L 10 :- Spirochetes, *Mycobacterium tuberculosis*, *Mycobacterium leprae*,

by

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Spirochetes

Spirochetes are long, slender, motile, flexible, undulating, gram-negative bacilli that have a characteristic helical shape. Depending on the species, they can be facultative anaerobic. Some spp can be grown in laboratory culture (either cell-free culture or tissue culture), whereas others cannot. Some spp are free-living, and some are part of the normal flora of humans & animals. Thy are important human pathogens are :

1. ***T. pallidum*** **(syphilis),**
2. ***Borrelia*** **(causes Lyme disease, & relapsing fever)**
3. ***Leptospira*** **(causes leptospirosis)**

Treponema Pallidum

Syphilis is a sexually transmitted disease (STD) caused by *T. pallidum*. Starting with a small lesion (**chancre**), several progressive stages of the disease can span a period of 30 years or more. The causative organism of syphilis is extremely fastidious and fragile. It cannot be cultured routinely in the laboratory, and is sensitive to disinfectants, heat, and drying. *T. pallidum* is so thin that it cannot be observed by light microscopy, but requires immunofluorescent or dark-field techniques. It is produce hyaluronidase that disrupts ground substance, and facilitates dissemination of the organism.

Pathogenesis & Clinical significance

Transmission of T. pallidum is almost always by sexual contact or translucently (congenital syphilis). The organism enters the body through a break in the skin, or by penetrating mucous membranes, such as those of the genitalia.

Syphilis: Syphilis occurs in three stages. The **first** symptom of primary stage syphilis is a hard genital or oral ulcer (chancre) that develops at the site of inoculation. The **secondary stage** may be accompanied by systemic involvement, causing hepatitis, meningitis, nephritis. In 40% of infected individuals, the disease progresses to a **tertiary stage**, characterized by degeneration of the nervous system, cardiovascular lesions such as **gummas lesions** in the liver, skin, and bones.

Congenital syphilis: It can be transmitted through the placenta to a fetus after the first 10 to 15 weeks of pregnancy.

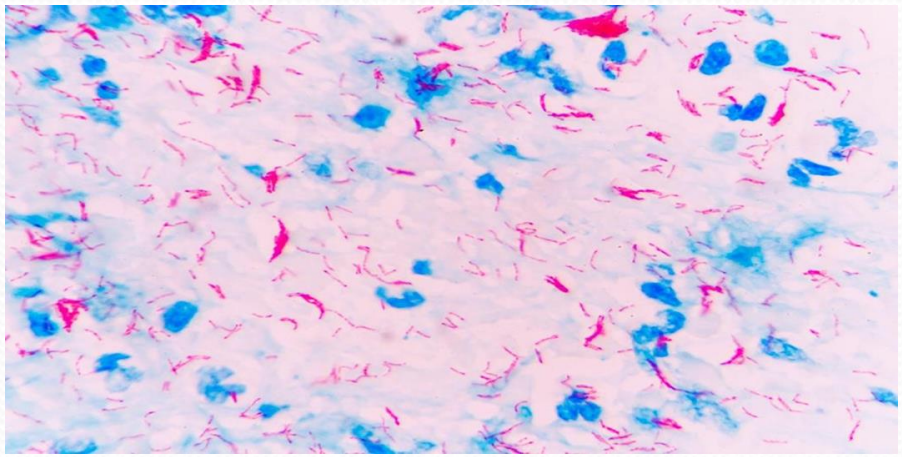
Laboratory identification: can be detected microscopically using immunofluorescent stain or dark-field illumination, syphilis is usually diagnosed serologically.

