Tishk International University
Faculty of Applied Science
Nutrition and Dietetics Department

Antimicrobial Sensitivity Test

Food Microbiology /5th Lab
2nd Grade / Spring Semester

Instructor: Adam Jalal Mohammed

Email: adam.jalal@tiu.edu.iq

What are Antimicrobials?

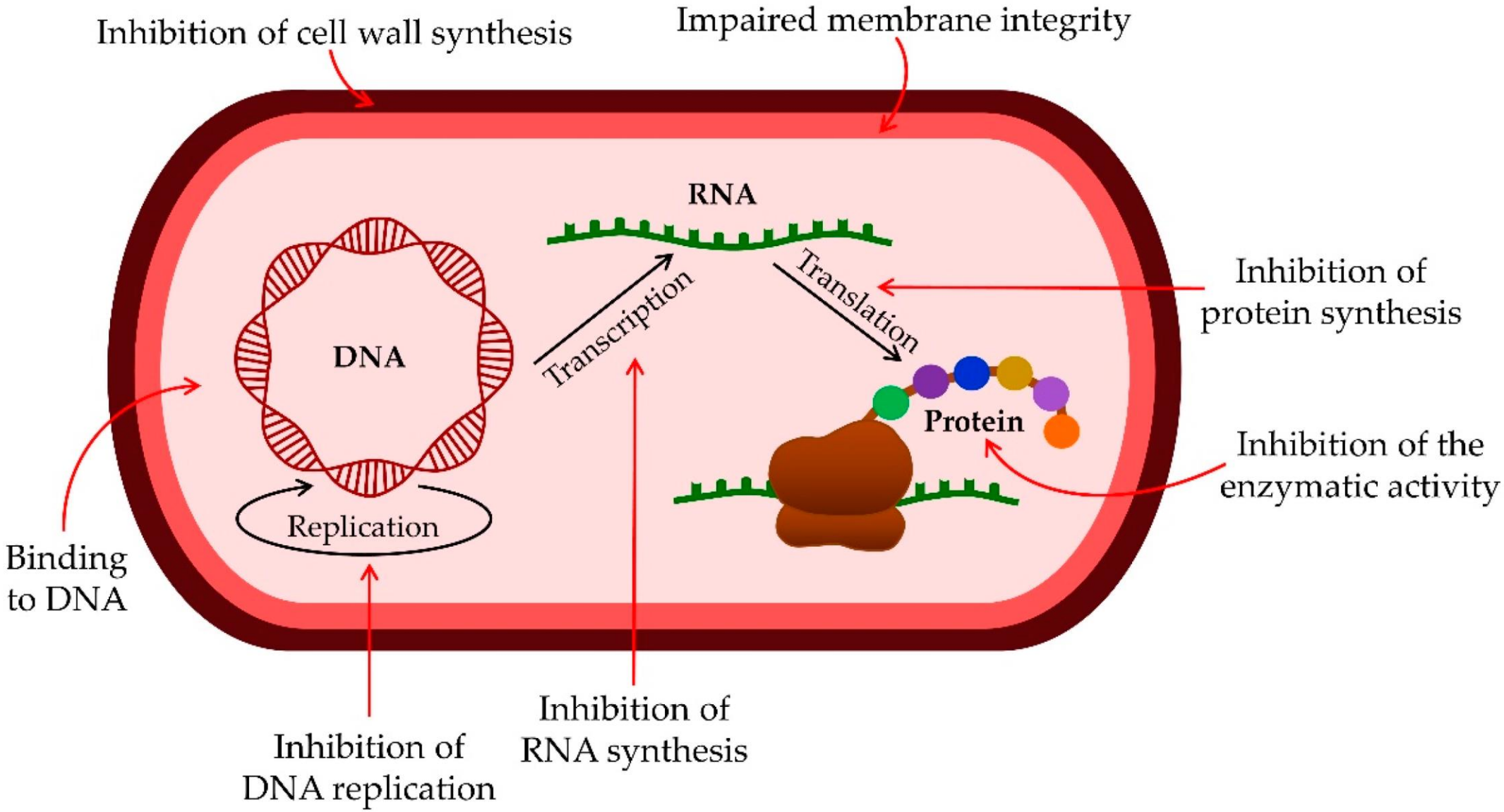
•Substances that kill or inhibit the growth of microorganisms.

