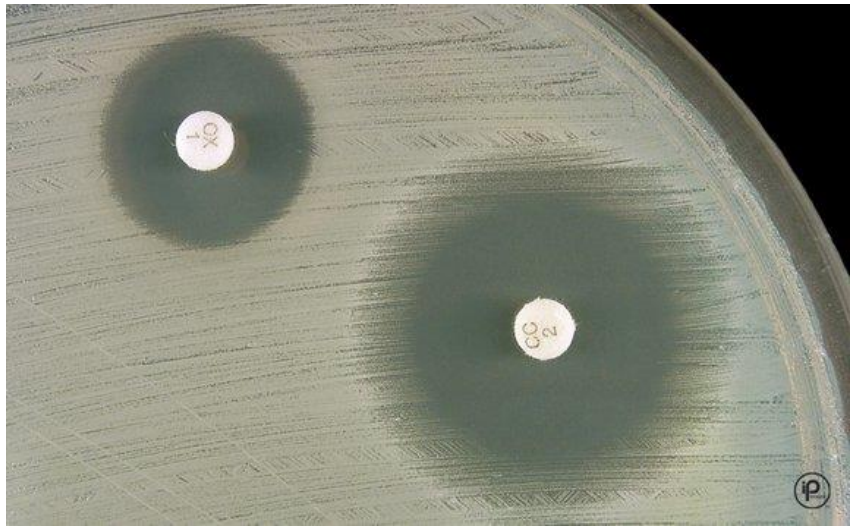
# Lecture 8



# Antimicrobial drug resistance

# Objectives

- Enumerate Mechanisms of Antimicrobial Drug resistance
- Interpret a cultured disc diffusion plate
- Explain the principle of the following susceptibility tests: Disc diffusion, serial dilution methods and E test
- Define ***Minimal inhibitory concentration and Minimal bactericidal concentration***

# Antimicrobial drug resistance

- It is the unresponsiveness of the organisms to the administered drug (antibiotics).

# Origin of resistance

**Intrinsic resistance**

- Some bacteria are **intrinsically resistant** to certain antibiotics.

  → Example: Gram-positive bacteria are much less susceptible to polymixins than Gram-negative bacteria.

**Acquired resistance**

- Many bacteria acquire resistance to one or more of the antibiotics to which they were **formerly susceptible**.

# Acquired resistance

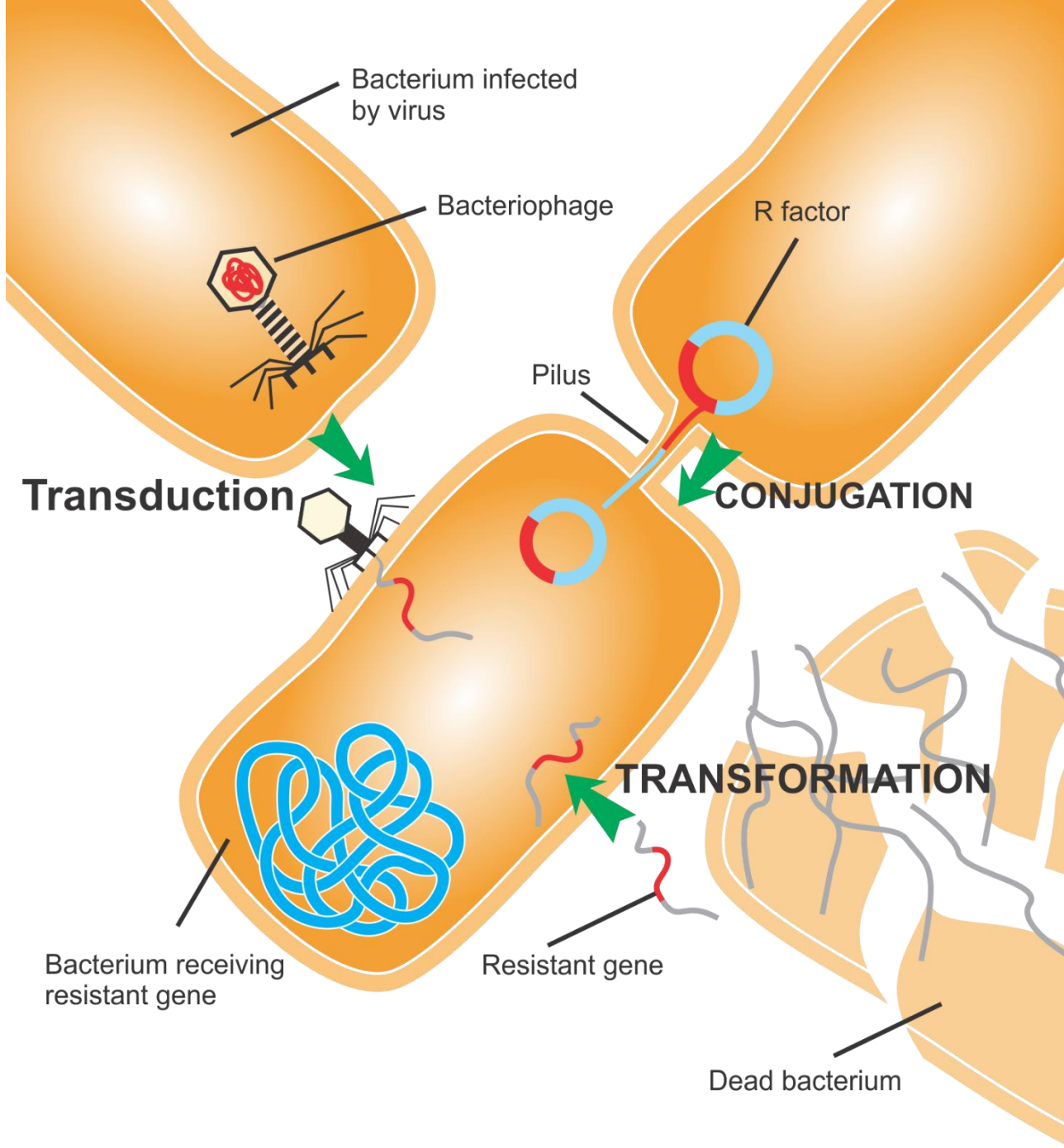
There are two mechanisms by which bacteria develops antibiotic resistance:

- **Mutation** of existing genes or
- **Acquisition of** new **resistance genes** from other bacteria that are already resistant to the antibiotic.