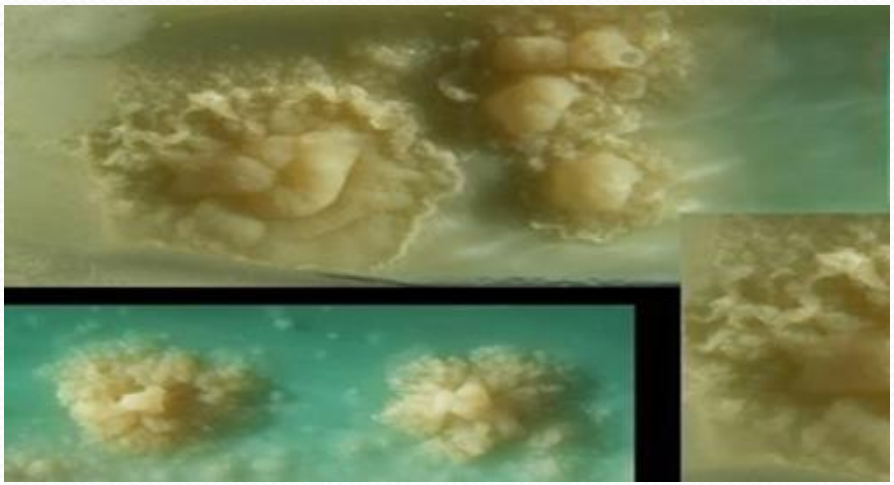
Treatment and prevention: penicillin is effective for primary and secondary syphilis. There is no vaccine against T. pallidum; **prevention** depends on safe sexual practices.

Mycobacterium tuberculosis or tubercle bacillus (TB)

TB is long, slender rods, strictly aerobic that are nonmotile and has not form spores. TB have thick cell walls, they are 60% lipid, (mycolic acids), therefore resistant to drying, but not to heat or ultraviolet irradiation.

The identification: A microscopic by using the Ziehl-Neelsen stain (acid-fastbacilli) is the most rapid test for mycobacteria. Culture: Lowenstein-Jensen medium.