➢ Types of Antimicrobials

- Antibacterial (Antibiotics): Effective against bacteria.
- Antifungal: Effective against fungi.
- Antiviral: Effective against viruses.
- Antiparasitic: Effective against parasites

General Antimicrobial Targets

- **Cell Wall Synthesis Inhibitors:** (e.g., Penicillins) - Interfere with building the bacterial cell wall.
- **Protein Synthesis Inhibitors:** (e.g., Tetracyclines) - Stop the production of essential bacterial proteins.
- **Nucleic Acid Synthesis Inhibitors:** (e.g., Quinolones) - Block the copying of bacterial DNA or RNA.
- **Cell Membrane Disruptors:** (e.g., Polymyxins) - Damage the bacterial cell membrane.
- **Disruption of metabolic pathways:** Blocking necessary biochemical reactions. (e.g., Sulfonamides).


Inhibition of cell wall synthesis
Impaired membrane integrity
RNA
DNA
Transcription
Translation
Inhibition of
protein synthesis
Protein
Inhibition of the
enzymatic activity
Replication
Binding
to DNA
Inhibition of
DNA replication
Inhibition of
RNA synthesis

Antimicrobial susceptibility testing (AST)

- Is a crucial laboratory procedure used to determine the ability of antimicrobials to inhibit or kill microorganisms, particularly bacteria.
- It helps clinicians to select the most appropriate antimicrobial agents for treating infections and to monitor the development of antimicrobial resistance.

Importance of AST

Importance of Antimicrobial Susceptibility Testing

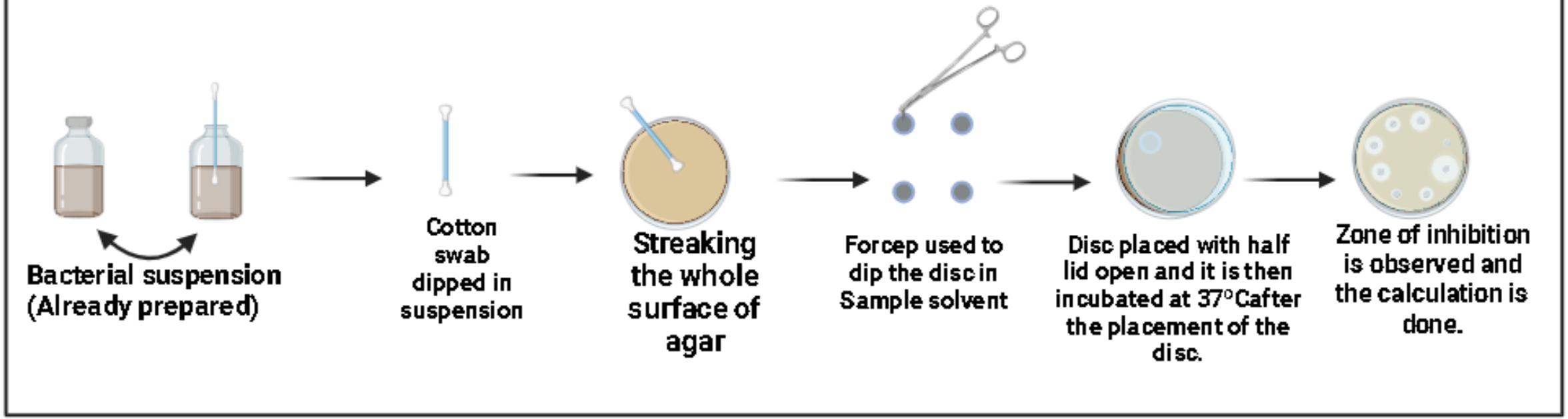
- **Guide Antimicrobial Therapy:** AST results help in choosing the most effective antibiotic, preventing treatment failure.
- **Prevent Resistance Development:** By using appropriate antibiotics, we can slow down the emergence and spread of antimicrobial resistance.
- **Patient Safety:** Ensures patients receive the right drug, at the correct dosage, reducing toxicity and side effects.

