Antimicrobial susceptibility testing

Antimicrobials: are agents kill or inhibit the disease-causing bacteria.

Antimicrobial susceptibility testing: is -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.

-The antimicrobial agents have two types of effect, either **bacteriostatic** or **bactericidal**.

-Some antimicrobial agents are active against several types of M.O. called (**Broad-spectrum**), whereas other are active against few types of M.O. called (**Narrow-spectrum**).

Mode of action:

Antibiotics have selective inhibition of growth of M.O. without damage to the host cell. This selectivity due to the differences between the metabolism and structure of M.O. and human. Mode of action according to the site of effect:

1. Inhibition of cell wall synthesis, ex, penicillins and cephalosporines.
2. Inhibition of cell membrane synthesis, ex, polymixine and garamicine.
3. Inhibition of nucleic acid synthesis, ex, rifampicine and quinolones.
4. Inhibition of protein synthesis, ex, erythromycin and tetracycline.

Methods of antimicrobial susceptibility testing:

Because the bacteria rapidly develop resistance, therefore; they should be tested for antimicrobial susceptibility by one of the following methods:

- **a. Diffusion testing**
- i. Kirby-Bauer
- ii. E-test (to estimate MIC -Minimum inhibitory concentration-)
- **b. Dilution testing**
- i. Broth dilution (Macro or microdilution method) to estimate MIC.
- ii. Agar dilution method
- iii. Schlichter (serum bactericidal test)

- **c. Beta-lactamase detection**
- **d. Extended-spectrum beta-lactamase detection**

Note: The purpose of the Kirby-Bauer disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds in order to assist a physician in selecting treatment options for his or her patients. Minimum inhibitory concentration (MIC) is determined when a patient does not respond to treatment thought to be adequate, relapses while being treated or when there is immunosuppression. MIC is the lowest concentration of antimicrobial agent that inhibits the growth of a bacterium.

Factors affecting antimicrobial susceptibility in vitro:

a) Type and components of the medium.
b) Type of inoculating method.
c) Number and activity of inoculated bacteria.
d) Stability and concentration of the used drug.
e) Temperature and time of incubation.

Disc diffusion method (Kirby & Bauer method)

Procedure:

i. Preparation of bacterial suspension by taking 3 colonies of young culture and adding it to tube contains 5 ml of normal saline.
ii. The turbidity of bacterial suspension will be compared with the standard McFarland tube (0.5) for determination of bacterial cell number that used in the inoculation. (McFarland standards are suspensions of either barium sulfate or latex particles that allow visual comparison of bacterial density, and 0.5 McFarland standard is equivalent to a bacterial suspension containing between 1 x 10^8 and 2 x 10^8 CFU/ml of *E. coli*).
iii. About 0.1 ml of the bacterial suspension is spread over a solid medium (Muller-Hinton agar medium) by swabbing (vertical and horizontal directions).

iv. Antibiotic-impregnated filter paper discs are placed, 4-6 discs on each plate by a sterile forceps. The plate will be incubated at 37°C for 18-24 hours.

v. The result will be recorded by measuring the inhibition zones (in mm). The interpretation of these results as sensitive or resistant according to a standard document of CLSI (clinical and laboratory standard institutes). The efficacy of drug combination can be estimated as **synergistic** (enhancement of efficacy of drug in the presence of other drug), or **antagonistic** (impairment of efficacy) or **indifferent** (no change in the presence of other drug).