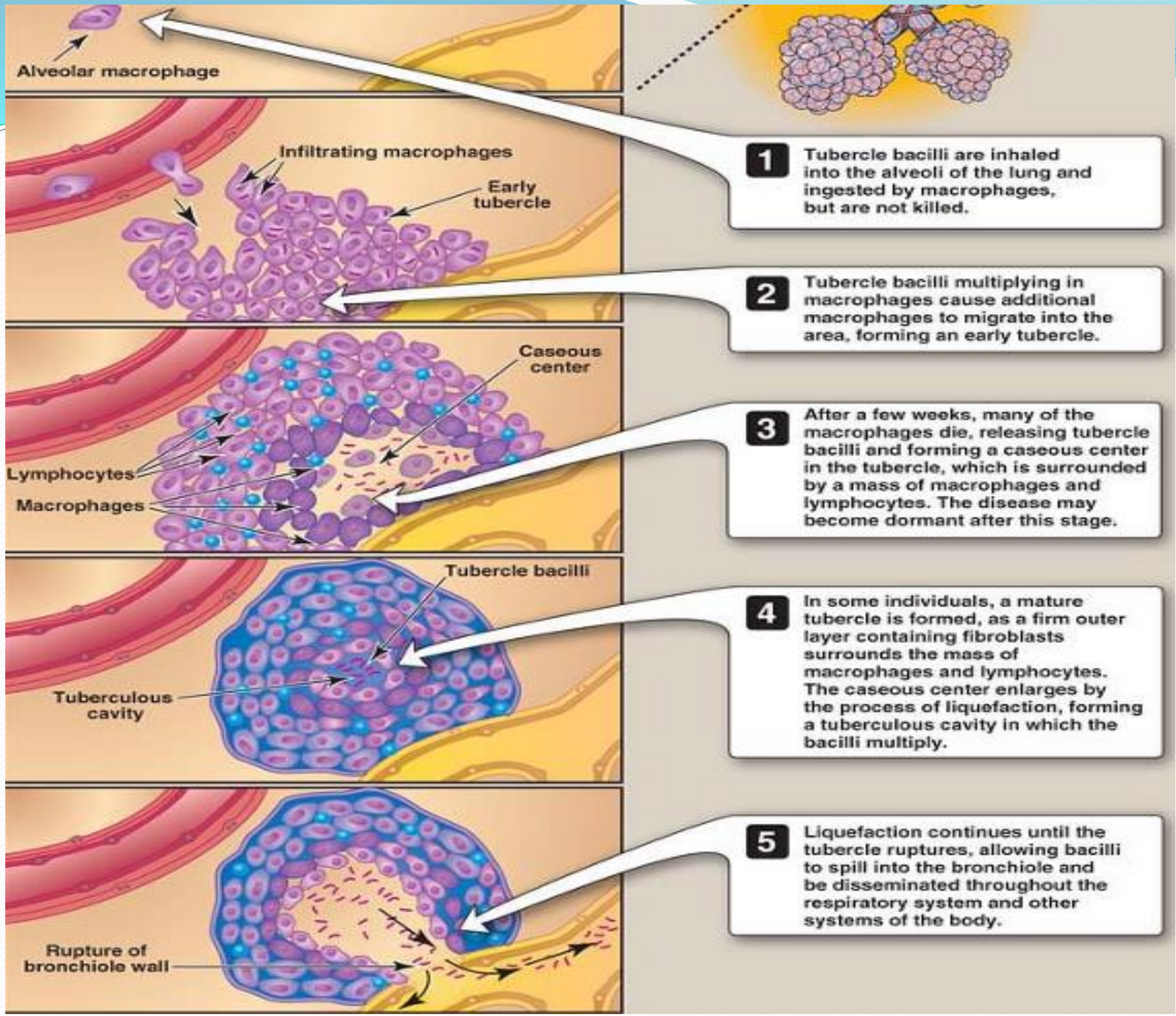




Pathogenicity & Clinical significance of TB: as shown in following figure.

Mycobacteria are emitted in droplets when infected persons cough, sneeze, or speak. The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages. Mycobacteria begin to multiply within macrophages. Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli. Pathogenic lesions associated with infection appear in the lung 1–2 months after exposure.Within 2 to 4 weeks, many bacilli are destroyed by the immune

system, but some survive and are spread by the blood to extrapulmonary sites. The virulence of TB rests with its ability to survive and grow within host cell, however, when engulfed by macrophages, bacteria inhibit the fusion of phagocytic vesicles with lysosomes. Primary tuberculosis occurs in a person who has no previous contact with the organism. For the majority of cases (about 95 %), the infection becomes arrested. The only evidence of tuberculosis may be a positive tuberculin test. A chest radiograph sometimes shows the initial pulmonary nodule, and some


Alveolar macrophage
Infiltrating macrophages
Early tubercle
Caseous center
Lymphocytes
Macrophages
Tubercle bacilli
Tuberculous cavity
Rupture of bronchiole wall
1
Tubercle bacilli are inhaled into the alveoli of the lung and ingested by macrophages, but are not killed.
2
Tubercle bacilli multiplying in macrophages cause additional macrophages to migrate into the area, forming an early tubercle.
3
After a few weeks, many of the macrophages die, releasing tubercle bacilli and forming a caseous center in the tubercle, which is surrounded by a mass of macrophages and lymphocytes. The disease may become dormant after this stage.
4
In some individuals, a mature tubercle is formed, as a firm outer layer containing fibroblasts surrounds the mass of macrophages and lymphocytes. The caseous center enlarges by the process of liquefaction, forming a tuberculous cavity in which the bacilli multiply.
5
Liquefaction continues until the tubercle ruptures, allowing bacilli to spill into the bronchiole and be disseminated throughout the respiratory system and other systems of the body.

Immunity: M. tuberculosis stimulates both a humoral and a cellular immune response.

The identification: Diagnosis of TB includes demonstration of clinical symptoms and abnormal chest radiographs, and confirmation by Identification of TB in clinical specimens: A microscopic search for acid-fast bacilli using the Ziehl-Neelsen stain is the most rapid test for mycobacteria. Culture: Lowenstein-Jensen medium.

Tuberculin reaction: In the routine procedure, a measured amount of PPD is injected intradermally in the forearm. It is read 48 to 72 hours later for the presence and size of an area of induration (hardening) at the site of injection, which must be observed for the test to be positive.

Treatment: Isoniazid, rifampin, ethambutol, streptomycin, and pyrazinamide

Vaccines: A vaccine is Bacille Calmette-Gurin (BCG), an attenuated strain of *M. bovis*. When injected intradermally, it can confer tuberculin hypersensitivity and an enhanced ability to activate macrophages that kill the pathogen.