# Acquired resistance -

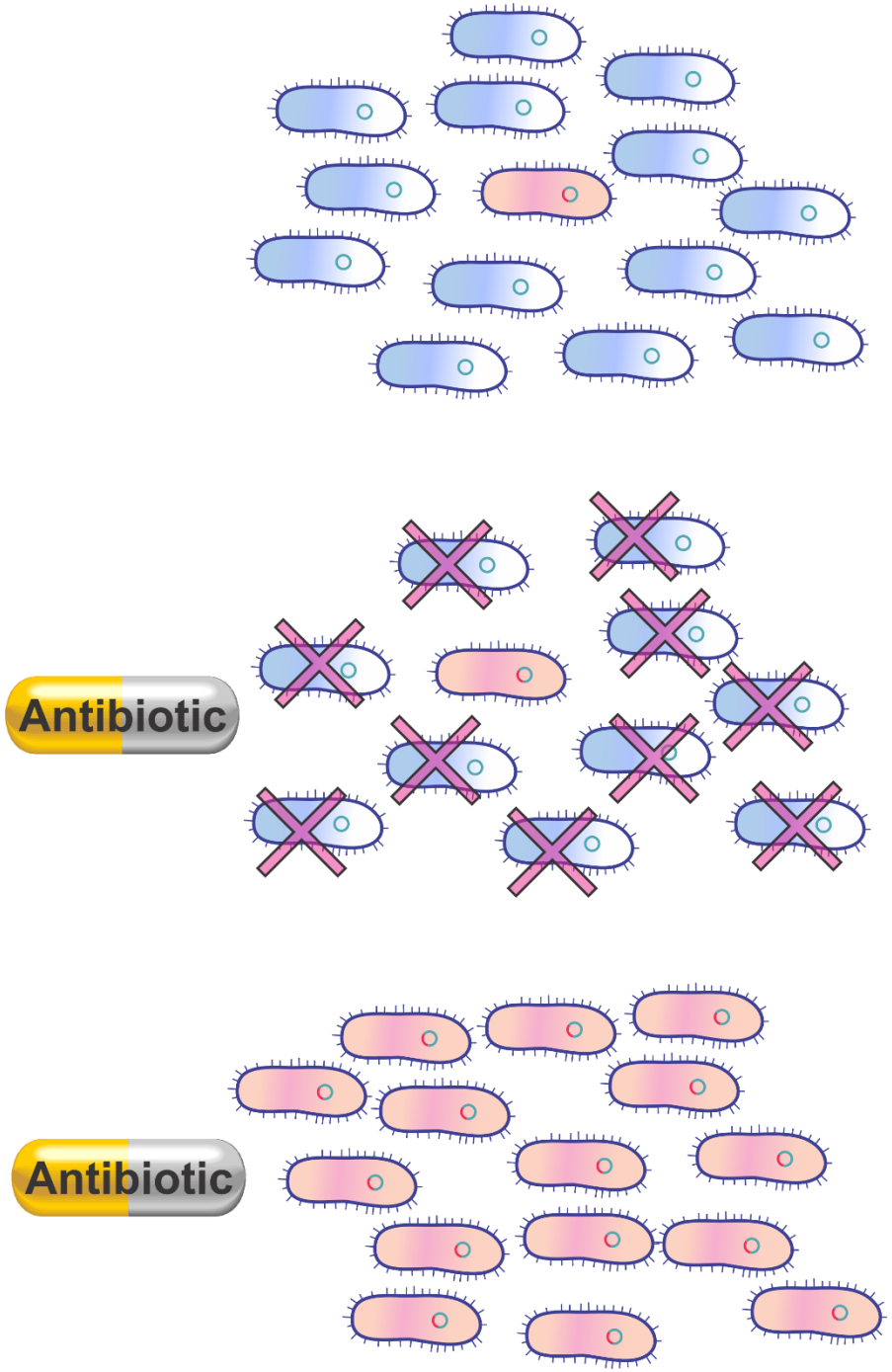
*Acquisition of new resistance genes*

- **R factors** are a class of plasmids that carry genes for resistance to one—and often several—antimicrobial drugs and heavy metals.
- Genetic material and plasmids can be transferred between bacteria by several mechanisms (**transduction**, **transformation**, and **conjugation**).



# Acquired resistance - *Natural selection*

- A bacterial population contains both drug-sensitive and drug-resistant cells.
- In antibiotic absence, the numbers of resistant cells will remain low because they have ***no growth advantage.***
- Exposure to antibiotic inhibits the sensitive cells; reduced competition from sensitive cells facilitates the multiplication of resistant cells.
- Eventually, resistant cells constitute the majority of the population.



# *Mechanisms of Antimicrobial Drug resistance*

❶ Microorganisms change their **permeability** to the drug

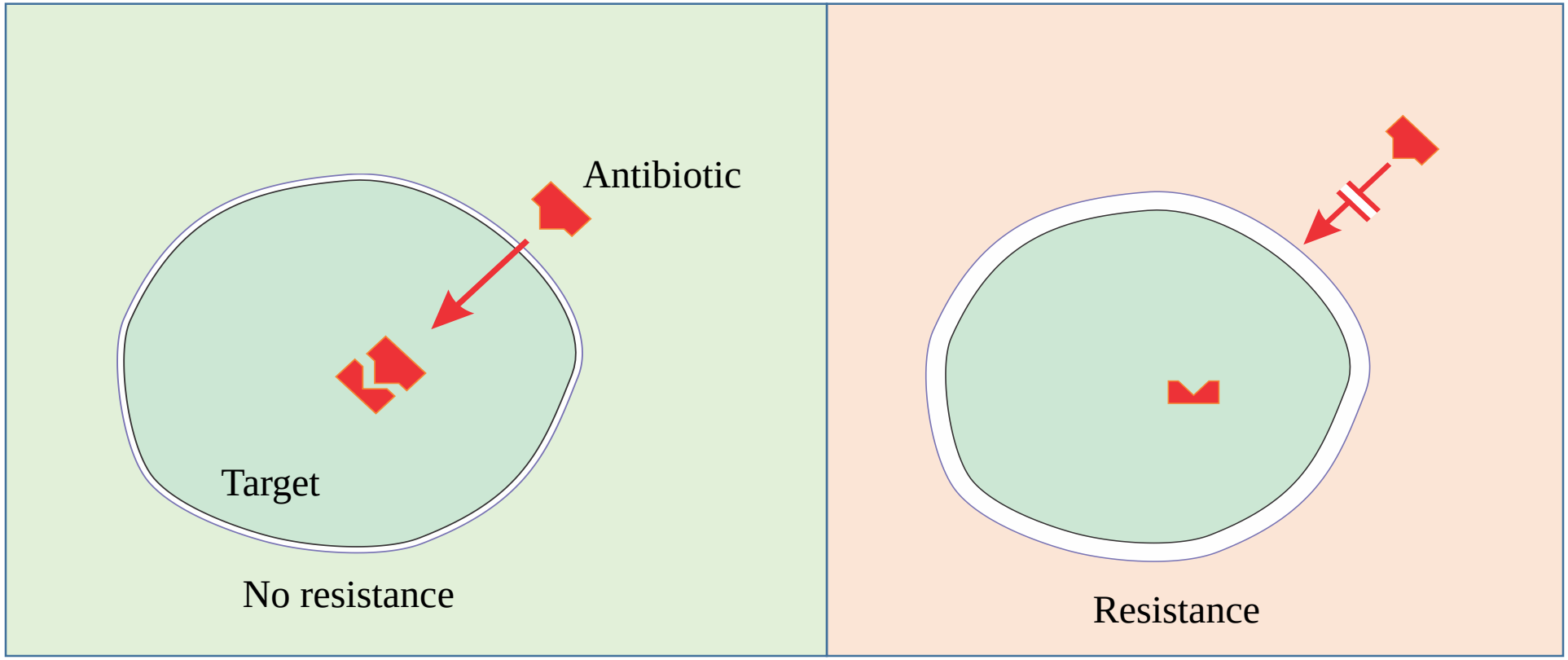
❷ **Pumping out** (Active efflux) of the drugs across the cell surface.

