Section 6

Antibiotic susceptibility tests

Antimicrobial agents are nontoxic antimicrobial therapeutic agents, which include antiseptics, antibiotics, preservatives, sterilants, and disinfectants; all have the capacity to kill or suppress the growth components of the practice of medicine. They are used to treat, prevent, and control the distribution of bacterial pathogens.

The term antibiotic has been traditionally reserved for compounds that are naturally produced by **living microorganisms,** such as bacteria and fungi.

The term has come to be more widely applied to any natural, semisynthetic, or synthetic molecule used to treat or prevent disease. Antibiotics target anabolic **cellular processes** such as:

1. Cell wall synthesis.

2. DNA replication.

3. RNA transcription.

4. Messenger RNA (mRNA) translation.

Antibiotic susceptibility testing is performed on bacteria **isolated** from **clinical specimens** to determine which antimicrobial agents might be effective in treating infections caused by the bacteria. Only bacteria that are likely to be contributing to an infection should be tested. Testing bacteria that are not involved in the infection would be misleading to the physician and could lead to a **more serious infection** with development of **antimicrobial resistance.** One of the major challenges in clinical microbiology is the identification of the bacterium that caused infections.

Often, these bacteria **need** to be **distinguished** from **normal flora** that may be present in at the site of the infection normally, although in some situations the microbial flora that reside at the site of the infection may be **contributing to the infection**. Therefore, thought needs to go into determining which bacteria from a specimen will be tested for susceptibility to antimicrobials.

Most microbiology laboratories have guidelines for determining when and on which bacteria susceptibility testing will be done. When in doubt about the significance of a bacteria from a specimen, it is best to discuss the **situation** with the attending **physician**.

In clinical laboratories, susceptibility testing is usually performed by a disk diffusion or and minimal inhibitory concentration [MIC] methods. Standards that describe these methods are published and frequently updated by the **Clinical and Laboratory Standards Institute (CLSI),** formerly the **National Committee for Clinical Laboratory Standards [NCCLS].**

After a pathogen is cultured, its sensitivity to specific antibiotics serves as a guide in choosing antimicrobial therapy Some pathogens, such as *Streptococcus pyogenes* and *N. meningitidis*, usually have predictable sensitivity patterns to certain antibiotics. In contrast, most gram-negative bacilli, enterococci, and staphylococcal species show unpredictable sensitivity patterns to various antibiotics and require susceptibility testing to determine appropriate antimicrobial therapy.

There are many methods for detecting this bacterial susceptibility pattern like:

1. Disk-diffusion method

The classic qualitative method to test susceptibility to antibiotics has been the Kirby-Bauer disk-diffusion method, in which disks with exact amounts of different antimicrobial agents are placed on culture dishes inoculated with the microorganism to be tested. The micro-organism's growth (resistance to the drug) or lack of growth (sensitivity to the drug) is then monitored (Figure-7).

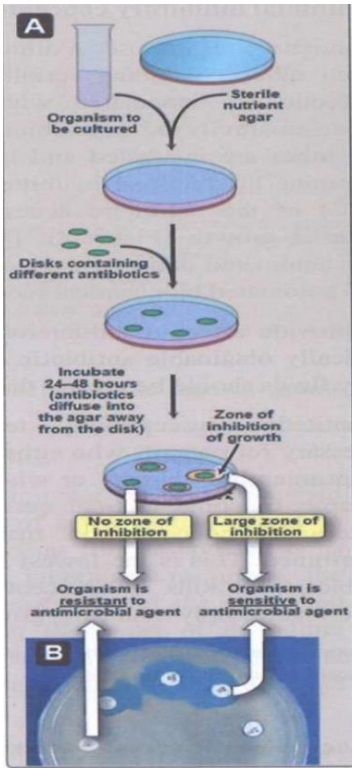
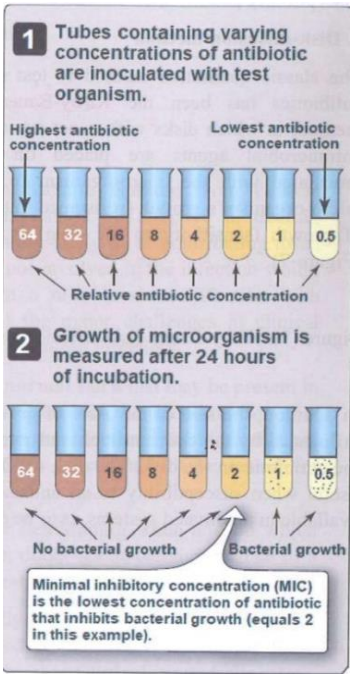


Figure (7): Kirby-Bauer disk-diffusion method

In addition, the size of the zone of growth inhibition is influenced by the concentration and rate of diffusion of the antibiotic on the disk. The disk - diffusion method is useful when susceptibility to an unusual antibiotic, not available in automated systems, is to be determined

2. Minimal inhibitory concentration



Quantitative testing uses a dilution technique in which tubes containing serial dilutions of an antibiotic are inoculated with the organism whose sensitivity to that antibiotic is to be tested. The tubes are incubated and later observed to determine the minimal inhibitory concentration (MIC) of the antibiotic necessary to prevent bacterial growth (Figure-8). [Note: MICS are now automated and often done simultaneously with automated biochemical identifications.]

To provide effective antimicrobial therapy, the clinically obtainable antibiotic concentration in body fluids should be greater than the MIC.

Quantitative susceptibility testing may be necessary for patients who either fail to respond to antimicrobial therapy or who relapse during therapy. In some clinical cases, the minimal bactericidal concentration may need to be determined. This is the lowest concentration of antibiotic that kills 100 percent of the bacteria, rather than simply inhibiting growth.

Figure (8): Determination of minimal inhibitory concentration (MIC) of an antibiotic.

3. Bacteriostatic versus bactericidal drugs

As noted above, antimicrobial drugs may be bacteriostatic or bactericidal.

Bacteriostatic drugs arrest the growth and replication of bacteria at serum levels achievable in the patient, thereby limiting the spread of infection while the body's immune system attacks, immobilizes, and eliminates the pathogens. If the drug is removed before the immune system has scavenged the microorganisms; enough viable organisms may remain to begin a second cycle of infection.

Figure (9) shows a laboratory experiment in which the growth of bacteria is arrested by the addition of a bacteriostatic agent. Note that viable organisms remain even in the presence of the bacteriostatic drug. By contrast, addition of a bactericidal agent kills bacteria, and the total number of viable microorganisms' decreases. Although practical, this classification may be too simplistic because it is possible for an antibiotic to be bacteriostatic for one organism and

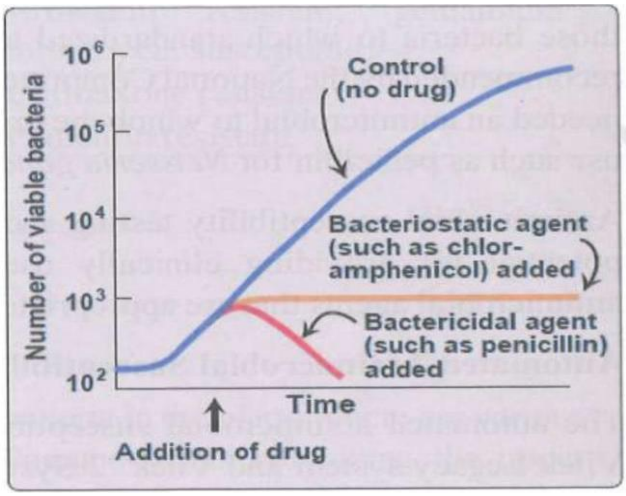


Figure (9): Effects of bactericidal and bacteriostatic drugs on the growth of bacteria in vitro

bactericidal for another (for example, chloramphenicol is bacteriostatic against gram-negative rods and bactericidal against pneumococci).

Laboratory strategies for antimicrobial susceptibility testing

The clinical microbiology laboratory is responsible for maximizing the positive impad that susceptibility, testing information can have on the use of antimicrobial agents to therapeutically manage infectious diseases. However, meeting this responsibility is difficult-because of de-mands for more efficient use of laboratory resources, the increasing complexities of important bacterial resistance profiles, and the continued expectations for high-quality results. To ensure quality in the midst of dwindling resources and expanding antimicrobial resistance, strategies for antimicrobial susceptibility testing must be carefully developed. These strategies should target accuracy, and communication as their goals (Table-I).

Table (1): Categorization of Bacteria According to Need for Routine Performance of Antimicrobial Susceptibility Testing.

Testing commonly	Testing OcCasionally	Testing Rarely required
Staphylococci *Streptococcus pneumoniae* *Viridans streptococci* *Pseudomonas aeruginosa* *Acinetobacter Spp*	*Haemophilus influenzae* *Neisseria gonorrhoeae* *Moraxel/a catarrhalis* *Anaerobic bacteria*	Beta-hemolytic streptococci (groups A, B, C,Fand G) *Neisseria meningitides Listeria monocytogenes*

Based on the assumption that the organism is clinically significant. Table () only includes those bacteria to which standardized testing procedures are available as outlined and recommended by the National Committee for Clinical Laboratory Standards. Testing only needed an antimicrobial to which the organisms are frequently resistant is considered for use such as penicillin for Neisseria gonorrhoeae.

Antimicrobial susceptibility testing should only be performed when there is sufficient potential for providing clinically useful and reliable information regarding those A antimicrobial agents that are appropriate for the bacterial isolate.

Automated Antimicrobial Susceptibility Test Systems.

The automated antimicrobial susceptibility test systems available for use include the Vitek Legacy system and Vitek 2 Systems (bioMerieux, Inc., Hazelwood, Mo), the Micro Scan Walkaway System (Dade International, Sacramento, Calif), and the Phoenix System (BD Microbiology Systems, Cockeysville, Md). These different systems vary with respect to the extent which inoculum preparation and inoculation are automated, the methods used to detect growth, and the procedures used to interpret and assign MIC values and categorical (i.e., susceptible, intermediate, resistant) findings.

In summary, this system facilitates standardized susceptibility testing in a closed environment with validated results and recognition of an organism's antimicrobial resistance mechanism in 6 to 8 hours for most clinically relevant bacteria.

Microbiologists must be aware of the strengths and weaknesses of their primary susceptibility testing methods (disk diffusion, commercial broth micro dilution, automation) for detecting relevant resistance patterns and know when assistant or supplemental testing is necessary. (Table-2)

Table (2): Examples of Susceptibility Testing Profiles.

