جامعة الانبار

كلية :الصيدلة

قسم :العلوم المختبرية السريرية

اسم المادة باللغة العربية:احياء مجهرية طبية **I**

اسم المدة باللغة الإنكليزية:**Medical Microbiology I**

المرحلة:الثانية

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عنوان المحاضرة باللغة العربية:اختبارات الحساسية للمضادات الحيوية

عنوان المحاضرة باللغة الإنكليزية: **Antimicrobial susceptibility test**

Antimicrobial susceptibility test

Antimicrobial susceptibility test measure the ability of an antibiotic to inhibit bacterial growth in vitro. This ability may be estimated either by dilution or diffusion method.

Indications for susceptibility testing

1. To guide the clinician in selecting the best antibiotic agent for an individual patient.
2. To control the use of inappropriate antibiotics in clinical practice
3. To accumulate epidemiological information on the resistance of microorganisms of public health importance within the community-.
4. When the susceptibility to the antimicrobial agents of choice is unpredictable and if the isolate is believed to be clinically significant.
5. If the patient is allergic to the antimicrobial agent of choice, testing susceptibility to alternative agents, such as erythromycin for *S. pyogenes*, is reasonable.
6. When an infectious process is likely to be fatal unless treated specifically (e.g., meningitis, septicemia).

7. -Bacteria have the ability to develop resistance following repeated or subclinical (insufficient) doses, so more advanced antibiotics and synthetic antibiotics are continually required to overcome them.
8. In certain infections where eradication of the infectious organism requires the use of drugs that are rapidly bactericidal, not merely bacteriostatic (e.g., infective endocarditis).

Quantitative susceptibility test

- ❖ Broth dilution method

A-Determination of minimal inhibitory concentration (MIC)

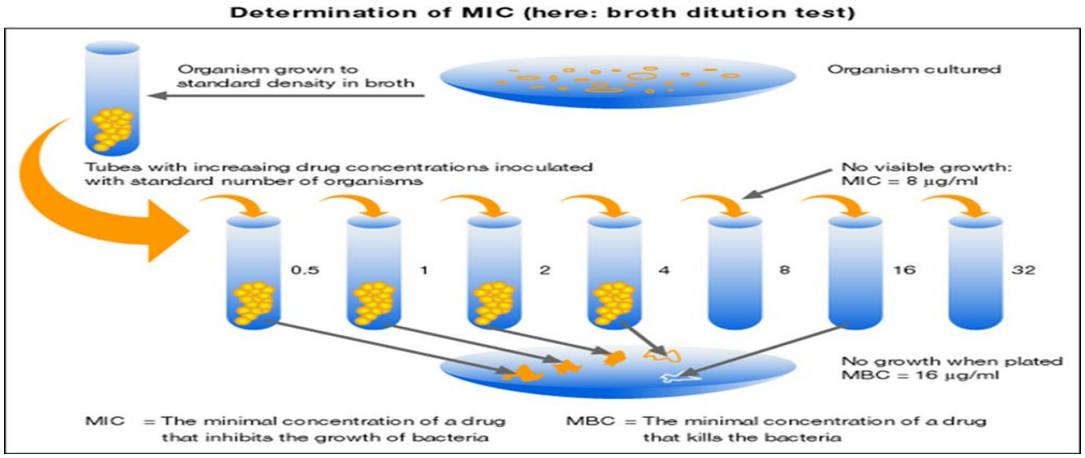
The quantitative determination of susceptibility to antimicrobial agents has proved to be the most reliable means of treating infection. Tube dilution method can be used to determine the MIC, this can be perform by either broth or agar dilution method. The lowest concentration that inhibits growth after overnight incubation is known as the minimum inhibitory concentration (MIC) of the agent.In this method serial dilutions of antimicrobial agent are prepared in several test tubes which contain broth medium ,equal amount of bacterial sample are added to each test tubes, a positive and negative control tests must be prepared to justify the result.The test tubes and control tubes are incubated at 37 ^{o} C for 18-24 hours in reading results turbidity is used as a sign of bacterial growth in the test tubes minimum inhibitory concentration (MIC) is recorded as it is the lowest concentration of antimicrobial agent that can inhibit visible growth of the test organism after overnight incubation

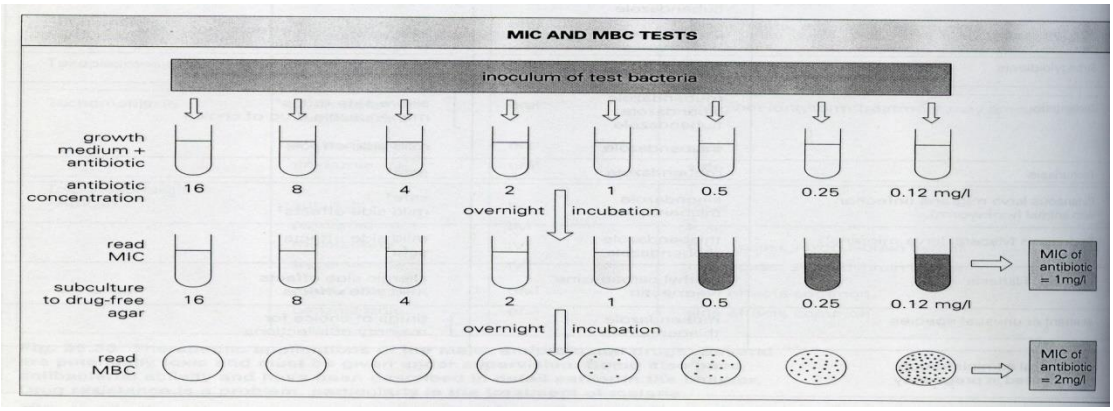
B-Determination of minimal bactericidal concentration (MBC)

