

## Practical Microbiology - Lecture 8

### Antimicrobial Susceptibility Testing

**Antibiotics:** are agents that are "selectively" toxic for bacteria (either killing them [bactericidal] or inhibiting their growth [bacteriostatic]) without harm to the patient. Antibiotics work most efficiently in conjunction with an active immune system to kill infecting bacteria in the host.

**Two principle methods available for doing antimicrobial sensitivity testing:**

#### **1-Disk diffusion Method (Kirby-Bauer):**

A daily procedure in bacteriology laboratories to determine susceptibility of microorganisms to different antimicrobial agents.

In this test, small filter paper disks (6 mm) impregnated with a fixed concentration of antibiotics are placed on the surface of Mueller-Hinton agar medium inoculated uniformly with a standard inoculum of tested bacteria. The plates are incubated overnight. In reading the results, the area around the disks are observed for bacterial growth. When a circular zone of no bacterial growth around the disks which called **inhibition zone** is formed, the diameter of inhibition zone is used as a measure of susceptibility of bacterium to antibiotic, by comparing the obtained zone diameter with the known zone diameter size for susceptibility (using reference tables) for example, a zone of certain size indicates susceptibility, zone of smaller diameter or no zone at all show that the bacterium is resistant to the antibiotic.

#### **2-Tube dilution method:**

This method is used to determine the sensitivity of bacteria to a single antimicrobial agent to determine the concentration of that agent which will cause either inhibition or death of bacteria under this test.

In this method serial dilutions of antimicrobial agent are prepared in several test tubes each tube contain an equal amount of a broth medium, equal amount of bacterial sample are added to the each test tubes, a positive and a negative control tests must be prepared to justify the test results. The test tubes and the control tubes are incubated at 37°C for 18-24 hours and 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 the antimicrobial agent that will cause inhibition of growth of the bacteria, subculturing from test tubes on solid agar may lead to bacteria growth due to the decreasing effect of the antimicrobial agent on the solid media, from this we record minimum bactericidal concentration (**MBC**) which is the lowest concentration of the antimicrobial agent that will cause no growth on the solid media.