employed currently: Hospital or community acquired infections.

Some terminologies that you should to know:

- **Pyelonephritis:** infection of the renal parenchyma, calyces, and pelvis
- **Nephrolithiasis:** stone in kidney
- **Ureterolithiasis:** stone in ureter.
- **Cystolithiasis:** stone in urinary bladder
- **Urethritis:** infection of the urethra
- **Ureteritis:** infection within the ureters.
- **Cystitis:** infection of the bladder.
- **Prostatitis:** Infection of prostate in males

Etiologic Agents

Bacterial species involved in community acquired UTI is :

1-***E. coli*** only those uropathogenic UPEC (have pili are responsible for UTIs).

2-Other microorganisms are ***Proteus spp., Klebseilla sp., Enterobacter sp.*** and ***Acinetobacter sp***.

Note: ***Proteus spp*.** that produce urease turns the environment alkaline which causing damage to tissues leading to renal stone (normal vaginal pH level is between 3.8-4.5).

3-On the other hand, ***Staph. saprophyticus*** is more efficient in attaching to UT epithelial cells than coagulase positive ***Staphylococcus*** or ***Staph. epidermidis*.**

Predisposing factors

1. Sex (male or female). Female usually gets infection, because she has shorter urethra and its closer to vaginal & anal opening also due to the way of wiping & cleaning while male rarely gets infection due to longer urethra.
2. Obstruction of urethra.
3. Any obstructions (Tumor and Stones).
4. Pregnancy
5. Diabetes mellitus
6. Immunosuppression and immunodeficiency
7. Catheterization

Routes of Infection

There are three routes for bacteria to gain excess to UT.:

1. **Ascending route** (passage of bacteria from urethra to bladder and kidney).

2. **Haematogenous route** (hematogenous).
3- **lymphatic route**

- ✓ Although the ascending route is the **most common** course of infection in females, it is association with instrumentation (e.g., urinary catheterization, cystoscopy) is the most common cause of hospital acquired UTIs in both sexes.

Note: The only part of UT has a limited number of resident bacteria is urethra, these microflorae colonize the epithelium in the distal portion.

The Host-Parasite Relationship

In most cases, the host defense mechanisms are able to eliminate the organisms through the following:

1. **Inhibitory effect of urine** (urethral flora).
2. **Urine properties**
3. The constant **flushing** of contaminated urine from the body
4. The **bladder mucosal surface** has antibacterial properties.
5. **Valvelike mechanism** at the junction of the ureter and bladder prevents the reflux (backward flow) of urine from the bladder to the upper urinary tract.
6. Activation of the host **immune response**
7. **Anti-adherence factor** synthesized exclusively by epithelial cells in kidney .
8. **Defensins**, a group of small antimicrobial peptides.

Type of infection

Urethritis

Symptoms associated with urethritis are, dysuria (painful or difficult urination), and frequency are similar to those associated with

lower UTIs. Urethritis is a common infection. Because ***Chlamydia trachomatis***, ***Neisseria gonorrhoeae***, and ***Trichomonas vaginalis*** are common causes of urethritis and considered to be sexually transmitted.

Ureteritis

Inflammation or infection within the ureters is considered in combination with kidney infections. UTI within the ureters indicates that organisms are in the process of ascending into the kidneys and should be treated to prevent further infection.

Cystitis

Patients with cystitis (infection of the bladder) complain of dysuria, frequency, and urgency (compelling need to urinate). These symptoms are due not only to inflammation of the bladder but also to multiplication of bacteria in the urine and urethra, here is pain and urine is bloody cloud and a bad odor. Because cystitis is a localized infection, fever and other signs of a systemic illness are usually not present.

Laboratory Diagnosis of Urinary Tract Infections

The diagnosis of UTI include :

1- general examination of urine

2- culture has done depending on findings of general examination.

3- Other parameters of diagnosis might aid the diagnosis of UTI :

A-biochemical parameters

B-hematological parameters aid the diagnosis by showing of elevation (raise) in number of leucocytes in general and neutrophils in specific.

Note : Culture, is on the **top of all diagnostic tools**, final decision is going to be taken according to the out-come of culture. Different culture media are used to full-fill this purpose. **Vitek system**, **PCR**, or other techniques come to confirm the diagnosis.

1-Specimen Collection and Transport

Prevention of contamination by normal flora is the most important consideration for collection of a clinically relevant urine specimen.

a) Clean-Catch Midstream Urine

b) Straight Catheterized Urine: collection of uncontaminated urine from bladder.

c) Suprapubic Bladder Aspiration: contamination-free urine specimen is withdrawn directly into a syringe through inserted needle.

d) Indwelling Catheter.

Bacterial counts remain constant for as long as **24 hours** by: Refrigeration at (**4°C**) or use Urine transport tubes.

2-Screening procedures

as many as 60% to 80% of all urine specimens will be negative on culture or contain contaminants, so use the following procedure:

Direct microscopic examinations:

WBCs, RBCs, Epithelial cells at general urine analysis. The presence of more than five WBCs and abundant epithelial cells per HPF (high-power field) supports infections.

Gram stain:

The presence of one bacterium in un-centrifuged gram stained urine confirms urinary tract infections.

Indirect Indices

Frequently, screening tests detect bacteriuria or pyuria by examining for the presence of bacterial enzymes or PMN enzymes rather than the organisms or PMNs themselves.

1-Nitrate Reductase Test

2-Leukocyte Esterase Test

3-Catalase

Automated and Semiautomated Systems

There are an instrument analyzes both the microscopic components (bacteria and leukocytes) and the chemistries of urine and body system

3-Urine culture

Most often, microbiologists use a calibrated loop designed to deliver a known volume, either 0.01 or 0.001 mL of urine. The (0.01 mL) loop is recommended to detect lower numbers of organisms in certain specimens.

Culture media:

- ❖ blood agar and MacConkey agar for general isolates
- ❖ chromogenic media for special isolates

Interpretation of Urine Cultures

dependent on:

- The type of urine submitted (e.g., voided, straight catheterization)
- The clinical history of the patient (e.g., age, sex, symptoms, antibiotic therapy).

Contaminated with normal flora, including *Enterobacteriaceae*

Laboratory diagnosis for Urethritis & Cervicitis / Vaginitis

1. Urethral and vaginal discharge

Urethritis: It manifests with urethral discharge, pain during urination and frequency of urination. These types are:

a. Gonococcal urethritis

Causative agent: *Neisseria gonorrhea*

Incubation period is 2-7 days. It accounts for 1/3 of urethritis cases.

Clinical findings: Yellowish purulent discharge and dysuria.

b. Non-gonococcal urethritis

Causative agents: *Chlamydia trachomatis* (50%); *Ureaplasma urealyticum* (30%); and *Mycoplasma hominis.*

Incubation period about 2-3 weeks.

Clinical findings: White mucoid discharge.

-**Specimen**: Urethral discharge or swab (Before urination or antibiotics)

-**Gram stain**: Gram-negative intracellular diplococci

-**Culture**: Modified thayer-martin medium

-**Biochemical and serology**: Species identification

2. Cervicitis / Vaginitis

It manifests with vaginal discharge.

Causative agents: *Neisseria gonorrhea* (Mucopurulent vaginal discharge).

Non-specific vaginitis (Yellowish homogenous vaginal discharge). It is caused by anaerobes and *Gardnerella vaginalis*

-**Specimen:** Vaginal discharge.