❸ Microorganisms change their **target receptor** for the drug

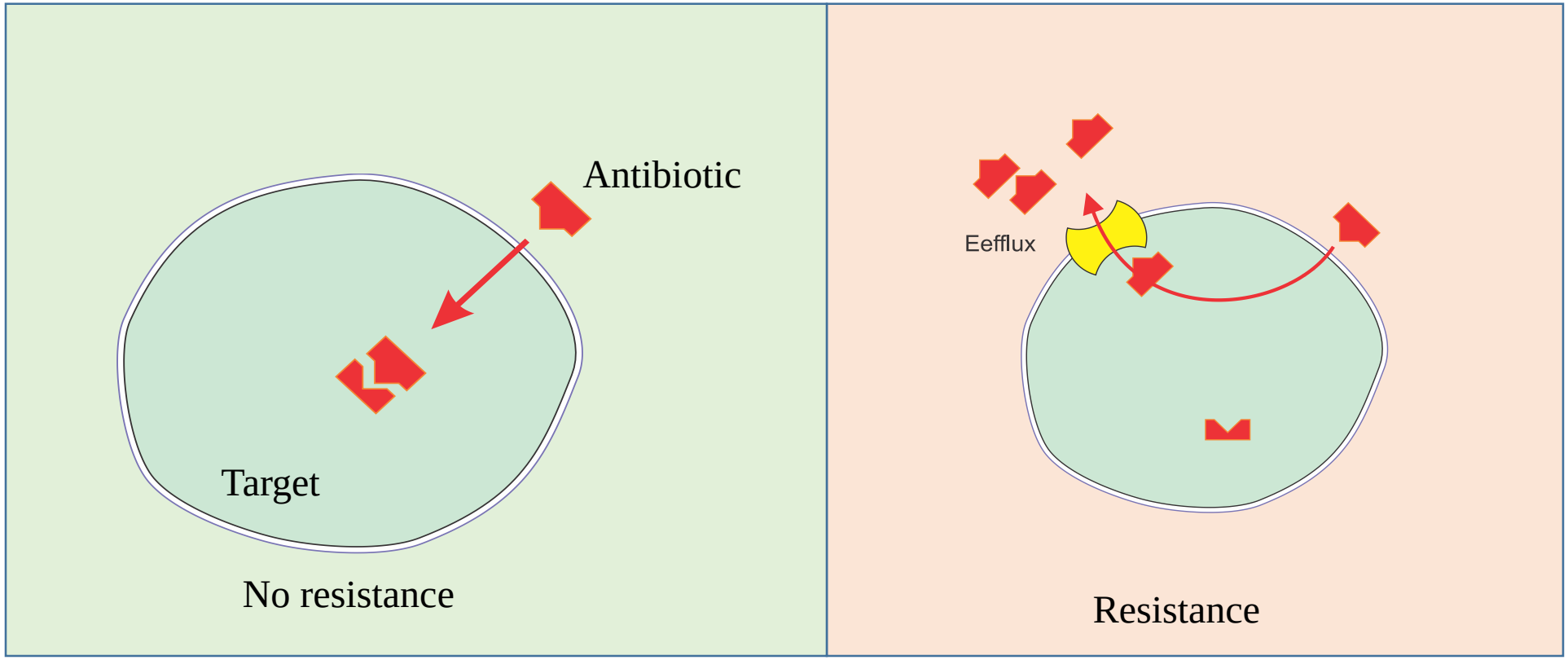
❹ Microorganisms produce enzymes that **destroy** **the drug**

❺ Microorganisms alter the **metabolic pathway** to bypass the reactions inhibited by the drug.

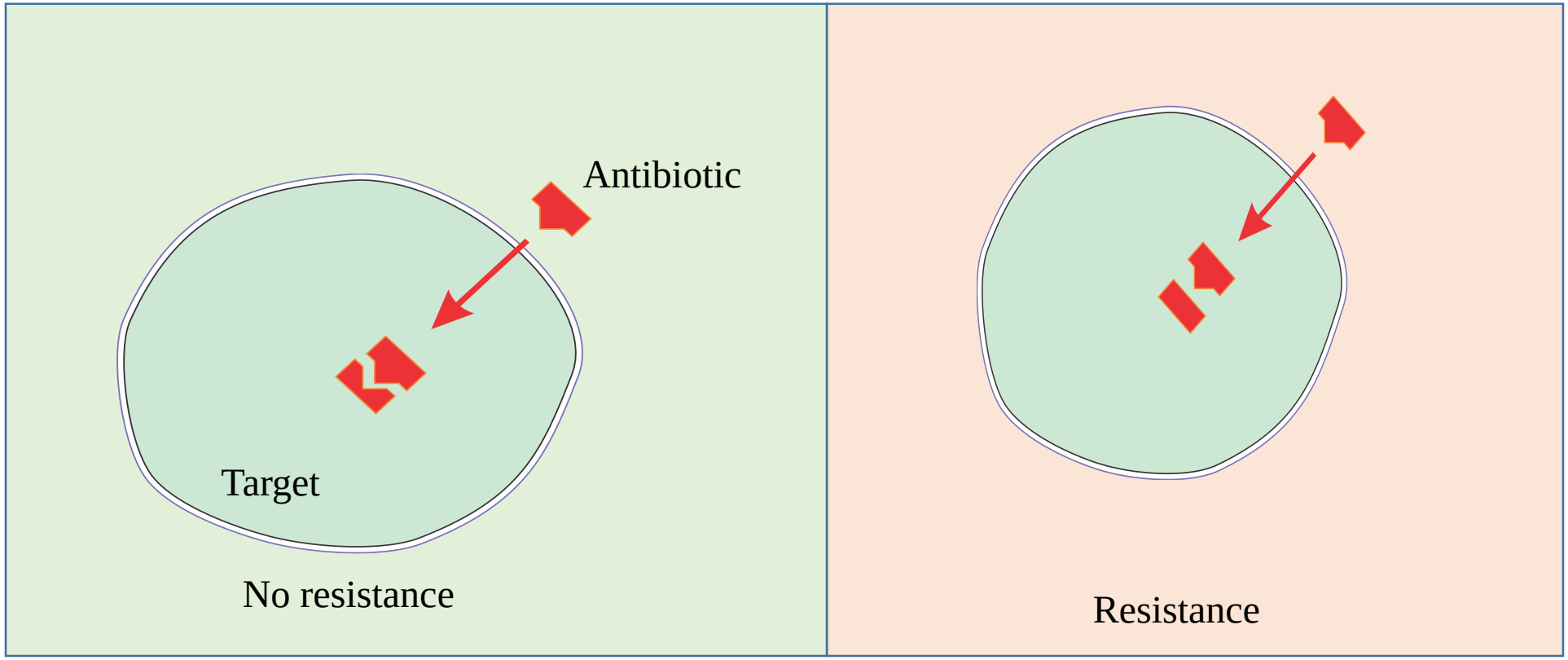
# 1. Microorganisms change their permeability to the drug



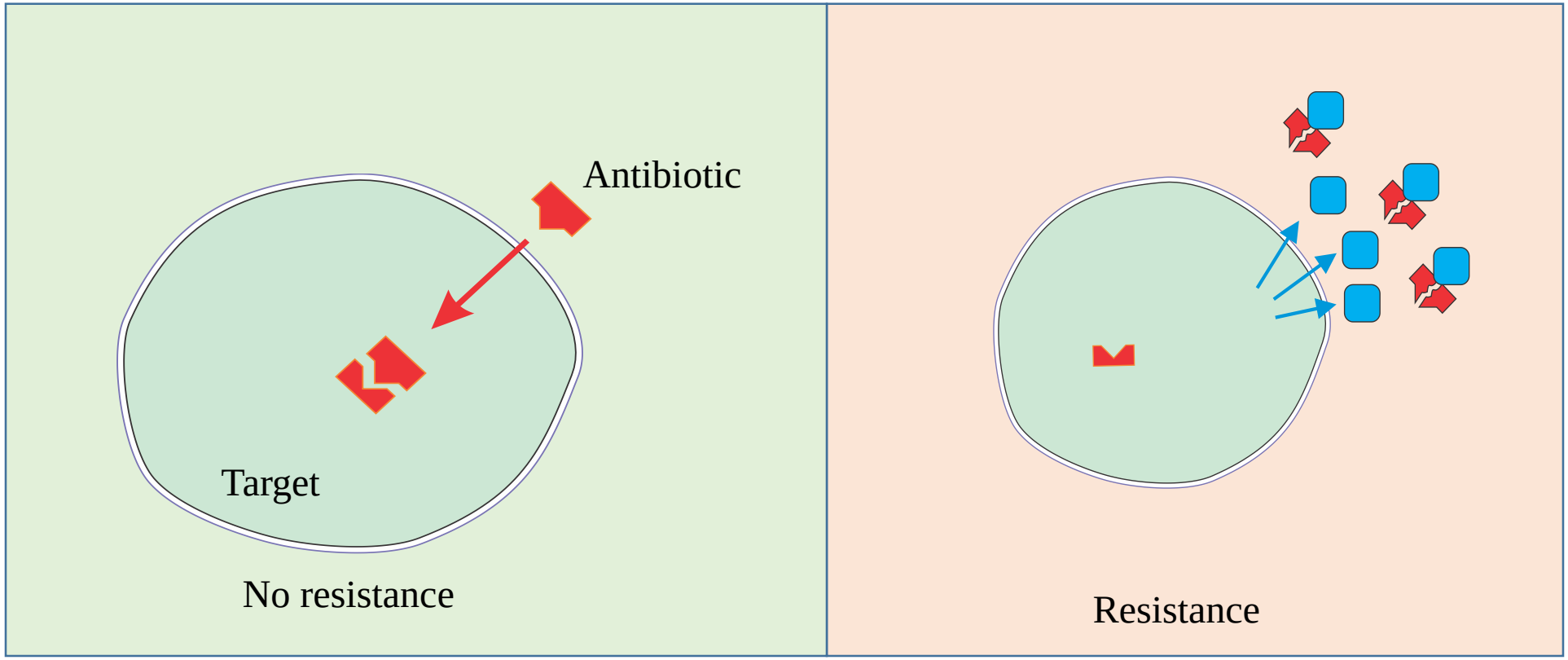
# 2. Pumping out (Active efflux) of the drugs across the cell surface.