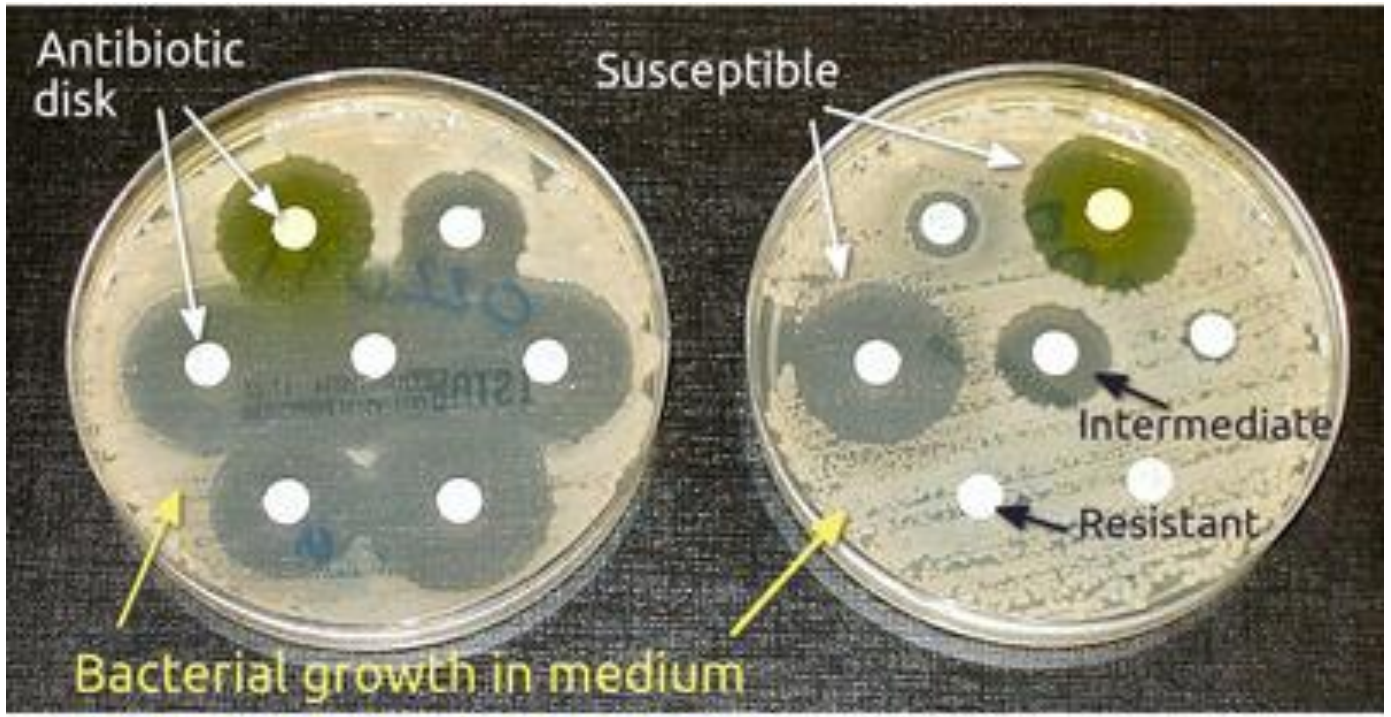
➢Antimicrobial Susceptibility Testing Methods

1. Kirby-Bauer Disc Diffusion Method

- Principle:
  - A standardized inoculum of the bacteria is spread on an agar plate (usually Mueller-Hinton agar).
  - Discs impregnated with specific concentrations of different antimicrobials are placed on the agar surface.
  - During incubation, the antimicrobials diffuse outward, creating a concentration gradient.
  - If the bacteria are sensitive to an antimicrobial, a clear zone of inhibition appears around the disc, where bacterial growth is inhibited.