-Wet mount: Clue (indication) cells that distorted vaginal epithelial cells coated heavily with gram-negative coccobacilli which are diagnostic of infection with *Gardnerella vaginalis*

-**Gram stain, culture, biochemical** and **serology** for species identification.

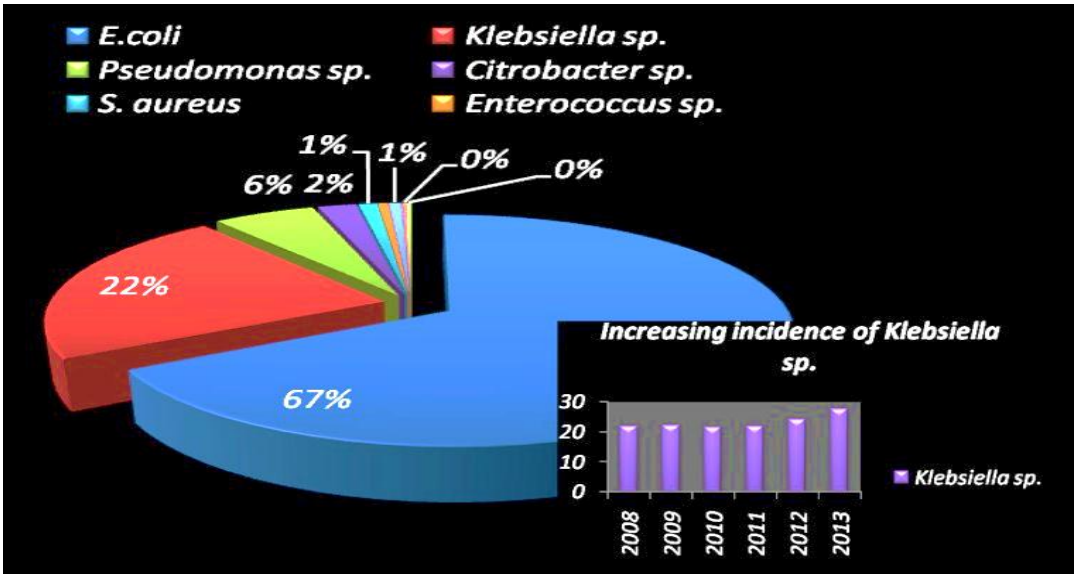


Figure (2): Percentages of UTIs bacterial causes

Genital tract infections

Anatomy :

The male reproductive system is located in close proximity to the urinary system, and the urethra is part of both systems. The testes are responsible for the production of sperm. The epididymis is a coiled tube that collects sperm from the testes and passes it on to the vas deferens. The epididymis is also the site of sperm maturation after they leave the testes. The seminal vesicles and prostate are accessory glands that produce fluid that supports sperm.

The female reproductive system is located near the urinary system .The external genitalia (vulva) in females open to the vagina, a muscular passage way that connects to the cervix. The cervix is the lower part of the uterus .The cervix is a common site of infection, especially for viruses that may lead to cervical cancer. The uterus leads to the fallopian tubes and eventually to the ovaries. Ovaries are the site of ova (egg) production, as well as the site of estrogen and progesterone production .

Resident microbial flora

The colonization of the surface by resident microbiota produces a biologic barrier preventing the adherence of pathogenic organisms. **Normal urethral microbiota** include coagulase-negative *Staphylococci* and *Corynebacteria*, as well as various anaerobes. The microbiota of the female genital tract varies with the pH and estrogen concentration of the mucosa, which depend on the host's age. The **females** of reproductive age may harbor large numbers of facultative bacteria such as ***Enterobacteriaceae, Streptococci***, and ***Staphylococci***, as

well as anaerobes such as ***Lactobacilli***, anaerobic non–spore-forming **bacilli** and **cocci**, and ***Clostridia***.

Note: The lactobacilli present in vaginal secretions metabolize glucose to lactic acid, resulting in a pH of approximately 4.0. The acidic pH coupled with the organism's ability to produce hydrogen peroxide prevents infection by exogenous sexually transmitted pathogens.

Sexually transmitted infections (STI), or sexually transmitted diseases (STD) and venereal diseases (VD):

are infections that are commonly spread by sex, especially vaginal contact, anal sex. Most STIs initially do not cause symptoms.

Symptoms and signs of disease may include **vaginal discharge**, **penile discharge**, **ulcers** on or around the **genitals**, and **pelvic pain**.

Bacterial STIs include:

1. Chlamydia (*Chlamydia trachomatis*)

2. Gonorrhea (*Neisseria gonorrhoeae*)

3. Granuloma inguinale or (*Klebsiella granulomatis*)

4. *Mycoplasma genitalium; Mycoplasma hominis*

5. Syphilis due to Spirochetes (*Treponema pallidum*)

6. Ureaplasma infection usually spread by sex,

Some STIs could also spread by **non-sexual contact** with contaminated blood and tissues, breastfeeding, or during childbirth.

Routes of Transmission

1- endogenous infections (genital microbiota)

2- exogenous infections

Clinical Manifestations

1- Asymptomatic

Although symptoms of genital tract infections generally cause the patient to seek medical attention, a patient with an STD, especially a female, may be free of symptoms (i.e., asymptomatic). For example, gonorrhea (*N. gonorrhoeae*) or chlamydia (*C. trachomatis*) infection is usually obvious in males because of a urethral discharge, yet females with either or both of these infections may have either minimal symptoms or no symptoms at all.

2-Dysuria

Although a common presenting symptom associated with urinary tract infection, dysuria (painful urination) can also result from an STD caused by organisms such as *N. gonorrhoeae, C. trachomatis*, and HSV.

3-Urethral Discharge

The presence of an inflammatory exudate at the tip of the urethral meatus is generally observed in males; the symptoms of urethral infection in females are not commonly localized. Most males complain of discomfort at the penile tip as well as dysuria. Urethritis (swelling and irritation of the urethra) may be gonococcal, caused by *N. gonorrhoeae*, or nongonococcal.

4-Lesions of the Skin and Mucous Membranes

Numerous organisms can cause genital lesions that are diverse in both their appearance and their associated symptoms but are most often associated with sexually transmitted diseases.The characteristics of the lesions may vary from one type of infectious process to another for the same organisms. For example, specific HPV genotypes infect mucosal cells in the cervix and anus.

5- Vaginitis

Inflammation of the vaginal mucosa, called vaginitis. Females who present with vaginal symptoms often complain of an abnormal discharge and additional symptoms such as an offensive odor or itching.

6- Cervicitis

Polymorphonuclear neutrophils (PMNs) are normally present in the endocervix; however, an abnormally increased number of PMNs may be associated with cervicitis (inflammation of the cervix). Therefore a purulent discharge from the endocervix can be observed in some cases of cervicitis. The endocervix is the site from which *N. gonorrhoeae* is most frequently isolated in females with gonococcal infections.