Organism	Susceptibility Profiles
Staphylococci	Vancomycin intermediate or resistant Clindamycin resistance; erythromycin susceptible
Viridans streptococci	Vancomycin intermediate or resistant
Streptococcus pneumoniae	Vancomycin intermediate or resistant
Beta-hemolytic streptococci	Penicillin intermediate or resistant
Enterobacteriaceae	Imipenem resistant
Enterobacter/Citrobacter/Serratia/Morganella	Susceptible to ampicillin or cefazolin
Pseudomonas aeruginosa	Amikacin resistant; gentamicin or tobramycin susceptible
Neisseria gonorrhoeae	Ceflriaxone resistant
Neisseria meningitidis	Penicillin resistant

Section 7

Blood stream infections

Which is primarily concerned with the presence of bacteria in the blood. There are various routes that organisms take to reach the blood. 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.

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). When native valve endocarditis is identified it can be straightforward to determine the

likely source of the organism. The patient with endocarditis caused by a streptococcus of the mouth flora, such as Streptococcus sanguinis, can have poor dentition, and this needs to be addressed as part of the patient's management, usually "involving the maxilla-facial surgical team.

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. The finding of Streptococcus gallolyticus in blood culture, often in the setting of endocarditis, is an alert to investigate this malignancy; if found this is removed before any valve surgery.

Bacteraemia defines the presence of bacteria as detected by the culture of blood. Septicaemia 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, multi-organ failure and leukocytosis, alert the clinician to the severity of the situation, and the need for immediate action in the management of the patient.

Bacteraemia can be defined as transient (a single episode lasting less than 30 minutes or so), intermittent or continuous. These definitions are important concepts in terms of the site from which they may arise. A transient bacteraemia can arise from a pneumococcal pneumonia, or pyelonephritis caused by *Escherichia coli*. *An intermittent bacteraemia implies* manipulation of an extravascular site, such as a Staphylococcus aureus abscess, where bacteria enter the lymphatics at irregular intervals, and from there, the blood. A continuous bacteremia implies an intravascular source, and endocarditis is the most important example.

Once bacteria enter the blood, they have the potential to settle in other sites of the body, and set up another focus of infection. A *Staphylococcus aureus* bacteremia arising from an infected peripheral venous cannula (PVC) site can result in bacteria attaching to a heart valve to initiate endocarditis, or settling in the spine and causing an abscess there. The bacteria can

cross the synovial membrane of a joint to initiate septic arthritis. These examples underline the critical importance of full clinical assessment of the septic or bacteremic patient.

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** Mycoplasma pneumoniae Neisseria spp. Gram-negative anaerobes Actinomyces spp. Nocardia spp. Rickettsia spp. Chlamydia trachomatis	Microscopy and culture from punctate DNA test from punctate if re- quired Serology; culture from punctate Microscopy and culture from punctate Serology

Section 8

Meningitis and other infections of the central nervous system

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.*

Bacterial Diagnosis of Cerebrospinal Fluid

Cerebrospinal (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 behaviours.

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 and adults.

• *Haemophilus influenzae* infect children 6 months to 5 years

• *Neisseria meningitidis* infect all ages

• *Streptococcus pneumonia* 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 10). 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.

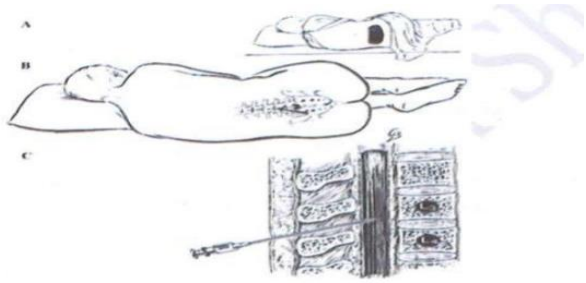


Figure (10): Collection of cerebrospinal fluid (CSF) by lumbar puncture.

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 11).

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).

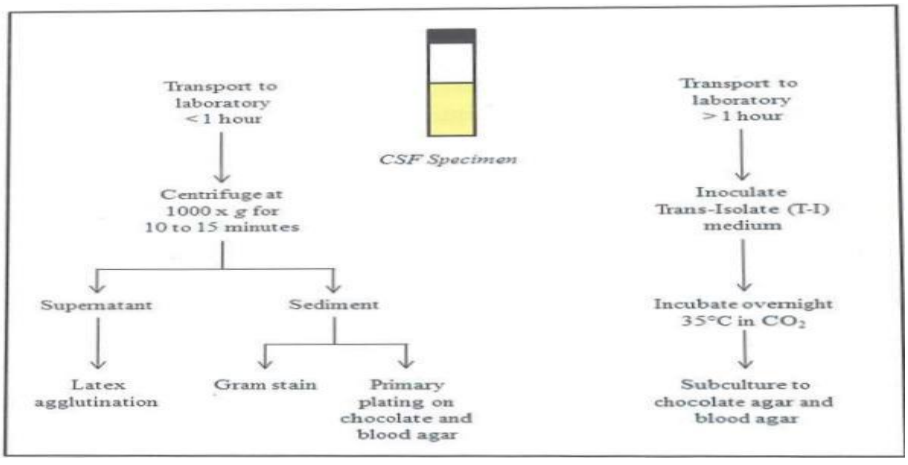


Figure (11): Cerebrospinal fluid (CSF) isolation and identification.

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 meningitidis are gram-negative, coffee-bean shaped diplococci that may occur intracellularly or extracellularly in polymorphic nuclei (PMN) leukocytes. (**PMNs or neutrophils are often more than 1000 WBCs/cu mm)**

Neisseria meningitidis is a fastidious organism, aerobic diplococci, which grows best at 35-37°C with ~5% CO2 (or in a candle-jar). It can grow on both a blood agar plate (BAP) and a chocolate agar plate (CAP). 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 12, 13).

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*, serological tests to identify the Serogroup

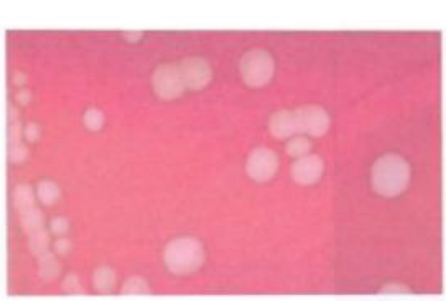


Figure (12): *N. meningitidis* colonies on a BAP

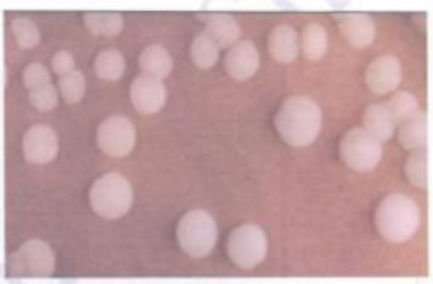


Figure (13): *N. meningitidis* colonies on a CAP

should performed. Additional methods for identification and characterization of ***N. meningitidis*** using molecular tools like PCR technique.

Strep. 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 alpha-hemolysis (green) (**Figure 14).**

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 15). When necessary, to obtain a pure culture. 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 16). ***Strep. pneumoniae*** can be identified using Gram stain, catalase (-), and optochin tests (see figure 17) (<14mm diameter) at the same time, with bile solubility (+) confirmatory test. If these tests indicate that, the isolate is Strep. pneumoniae, then as a 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.

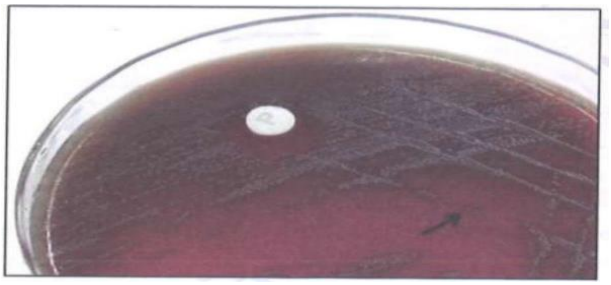


Figure (14): Strep. pneumoniae colonies with a surrounding green zone of alpha- hemolysis (black arrow) on a Blood Agar Plate.

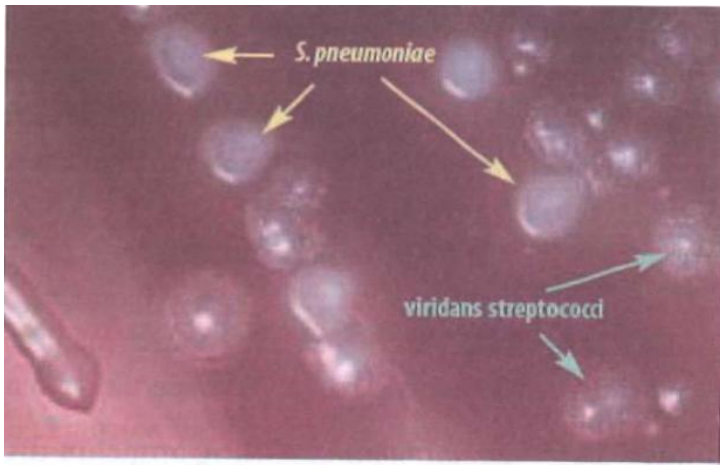


Figure (15): Strep. pneumoniae colonies have a flattened and depressed center after 24-48 hours of growth on a BAP, whereas the viridans streptococci retain a raised center.

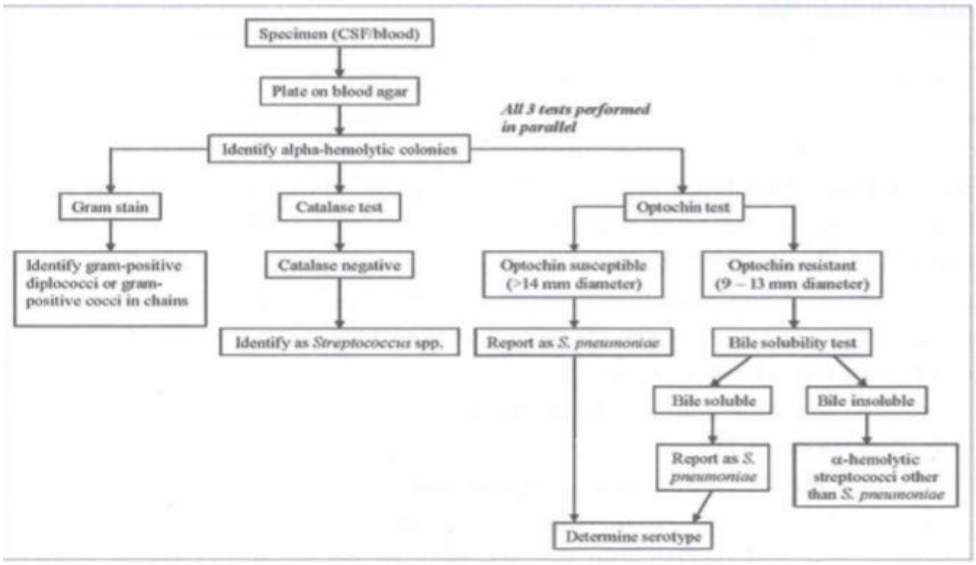


Figure (16): Flow chart for identification and characterization of-a Strep. pneumoniae isolate.