Mycobacterium leprae

Mycobacterium leprae is an obligate intra-cellular parasite in man, multiplying mainly in histiocytes and Schwann cells. The entry of the bacilli into the Schwann cells causes peripheral neuropathy. *M. leprae* is a bacterium that causes **leprosy**, also known as "Hansen's disease

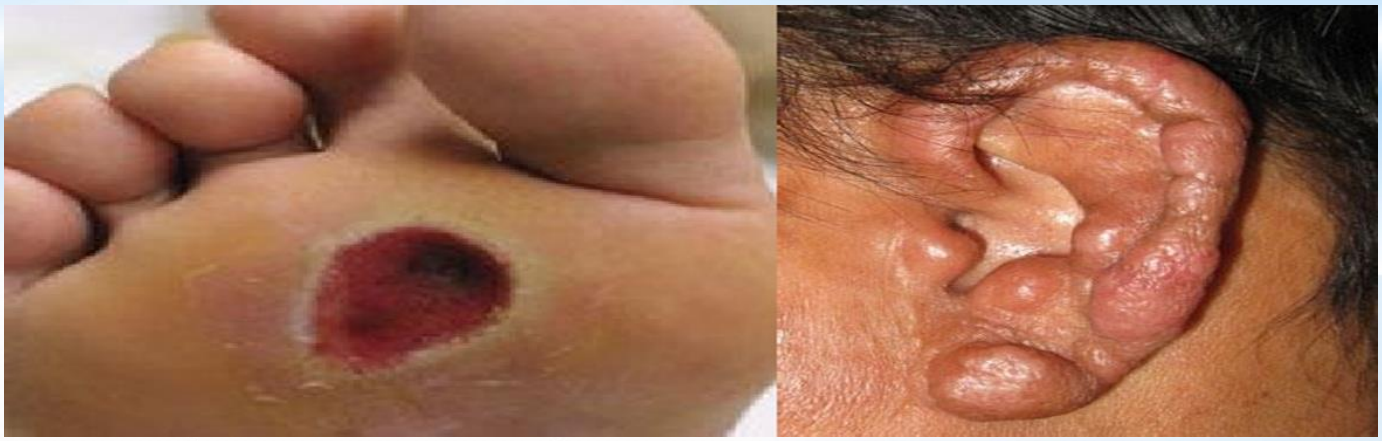
Leprosy is a chronic infectious disease caused by *Mycobacterium leprae*. It damages peripheral nerves and can affect the skin, eyes, nose and muscles. Nerve injury in leprosy can cause severe disabling deformities.

People who develop leprosy usually incubate the infection for 3–5 years before manifesting illness.

Treatment of *M. leprae*

Current treatment of leprosy involves use of 3 drugs: rifampicin (rifampin); clofazimine; and **dapsone**.

The duration of therapy was recently reduced from 24 to 12 months.



Antibiotics and antimicrobial agents

Antimicrobials is the treatment of infectious diseases by administration of drugs (antibiotics) which are lethal or inhibitory to the causative organisms.

Types of action of antimicrobial chemotherapeutics

From their behavior toward bacterial populations antibacterial agents are divided into two classes:

1- Bactericidal drugs: these have a rapid lethal action against the pathogenic agents e.g. penicillins, andcephalosporins

2- Bacteriostatic drugs: these merely inhibit the division of the pathogenic agents i.e. growth of organisms e.g. sulphonamides, and tetracycline

Range of Action of Antimicrobial Chemotherapeutics:

Antibiotics fall into three main categories:

1.Active mainly against gram-positive organisms e.g. penicillin, erythromycin and lincomysin.

2.Active mainly against gram-negative organisms e.g. polymyxin and nalidixic acid.

3.Active against both gram-positive and gram-negative organisms (broad-spectrum activity) e.g. tetracyclines, chloramphenicol, and ampicillin.

Broad Spectrum Antibiotics

Broad-spectrum antibiotics are antibiotics which are designed to work against a broad spectrum of bacteria, rather than **narrow-spectrum antibiotics**, which are only effective against a smaller range of bacteria.

Some examples of broad-spectrum antibiotics include:

penicillin, cephalosporin, tetracycline, ciprofloxacin and levofloxacin

Mechanism of Action of Antimicrobial Chemotherapeutics

Several mechanisms are known:

1- Inhibition of cell wall synthesis: Some antibiotics e.g. **penicillin, andcephalosporins** which interfere with cell wall synthesis and cause bacteriolysis.

2- Inhibition of cytoplasmic membrane function: Some antibiotics cause disruption of the cytoplasmic membrane such as Polymyxins,.