Lower Genital Tract Infections. Urethritis, Cervicitis, and Vaginitis

1-Urethral

Urethral discharge may occur in both males and females infected with pathogens such as *N. gonorrhoeae* and *T. vaginalis*. The presence of infection is more likely to be asymptomatic in females, because the discharge is usually less profuse and may be masked by normal vaginal secretions. to obtain a urethral specimen, a swab is inserted approximately 2 cm into the urethra and rotated gently before withdrawing. Because *Chlamydiae* are intracellular pathogens, it is important to remove epithelial cells (with the swab) from the urethral mucosa. When profuse urethral discharge is present, particularly in males, the discharge may be collected externally without inserting a sampling device into the urethra.

cervicitis and vaginitis

Organisms that cause purulent vaginal discharge (vaginitis) include *T. vaginalis*, *gonococci,* and, rarely, beta-hemolytic *Streptococci*.

The same organisms that cause purulent infections in the urethra may also infect the epithelial cells in the cervical opening , as can HSV. Mucous is removed by gently rubbing the area with a cotton ball. The urethral swab is inserted into the cervical canal and rotated and moved from side to side for 30 seconds before removal.

Direct Microscopic Examination

In addition to culture, urethral discharge may be examined by Gram stain for the presence of gram-negative intracellular diplococci , usually indicative of gonorrhae in males. After inoculation to culture media, the swab is rolled over the surface of a glass slide, covering an area of at least 1 cm^2 . Specimens collected from within the urethra may contain small cuboidal epithelial cells with a large nucleus. cultures of urethral discharge need not be performed.

Culture. Modified Thayer-Martin medium is most often used, although New York City (NYC) medium has the added advantage of supporting the growth of mycoplasmas and gonococci.Specimens must be inoculated to additional media for isolation of yeast, streptococci, and mycoplasmas. Yeast grows well on Columbia agar base with 5% sheep blood and colistin and nalidixic acid (CNA), although more selective media are available.

Example on bacterial STIs include:

***1-Chlamydia trachomatis*:**

The disease chlamydia or chlamydial urethritis is caused by *Chlamydia trachomatis*, an exceptionally small (0.35 μm), round to ovoid-shaped organism. Being an obligate, intracellular parasite, it has one of the smallest bacterial genomes, having about 600 genes (*Escherichia coli* has around 4,200 genes).

***C. trachomatis* is transmitted** by any sexually active individual can be infected through sexual contact with an infected individual. The disease has an incubation period of about 1 to 3 weeks. *Chlamydia* often is referred to as the "silent disease" because the organism does not cause extensive tissue injury directly. Chlamydial pharyngitis or inflammation of the anus (proctitis) is possible through anal intercourse.

Laboratory identification

Chlamydia trachomatis could have demonstrated in clinical material by several direct procedures and by culturing in human cell lines (tissue culture). Samples, particularly from the urethra and cervix in urogenital tract infection and conjunctivae in ocular disease, should obtained by cleaning away overlying exudate and gently scraping to collect infected epithelial cells.

1. **Direct tests:** Microscopic examination using direct fluorescent antibody staining reveals characteristic cellular cytoplasmic inclusions.*C. trachomatis* infections could have detected with high sensitivity and specificity using DNA amplification performed on urine specimens.

2. **Culturing methods:** *Chlamydia trachomatis* could have cultivated by tissue culture in several human cell lines. The presence of chlamydial inclusions could have demonstrated after 2 to 7 days of incubation.

3. **Detection of serotypes:** Serotypes of *Chlamydia trachomatis* could determine by immunofluorescence staining with monoclonal antibodies.

2-*Neisseria gonorrhoeae*

One of the most common STIs in men and women is gonorrhea caused by *Neisseria gonorrhoeae*. The organism, commonly known as the gonococcus .The great majority of cases of gonorrhea are transmitted during sexual intercourse.

Gonorrhea is the second most frequently reported nationally. Virulence Factors :

1. Receptors for human transferrin
2. Capsule (*N. meningitidis*)
3. Pili (fimbriae)
4. Cell membrane proteins
5. Lipooligosaccharide (LOS) or endotoxin.

Clinical Presentation

Following attachment of *N. gonorrhoeae* by pili to the genital tract, the incubation period for gonorrhea ranges from 2 to 6 day . Patients often report abdominal pain and a burning sensation on urination, and the normal menstrual cycle might be interrupted.

Symptoms of gonorrhea tend to be more acute in males than in females, and males thus tend to seek diagnosis and treatment more readily. In the male, the finding of numerous neutrophils containing gram negative diplococci in a smear of **urethral exudate** permits a temporary diagnosis of gonococcal infection and indicates that the individual should treated.

1. Growth conditions for culture: *N. gonorrhoeae* grows best under aerobic conditions, and most strains require enhanced CO2. *N. gonorrhoeae* utilizes glucose as a carbon and energy source but not maltose, lactose, or sucrose .All members of the genus are **oxidase-positive**, that used to identify *Neisseriae.*

2. Selective media: Gonococci, like pneumococci, are very sensitive to heating or drying.Thayer-Martin medium (chocolate agar supplemented with several antibiotics that suppress the growth of nonpathogenic *Neisseriae* and other normal and abnormal flora) has typically used to isolate gonococci. Culture of *N. gonorrhoeae* on Thayer-Martin agar remains the “gold standard” for diagnosis.

3-*Treponema pallidum*

Syphilis is caused by *Treponema pallidum* .This spirochete moves by means of endoflagella. Humans are the only host for *T. pallidum,* so the organism must spread by direct human-to-human contact, usually during sexual intercourse.

The incubation period for syphilis varies greatly (10 to 90 days), but it averages about 3 weeks.

_ **Primary Syphilis.** A lesion, called a chancre, which is a painless circular, purplish ulcer with a small, raised margin with hard edges is typical of primary syphilis .The chancre develops at the site of entry of the spirochetes, often the genital organs. However, any area of the skin can be affected, including the pharynx, rectum, or lips.

_ **Secondary Syphilis.** Several weeks after the chancre of primary syphilis has healed, the patient develops a fever and a flu-like illness as well as swollen lymph nodes. With secondary syphilis, a skin rash develops, which can be mistaken for measles, rubella, or chickenpox. The rash appears as reddish-brown spots on the palms, face, and trunk .Transmission can occur if there are moist lesions.

Tertiary Syphilis. About 40% of untreated patients develop tertiary syphilis. This stage occurs in many forms, but most commonly,it involves the skin, skeletal, or cardiovascular and nervous systems. The hallmark of tertiary syphilis is the gumma, a soft, painless,gummy noninfectious granular lesion.

Congenital syphilis: is a serious problem in pregnant women because the *Treponema* spirochetes penetrate the placental barrier after the third or fourth month of pregnancy. Infection in the fetus can lead to death (stillbirth); surviving infants can develop skin lesions and open sores. Affected children often suffer poor bone formation, meningitis, or

Hutchinson's triad, a combination of deafness, impaired vision, and notched, peg-shaped teeth.

Laboratory identification:

Definitive diagnosis of syphilis has complicated by the inability to cultivate *Treponema pallidum* subsp *pallidum* in vitro. Clinical manifestations, demonstration of treponemes in lesion material, and serologic reactions have used for diagnosis. If manifestations include one or more cutaneous exudative lesions, motile treponemes could visualized within lesion exudate by dark-field microscopy.

Treponema pallidum subsp *pallidum* is a fastidious organism that exhibits narrow optimal ranges of pH (7.2 to 7.4) and temperature (30 to 37°C). It is rapidly inactivated by mild heat, cold, desiccation, and most disinfectants.