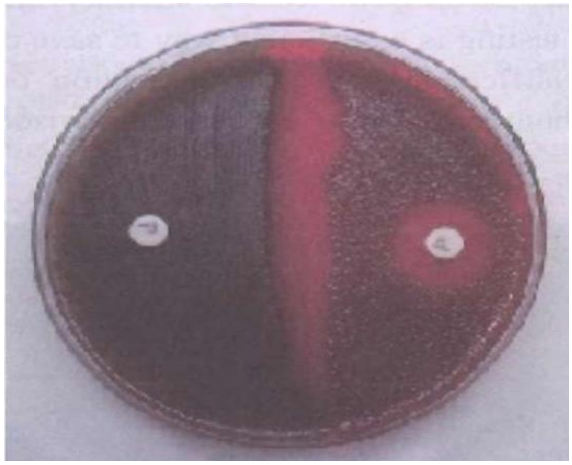


Figure (17): Optochin test for Strep. pneumoniae using optochin disks. The strain on the left is resistant to optochin with no zone of inhibition, and therefore is not a pneumococcus. The strain on the right is susceptible to optochin and is Strep. pneumoniae.

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% CO2 (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 18). ***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 19).

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. All these causes summarized at table (4).

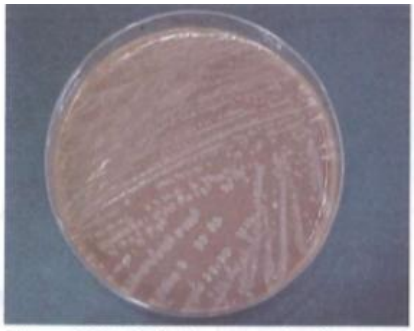


Figure (18): *H. influenzae* colonies on a CAP.

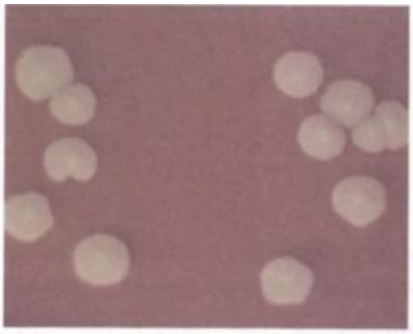


Figure (19): *H. influenzae* colonies on a CAP

Infection	Most important Pathogens	Laboratory Diagnosis
Meningitis Bacteria	Neisseria meningitidis (~ 20 %) Streptococcus pneumoniae (~ 30 %) Haemophilus influenzae b (Less frequent now due to Vaccination in children) Rare : Enterobacteriaceoe (senium) Mycobacterium tuberculosis Leptospira interrogans Listeria monocytogenes Neonates : E. coli Group B streptococd	**Microscopy and culture from cerebrospinal fluid; antigen detection if required (rapid test)**
Bacteria	.Rickettsia spp. Brucella spp	**Serology**
	Borrelia burgdorferi	**Serology and PCR ; culture in biopsy if required**
	Leptospira interrogans	**Serology and culture in biopsy if required**

	Treponema pallidum	**Syphilis serology**
	Listeria monocytogenes	**Try microscopy and culture from cerebrospinal fluid and blood**
	Mycobacterium tuberculosis	**Microscopy and culture from cerebrospinal fluid; DNA test if required**
Cerebral abscess Epidural abscess Subdural empyema	Streptococcus milleri Gram-negative anaerobes Enterobacteriacege Staphylococcus aureus	**Microscopy and culture for bacteria from pus**
	Mucorales Aspergillus spp. .Candida spp	**Microscopy and culture for fungi from pus; serology**
	Toxoplasma gondi	**Serology. Microscopy; DNA test (in cerebrospinal fluid)**
Tetanus	Clostridium tetani	**Toxin (animal test, PCR) in material excised from wound. Try microscopy and culture from excised material**
Botulism	Clostridium botulinum	**Toxin detection in blood or food (animal test, PCR)**
Leprosy (peripheral nerves)	Mycobacterium leprae	**Microscopy of biopsy specimen. or scrapings from nasal mucosa**

Table (4): summary of bacterial nervous system infections.

Section 9

Bacterial infections of respiratory tract

Respiratory system has divided into two major parts:

■ Upper 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 opportunistic disease.** 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.

Sore throat 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** (Table 5)

Disease	Bacterial causes	Lab Diagnosis
Sinusitis	*Streptococcus pneumoniae* *Haemophilus influenzae* *Staphylococcus aureus* *Moraxella catarrhalis* (children) *Streptococcus pyogenes* rarely: anaerobes	Microscopy and culturing from sinus secretion/pus (punctate) or sinus lavage

Bacteria	*Streptococcus pyogenes*, rarely: streptococci of groups B, C, or G	Culture from swab; rapid antigen detection test for A-streptocci in swab material if required
Plaut-vincent angina	*Treponema vincentii* + mixed anaerobic flora	Microscopy from swab
Acute necrotic ulcerous gingivostomatitis	*Treponema vincentii* + mixed anaerobic flora	Microscopy from swab
Diphtheria	*Corynebacterium diphtheriae*	Culture from swab
Epiglottitis	*Haemophilus influenzae* (usually serovar “ b") Mbre rarely: *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*	Blood culture. Culture from swab (caution: respiratory arrest possible in taking the swab)
Acute bronchitis. Acute bronchiolitis (small children)	*Mycoplasma pneumoniae* *Chlamydia pneumoniae*	Serology Serology
Acute exacerbation of "chronic obstructive pulmonary disease"| (COPD)	*Streptococcus pneumoniae* *Haemophillus influenzae* *moraxella catarrhalis*	Culture from Sputum or bronchial secretion
Tuberculosis	***Mycobacterium tuberculosis*** other mycobacteria	Microscopy and culture (time requirement : 3-6-8 weeks)
Bacteria (80-90 %)/ "Community-acquired "pneumonía	*Streptococcus pneumoniae* (30%) *Haemophilus influenzae* (5%) *Staphylococcus aureus*	Microscopy and culturing from expectorated sputum, or better yet from transtracheal or bronchial

	(5%) *Klebsiella pneumoniae Legionella pneumophila* Mixed anaerobic flora (aspiration pneumonia) *Mycoplasma pneumoniae* (10%) *Coxiella burnetii* *Chlamydia psittaci* *Chamydia pneumoniae*	aspirate, from bronchoalveolar lavage or biopsy material. If anaerobes are suspected use special transport vessels Serology Serology Serology: CFT can detect only antibodies to genus. Microimmunofluorescence (MIF) species-specific Serology: MIF
Hospital-acquired " "pneumonia	***Enterobacteriaceae*** ***Pseudomonas aeruginosa*** ***Staphylococcus aureus***	Laboratory procedures see above at "community- "acquired pneumonia
Pulmonary abscess Necrotizing pneumonia	Usually endogenous infections with Gram-negative/Gram-positive mixed anaerobic flora Aerobes also possible	Microscopy and culture from transtracheal or bronchial aspirate, bronchoalveolar lavage or lung biopsy. Transport in medium for anaerobes

Table (5): 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 pneumonia; 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. pneumonia, Staph. aureus, Haemophillus influenzae and Mycoplasma pneumonia***.

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, WBCS,** 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, **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.

Section 10

Bacterial infections of urinary tract systems

Urinary tract consists of the kidney, ureters, bladder and urethra as shown in figure (2). The function of it is produce and process urine, which is **normal sterile**. Urinary tract infection has classified as; **upper** or **lower tract** infections based on the location of infections. The upper urinary tract consists of the kidneys and the ureters, and the lower urinary tract consists of the bladder and the urethra.

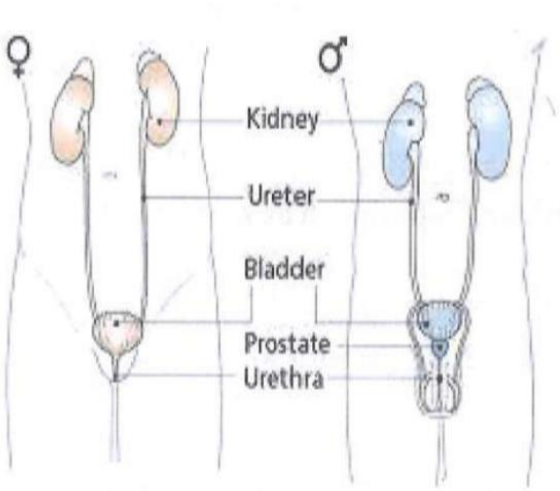


Figure (2): Male and female urinary tract system.

They are one of the most common types of infection and account for around 8.1 million visits to a physician every year. A proper classification has employed currently: Hospital or community acquired infections, whatever the classification is an infection could affect ureters (ureteritis), or renal parenchyma cells (pyelonephritis), or urethra (urethritis), or the bladder (cystitis). Sometimes prostate gland might involve (prostitis). There are **three routes for bacteria to gain excess to UT**.: ascending, hematogenous and lymphaties.

Routes of infection

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

2. Haematogenous route (source of infection is blood).

- Ascending route is the commonest route infection of the urinary tract.

Females are susceptible to get infection than men because of shorter urethra that allow pathogens to reach different site of urinary tract. The **only part of UT** has a

limited number of resident bacteria is urethra, these microflorae colonize the epithelium in the distal portion.

Most UTIS go away after treatment. **Chronic UTIs** either don't go away after treatment or keep recurring. Recurrent UTIS are common among women.

Bacterial species involved in community acquired UTI is by far *E. coli*, yet not all E. coli are capable causing UTI, **only those uropathogenic E. coli equipped** by pili are responsible for UTIS. Other microorganisms incriminated with UTIS are *Proteus sp., Klebseilla sp., Enterobacter sp. and Acinetobacter sp.*

On the other hand, *Staph. saprophyticus* is more efficient in attaching to UT epithelial cells than **coagulase positive Staphylococcus or Staph. Epidermidis.** The former is associated with UTIS among females in reproductive ages. *Proteus sp*. 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).**

Lab. diagnosis

The diagnosis of UTI include **general examination** of urine then **culture** has done depending on findings of general examination. Other parameters of diagnosis might aid the diagnosis of UTI, **biochemical parameters** are of great importance in diagnosis of complicated UTI, **hematological parameters** aid the diagnosis by showing of elevation (raise) in number of **leucocytes in general and neutrophils in specific**.

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** in most cases.