# 3. Microorganisms change their target receptor for the drug.

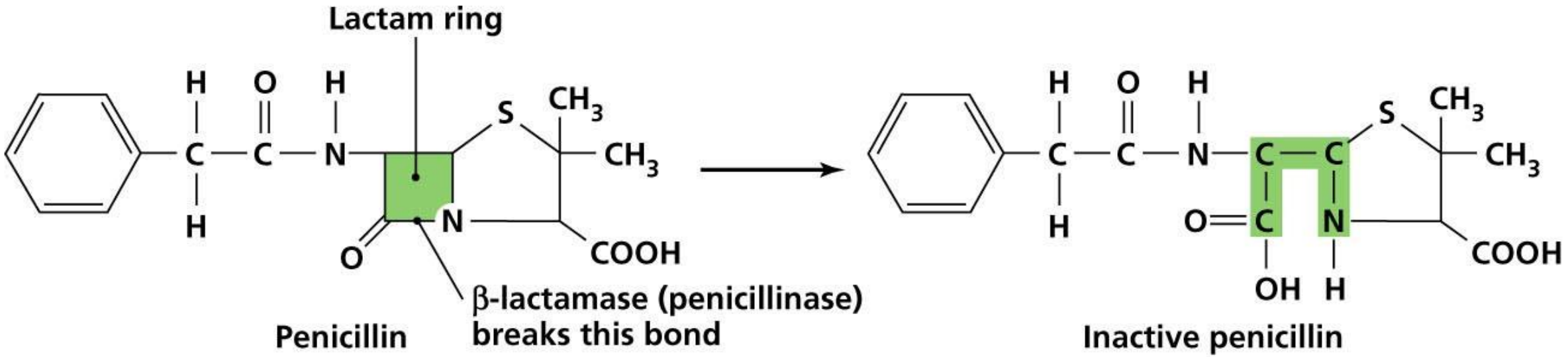


# 4. Microorganisms produce enzymes that destroy the drug.

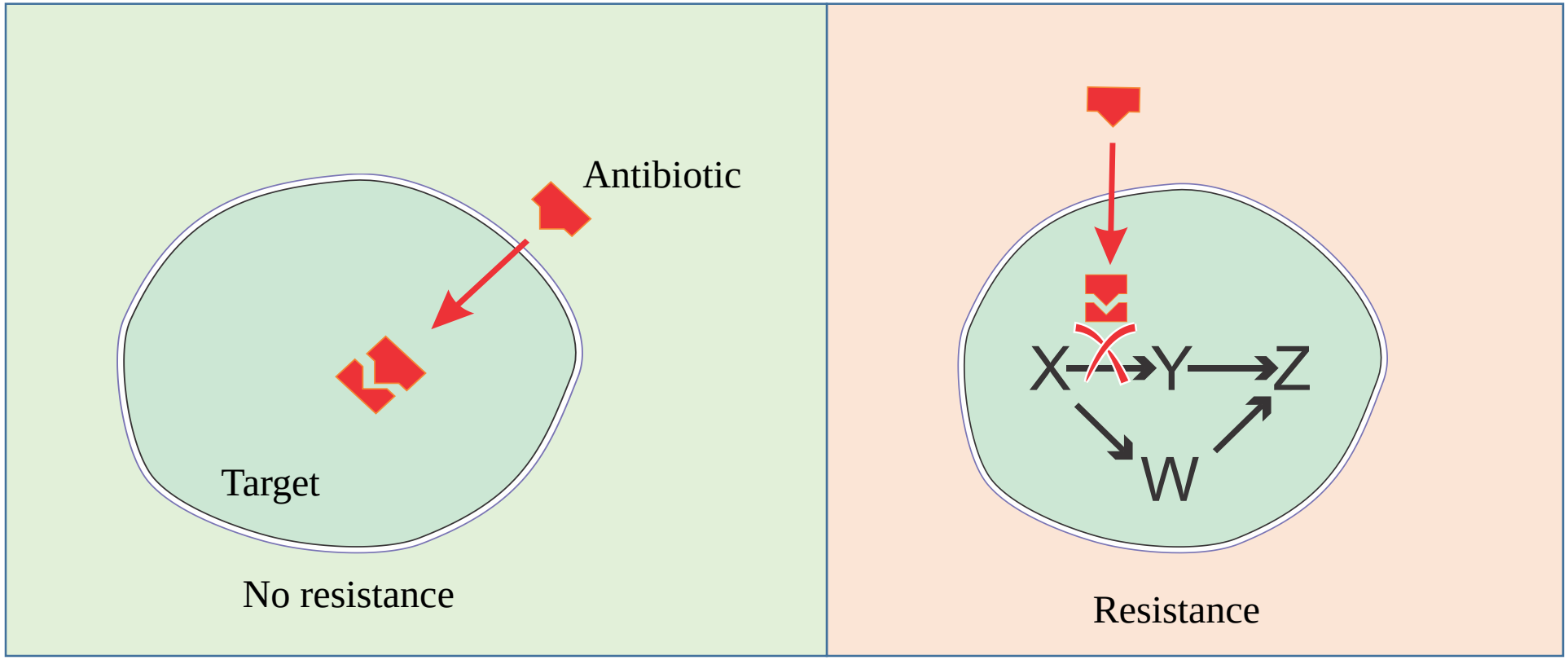


# Example ⟶ beta lactamase enzyme

**Beta-lactamases** are enzymes produced by some bacteria and are responsible for their resistance to **beta-lactam** antibiotics like penicillin.