The in vivo generation time is relatively long (30 hours). *T.pallidum* subsp *pallidum* had not successfully cultured in vitro. Viable organismscan be maintained for 18 to 21 days in complex media, while limited replication has been obtained by co-cultivation with tissue culture cells.

Blood tests

Blood tests have divided into non-treponemal and treponemal tests.

Because of the possibility of false positives with non-treponemal tests, confirmation is required with a treponemal test, such as treponemal pallidum particle agglutination (TPHA) or fluorescent treponemal antibody absorption test (FTA-Abs).

Treponemal antibody tests usually **become positive two to five weeks** after the initial infection. **Neurosyphilis is diagnosed** by finding high numbers of **leukocytes** (predominately lymphocytes) and high protein

levels in the cerebrospinal fluid (CSF) in the setting of a known syphilis infection.

Direct testing

Dark ground microscopy of serous fluid from a chancre (painless ulcer) may be used to make an immediate diagnosis. Sensitivity has reported to be nearly 80%; therefore, the test can only use to confirm a diagnosis.

Two other tests can carried out on a sample from the chancre: direct fluorescent antibody testing and nucleic acid amplification tests.

Direct fluorescent testing uses antibodies tagged with fluorescein, which attach to specific syphilis proteins, while nucleic acid amplification uses techniques, such as the **polymerase chain reaction**, to detect the presence of specific syphilis genes.

Lec 10 - 11

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.**

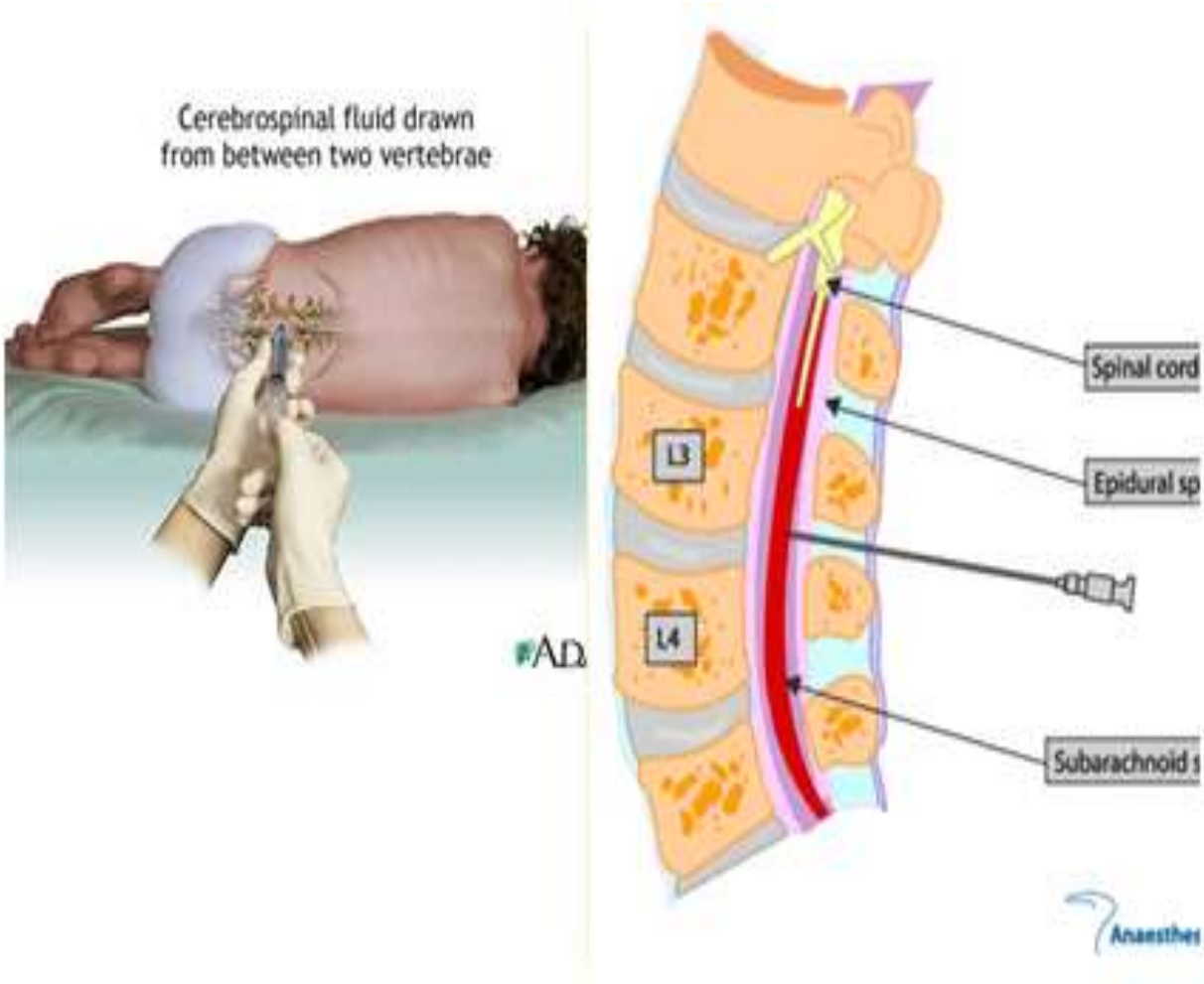


Figure (1): Collection of cerebrospinal fluid (CSF) by lumbar puncture.

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).