The **existence of pus** cells is the **guide** to culture urine sample, yet, this is not valid for every case, pus cells sometimes may reflect an inflammation act, i.c. culture shows no bacterial growth, or absence of pus **does not mean that the patient has no UTIS**. Proteus spp., Staphylococcus spp. or any bacterial species produces urease enzyme may destroy pus cells and give false negative results. Mycoplasma, ***Chlamydia spp.*** are produced pus with no growth on eulturing routinely, so it very important to take care towards **these observations.** (Pus consists of a thin, protein-rich fluid and dead **leukocytes** from the body's **immune response** (mostly **neutrophils)**).

Conclusion:

The commonest causative agents of UTIS are gram-negative rods (as listed in figure 20). These are: **Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Proteus spp., Enterobacter aerogens.**

Other important causative agents: ***Enterococci*** and ***Staphylococcus saprophyticus***

Laboratory diagnosis:

- Specimens: Clean midstream urine, Catheterized urine, Suprapubic aspiration.
- 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 of urinary tract.

- Gram stain: The presence of one bacterium in un-centrifuged gram stained urine confirms urinary tract infections.
- Culture: Blood agar medium, MacConkey agar medium (see figure 21).

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

Laboratory diagnosis:

- 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

Laboratory diagnosis:

- ❖ 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.

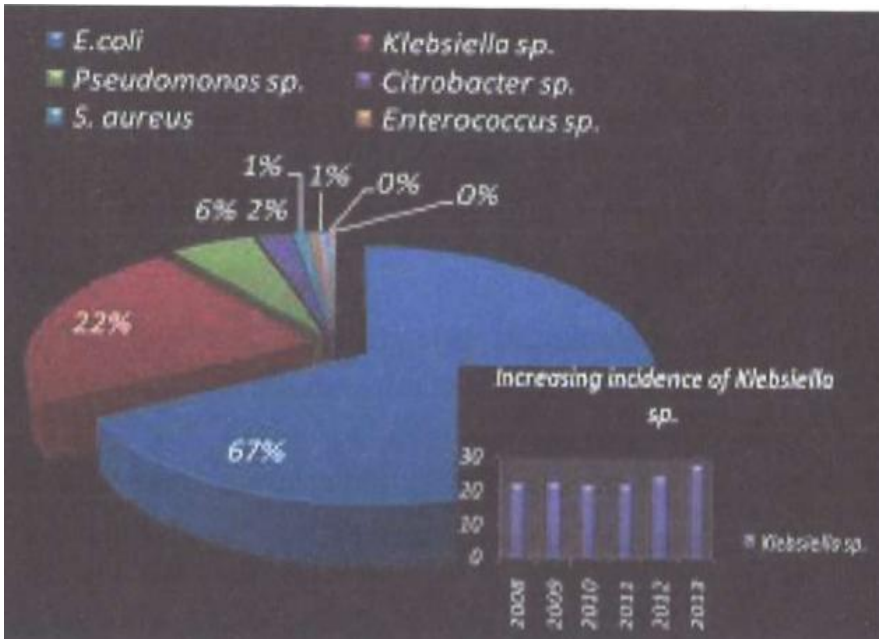


Figure (20): Percentages of UTIS bacterial causes.

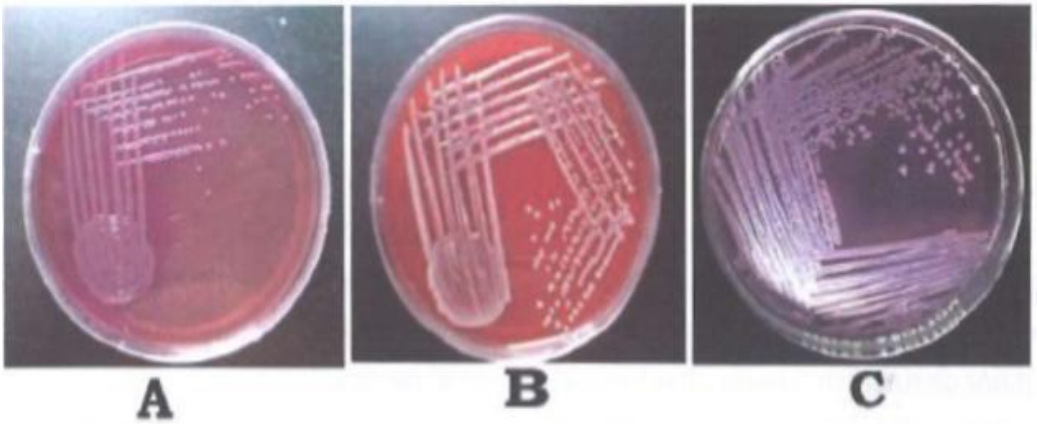


Figure 1: A) Mac Conkey Agar shows fermenting colonies of E. coli. B) Blood Agar shows non haemolytic colonies of E. coli. C) Eosine Methyl Blue agar showing metallic sheen around the colonies of E. coli.

Figure (21): *E. coli* on blood and MacConkey agar.

Section 11

Bacterial infections of genital tract

Sexually transmitted infections (**STI**), also referred to as sexually transmitted diseases (**STD**) and venereal diseases (**VD**), are infections that are commonly **spread** by **sex**, especially vaginal contact, anal sex and oral 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. Some STIS may cause problems with the ability to get pregnant.

More than 30 different bacteria, viruses, and parasites can cause STIS. **Bacterial STIs include:**

- Chlamydia (*Chlamydia trachomatis*)
- Gonorrhea (*Neisseria gonorrhoeae*)
- Granuloma inguinale or (*Klebsiella granulomatis*)
- *Mycoplasma genitalium; Mycoplasma hominis*
- Syphilis due to Spirochetes (*Treponema pallidum*)
- Ureaplasma infection

While usually spread by sex, some STIS could also spread by non-sexual contact with contaminated blood and tissues, breastfeeding, or during childbirth.

NEISSERIA

The genus Neisseria consists of gram-negative, aerobic cocci. Two Neisseria species are pathogenic for humans ***Neisseria gonorrhoeae*** (commonly called **gonococcus**), the causal agent of gonorrhea and *Neisseria meningitidis* (commonly called meningococcus), a frequent cause of meningitis. Gonococci and meningococci are **nonmotile diplococci** that could not distinguish from each other under the microscope.

However, they could differentiate in the laboratory by sugar-use patterns, and the sites of their primary infections. Both bacteria have classified as pyogenic cocci because infections by these organisms have also characterized by the **production of purulent (pus like) material** comprised largely of white blood cells.

Neisseria gonorrhoeae

Gonorrhea is one of the most frequently reported infectious diseases. The causal agent, *N. gonorrhoeae*, a gram-negative diplococcus, has frequently observed within the **polymorphonuclear leukocytes** of clinical samples obtained from infected patients. *N. gonorrhoeae* has usually transmitted during sexual contact or, more rarely, during the passage of a baby through an infected birth canal. It **does not survive long outside** the human body because it is **highly sensitive** to dehydration.

Structure:

Gonococci are unencapsulated (unlike meningococci), piliated, and nonmotile, and they resemble a pair of kidney beans.

Laboratory identification:

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. In contrast, a positive culture has needed to diagnose gonococcal infection in the female as well as at sites other than the urethra in the male.

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. [Note: N. meningitidis utilizes both glucose and maltose. 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. Cultures might have plated quickly or, if this is not possible, transport media might use to extend the viability of the organism to be cultured. 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. On nonselective media, the normal flora overgrows the gonococci. Culture of *N. gonorrhoeae* on Thayer-Martin agar remains the "gold standard" for diagnosis.

• Chlamydia is a sexually transmitted infection caused by the bacterium *Chlamydia trachomatis.* **In women**, symptoms may include abnormal vaginal discharge, burning during urination, and bleeding in between periods, although most women do not involvement any symptoms. **Symptoms in men** include pain when urinating, and abnormal discharge from their penis. If left untreated in both men and women, Chlamydia can infect the urinary tract and potentially lead to pelvic inflammatory disease (PID). However, Chlamydia could treated with antibiotics.

Chlamydia trachomatis

Chlamydia trachomatis has divided into a number of serotypes, which correlate with the clinical syndrome they cause. *C. trachomatis*, the major causal agent of sexual transmitted diseases, which is currently the most common reportable infectious disease in the world. In addition, C. trachomatis could **cause eye infections**, with symptoms ranging from irritation to blindness.

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 specificit 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. However, the procedure is not widely used because it enhances little to clinical effects. **Serologic testing** for specific antibodies is similarly not helpful except in suspected lympho- granuloma venereum (LGV), in which a single high-titer response is diagnöstic.

- **Syphilis** is a sexually transmitted infection caused by the bacterium *Treponema pallidum* subspecies *pallidum*. The signs and symptoms of syphilis vary depending in which of the four stages it presents (primary, secondary, latent, and tertiary). The primary stage classically presents with a single chancre (**painless ulcer**). In **secondary syphilis** a diffuse rash occurs, which frequently involves the palms of the hands and soles of the feet. There may also be sores in the mouth or vagina. **In latent** syphilis, which can last for years, there are little to **no symptoms.** In tertiary syphilis there are gummas (soft non-cancerous growths), neurological, or heart symptoms.
- Syphilis has most commonly spread through sexual activity. It has also transmitted from mother to baby during pregnancy or at birth, resulting in congenital syphilis.

Diagnosis has usually made by using blood tests; the bacteria can also be detected using dark field microscopy.

Treponema pallidum

Treponema pallichum subspecies pallidum is a **spiral-shaped, Gram-negative,** highly movable bacterium. Humans are the only known **natural reservoir** for subspecies *pallidum*. It is unable to survive without a host for more than a few days.

Laboratory identification:

Syphilis is difficult to diagnose clinically early in its presentation. Confirmation is via either blood tests or direct **visual inspection** using microscopy. **Blood tests** have more commonly used, as they are easier to perform. Diagnostic tests are unable to distinguish between the stages of the disease.

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.

Traditionally this organism had considered a strict anaerobe, but it is now known to be microaerophilic. The in vivo generation time is relatively long (30 hours). *T. pallidum* subsp *pallidum* had not successfully cultured in vitro. Viable organisms can be maintained for 18 to 21 days in complex media, while limited replication has been obtained by co-cultivation with tissue culture cells.

Conclusion: Bacterial causative agent's diseases of genital tract which, are Genital tract infection manifests as either genital discharge or genital ulceration with or without inguinal lymphadenitis such as:

1. *Neisseria gonorrhea* Gonorrhea
2. *Chlamydia trachomatis* Urethritis, cervicitis, LGV
3. *Ureaplasma urealyticum* Urethritis

4. *Gardenella vaginalis* Vaginitis

5. *Treponema pallidum* Syphilis

Blood tests

Blood tests have divided into non-treponemal and treponemal tests. Because of 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. These tests are not as time-sensitive, as they do not require living bacteria to make the diagnosis.