# 5. Microorganisms change the metabolic pathway to bypass the reactions inhibited by the drug.



# Antimicrobial susceptibility tests

# AIM of susceptibility tests

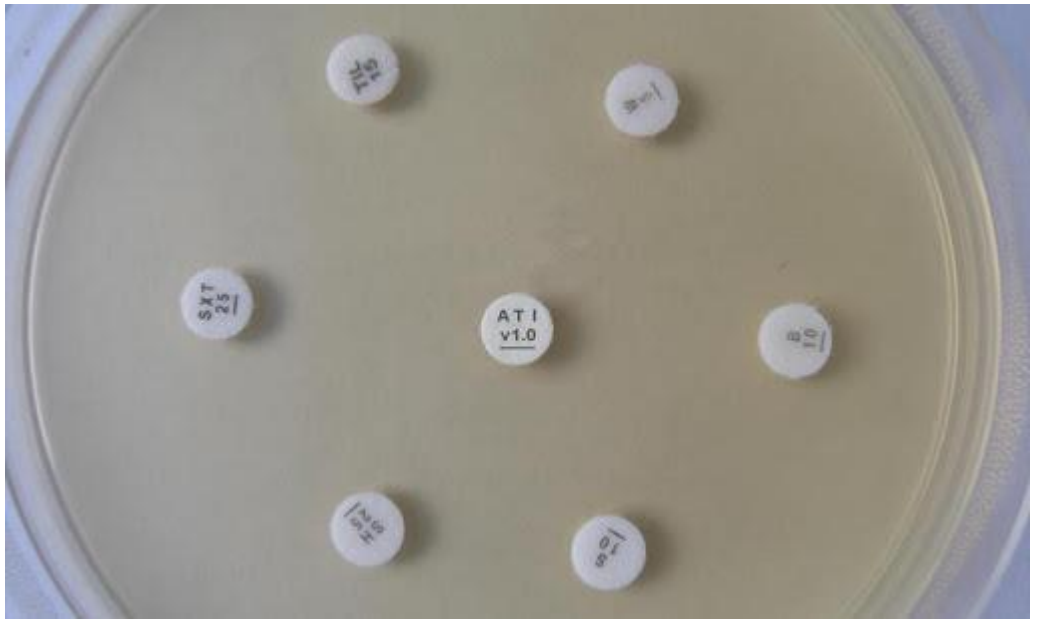
To know bacterial sensitivity to known concentration of the Antibiotic.

# Types of antimicrobial susceptibility tests

1. Disk-diffusion method (Kirby-Bauer)
2. Dilution Method
3. E test

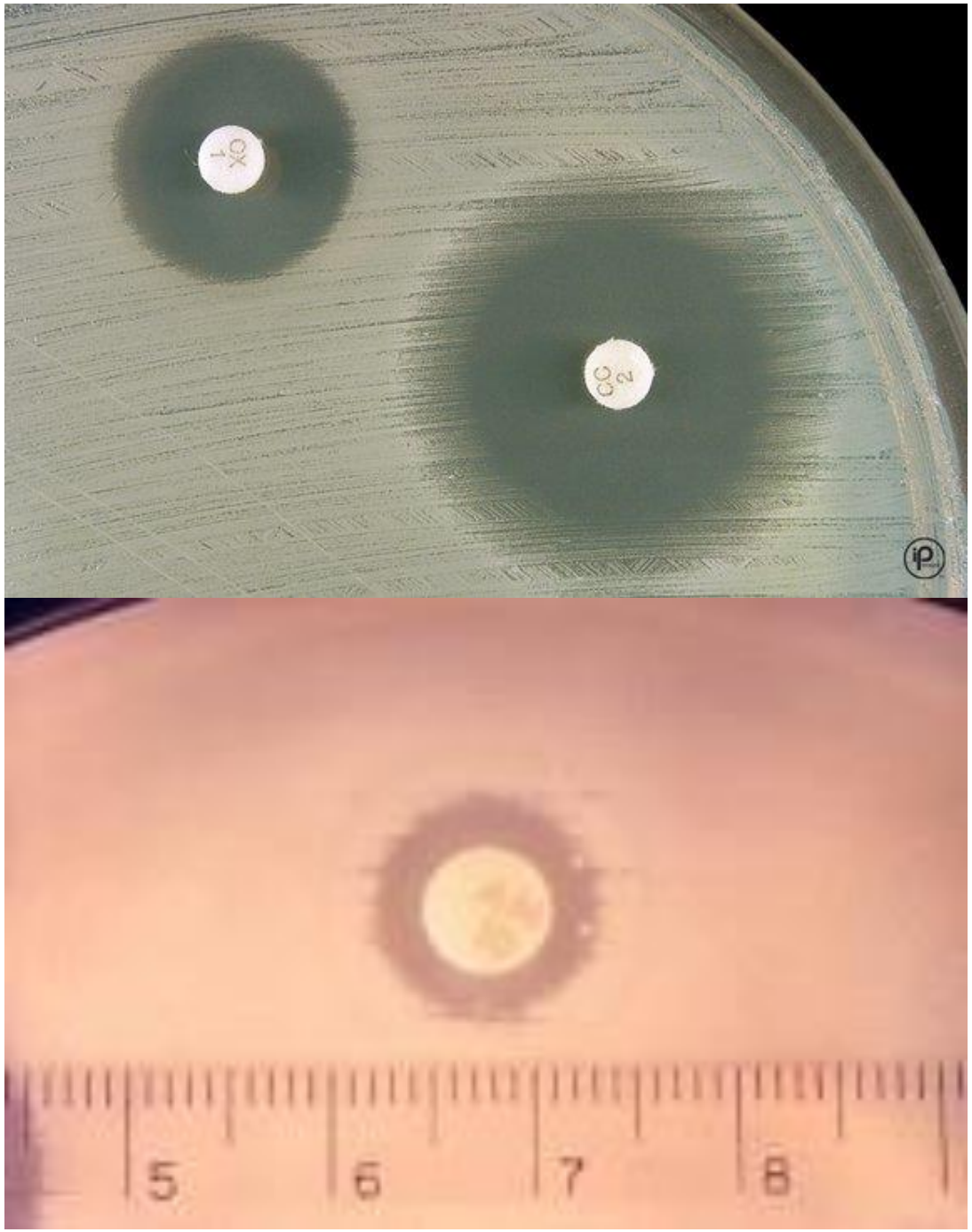
# *1. Disk-diffusion method (Kirby-Bauer):*

- An agar plate is uniformly inoculated with the test organism.
- A paper disk impregnated with a fixed concentration of an antibiotic is placed on the agar surface.



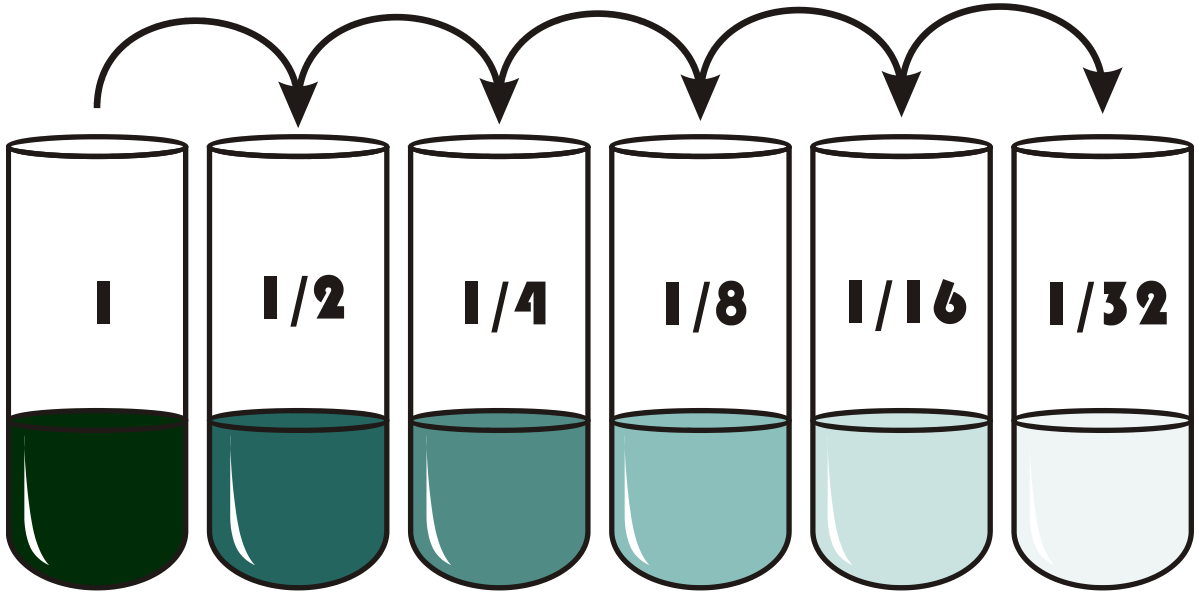
# *Disk-diffusion method*

- If the test organism is susceptible to the antibiotic, the growth of the test organism will be inhibited around the disk (**inhibition zone**).
- The **diameter** of **inhibition zone** correlates with susceptibility of the organism.
  → A larger zone indicates a more susceptible organism.