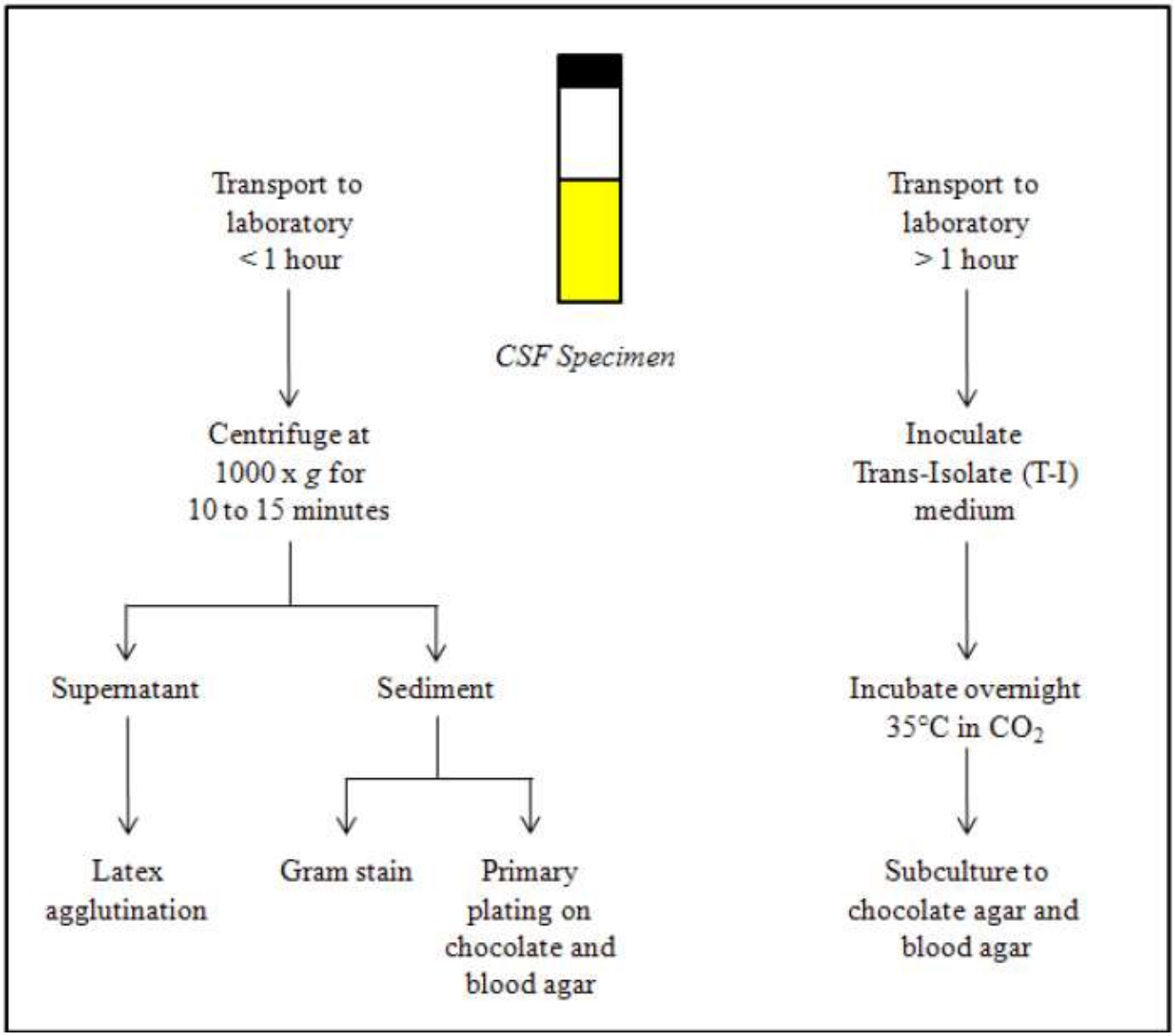


Figure (2): Cerebrospinal fluid (CSF) isolation and identification.

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 3- may occur intracellularly or extracellularly in polymorphic nuclei (PMN) leukocytes. 4- (PMNs or neutrophils are often more than 1000 WBCs/cu mm).**

5- ***Neisseria meningitidis*** is a **fastidious organism, aerobic diplococci,** which **6-** grows best at 35-37°C with ~5% CO2 (or **in a candle-jar**). **7-** It can grow on both a blood agar plate (BAP) and chocolate agar plate (CAP). **8-** 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 **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*, **10- serological tests** to identify the serogroup should performed. Additional methods for identification and characterization of ***N. meningitidis*** using molecular tools like **11-** PCR technique.

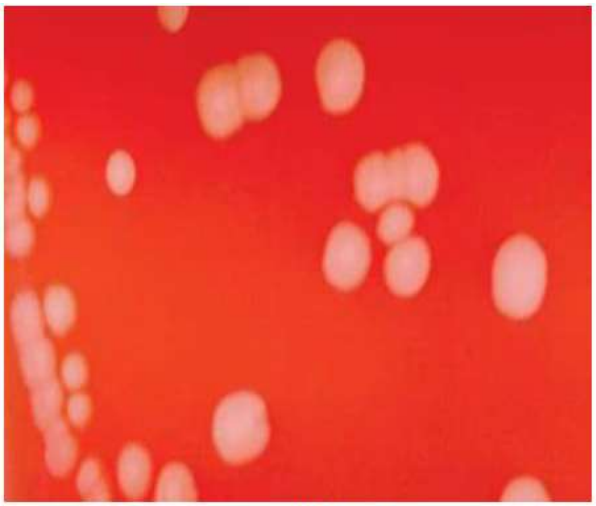


Figure (3): *N. meningitidis* colonies on a BAP

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 susceptible to 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**.

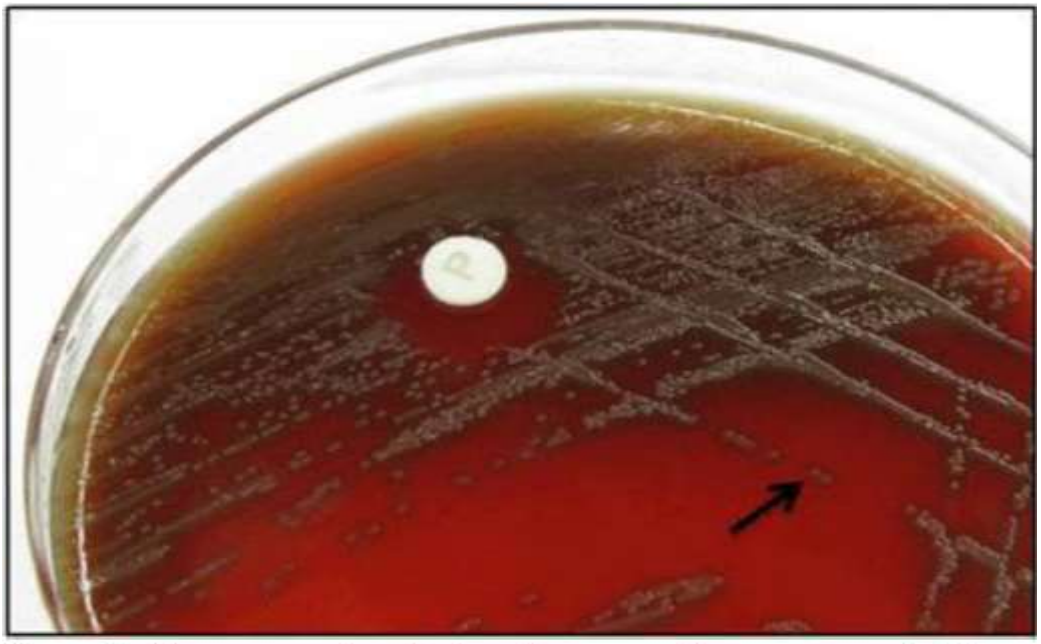


Figure (5): *Strep. pneumoniae* colonies with a surrounding green zone of alpha-hemolysis (black arrow) on a Blood Agar Plate.

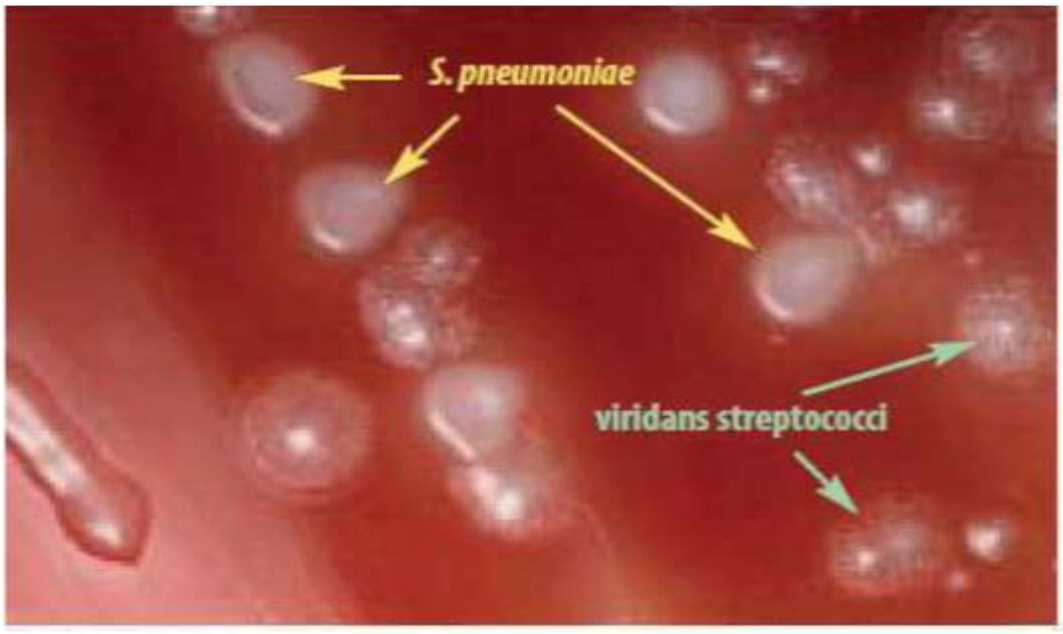


Figure (6): *Strep. pneumoniae* colonies have a flattened and depressed center afte

