Antimicrobial sensitivity test

Antimicrobial susceptibility tests are used to determine which specific antibiotics a particular bacteria or fungus is sensitive to. Most often, this testing complements a Gram stain and culture, the results of which are obtained much sooner. Antimicrobial susceptibility tests can guide the physician in drug choice and dosage for difficult-to-treat infections**.**

Results are commonly reported as the minimal inhibitory concentration (MIC), The interpretation usually categorizes each result as susceptible (S), intermediate (I), resistant (R), sensitive-dose dependent (SD), or no interpretation (NI).

Bacteriostatic vs. Bactericidal Agents

Bacteriostatic agents, such as tetracycline, inhibit the growth and multiplication of bacteria. cells in a susceptible population stop dividing. However if the agent is removed, the cells once again multiply.

Bactericidal agents, such as fluoroquinolones, not only inhibit the growth of cells but also trigger pathways within the cell that lead to cell death.

Modes of Antimicrobial Action

Antimicrobial agents are classified by their specific modes of action against bacterial

cells.

A- Interference with cell wall synthesis:

1- Antimicrobial agents that interfere with cell wall synthesis block peptidoglycan synthesis and thus are active against growing bacteria. This includes penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems.

2- Activity of beta lactams on **gram-negative bacteria:** In susceptible cells, beta-lactam molecules bind to penicillin binding proteins (PBPs) that are enzymes required for cell wall synthesis.

The attachment located on the surface of the cytoplasmic membrane, blocks their function. This causes weakened or defective cell walls and leads to cell lysis and death.

3- Activity of beta lactams on **gram-positive bacteria:** Since gram-positive bacteria do not possess an outer membrane, beta lactam antimicrobials diffuse throughthe cell wall. The next steps are similar to those in gram-negative bacteria

B- Interference with the cytoplasmic membrane: Polymyxin molecules diffuse through the outer membrane and cell wall of susceptible cells to the cytoplasmic membrane. They bind to the cytoplasmic membrane and disrupt and destabilize it. This causes the cytoplasm to leak out of the cell resulting in cell death.

C- Interference with protein synthesis by binding to the 30S ribosomal subunit.

1- Tetracyclines (e.g. tetracycline, minocycline and doxycycline) bind to the 30S subunit of the ribosome and block the attachment of transfer RNA (tRNA).

2- Aminoglycosides (e.g. gentamicin, tobramycin, amikacin, and streptomycin)also bind to the ribosomal subunit and can block protein synthesis in two different ways. First they can attach to the 30S subunit of the ribosome and prevent the 30S subunit from attaching to messenger RNA (mRNA). Second, the presence of the aminoglycoside on the ribosome may cause misreading of the mRNA. This leads to the insertion of the wrong amino acid into the protein or interference with the ability of amino acids to connect with one another.

D- Inhibition of protein synthesis by binding to the 50S ribosomal subunit

1- Macrolides (e.g. erythromycin, azithromycin and clarithromycin) and lincosamides (e.g. clindamycin) attach to the 50S ribosomal subunit causing termination of the growing protein chain and inhibition of protein synthesis.

2- Chloramphenicol also binds to the 50S subunit of the ribosome and interferes with binding of amino acids to the growing protein.

E- Interference with nucleic acid synthesis is caused by two classes of drugs

1- Fluoroquinolones (e.g. nalidixic acid, ciprofloxacin, levofloxacin and gemifloxacin) interfere with DNA synthesis by blocking the enzyme DNA gyrase. Which leads to cell death.

2- Rifampin binds to DNA-dependent RNA polymerase, which blocks the synthesis of RNA and results in cell death.

F- Inhibition of the metabolic pathway for folic acid synthesis is caused by the sulfonamides and trimethoprim: For many organisms para-aminobenzoic acid (PABA) is an essential metabolite and is involved in the synthesis of folic acid, an important precursor to the synthesis of nucleic acids. Sulfonamides are structural analogs of PABA and compete with PABA for the enzyme dihydropteroate synthetase. Trimethoprim acts on the folic acid synthesis pathway at a point after the sulfonamides. It inhibits the enzyme dihyrofolate reductase**.**

MECHANISMS OF ANTIMICROBIAL RESISTANCE

There are a number of ways by which microorganisms are resistant to antimicrobial agents. These include:

1) the bacteria produce enzymes that either destroy the antimicrobial agent before it reaches its target or modify the drug so that it no longer is recognized by the target; **such as Beta-lactamases** are enzymes that

hydrolyze beta-lactam drugs This includes penicillin derivatives (penams), cephalosporins (cephems),

2) the cell wall becomes impermeable to the antimicrobial agent; include **Alteration of porins in gram-negative bacteria** such as beta-lactams cannot reach the PBPs, the cell is resistant.