Section 12

Bacterial infections of gastrointestinal tract

Bacterial gastrointestinal tract infection has many causes, can range from mild to severe, and typically manifests with symptoms of nausea, vomiting, diarrhea, and abdominal discomfort, In reality, most such attacks have caused by enterotoxins, drugs, or systemic illnesses.

The lower bowel has an especially large normal bacterial microbiota. The most prevalent organisms are anaerobes (*Bacteroides*, gram-positive rods, and gram-positive cocci), gram-negative enteric organisms, and *Enterococcus faecalis.* Any effort to improve pathogenic bacteria from feces involves separation of pathogens from the normal microbiota, usually through using of differential selective media and enrichment cultures. Important causes of **acute gastroenteritis** include viruses, toxins (of staphylococci, clostridia, vibrios, toxigenic E. coli), invasive enteric gram-negative rods, slow lactose fermenters, shigellae, salmonellae, and campylobacters (figure 22).

Diagnosis Gastrointestinal Tract Specimens

Specimens (on general)

Feces and rectal swabs are the most readily available specimens. The presence of blood, mucus, or helminths must note on gross inspection of the specimen. Leukocytes seen in suspensions of stool examined microscopically which are useful means of differentiating invasive from noninvasive infectious diarrheas. However, it is important to note that leukocytes may be present in non-infectious, inflammatory conditions of the gastrointestinal tract.

Culture Media:

Specimens have suspended in broth and cultured on ordinary as well as differential media (MacConkey agar, EMB agar) to permit separation of non-lactose fermenting gram- negative rods from other enteric bacteria. If salmonella infection has suspected, the specimen has also placed in an enrichment medium (selenite broth) for 18 hours before has plated on differential media (Hektoen enteric or Shigella- Salmonella agar). Vibrios grow best on thiosulfate citrate bile salts sucrose agar. Thermophilic campylobacters are isolated on Skirrow's selective medium incubated at 40-42°C in 10% CO2.

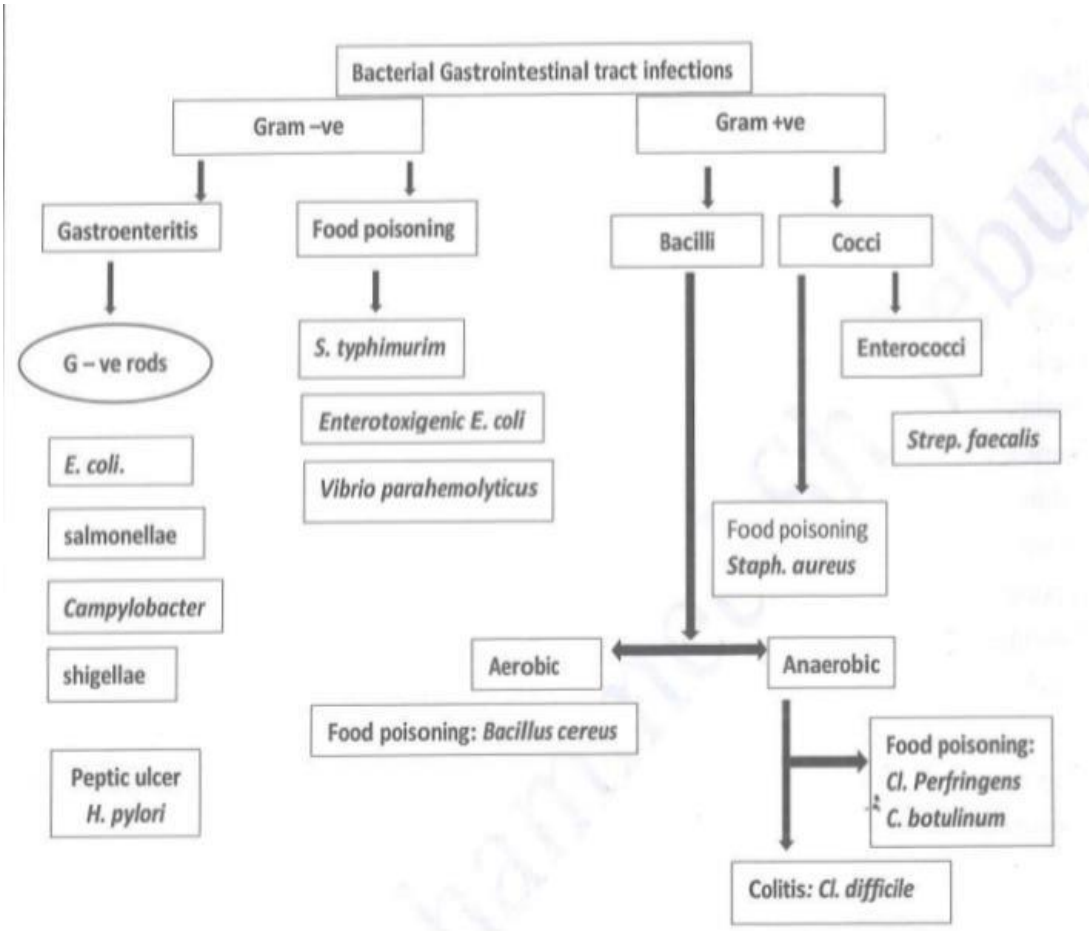


Figure (22): Bacterial Gastrointestinal tract causes.

Gastrointestinal Gram-negative rods

All organisms that have routinely found in the gastrointestinal (GI) tract of humans or other animals. Many also have altenative habitats in soil or water. All are relatively hardy but are sensitive to drying, and all grow in the presence or absence of oxygen, being facultative anaerobes. They contain lipopolysaccharide (LPS), which is both antigenic and an important virulence factor (endotoxin).

Different enteric gram-negative rods cause diseases within the GI tract, outside the GI tract, or in both locations.

Fecal contamination is commonly important in the transmission of those organisms that cause GI diseases.

Escherichia coli

Escherichia coli is part of the normal flora of the colon in humans and other animals but can be pathogenic both within and outside of the GI tract.

E. coli has fimbriae or pili that are important for adherence to host mucosal surfaces, and different strains of the organism may be motile or non-motile. Most strains can ferment lactose (Lac+) in contrast to the major intestinal pathogens, Salmonella and some strains of Shigella, which cannot ferment lactose (Lac -). ***E. coli*** produces both acid and gas during fermentation of carbohydrates.

Transmission of intestinal disease is commonly by the fecal-oral route, with contaminated food and water serving as vehicles for transmission. At least five types of *E. coli* that differ in pathogenic mechanisms have identified as:

1. Enterotoxigenic (ETEC), a common cause of traveler's diarrhea.
2. Enteropathogenic (EPEC), an important cause of diarrhea in infants.
3. Enterohemorrhagic (EHEC), associated with acute bloody diarrhea.
4. Enteroinvasive (EIEC), cause a dysentery-like syndrome.
5. Enteroaggregative (EAEC), cause traveler's diarrhea.

Laboratory identification

Intestinal disease: Because ***E. coli*** is normally part of the intestinal flora, detection in stool cultures of disease-causing strains is generally difficult. Many strains have detected on media such as MacConkey agar (**figure 23, 24**). Strains of ***E. coli*** could further characterize based on serologie tests. Current molecular techniques, such as PCR technique, might employed to identify E. coli strains. General biochemical tests of E. coli are:

Short rods, Facultative anaerobe, Ferments glucose, Most strains ferment lactose, Catalase positive, Oxidase negative and Culture on MacConkey agar.

Figure (23): E. coli colony

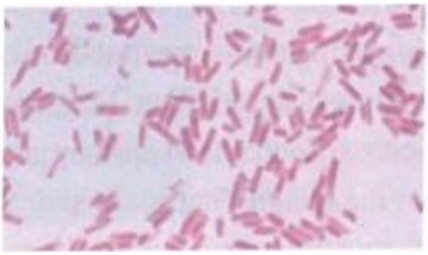


Figure (24): E. coli Gram stain

SALMONELLA (Salmonellosis)

Members of the genus Salmonella can cause a variety of diseases, including gastroenteritis and enteric (typhoid) fever. Although Salmonella classification has undergone numerous modifications, currently, all strains affecting humans have grouped in a single species, *Salmonella enteritidis*, which has approximately 2,500 different serotypes, or serovars, including the clinically significant serotypes Typhimurium and Typhi.

A **serotype or serovar** is a isolated variation within a species of bacteria or virus or among immune cells of different individuals. These microorganisms, viruses, or cells have classified together based on their cell surface **antigens**, allowing the **epidemiologic classification** of organisms to the sub-species level.

Serotyping often play an essential role in determining **species** and **subspecies**. ***Salmonella genus*** of **bacteria**, for example has determined to have over 2600 serotypes, including *Salmonella enterica* serovar **Typhimurium**, *S. enterica* serovar **Typhi**, and *S. enterica* serovar **Dublin**.

Most strains of Salmonella are Lactose negative and produce acid and gas during fermentation of glucose. They also produce H2S from sulfur-containing amino acids.

Transmission

Salmonella are widely distributed in nature. Serovar Typhi is an absolutely human pathogen, whereas other strains are associated with animals and foods (for example, eggs and poultry). **Fecal-oral** transmission occurs and Salmonella serovar Typhi may involve **chronic carriers.** Young children and older adults are particularly susceptible to Salmonella infections. Individuals in crowded areas may also be involved in Salmonella epidemics.

Salmonella infection can cause both intestinal and extraintestinal diseases.

1. **Gastroenteritis**: This localized disease (salmonellosis) has causcd primarily by serovars Enteriditis and Typhimurium. Salmonellosis is characterized by nausea, vomiting, and diarrhea (usually non-bloody), which develop generally within **48 hours** of ingesting contaminated food or water. Fever and abdominal cramping are common. More than 95 % of cases of Salmonella infection are foodborne and salmonellosis accounts for approximately 30 % of deaths resulting from foodborne illnesses in the United States.

2. **Enteric or typhoid fever**: This is a severe, life-threatening systemic illness, characterized by fever and, frequently, abdominal symptoms. It has caused primarily by **serovar Typhi**. About 30 % of patients become weak and having **transient skin rose spots**. The incubation period varies from 5 to 21 days. **Untreated**, mortality is approximately 15 %. Complications can include intestinal hemorrhage. **Typhoid fever remains a global health problem.**

Laboratory identification

In patients with diarrhea, Salmonella can typically be isolated from stools on **MacConkey agar (see figure 25) or selective media.** For patients with enteric fever, appropriate specimens include **blood, bone marrow, urine, stool, and tissue** from typical rose spots (if they are present).