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

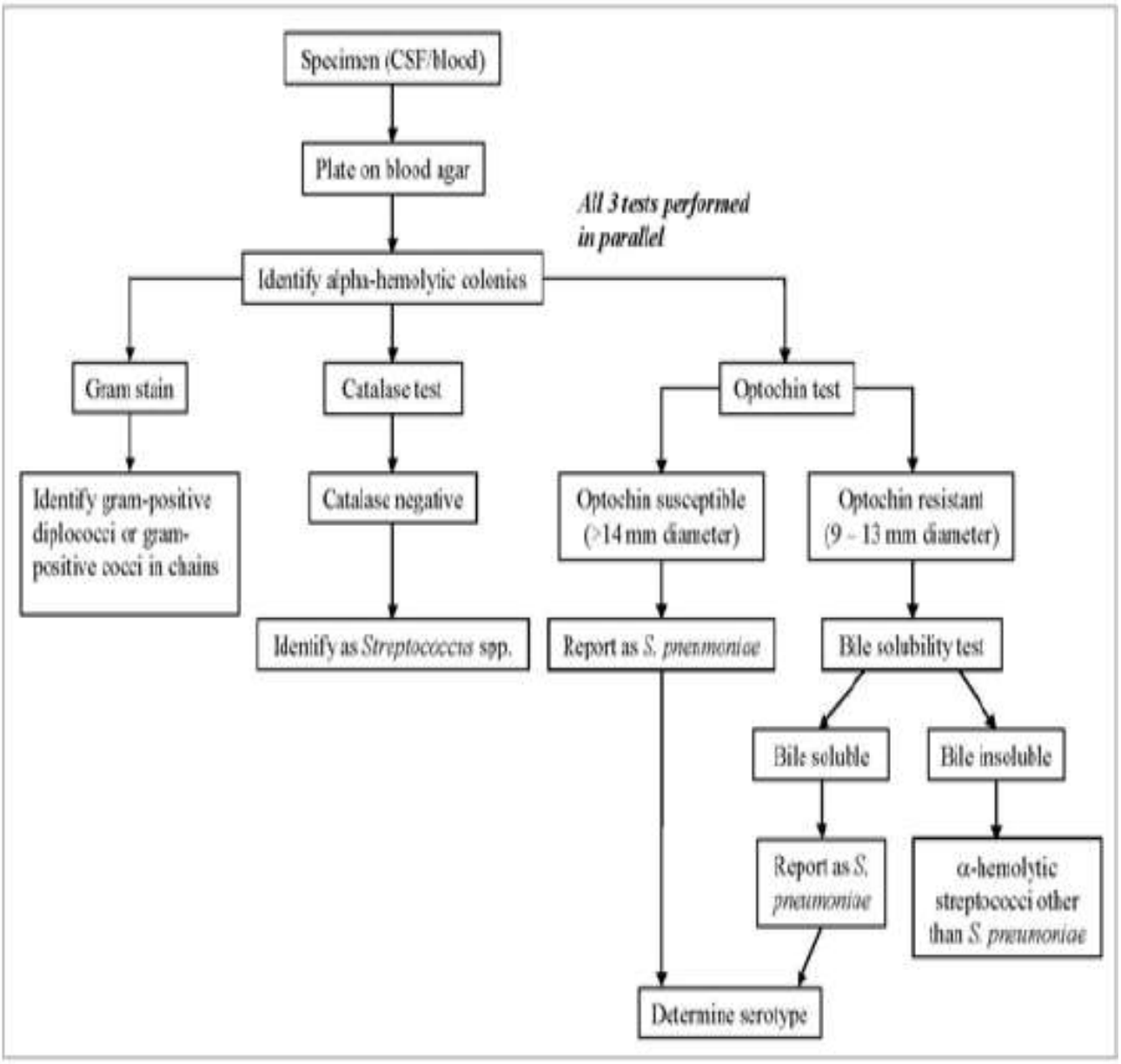


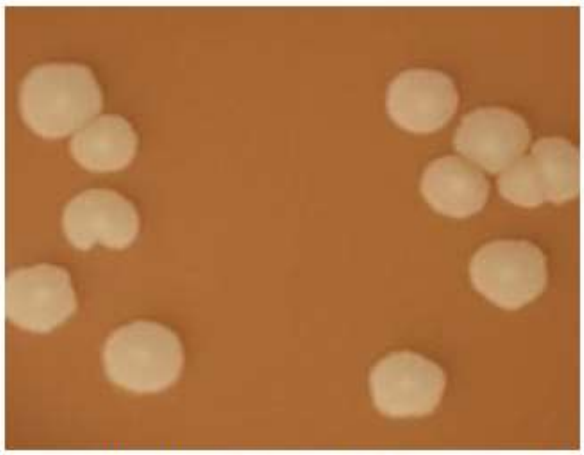
Figure (7): Flow chart for identification and characterization of a *Strep. pneumo* isolate.

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*.

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. 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 non-hemolyzed 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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Diagnostic Microbiology Lec 12 -13

Bacterial infections of eyes, ears and sinuses infections

Eye Infections

Anatomy

Eye (ocular) infections can be divided based on the area of the eye into external structures or the internal sites of the eye. **The external structures of the eye include eyelids, conjunctiva, sclera, and cornea. The eyeball comprises three layers. From the outside in, these tissues are the sclera, choroid, and retina. large interior space of the eyeball is divided into two sections: the anterior and posterior cavities.** The anterior cavity is filled with a clear and watery substance called **aqueous humor; the posterior cavity is filled with a soft, gelatin-like substance called vitreous humor.** Normally eyes are quite sterile sites of infections because of many defense mechanisms such as tear through lacrimation. **Tears** in eyes decreases the number of microorganisms that may find its way to eye because its content of **lysozyme that destroys bacterial cells.**

Resident Microbial Flora: *Staphylococcus aureus*, ***Staphylococcus epidermidis, Lactobacillus* spp. *Propionibacterium acnes, Haemophilus influenzae* and *Enterobacteriaceae*.**

While *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most common pathogenic causes of eye infections, while *Streptococcus* spp. are less common.