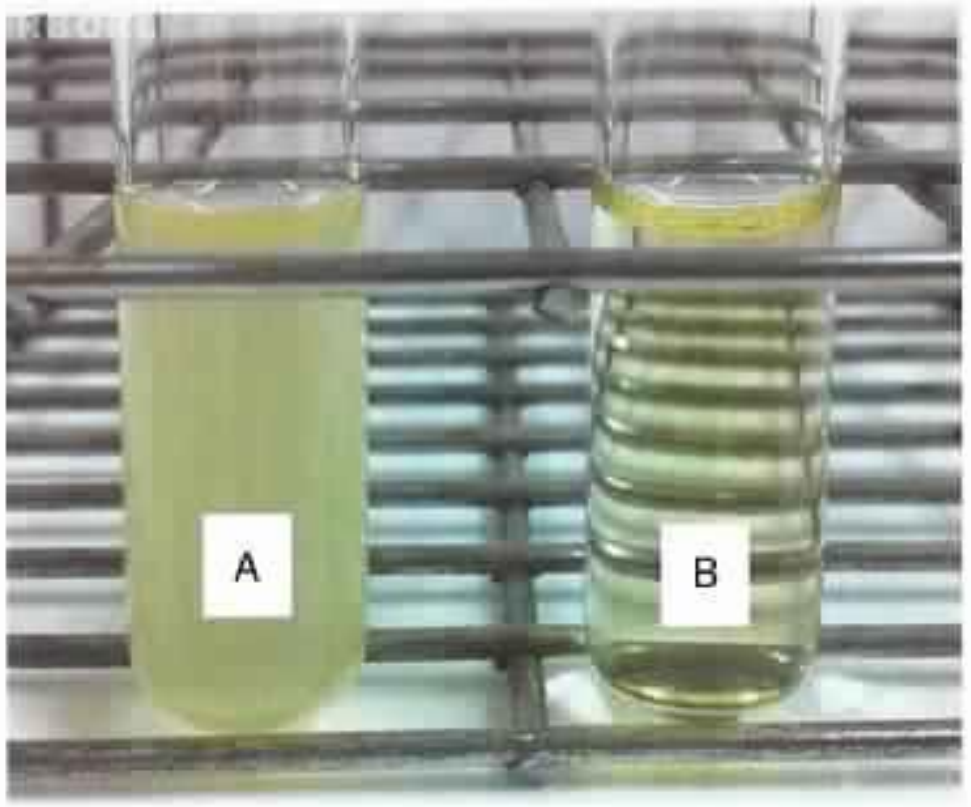
# 2. Dilution Method

1. Serial dilutions of the antibiotic are made in a liquid medium.
2. A standardized number of bacteria is added to each dilution.



