Ministry of Higher Education
& Scientific Research
University of Sawah
Dep. Of laboratories

Grade: 4

Lecture : 11

Antibiotic susceptibility tests

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Antibiotic susceptibility tests

- Antimicrobial agents are nontoxic antimicrobial therapeutic agents, which include antiseptics, antibiotics, preservatives, sterilants, and disinfectants; all have the capacity to kill or suppress the growth of microorganisms. Antimicrobial agents are an essential components of the practice of medicine. They are used to treat, prevent, and control the distribution of bacterial pathogens. The term antibiotic has been traditionally reserved for compounds that are naturally produced by living **microorganisms**, such as bacteria and fungi. The term has come to be more widely applied to any natural, semisynthetic, or synthetic molecule used to treat or prevent disease.

Mode of action of antibiotics

Cell wall synthesis inhibitors

β-lactams
Glycopeptides
Fosfomycin
Bacitracin
Alafosfalin

Cell membrane synthesis disruptors

Lipopeptides

Folate synthesis inhibitors

Sulfonamides

DNA gyrase inhibitors

Quinolones
Coumermycin antibiotics

Inhibition of DNA-dependent RNA polymerase

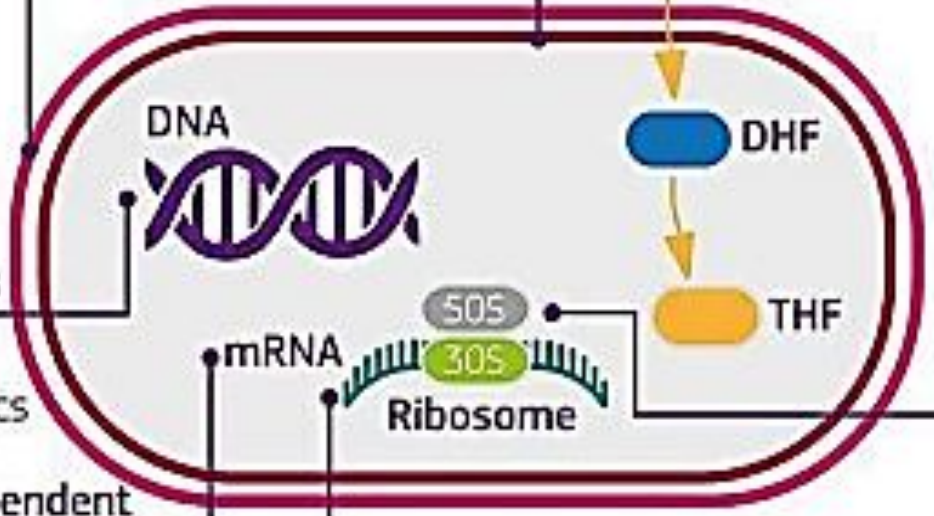
Rifampicin

RNA synthesis inhibitors

Ansamycines

Protein synthesis (30S and 50S inhibitors)

Tetracyclines
Aminoglycosides
Macrolides
Lincosamides
Amphenicols
Pleuromutilins
Oxazolidinones



Antibiotic susceptibility testing is performed on bacteria isolated from clinical specimens to determine which antimicrobial agents might be effective in treating infections caused by the bacteria. Only bacteria that are likely to be contributing to an infection should be tested. Testing bacteria that are not involved in the infection would be misleading to the physician and could lead to a more serious infection with development of antimicrobial resistance. One of the major challenges in clinical microbiology is the identification of the bacterium that caused infections. Often, these bacteria need to be distinguished from normal flora that may be present in at the site of the infection normally, although in some situations the microbial flora that reside at the site of the infection may be contributing to the infection. Therefore, thought needs to go into determining which bacteria from a specimen will be tested for susceptibility to antimicrobials. Most microbiology laboratories have guidelines for determining when and on which bacteria susceptibility testing will be done. When in doubt about the significance of a bacteria from a specimen, it is best to discuss the situation with the attending physician.

In clinical laboratories, susceptibility testing is usually performed by a disk diffusion or and minimal inhibitory concentration [MIC] methods. Standards that describe these methods are published and frequently updated by the

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CLSI

Clinical and Laboratory Standards Institute

02

NCCLS

National Committee for Clinical Laboratory Standards

After a pathogen is cultured, its sensitivity to specific antibiotics serves as a guide in choosing antimicrobial therapy. Some pathogens, such as Streptococcus pyogenes and N. meningitidis, usually have predictable sensitivity patterns to certain antibiotics. In contrast, most gram-negative bacilli, enterococci, and staphylococcal species show unpredictable sensitivity patterns to various antibiotics and require susceptibility testing to determine appropriate antimicrobial therapy. There are many methods for detecting this bacterial susceptibility pattern like