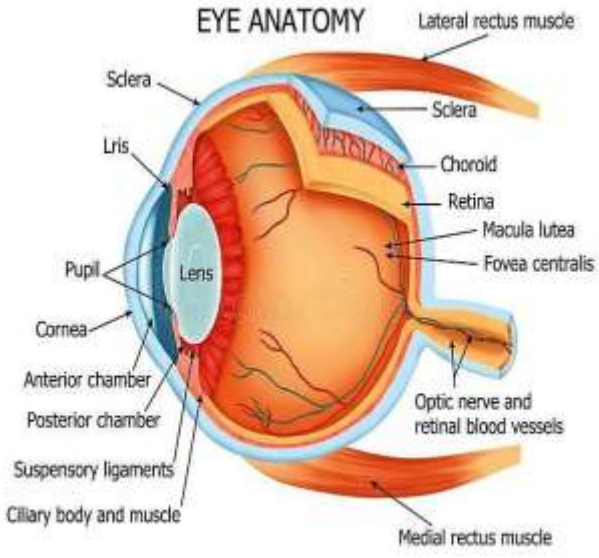

Fig.1: Anatomy of eye

Eye infection and etiology

1- Blepharitis

Blepharitis is a bump that appears on the eyelid that is red, swollen, and resembles a pimple. Most bumps on the eyelid are caused by an **inflamed oil gland on the edge of the eyelid** commonly referred to as a **sty**. ***Staphylococcus aureus* and *S. epidermidis* are the most common infectious agents associated with blepharitis.** Symptoms include burning, itching, the sensation of the presence of a foreign body, and crusting of the eyelids. Herpes simplex virus (HSV) produces vesicles on the eyelids that typically crust and heal with scarring within 2 weeks.

2- **Conjunctivitis**: **Bacterial conjunctivitis, commonly referred to as <u>"pink eye,"</u>** is the most common type of ocular infection and may be caused by allergies or **bacterial** or **viral infection**. **In neonates, Neisserial and chlamydial infections are frequent and are acquired during passage through an infected vaginal canal.** However, ***Chlamydia trachomatis*** **remains responsible for one of the most important types of conjunctivitis**, **<u>trachoma</u> is one of the leading causes of <u>blindness</u> in the world. In children** the most common causes of bacterial conjunctivitis include ***Haemophilus influenzae, S. pneumoniae, S. aureus*** **and** ***H. aegyptius.***

3- **Keratitis:** Keratitis (corneal infection) may be caused by a variety of infectious agents such as *Pseudomonas aeruginosa* and *S. aureu*s. With *P. aeruginosa* and *Neisseria gonorrhoeae* are responsible for the corneal destruction. The principal causes of eye infections are listed in the following "table-1":

Table-1: Infection of the eye and the causative agents

Infection	Description	Bacteria	Viruses	Fungi	Parasites
Blepharitis	Inflammation of the margins (edges) of the eyelids; (eyelids, eye lashes or associated pilosebaceous glands or meibomiam glawds) symptoms include irritation, redness, burning sensation, and occasional itching. Condition is typically bilateral	*Staphylococcus aureus*	Herpes simplex virus	Staphylococcues epidermidrs Malassezia furfar	Phthirus pulis
Conjunctivitis	Inflammation of the conjunctiva; symptoms vary according to the etiologic agent, but most patients have swelling of the conjunctiva, inflammatory exudates, and burning and itching	*Streptococcus pneumoniae, Haemophilus influenzae, S. aureus, Haemophilus* spp., *Chlamydia trachomatis, Neisseria gonorrhoeae, Streptococcus pyogenes, Moraxella* spp., *Corynebacterium* spp.	Adenoviruses, herpes simplex (HSV), varicella zoster. Epstein-Barr virus (EBV) influeza vius, pararryxovirus, rubella, HIV enterovirus, coxscukie A		
Keratitis	Inflammation of the cornea; although there are no specific clinical signs to confirm infection, most patients complain of pain and usually some decrease in vision, with or without discharge from the eye	*S. aureus S. pneumoniae, Pseudomonas, aeruginosa Moraxella lacunata, Bacillus* spp.	HSV, adenoviruses, varicella zoster	*Fusarium solani, Aspergillus* spp., *Candida* spp., *Acremonium, Curvularia*	*Acanthamoeba* spp.

Laboratory diagnosis

Specimen Collection: P**urulent material from the surface of the lower conjunctiva sac and inner canthus (angle) of the eye is collected on a sterile swab for Gram stain and cultures**. **Both eyes should be cultured separately.**

Chlamydial cultures are taken with a dry calcium alginate swab and placed in 2-SP (2-sucrose phosphate) transport medium. An additional swab may be rolled across the surface of a slide, fixed with methanol, and examined **by direct fluorescent antibody (DFA) chlamydia stains are used for detection**.
Multiple inoculations with the spatula are made to blood agar, chocolate agar, an agar for the isolation of fungi, thioglycollate broth, and an anaerobic blood agar plate.

Direct Visual Examination
All material submitted for culture should be smeared and examined directly by Gram stain or other appropriate microscopic techniques. In bacterial conjunctivitis, polymorphonuclear leukocytes predominate; in viral infection, the host cells are primarily lymphocytes and monocytes.

Culture
Because of the constant washing action of the tears, the number of organisms recovered from cultures of eye infections may be relatively low. Unless the clinical specimen is obviously purulent, using a relatively large inoculum and a variety of media is recommended to ensure recovery of the etiologic agent. Conjunctival scrapings placed directly onto media yield the best results. At a minimum, blood and chocolate agar plates should be used for isolation and identification of fastidious bacteria. PCR and ELISA techniques may be used also.

EARS Infections

Anatomy
The ear is divided into three anatomic parts: the external, middle, and inner ear. The middle ear is part of a continuous system including the nares, nasopharynx, auditory tube, and the mastoid air spaces. cells, mucus-secreting goblet cells. The middle and inner ear are normally sterile, while outer ear and auditory canal contain the normal flora of mouth, nose and skin. When a person coughs, sneezes or blow his nose these microorganisms may reach middle or inner ear and causing infection.

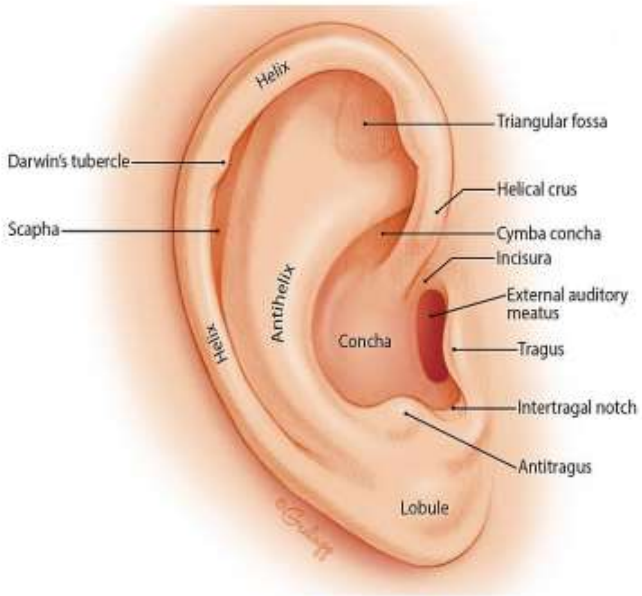

Fig.2: Anatomy of ear

Resident Microbial Flora: such as pneumococci, ***Streptococcus pneumoniae, Propionibacterium acnes, Staphylococcus aureus, Pseudomonas aeruginosa and Enterobacteriaceae*.** While the most common bacteria that cause ear infections are: **coagulase positive staphylococci, alpha & beta hemolytic Streptococci, Proteus spp. *Pseudomonas aeruginosa* and *E. coli***.