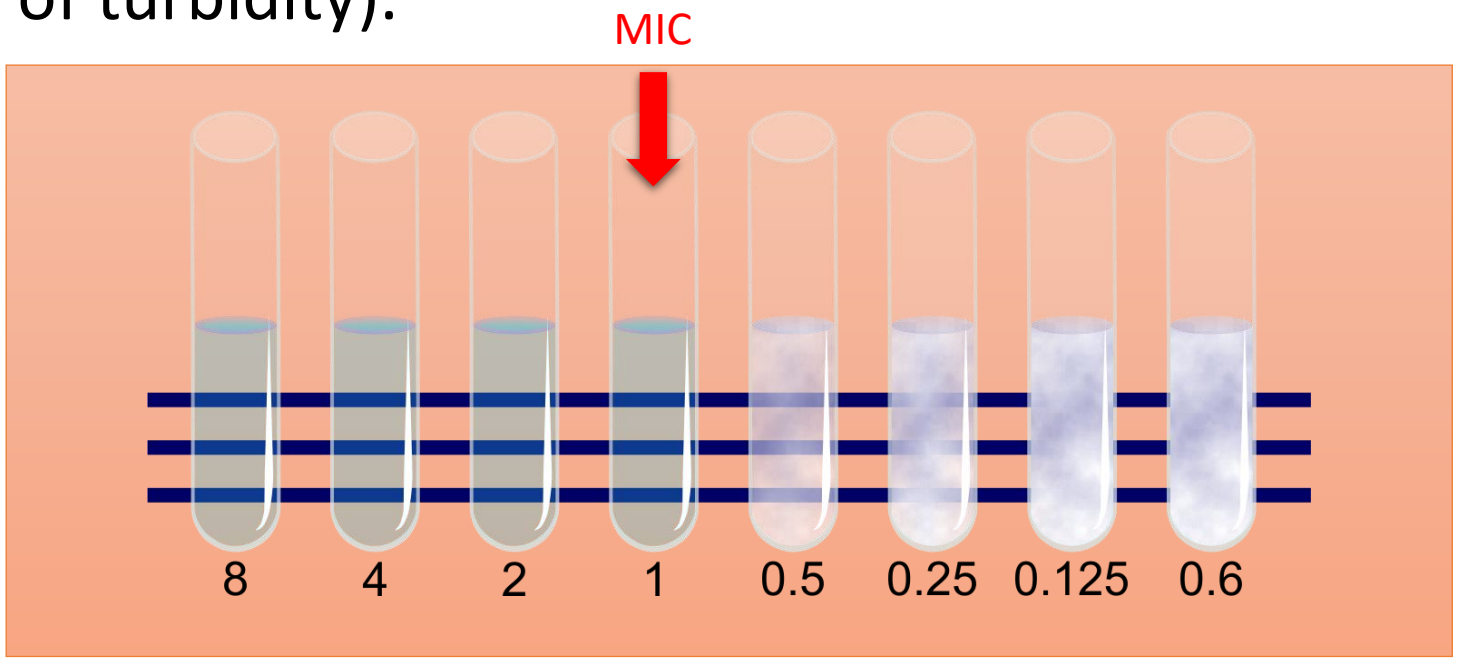
# 2. Dilution Method

3. After incubation the tubes are examined for visible bacterial growth (i.e. turbidity).



# *Minimal inhibitory concentration*

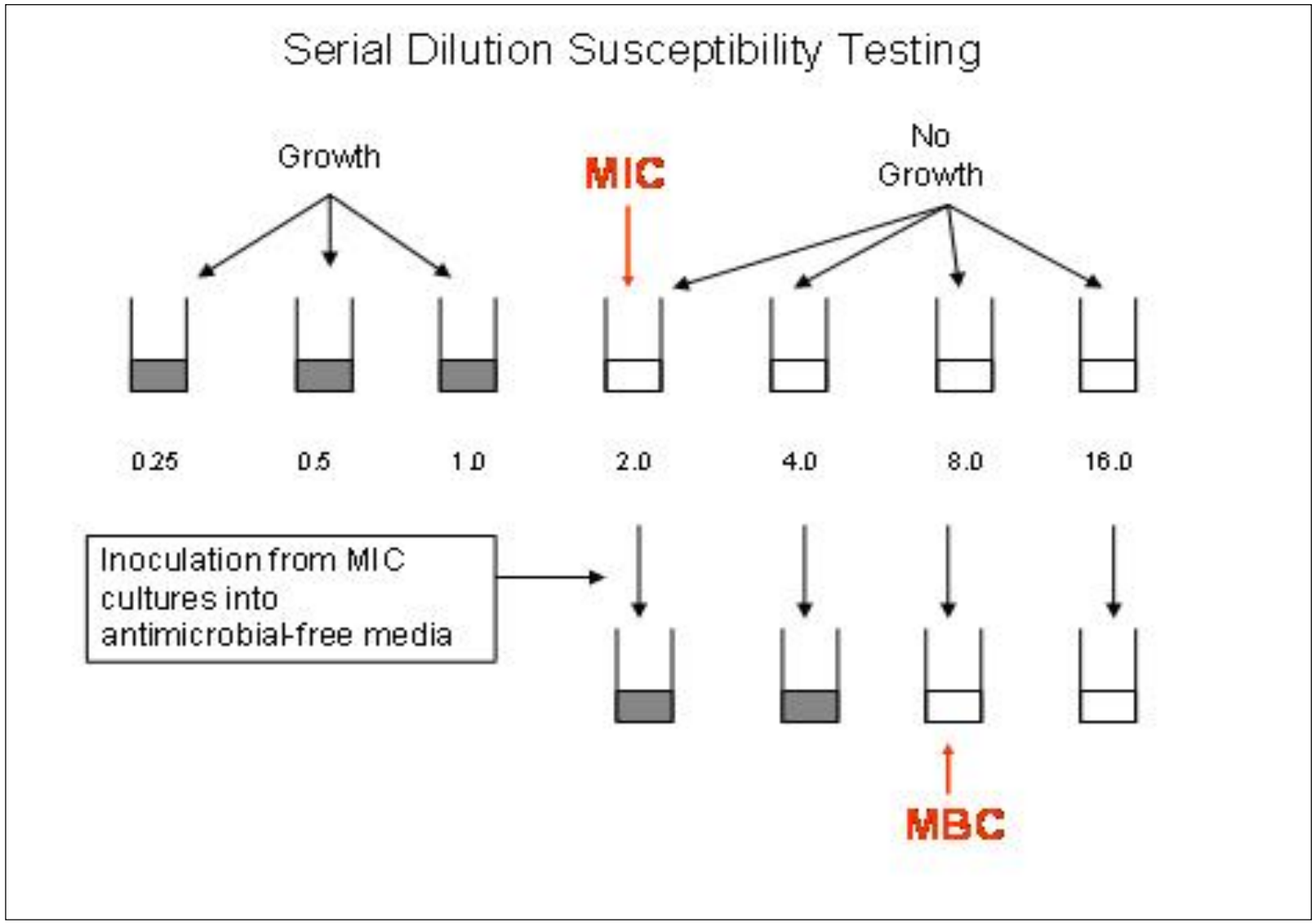
***Minimal inhibitory concentration (MIC):*** The **lowest** concentration of antibiotic that **inhibit** the growth of the bacteria (i.e. preventing appearance of turbidity).




In this figure, the lowest concentration that inhibit the growth of bacteria is 1 ug/ml.

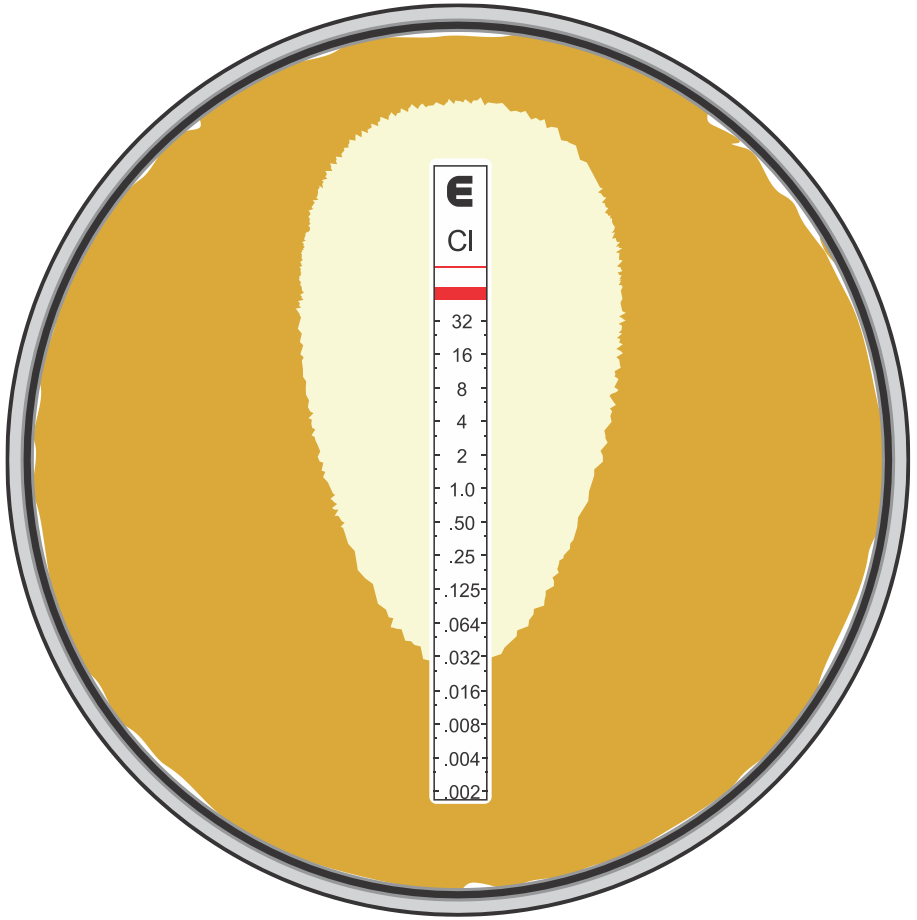
# *Minimal bactericidal concentration*

- ***Minimal bactericidal concentration (MBC)***: The **lowest** concentration of antibiotic required to **kill** the bacteria.
- **MBC** can be determined by subculturing the contents of the tubes with no turbidity onto antibiotic-free medium and examining for bacterial growth. ***(MBC ≥ MIC)***


Serial Dilution Susceptibility Testing
Growth
MIC
No Growth
0.25
0.5
1.0
2.0
4.0
8.0
16.0
Inoculation from MIC cultures into antimicrobial-free media
MBC

# E test

- The E test consists of a strip containing a concentration gradient of one antibiotic.
- An agar plate is inoculated with an organism, a strip is placed on the plate.
- After overnight incubation, the **intersection** of the growth on the scale is read to determine the MIC.