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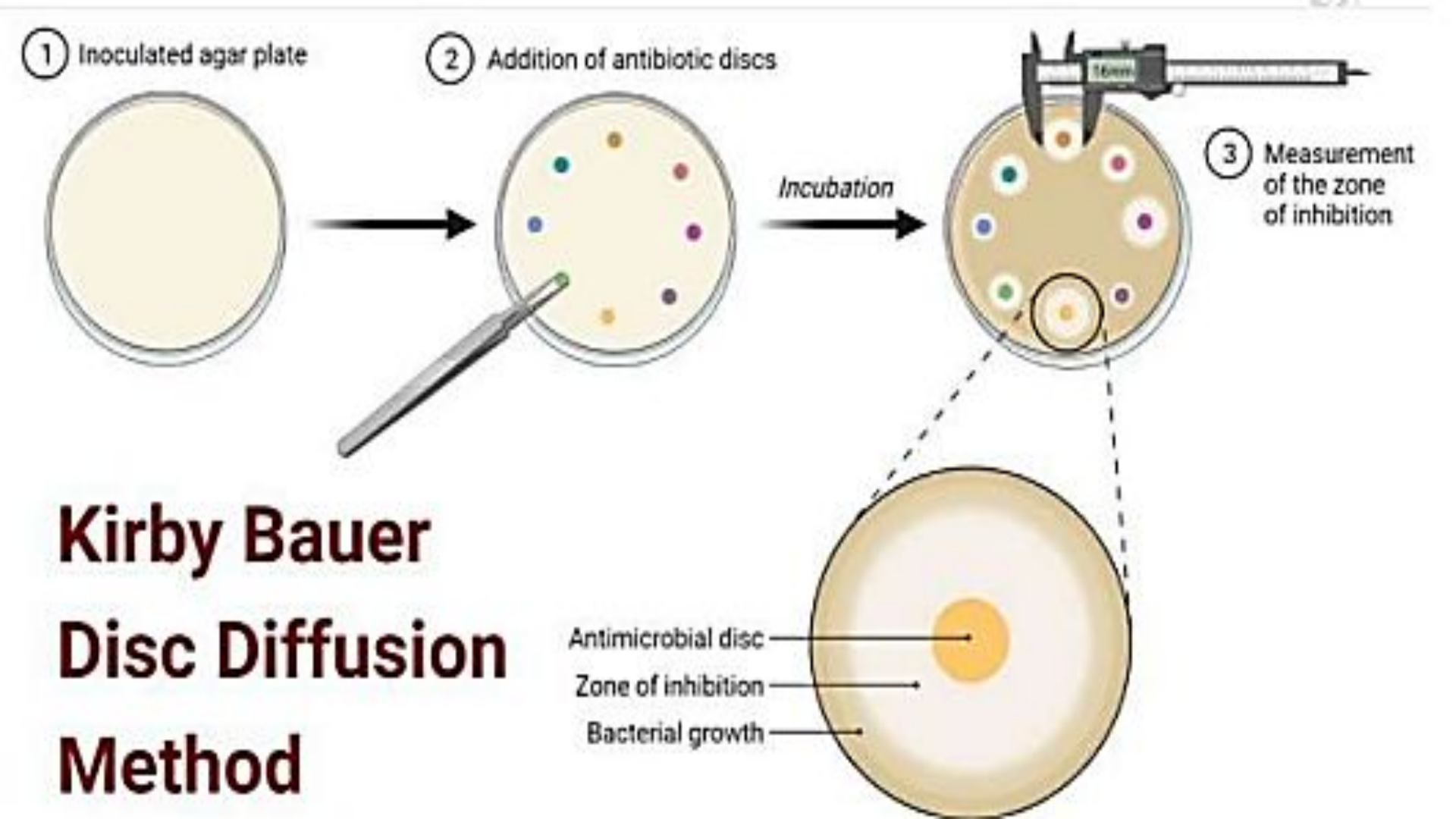
Disk-diffusion method

The classic qualitative method to test susceptibility to antibiotics has been the KirbyBauer disk-diffusion method, in which disks with exact amounts of different antimicrobial agents are placed on culture dishes inoculated with the microorganism to be tested. The micro-organism's growth (resistance to the drug) or lack of growth 2.Minimal inhibitory concentration (M


1 Inoculated agar plate
2 Addition of antibiotic discs
Incubation
3 Measurement of the zone of inhibition
Kirby Bauer Disc Diffusion Method
Antimicrobial disc
Zone of inhibition
Bacterial growth