The main advantage of the broth dilution method is that it can be readily converted into a bactericidal test. In this case dilutions and inoculations are prepared in the same manner as described for the determination of MIC. The control tube containing no antibiotic is immediately subcultured (Before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism and incubated at 37 ^{o}C overnight. The tubes are also incubated

overnight at 37 ^{o} C. The MIC is then recorder, subculture all tubes not showing visible growth in the same manner as the control tube described above and incubate at 37 ^{o}C overnight. Compare the amount of growth from the control tube before incubation, which represents the original inoculum. These subcultures may show a similar number of colonies, indicating bacteristasis only, a reduced number of colonies, indicating partial or slow bactericidal activity, or no growth if the whole inoculum has been killed .

MBC is the lowest concentration of the antimicrobial agent which kills 99.9% of the tested bacteria after overnight incubation .



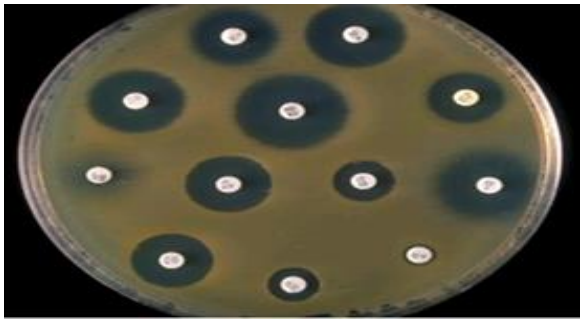


Qualitative susceptibility test

The diffusion test **(kirby-bauer test for antibiotic susceptibility**)

Paper disk, impregnated with the antibiotic are placed on agar medium uniformly seeded with the test organism. A concentration gradient of the antibiotic forms by diffusion from the disk and the growth of the test organism is inhibited at a distance from the disc . If the organism is

killed or inhibited by the concentration of the antibiotic, there will be NO growth in the immediate area around the disc: This is called the zone of inhibition. The zone sizes are looked up on a standardized chart to give a result of susceptible, resistant, or an intermediate.



Kirby-Bauer Method

Materials

- Mueller-Hinton Agar
- Antibiotic Disks
- Turbidity Standard
- Swabs

Procedure

1. To prepare the inoculum from the primary culture plate, touch with a loop the tops of each of 3–5 colonies, of similar appearance, of the organism to be tested.
2. Transfer this growth to a tube of saline.
3. Compare the tube with the turbidity standard and adjust the density of the test suspension to that of the standard by adding more bacteria or more sterile saline.
4. Inoculate the plates by dipping a sterile swab into the inoculum.
5. Streak the swab all over the surface of the medium three times, rotating the plate through an angle of 60^0 after each application
6. Finally, pass the swab round the edge of the agar surface.
7. Leave the inoculum to dry for a few minutes at room temperature with the lid closed.
8. The antibiotic discs may be placed on the inoculated plates using sterile forceps, template, sterile needle tip and antibiotic disc dispenser. Incubate the plates for 16 to 18 hours at 35°C.

Interpretation

Measure the diameters of inhibition zone in mm

1- Place the ruler across the zone of inhibition and measure from one edge of the zone to other.

2- Zone diameter is reported in millimeters, looked up on chart and result reported (sensitive), (resistant), or (intermediate).

❖ **Synergism** when the activity of both drugs is significantly greater than that of either acting alone in the same concentration.

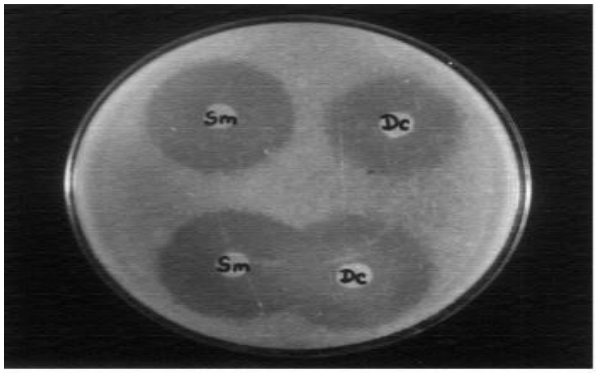


Figure 2. Synergism between diclofenac (50 µg/ml) and streptomycin (10 µg/ml) against *Mycobacterium smegmatis* 798.