3- Inhibition of protein synthesis: Many antimicrobial chemotherapeutics block protein synthesis by acting on the 30s or 50s subunits of the bacterial ribosome. Examples are chloramphenicol**,** **tetracycline**, **erythromycin** and the aminoglycosides e.g. tobramycin, gentamycin and streptomycin.

4- Inhibition of nucleic acid synthesis: These can act on any of the steps of DNA or RNA replication e.g. **quinolones**, trimethoprim, **rifampicin**, nalidixic acid, novobiocin and metronidazole.

Mechanisms of Resistance to Antimicrobial Agents

The major mechanisms that mediate bacterial resistance to drugs:

1. Certain bacteria produce enzymes that destroy the drug, ex**, Beta-lactamase enzymes** can inactivate penicillines and cephalosporines by cleaving the beta-lactam ring of the drug.

2. Certain bacteria synthesize modified target site of drug action.

3. Certain bacteria change their permeability to the drugs.

4. Certain bacteria increase the export of drug to the outside of the M.O

Notes:

A.Bacteria have the ability to develop resistance following repeated or insufficient doses, so more advanced and synthetic antibiotics are continually required to overcome them.

B.Certain bacteria are not only resistant to drug but require it for growth, called drug-dependent bacteria.

C.Most drug resistance is due to a genetic change in bacteria(1)due to mutation in bacterial chromosome, inherited (2) acquired resistance due to acquisition of genetic materials

Origin of Resistance to Antimicrobial Agents

These mechanisms may be of non genetic or genetic origin:

A- Non genetic Drug Resistance:

i. Metabolic inactivity.

ii. Loss of target structure

B- Genetic Drug Resistance

i- **Plasmid mediated resistance**

Resistance (R) factors are a class of plasmids that mediate resistance to one or more antimicrobial agent. Plasmids frequently carry genes that code for the production of enzymes that inactivate or destroy antimicrobial agents e.g. β-Iactamase which destroys the p-lactam ring.

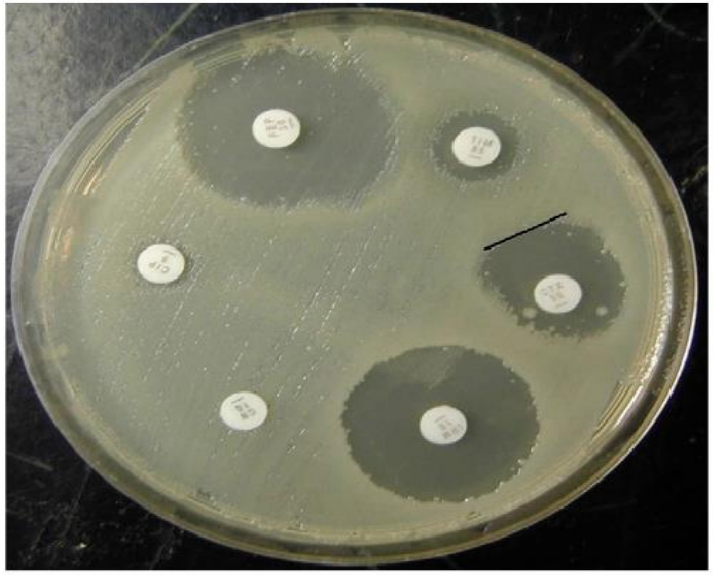
ii. **Transposon-mediated resistance**

Transposons are sequences of DNA that can move or transpose themselves to new positions within the genome of a single cell. Many transposons carry genes that code for drug resistance.

iii. Chromosomal drug resistance This develops as a result of spontaneous mutation in a gene that controls susceptibility to an antimicrobial agent.

Antimicrobial susceptibility testing: An in vitro test; done to check the effectiveness of a drug against a bacterium and to select the best drug that acts against the bacterium.

Fig. 1. **Antimicrobial susceptibility test using the disc method**



This blood isolate of extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* came from a septic patient who died from the infection. There is resistance to ceftriaxone (CTX), ciprofloxacin (CIP), ticarcillin-clavulanate (TIM) and gentamicin (CN). A "keyhole" (partial zone enlargement) is present between CTX and TIM, indicating the presence of an ESBL enzyme. The size of the zone around cefepime (FEP 4th generation cephalosporin) and imipenem (a carbapenem) is sufficient to indicate susceptibility.