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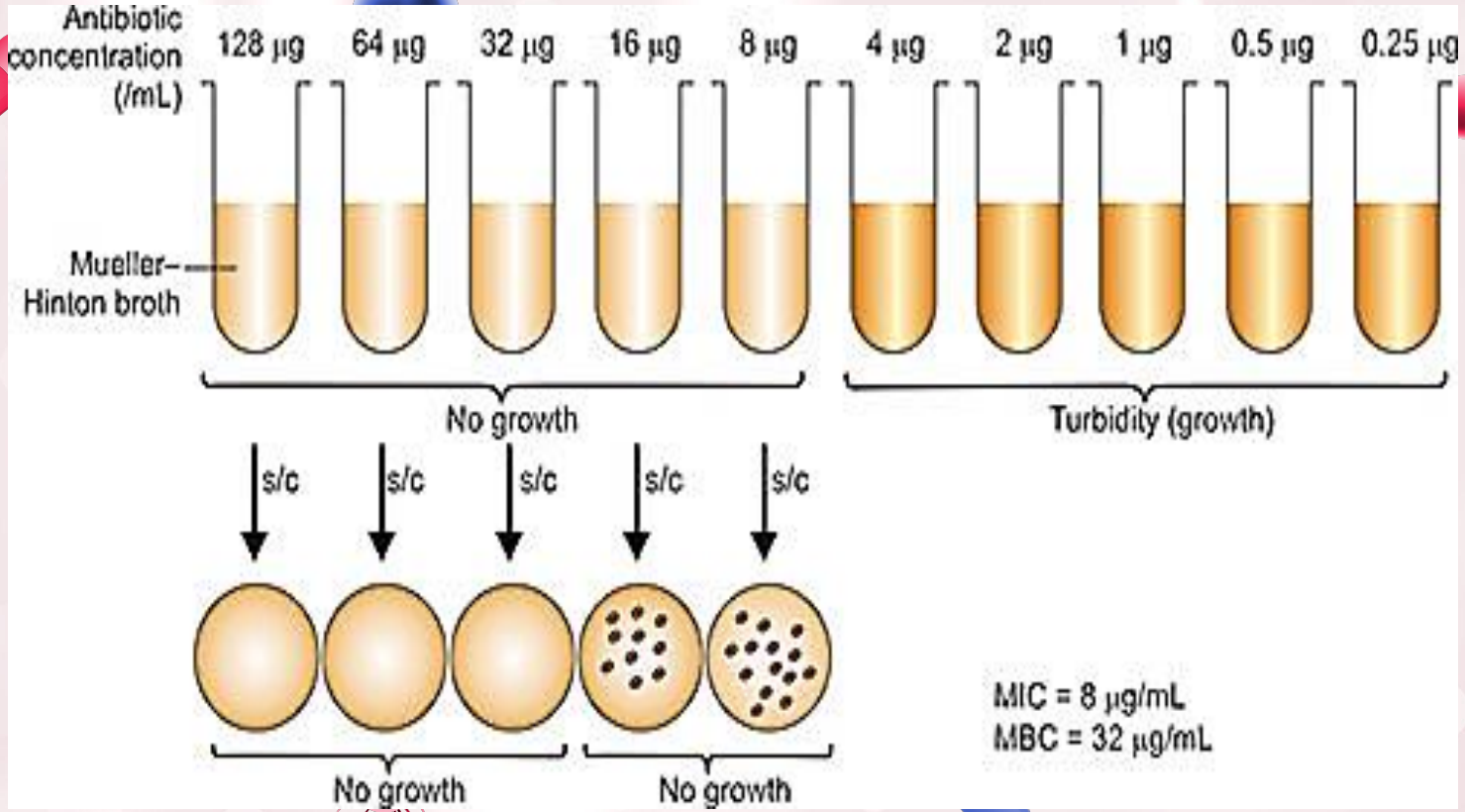
Minimal inhibitory concentration (MIC)

a- Broth Dilution : In broth dilution testing method, each antimicrobial agent is tested using a range of concentrations (µg of active drug/mL. of broth). Typically, the range of concentrations examined for each antibiotic is a series of doubling dilutions (16, 8, 4, 2, 1, 0.5, 0.25 µg/mL); the lowest antimicrobial concentration that completely inhibits visible bacterial growth, as detected visually or with an automated method, is recorded as the minimal inhibitory concentration (MIC).

02

Minimal inhibitory concentration (MIC)

B- Tube dilution (quantitative or macrodilution susceptibility testing): In this method, tubes containing serial dilutions of an antibiotic are inoculated with the tested organism. The tubes are incubated and later observed to determine the 4 minimal inhibitory concentration (**MIC**) of the antibiotic necessary to prevent bacterial growth (Figure-3). the minimal bactericidal concentration (**MBC**) may need to be determined. This is the lowest concentration of antibiotic that kills 100% of the bacteria, rather than simply inhibiting growth.


Antibiotic concentration (/mL)
128 µg
64 µg
32 µg
16 µg
8 µg
4 µg
2 µg
1 µg
0.5 µg
0.25 µg
Mueller–Hinton broth
No growth
Turbidity (growth)
s/c
s/c
s/c
s/c
s/c
No growth
No growth
MIC = 8 µg/mL
MBC = 32 µg/mL

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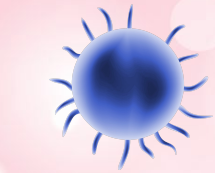
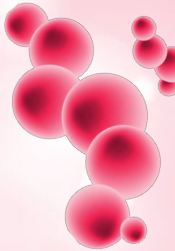
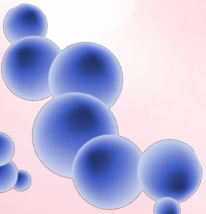
Automated Antimicrobial Susceptibility Test Systems

The automated antimicrobial susceptibility test systems available for use include the Vitek Legacy and Vitek 2 system.


RENDER
MA120




THANK YOU