General biochemical tests of Salmonella are:

Short, flagellated rods, Facultative anaerobes, Ferment glucose, Do not ferment lactose, Catalase positive, Oxidase negative, Culture on MacConkey agar

Figure (25): Salmonella typhi on MacConkey agar

CAMPYLOBACTER

Members of the genus Campylobacter are curved, spiral, or S-shaped organisms that microscopically resemble vibrios. A single, polar flagellum provides the organism with its characteristic darting (running) motility. Somatic, flagellar, and capsular antigens all contribute to the numerous serotypes. Most Campylobacter are microaerophilic (that is, they require oxygen but at lower concentrations than that found in air).

Campylobacter infect the intestine and can cause ulcerative, inflammatory lesions in the jejunum, ileum, or colon. Also bacteremia may occur.

Campylobacter are **transmitted** to humans primarily via the fecal-oral route through direct contact, exposure to contaminated meat (especially poultry), or contaminated water supplies.

Laboratory identification

Campylobacter can be isolated from feces using special selective media (**blood agar containing antibiotics to inhibit growth of other fecal flora**) (**see figure 26, 27**) and mieroaerophilic conditions. Members of this genus do not ferment carbohydrates. Possible diagnosis could have based on finding curved organisms with rapid, darting motility in a wet mount of feces.

Figure (26): ***Campylobacter jejuni*** **(Preston selective medium)**

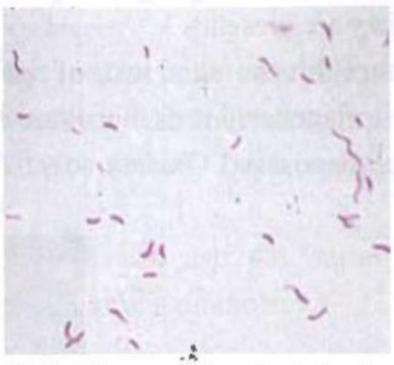


Figure (27): ***Campylobacter jeiuni***

SHIGELLA (shigellosis)

Shigella species cause shigellosis (bacillary dysentery), a human intestinal disease that occurs most commonly among young children.

Shigellae are **non-motile,** un-encapsulated, and **Lactose negative**. Most strains do not produce gas in a mixed-acid **fermentation of glucose.**

Shigella have typically spread from person to person, with contaminated stools serving as a **major source** of organisms. **Humans** are the only natural host for Shigella species. **Flies** and **contaminated food or water** can also transmit the disease. Shigellosis has a **low infectious dose:** Approximately **10-100** viable organisms are sufficient to cause discase. Therefore, secondary cases within a family are common, particularly under conditions of **crowding or poor sanitation.**

Shigella dysenteriae causes the most serious infections, so Shigellae invade and destroy the mucosa of the large intestine.

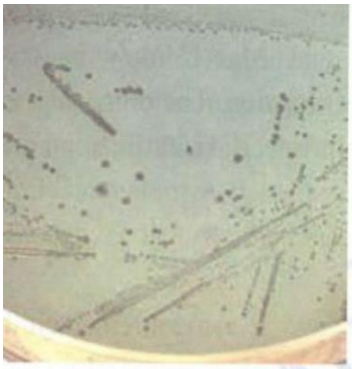
S. dysenteriae type 1 produces Shiga toxin, which is structurally and genetically very similar to Shiga-like toxins 1 and 2 produced by ***E. coli***.

Shigellae cause classic bacillary dysentery, characterized by bloody diarrhea and mucus ("currant jelly" stools), with painful abdominal cramping. The disease is generally most severe in the very young; older adults; and among emaciated individuals, in whom shigellosis may lead to **severe dehydration** and, sometimes, death.

Laboratory identification

During acute illness, organisms can be cultured from stools using differential, selective Hektoen agar (**figure 28, 29**) or other media specific for intestinal pathogens.

Other **biochemical** features of Shigellae are non-motile, non-encapsulated, cannot ferment lactose, most strains do not produce gas in a mixed-acid fermentation of glucose.



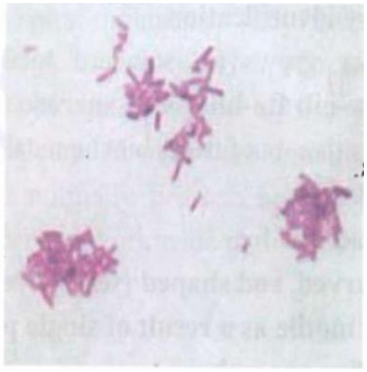


Fig. (28): Shigella species (Hektoen agar). Fig. (29): *Shigella on Gram stain*

VIBRIO

Members of the genus Vibrio are **short, curved, rod-shaped** organisms. Vibrios are closely related to the family Enterobacteriaceae. They are rapidly motile by means of a single polar flagellum. This contrasts with the peritrichous flagella (distributed all over the surface) of the motile Enterobacteriaceae. O and H antigens are both present, but only **O antigens** are useful in distinguishing strains of vibrios that cause **epidemics**. Vibrios are facultative anaerobes. The growth of many Vibrio strains either requires or is **stimulated by NaCl.** Pathogenic vibrios include:

1. *Vibrio cholerae*, serogroup O1 and O139 strains that are associated with **epidemic and pandemic cholera.**
2. Non-Oland nonO139 *V. cholerae* and related strains that **cause sporadic cases of choleralike** and other illnesses (extraintestinal infection).
3. *Vibrio parahaemolyticus* and other halophilic vibrios, which cause **gastroenteritis** and **extraintestinal** infections.

Clinical significance

Cholera is characterized by **massive loss of fluid** and **electrolytes** from the body. After an incubation period ranging from **hours to a few days**, profuse (excessive) watery diarrhea ("**rice-water" stools**) begins. Untreated, death from **severe dehydration** causing hypo - volemic shock may occur in hours to days, and the death rate may exceed 50 %. They also cause milder illness, comparable to that caused by enterotoxigenic ***E. coli***, such as gastrointestinal diseases.

Laboratory identification

V. cholerae grows on standard media such as **blood** and **Mac-Conkey** agars. **Thiosulfate-citrate-bile salts-sucrose** medium can enhance isolation. The organism is oxidase positive, but further biochemical testing is necessary for specific identification of *V. cholerae*.

It is characterized by:

- **Short, curved, rod shaped (see figure 30).**
- **Rapidly motile as a result of single polar flagellum.**
- **Facultative anaerobes.**
- **Growth of many Vibrio strains requires or is stimulated by NaCl.**
- **Culture on blood or MacConkey agar.**

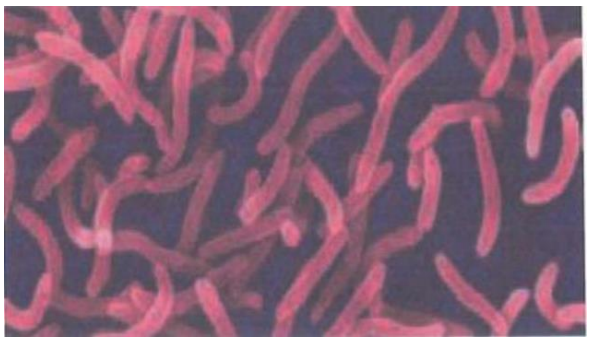


Figure (30): Gram stain of *Vibrio cholerae*

Vibrio parahaemolyticus and other halophilic, non-cholera vibrios

These organisms are characterized by a requirement for higher than usual concentrations of NaCl and their ability to grow in 10 % NaCl. They are common in seawaters. ***Vibrio parahaemolyticus*** is associated with **outbreaks** of **Gastrointestinal** illness that result from ingestion of contaminated and inadequately cooked seafood, especially shellfish and crustaceans.

Other halophilic, **noncholera vibrios** are associated with soft tissue infections and septicemia resulting either from contact of **wounds** with contaminated seawater or from ingestion of **contaminated seafood.** For soft tissue infections, prompt administration of antibiotics, such as tetracycline, fluoroquinolones or cefotaxime, is important, and surgical drainage may be required.

HELICOBACTER

Members of the genus Helicobacter are curved or spiral organisms. They have a rapid, corkscrew (coiled) motility resulting from multiple polar flagella. ***Helicobacter pylori***, the species of **human significance,** is microacrophilic, and produces urease. It causes **acute gastritis** and 95% of **duodenal** and **gastric ulcers**. ***H. pylori*** are **unusual** in their ability to **colonize the stomach**, where low pH normally protects against bacterial infection. *H. pylori* infections are relatively common and worldwide in distribution.

Clinical significance

Initial infection with ***H. pylori*** causes acute gastritis, sometimes with diarrhea that lasts about 1 week. The infection usually **becomes chronic**, with diffuse, superficial gastritis that may be associated with epigastric discomfort. ***H. pylori*** infection appears to be a risk factor for development of gastric carcinoma and gastric B-cell lymphoma.

Laboratory identification

Noninvasive diagnostic tests include serologic tests (enzyme-linked immunosorbent assay, commonly known as **ELISA**, for serum antibodies to ***H. pylori)*** and breath tests for urease. [Note: Breath tests involve administering radioactively labeled urea by mouth. If *H. pylori* are present in the patient's stomach, the urease produced by the organism will split the urea to CO2 (radioactively labeled and breathe out) and NH3.] Invasive tests involve gastric **biopsy specimens** obtained by **endoscopy**.

It is characterized by:

- **Curved or spiral rods (figure 31).**
- **Multiple polar flagella, which give organism rapid, corkscrew motility.**
- **Urease positive.**
- **Culture on selective medium containing antibiotics (figure 32).**

H. pylori can be detected in such specimens histologically, by culture, or by a for urease.

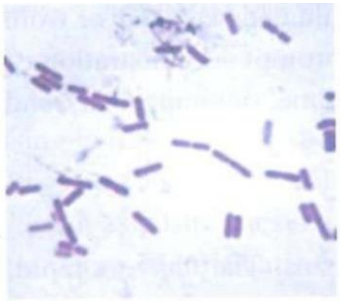


Fig. (31): *Helicobacter pylori*

(Note: Young cultures grown in

***vitro frequently* stain gram-positive)**

Figure (32): *Helicobacter pylori* colonies on an agar plate

OTHER ENTEROBACTERIACEAE

Other genera of Enterobacteriaceae, such as **Klebsiella, Enterobacter, Proteus**, and **Serratia**, which can be found as normal inhabitants of the large intestine, include organisms that are primarily opportunistic and often nosocomial pathogens.

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) 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.

Clostridium perfringens

C. perfringens is a large, nonmotile, gram-positive, encapsulated bacillus. It is universal in nature, with its vegetative form as part of the normal flora of the vagina and gastrointestinal (GI) tract. Its spores are found in soil. [Note: Spores are rarely seen in the body or following in vitro cultivation.] When introduced into tissue. Clostridium Granm stain. Individual gram-positive bacilli are present. Many are in chains. Some of the bacilli have spores, which are the unstained or clear ovoid shapes as shown in figure (33).