3) The target site is altered by mutation so that it no longer binds the antimicrobial agent;

4) The bacteria possess an efflux pump that expels the antimicrobial agent from the cell before it can reach its target;

5) Specific metabolic pathways in the bacteria are genetically altered so that the antimicrobial agent cannot exert an effect.

Minimum inhibitory concentration

It is mean the lowest concentration of a chemical that prevents visible growth of a bacterium (in other words, at which it has bacteriostatic activity).

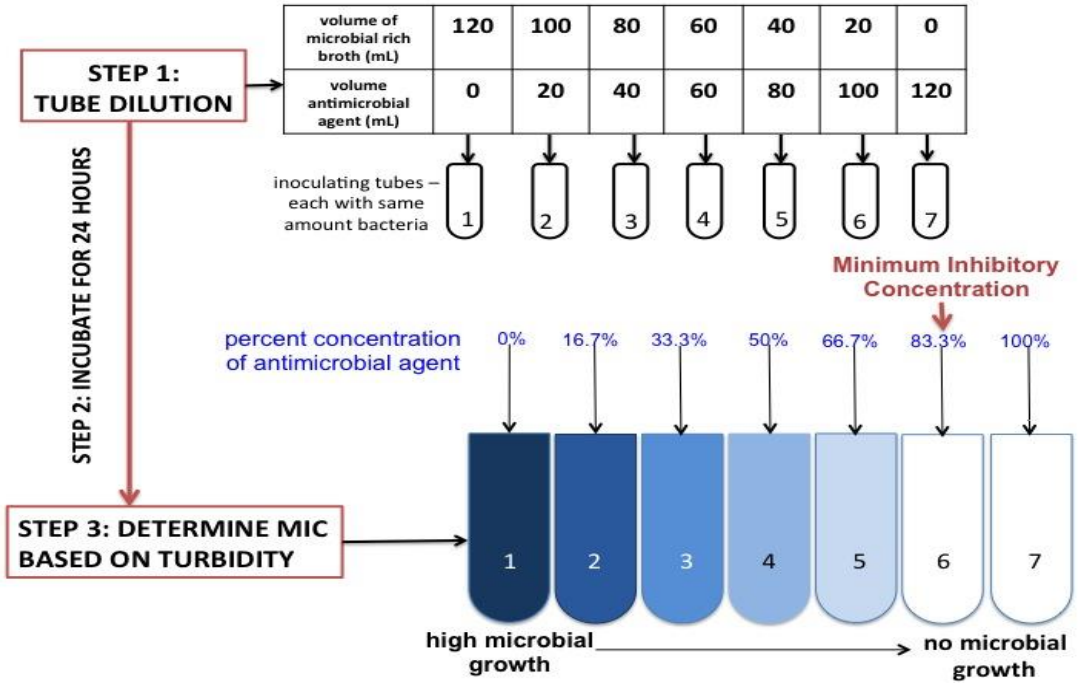
Minimum bactericidal concentration

(MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium.

the MIC and MBC of a chemical is determined by preparing solutions of the chemical at increasing concentrations, incubating the solutions with the separate batches of cultured bacteria, and measuring the results using agar dilution or broth microdilution.

In medicine is important for identifying the correct drug to actually give to the patient.

A- Broth dilution methods



Minimum Inhibitory Concentration: Tube Dilution Assay is performed by constantly increasing the percent concentration of antimicrobial agent to microbial rich broth in a series of tubes. It is used to measure the Minimum Inhibitory Concentration [MIC] of an antimicrobial agent, which is the lowest concentration of antimicrobial agent that will inhibit the growth of microbes. The turbidity of the tubes indicates the amount of microbe growth, with the least turbid, or clear, tubes (tubes 6 and 7) correlating with the absence of microbes. The tube with no antimicrobial agent (tube 1) presents as opaque and most turbid because the microbes are able to flourish. As antimicrobial concentration increases, the turbidity decreases until the MIC is reached and microbes can no longer survive. Antimicrobials with low MICs are more effective than those with high MICs, as only a low dosage is necessary to eradicate microbes.

B- DISK DIFFUSION METHOD

Because of convenience, efficiency and cost, the disk diffusion method is probably the most widely used method for determining antimicrobial resistance in private veterinary clinics.

A growth medium, usually Mueller-Hinton agar, is first evenly seeded throughout the plate with the isolate of interest that has been diluted at a standard concentration and used antibiotic disk**.**

The zone around an antibiotic disk that has no growth is referred to as the zone of inhibition since this approximates the minimum antibiotic concentration sufficient to prevent growth of the test isolate. This zone is then measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant. MIC measurement cannot be determined from this qualitative test, which simply classifies the isolate as susceptible, intermediate or resistant.