Infection of middle ear and sinuses

1. Acute infection

a. Acute otitis media

Causative agent: ***Hemophillus influenzae***, ***Strep. pneumoniae, Morexella catarrhalis***

Source: Endogenous; normal flora of the oropharynx.

Lab. diagnosis:

Specimen: Ear discharge (pus)

Procedures: Gram staining, culture, biochemical testing, serological testing, sensitivity testing

b. Acute sinusitis: Acute infections of middle ear and sinuses are often due to secondary bacterial invasion following a viral infection of respiratory tract.

Causative agent: *Hemophillus influenzae; Strep. pneumoniae; Strep. pyogenes*.

Source: Endogenous: normal flora of the nasopharynx.

Lab. Diagnosis:

Specimen: Lavage/drainage of sinuses.

Procedure: Gram staining, culture, biochemical testing for bacterial isolation, serological testing and sensitivity testing.

2. Chronic infection

a. Chronic suppurative otitis media.

Risk factors: History of acute or chronic otitis media; Parental (source) history of otitis media.

Causative agent: ***Pseudomonas aeruginosa, Strep. pneumoniae***

Laboratory diagnosis:

Specimen: Swabs of pus from the infected ear.

Procedure: Gram staining, culture, biochemical and serological test for microbe identification, nonculture methods include conventional and real-time PCR.

b. Chronic sinusitis

Painful sinuses and head ache are prominent symptoms; often associated with mucoid or purulent nasal discharge and nasal obstruction. Causal organisms are same as those implicated in acute sinusitis.

Laboratory diagnosis:

Specimen: Saline washings from the affected sinus.

Procedure: Gram staining, culture, biochemical and serological test for microbe identification, nonculture methods include conventional and real-time PCR.

Note: The external ear should be cleansed with a mild germicide to reduce the numbers of contaminating skin flora before obtaining the specimen (Specimens should be transported anaerobically

Diagnostic Microbiology Lec 14 - 15

Normally Sterile Body Fluids, Bone and Bone Marrow, and Solid Tissues

The human body is divided into five main body cavities: cranial, spinal, thoracic, abdominal, and pelvic. Each cavity is lined with membranes, and within the body wall and these membranes, or between the membranes and organs, are small spaces filled with minute amounts of fluid. The purpose of this fluid is to bathe the organs and membranes, reducing friction between organs. Bacteria, fungi, viruses, or parasites can invade any body tissue or sterile body fluid site.

Specimens from Sterile Body Sites

Fluids: In response to infection, fluid may accumulate in any body cavity. Infected solid tissue often presents as cellulitis or with abscess formation. Areas of the body from which fluids are typically sent for microbiologic studies (in addition to blood and CSF).

A- Pleural Fluid:

1. Lining the entire thoracic cavity of the body is a serous membrane called the parietal pleura.

2. Covering the outer surface of the lung is another membrane called the visceral pleura.

3. Within the pleural space between the lung and chest wall is a small amount of fluid called pleural fluid that lubricates the surfaces of the pleura (the membranes surrounding the lungs and lining of the chest cavity).

Table -1: Microbiology Laboratory Body Fluid Collection Sites

Body Area	Fluid Name(s)
Thorax	Thoracentesis or pleural or empyema fluid
Abdominal cavity	Paracentesis or ascitic or peritoneal fluid
Joint	Synovial fluid
Pericardium	Pericardial fluid

B- Peritoneal Fluid

The peritoneum is a large, moist, continuous sheet of serous membrane lining the walls of the abdominal-pelvic cavity and the outer coat of the organs contained within the cavity.

- Normal peritoneal fluid contains as many as 300 white blood cells per milliliter, but the protein content and specific gravity of the fluid are low.
- During an infectious or inflammatory process, increased amounts of fluid accumulate in the peritoneal cavity, a condition called ascites. Most cases of ascites are caused by liver disease, and in severe cases, the abdomen is often distended.
- The fluid can be collected for testing by paracentesis (the insertion of a needle into the abdomen and removal of fluid).

❖ Primary Peritonitis results when the peritoneal membrane becomes inflamed and can be either primary or secondary. The most common etiologic agents in children are *Streptococcus pneumoniae* and group –A streptococci, *Enterobacteriaceae*, other gram-negative bacilli, and staphylococci.

❖ In adults, *Escherichia coli* is the most common bacterium, followed by *S. pneumoniae* and group A streptococci.

- ❖ Among sexually active young women, ***Neisseria gonorrhoeae*** and ***Chlamydia trachomatis*** are common etiologic agents of peritoneal infection, often in the form of a perihepatitis (inflammation of the surface of the liver, called Fitz-Hugh–Curtis syndrome).

2- Secondary peritonitis is a complication of a perforated viscus (organ), surgery, traumatic injury, loss of bowel wall integrity after a destructive disease (e.g**., ulcerative colitis, ruptured appendix, carcinoma**), obstruction, or a preceding infection (liver abscess, **salpingitis**, **septicemia**).

C- Pericardial Fluid: The heart and contiguous major blood vessels are surrounded by the pericardium, a protective tissue.

1-The area between the epicardium, which is the membrane surrounding the heart muscle, and the pericardium is called the pericardial space and normally contains 15 to 20 mL of clear fluid.

2-Agents of pericarditis (inflammation of the pericardium) are usually viruses, especially coxsackie virus. Parasites, bacteria, certain fungi.

Myocarditis (inflammation of the heart muscle itself) may accompany or follow pericarditis. The pathogenesis of disease involves the host inflammatory response contributing to fluid buildup as well as cell and tissue damage. **Common causes of myocarditis include viral infections with coxsackie virus, echoviruses, or adenovirus.**

D- Bone: Bone Marrow Aspiration or Biopsy:

1- **Diagnosis of diseases**, including brucellosis, histoplasmosis, blastomycosis, tuberculosis, and leishmaniasis, can sometimes be made by detection of the organisms in bone marrow.
2- ***Brucella*** **spp**. can be isolated on culture media, as can fungi, but parasitic agents must be visualized in smears or sections made from bone marrow material.
3- **Many of the etiologic agents associated with disseminated infections** in patients with human immunodeficiency virus (HIV) may be visualized or isolated from bone marrow.

Some of these organisms include **cytomegalovirus**, **Cryptococcus neoformans**, and **Mycobacterium avium complex**.

E- Bone Biopsy

A small piece of infected bone is occasionally sent to the microbiology laboratory to identify the etiologic agent of osteomyelitis (infection of bone).

- ✓ **Patients develop osteomyelitis from hematogenous** spread of an infectious agent, invasion of bone tissue from an adjacent site (e.g., joint infection, dental infection), breakdown of tissue caused by trauma or surgery, or lack of adequate circulation followed by colonization of a skin ulceration with microorganisms.
- ✓ ***S. aureus,* seeded during bacteremia**, is the most common etiologic agent of osteomyelitis among patients of all age groups.
- ✓ Other organisms recovered **from hematogenously acquired osteomyelitis** include ***Salmonella*** spp., ***Haemophilus*** spp., ***Enterobacteriaceae***.
- ✓ **Nucleic acid–based testing**, such as polymerase chain reaction (**PCR**), may be useful in determining the infectious organism.