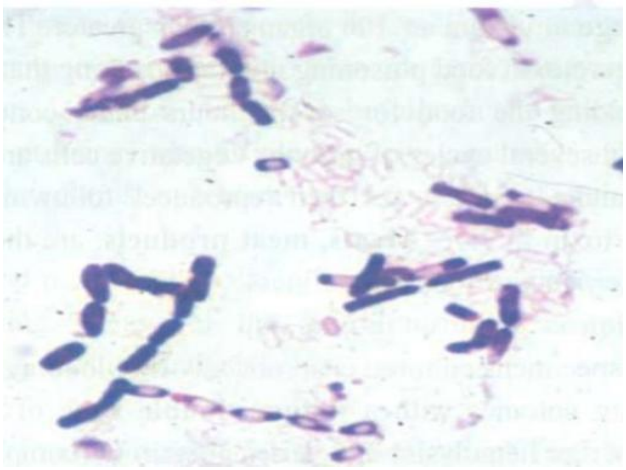


Figure (33): Gram stain of *Clostridium perfringens*

However, *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. a 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 I to 2 days. The occurrence of clinical symptoms requires a large inoculum of 10^8 organisms or greater. Therefore, a typical episode of clostridial enterotoxin food poisoning involves cooking that fails to inactivate spores, followed by holding the food for several hours under conditions that allow bacterial germination and several cycles of growth. 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 Figure (34). In food infection, the organism can be **required** in suspected food and the patient's feces. Gram stain and other laboratory findings greatly help planning of antibiotic therapy in patients (figure 35).

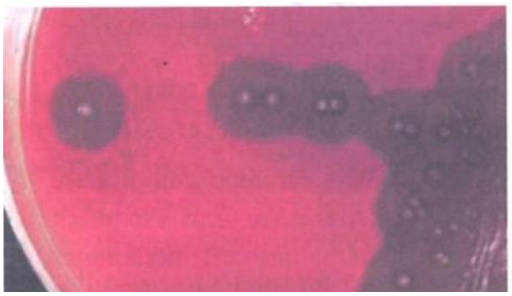


Figure (34): C. perfringens. on blood agar showing double zone of hemolysis.

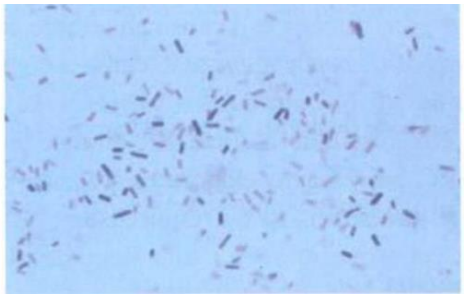


Figure (35): Gram stain of C. perfringens

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 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 **neurotransmission**. 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. Also MacConkey agar used as **Selective media**.

Clostridium difficile

Diarrhea, a common complication of antimicrobial drug treatment, can range from loose stools to life-threatening pseudomembranous colitis (PMC). ***C. difficile*** is estimated to be responsible for at least one fourth of antibiotic-associated diarrheas (AADS) in hospitalized patients and almost all cases of PMC. After its introduction to a site, the environment (that is, dust, bedding, toilets, etc.) becomes persistently **contaminated with spores**. They are then at higher risk for developing the adverse intestinal effect of antibiotic treatments.

Pathogenesis of *C. difficile* is a minor component of the **normal flora** of the large intestine. When antimicrobial treatment suppresses more predominant species in this community, *C. difficile proliferates*.

Pathogenic strains produce two toxic polypeptides, designated toxins A and B. Toxin A is an enterotoxin that causes **excessive fluid secretion**, but also stimulates an inflammatory response, and has some cytopathic effect in tissue culture. Toxin B is a cytotoxin.

Clinical significance

Fundamentally **all antimicrobial** drugs have been reported as predisposing to clostridial AAD (antibiotic-associated diarrhea) and colitis. The three drugs most commonly implicated are clindamycin, ampicillin, and the cephalosporins. The pseudo membranous exudate, composed of mucus, fibrin, inflammatory cells, and cell debris overlying an **ulcerated epithelium,** is best demonstrated by endoscopy.

Laboratory identification

C. difficile can be cultured from stools and identified by routine anaerobic procedures, but the more rapid and useful tests are directed at **demonstrating toxin** production in **stool extracts**. Enzyme immunoassays (ELISA) for exotoxins A andB have replaced earlier immunologic or tissue culture cytotoxicity assays. Polymerase chain reaction-based detection strategies are also widely available.

BACILLUS SPECIES

Species of the genus Bacillus are **gram-positive**, form **endospores**, and are either strict aerobes or aerotolerant anaerobes (that is, they can grow in the presence of oxygen, **but do not require it)**. Most of the 70 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

Bacillus cereus 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, the **emetic type,** which is associated with **cooked** rice, and 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 within 24 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 (**see figure 40)** bacterium commonly found in soil (on vegetables) and food (raw and processed). ***B. cereus*** bacteria are facultative anaerobes, and like other members of the genus Bacillus, can **produce** protective **endospores**. Its **virulence factors** include cercolysin 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 10^5 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.

Figure (40): B. cereus on blood agar with characteristic blood hemolysis.

Section 13

Bacterial infections of eyes, ears and sinuses

Ear infections:

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.

The most common bacteria that cause ear infections are coagulase positive **Staphylococci**, beta hemolytic **Streptococci**, alpha hemolytic Streptococci (***Strep. pneumonia***) (figure 36), ***Proteus spp., Pseudomonas aeruginosa*** and ***E. coli***. While ***Klebsiella pneumonia*** (less common) and **anaerobic** bacteria are **rare**.

Ear infection, may be the way for enteric bacteria to reach to un limited area in respiratory tract or nervous systems, *E. coli* meningitis is one example among many of such cases.

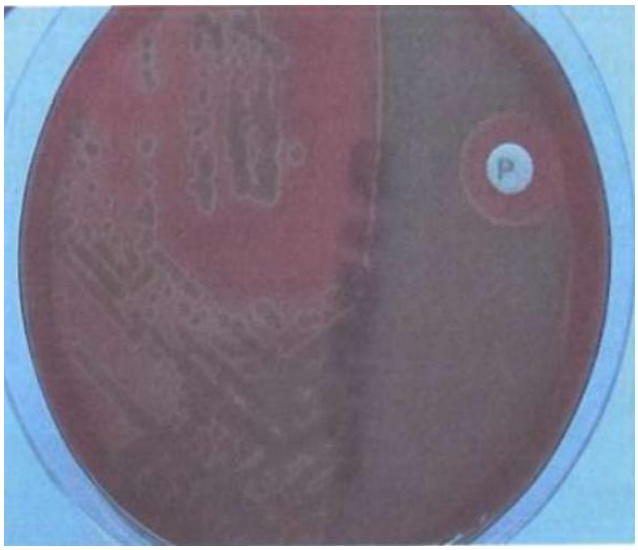


Figure (36): Alpha hemolytic streptococci grown on a blood agar plate.

Alpha hemolysis presents with a dark green color.

Outer Ear

P. aeruginosa is a Gram-negative rod-shaped bacterium with a flagellum at one pole. *P. aeruginosa* is a facultative anaerobe, but prefers aerobic respiration. This makes it well

suited for life on the skin and the outer ear which is exposed to the oxygen-filled atmosphere.

P. aeruginosa is able to utilize a wide variety of metabolites. *P. aeruginosa* is also an opportunistic pathogen that causes multiple different diseases such as pneumonia, UTIS, and other skin diseases including **acute** diffuse **otitis externa** **(figure 37)**.

S. epidermidis is found in the outer ear due to the similarity in environments to human skin, that may cause ear infection.

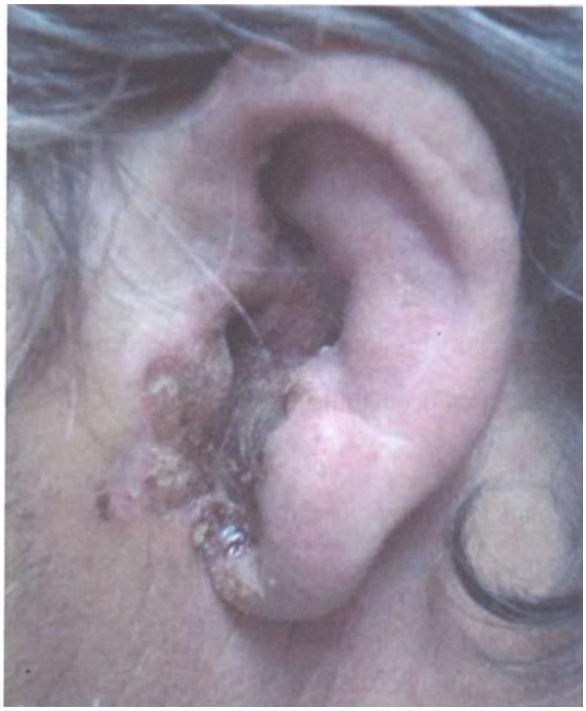


Figure (37): Acute diffuse otitis externa.

Streptococcus pneumoniae; Haemophilus influenzae & Moraxella catarrhalis these microbes are the most common cause for acute otitis media. *Strep. pneumoniae, H. influenzae* and *M. catarrhalis* are all associated with the **upper respiratory tract** of humans. (figure 38)

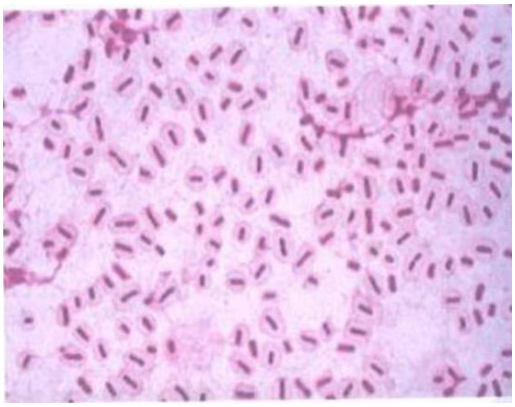


Figure (38): *Haemophilus influenzae* Gram stain under light microscopy.

Beneficial Ear Microbiota:

More and more scientists are beginning to realize that the interplay between the human body and naturally occurring biological flora can be beneficial for both the host and the microbe. In order to restore proper gut health fecal transplants were performed in order to replace the **beneficial bacteria**. This logic has been extended to the treatment of ear infections. Alpha hemolytic streptococci are **present in the middle ear naturally** and evidence suggests that these bacteria effectively interfere with the activity of *Strep. pneumonia, H. influenza*, and *M. catarrhalis* and essentially crowd out the growth of these pathogens. In this way alpha hemolytic streptococci and other **probiotics** can be utilized to combat irritating ear infections by essentially replacing the naturally occurring bacterial flora in the middle ear in this case.