KIRBY BAUER DISC- DIFFUSION METHOD




Antibiotic disk
Susceptible
Intermediate
Resistant
Bacterial growth in medium

2. Broth Dilution Method (MIC)

This method determines the **minimum inhibitory concentration (MIC)** and **minimum bactericidal concentration (MBC)** of an antimicrobial.

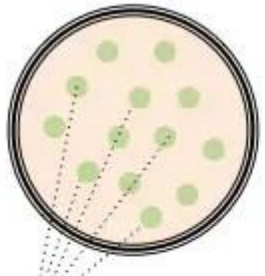
- MIC: The lowest concentration of an antimicrobial that inhibits the visible growth of a microorganism after incubation.

❑ Procedure:

1. **Prepare Antibiotic Dilutions**
   - Prepare a series of test tubes containing nutrient broth.
   - Add the antibiotic in **decreasing concentrations.**
2. **Add Bacterial Inoculum**
   - Inoculate each tube with a **standardized bacterial suspension** .
   - Incubate all tubes at **35–37°C for 18–24 hours**.
3. **Check for Bacterial Growth**
   - After incubation, examine each tube for **turbidity** (cloudiness).
   - **Turbid = growth of bacteria**
   - **Clear = no growth of bacteria**
4. **Determine the MIC**
   - Identify the **lowest concentration** of antibiotic that **prevents visible bacterial growth**.
   - This is the **MIC value**.

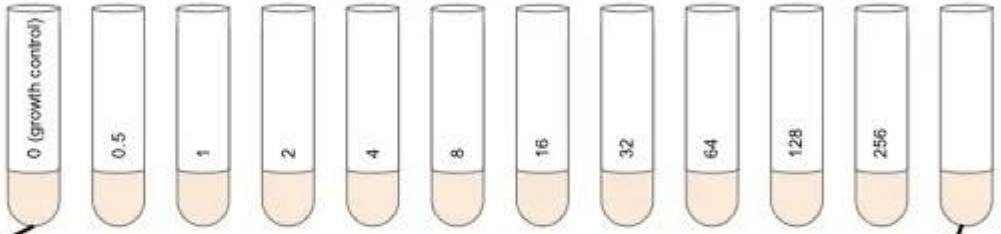
Broth dilution method for measuring minimum inhibitory concentration of antibiotics

1. Obtain isolated colonies of bacterial strain to test.



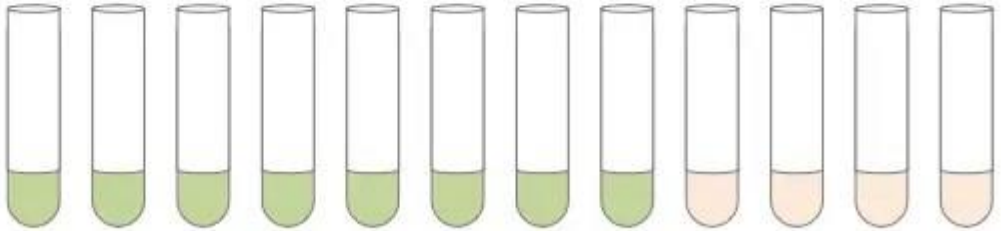
2. Combine 4-5 colonies and culture overnight in rich media broth.

3. After overnight incubation shown at left, add rich broth with appropriate dilution series of test antibiotic to test tubes. Example concentrations (mg/L) are shown below. Inoculate bacteria to a final density of 5 x 10^5 cfu/ml.



No bacteria; broth control

4. Plate aliquot of growth control (i.e., no antibiotic added) to verify cfu/ml counts of viable bacteria. Incubate overnight and count colonies.



5. After overnight incubation, check cultures for growth. The MIC is the lowest concentration of antibiotic that prevents visible growth. In this example, the MIC is 64 mg/L.

Factors Affecting Antimicrobial Susceptibility Testing

- **Inoculum Density:** Too high or too low inoculum can affect zone sizes or MIC values.
- **Growth Medium:** The type and pH of the medium can influence antimicrobial activity.
- **Incubation Conditions:** Temperature and incubation time must be controlled.
- **Antimicrobial Concentration:** Disc content must be accurate.
- **Disc Spacing:** Discs must be placed far enough apart to prevent overlapping zones.