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 influenza; Strep.pneumoniae; Strep. pyogenes*

Source: Endogenous: normal flora of the nasopharynx

Clinical features: Discomfort over the frontal or maxillary sinuses, Pain and tenderness of sinuses with purulent nasal discharge.

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

Long standing ear disease characterized by periods of exacerbation with profuse ear discharge and pain; and remission with relatively dry ear.

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

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.

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.

Diagnosis of bacterial eye infections

Ear, eye and nose are all share common canal, so any infection of one of these parts may cause infection to others.

Eye Infections:

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. External part of eye considers as a part of skin, so any bacterial infections of skin is expected to cause infection to the outside part of eye.

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

A. Conjunctivitis (pinkeye)

1. Infection of the conjunctiva.

2. Bacterial conjunctivitis is often caused by ***Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes, and Neisseria gonorrhoeae (*****infant*****)***.

3. Symptoms include a sensitivity to bright lights, swelling of the eyelids, increased tears, redness, and large amounts of pus (Bacterial infections- **milky discharge).**

B. Keratitis

1. More serious infection than conjunctivitis.
2. Invasion of **deeper eye tissues o**ccurs, can lead to **complete corneal destruction.**
3. Also cause conjunctivitis, sharp pain, and sensitivity to light.
4. Can lead to blindness.

Diagnosis of bacterial Nose infections

Nose infections:

Nasal cavity considers as a reservoir for Genus ***Staphylococcus*** along with other Gram positive bacteria such as alpha and beta **hemolytic Streptococci.** Nasal cavity is the pathway for deeper parts of respiratory tract, so that resident bacteria of nasal cavity may and will find its way to the system causing problems here and there in respiratory tract or from this location to nervous system such as meningitis, which could happen due to infection with bacteria resident in nasal cavity.

Enteric bacteria such as ***E. coli, Klebsiella spp***. and ***Proteus spp***. are either transit or resident which depend on the hygienic and immunologic status of individual. ***Pseudomonas aeruginosa*** is less common because it is less competitive.

Laboratory diagnosis

Bacterial parameter could be enough for diagnosis of classical infections with the aid of **CBC** looking for **raising in number of leucocytes** in general, and neutrophils in particular, biochemical exams may be of little assistant in this regard. Mycoplasmal and chlamydial infection cannot be diagnose using bacterial parameter since there is no suitable media to isolate them routinely, for that, serological or immunological parameters are prime diagnostic tool in this aspect, again CBC, and differential blood count could aid the diagnosis.

Table (5): Summary for bacterial diagnosis of Ear, Eye and Nose infection

Infection	Most important pathogens	Laboratory diagnosis
Otitis externa (Bacteria involved in this aspect will just that of skin infections)	***Pseudomonas aeruginosa*** ***Staphylococcus aureus*** ***Streptococcus pyogenes***	Culture and Microscopy for bacteria of swab material
Otitis media	***Streptococcus pneumonia*** ***Haemophilus influenzae*** ***Streptococcus pyogenes*** ***Staphylococcus aureus*** ***Moraxella catarrhalis (children)***	Culture and Microscopy for bacteria of middle ear
Keratitis	***Staphylococcus spp.*** ***Streptococcus spp.*** ***Neisseria gonorrheae*** ***Pseudomonas spp.*** ***Bacillus spp. Mycobacterium spp. Moraxella lacunata*** ***Actinomyces spp.*** ***Nocardia spp.*** ***Treponema pallidum Chlamydia trachomatis***	Culture and Microscopy for bacteria swab or corneal scrapings Diagnostic procedures with corneal swab or scrapings Serology Diagnostic procedures with corneal swab or scrapings see at "trachoma"
Trachoma	***Chlamydia trachomatis, serovars A, B, Ba, C***	Microscopical detection of inclusions in conjunctival cells (Giemsa stain); direct immunofluorescence; cell culture; PCR. Serology: recombinant immunoassay for antibodies to genus-specific antigen (LPS).

Conjunctivitis	*Neisseria spp. Streptococcus spp. Staphylococcus aureus Haemophitus spp. Pseudomonas spp. Mycobacterium spp. Moraxella lacunata* *Treponema pallidum Chlamydia trachomatis*	**Microscopy and culture for bacteria in conjunctival secretion or in scrapings** **Serology (basic diagnostics) See at "trachoma"**
Endophthalmitis	***Staphylococcus spp. Streptococcus spp. Neisseria gonorrhoeae Pseudomonas spp. Bacillus spp. Mycobacterium spp. Moraxella lacunata Nocardia spp. Chlamydia trachomatis Treponema pallidum***	Microscopy (Gram) and culture for aerobic and anaerobic bacteria and Mycobacterium.

Section 14

Bacterial infections of skin, soft tissues and wounds:

The skin is an essential component of the nonspecific immune system, protecting the host from potential pathogens in the environment. Breaches in this protective barrier thus represent a form of immuno-compromise that predisposes a patient to infection (Figure 39 types of wounds).

Effective factors of **wound infections** are:

1) Virulence of microbes.
2) Number of microbial cells in wound.
3) Status of host's immune system.
4) Type of wound, especially whether tissues are crushed or contained foreign matter, which are:
   - Incisions: Produced by knife or other sharp object.
   - Punctures: Result from penetration by small sharp object, such as needle or nail.
   - Lacerations: Occur when tissue is torn.
   - Contusions: Produced by blow that crushes tissue.
   - Abrasions: Occur when the epidermis is scraped off.
   - Gunshot wounds: Caused by bullets or other projectiles.

Quite few pathogens are existing normally on the skin, yet under control of natural balance, when this balance is unbalanced, then these bacteria become active and causing diseases, these are **opportunistic** bacteria.

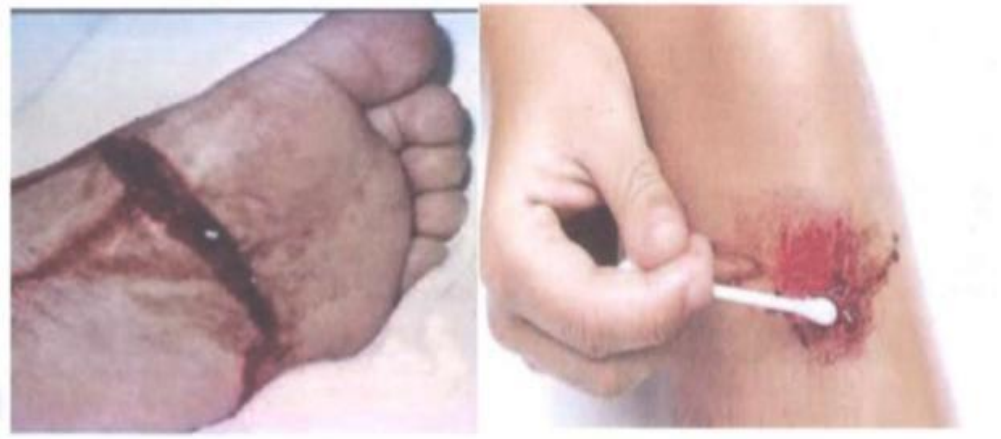


Figure (39): Types of wounds.

Abscess has localized collection of pus surrounded by inflamed body tissue, so form as result of body's immune defenses and indicate an infection.

Pus has composed of dead and living leukocytes, tissue debris, and proteins. Mechanism of abscess Formation

1. Microorganisms enter tissue from a wound or from bloodstream.
2. Blood vessels dilate, and leukocytes migrate to the area of the developing infection.
3. Pus forms and an abscess develops, clotting occurs in adjacent blood vessels.
4. Buildup of pressure causes abscess to expand in direction of least resistance; if it reaches body surface, it may rupture and discharge its contents.

Deep wounds

- Allows growth of obligate anaerobes such as ***Clostridium tetani***

- Wounds that have extensive tissue damage, are contaminated with dirt or are small in diameter but deep, such as punctures. Also often created when different microbial species grow in wound (polymicrobial infections), because acrobic and facultative anaerobes use up available oxygen.

Pseudomonas aeruginosa infections which are:

- Opportunistic pathogen.

- Major cause of healthcare-associated infections.

- Common cause of wound infections, especially of thermal burns.

- Very difficult to treat.

- Also occasionally cause community-acquired infections.

- Characteristic green color, caused by water-soluble pigments produced by organisms.

Pseudomonas aeruginosa causative agent characteristics are:

- Motile Gram-negative rod with single polar flagellum.

- Found in soil and water.

- Aerobe.

- Also respires anaerobically in absence of oxygen if nitrate is present.

- Develop biofilms

Pseudomonas aeruginosa pathogenesis are:

- Infection causes additional tissue damage, delays healing, and increases risk of septic shock.

- Virulence depends on production of 2 extracellular proteins: exotoxin A (stops host cell protein synthesis) and exoenzyme A (hydrolyze/destroy lecithin to cause membrane disruption and cell death).
- Derivative of pigment pyocyanin produced acts as siderophore, helping pathogen acquire iron from environment, thereby inhibiting competing bacteria by reducing their available iron.
- Pigment also impairs function of human nasal cilia and disrupts respiratory epithelium.

Tetanus (lockjaw)

-Caused by anaerobic bacterial wound infections, which often fatal and exposure to causative organism cannot be avoided because it produces endospore that are widespread in dust and dirt, frequently contaminating clothing, skin, and wounds

Tetanus signs and symptoms:

- Continuous, painful, uncontrollable cramp like muscle spasms
- Spasms begin with jaw muscles.
- As more muscles go into sustained contraction (tetany), breathing becomes difficult, abnormal heart rhythms occur, and bones can fracture.
- Infected person often dies of pneumonia or from lung damage caused by regurgitation of stomach contents into lung.
- Healthcare associated infections can also develop because people are in hospitals for long periods of time.

Tetanus causative agent is ***Clostridium tetani*** which is anaerobic, spore-forming, Gram-positive, rod-shaped bacterium and 2 characteristic features:

1. Spherical endospore that forms at end of cell (figure 40).
2. Swarming growth that quickly spreads over surface of solid media.

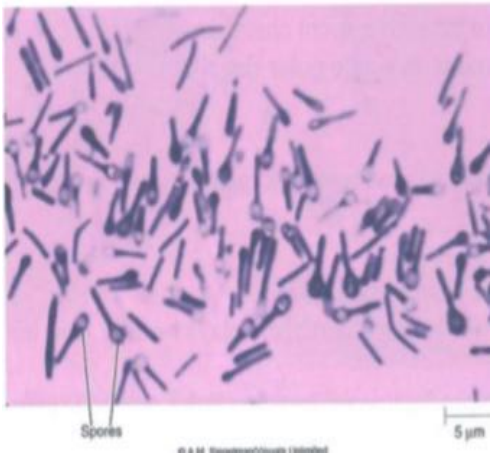


Figure (40): Microscopic appearance of *Clostridium tetani*