# Quizzes

# 1. MBC is :

A. The **lowest** concentration of antibiotic required to **inhibit** the growth of the bacteria

B. The **highest** concentration of antibiotic required to **inhibit** the growth of the bacteria

C. The **lowest** concentration of antibiotic required to **kill** the bacteria

D. The **highest** concentration of antibiotic required to **kill** the bacteria

# 2. MIC is :

A. The **lowest** concentration of antibiotic required to **inhibit** the growth of the bacteria
B. The **highest** concentration of antibiotic required to **inhibit** the growth of the bacteria
C. The **lowest** concentration of antibiotic required to **kill** the bacteria
D. The **highest** concentration of antibiotic required to **kill** the bacteria

3. Beta lactamase enzyme is used by some bacteria to resist:

A. Penicillin
B. Tetracycline
C. Polymixin
D. Rifampicin

# 4. In Disc diffusion method of antibiotic susceptibility tests, increase in the diameter of the inhibition zone means

A. More resistance to the antibiotic

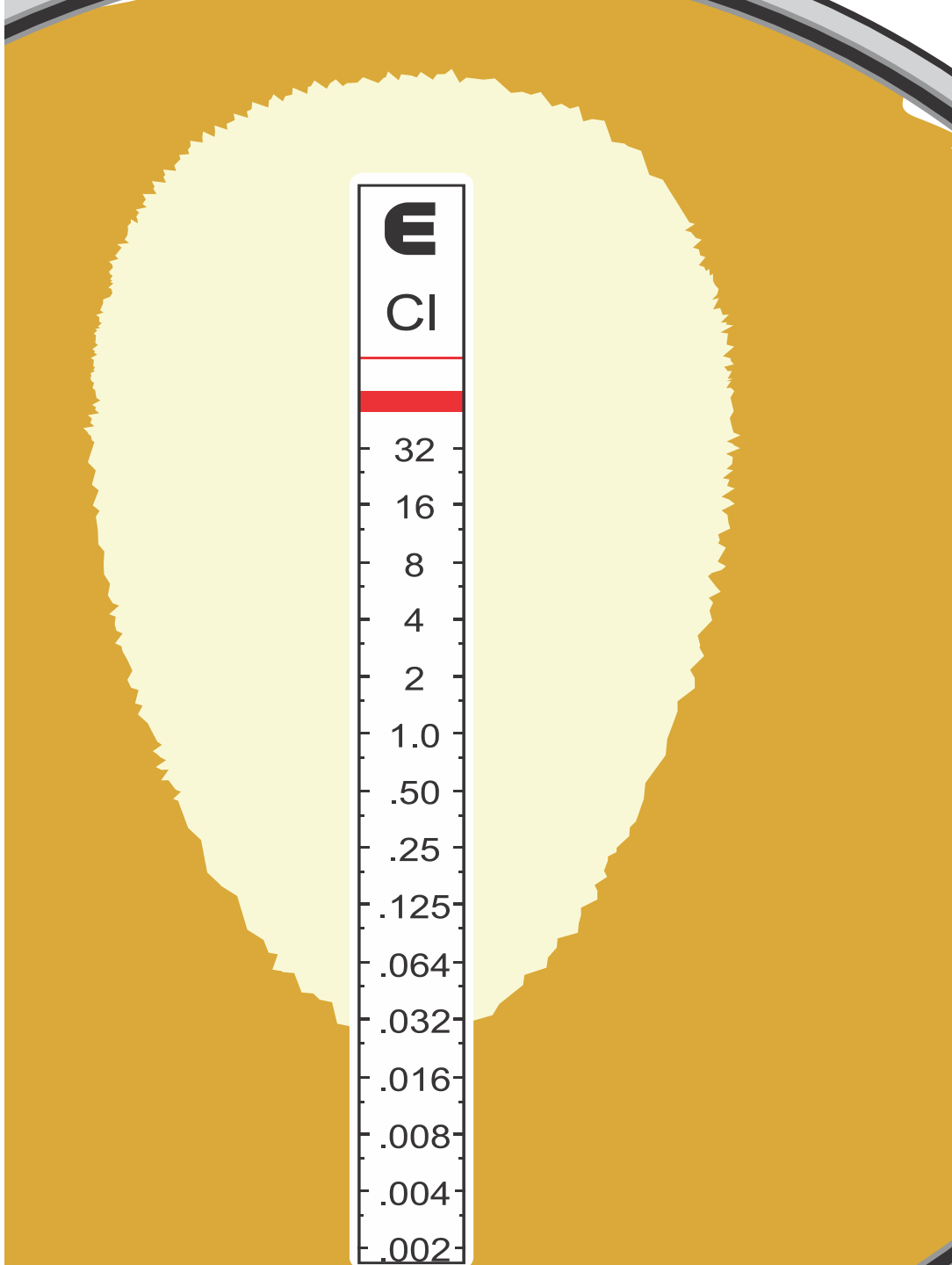
B. More susceptibility to the antibiotic

# 5. The name of this test

A. Disc diffusion Method

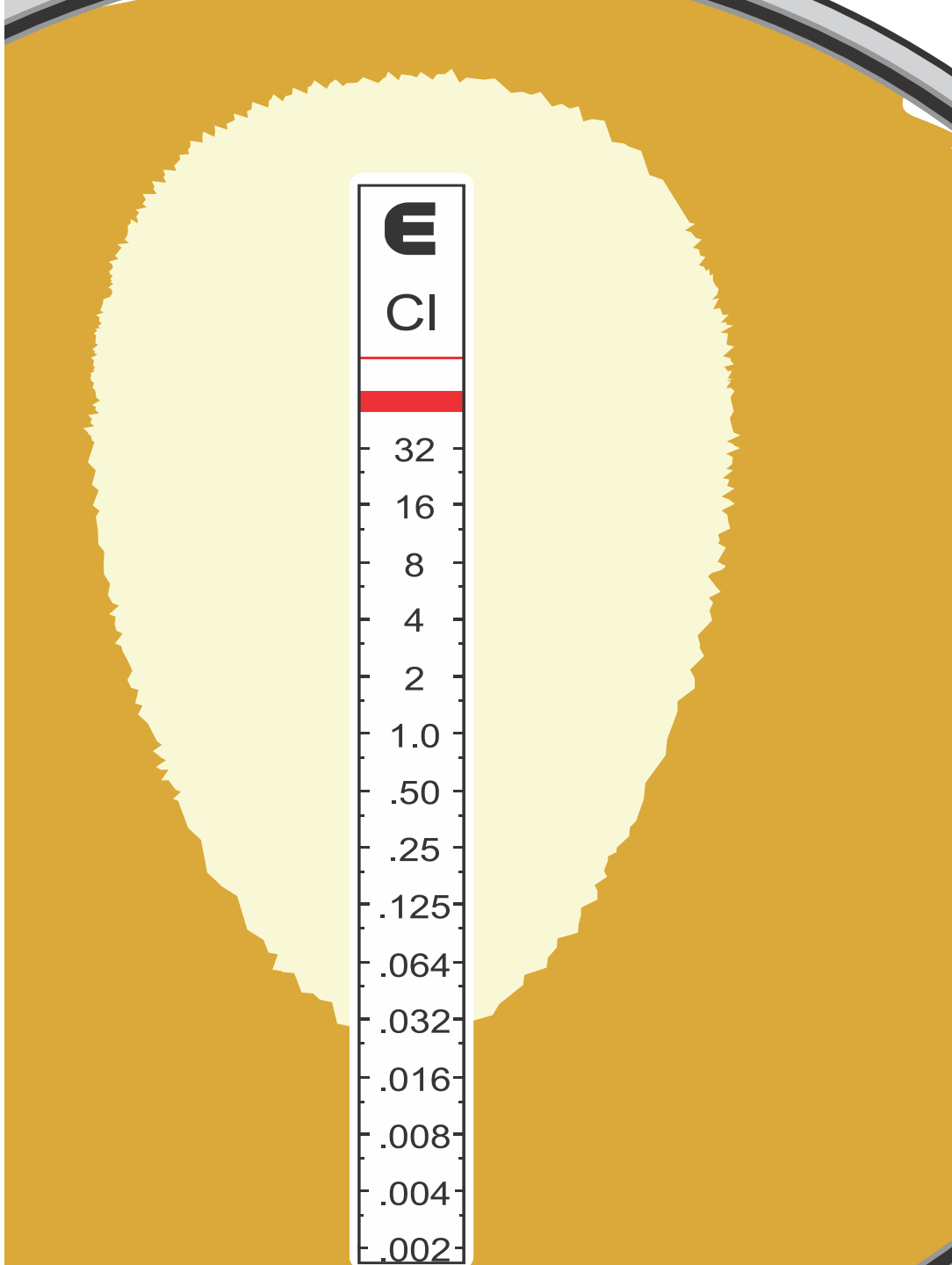
B. E test

C. Broth dilution Method



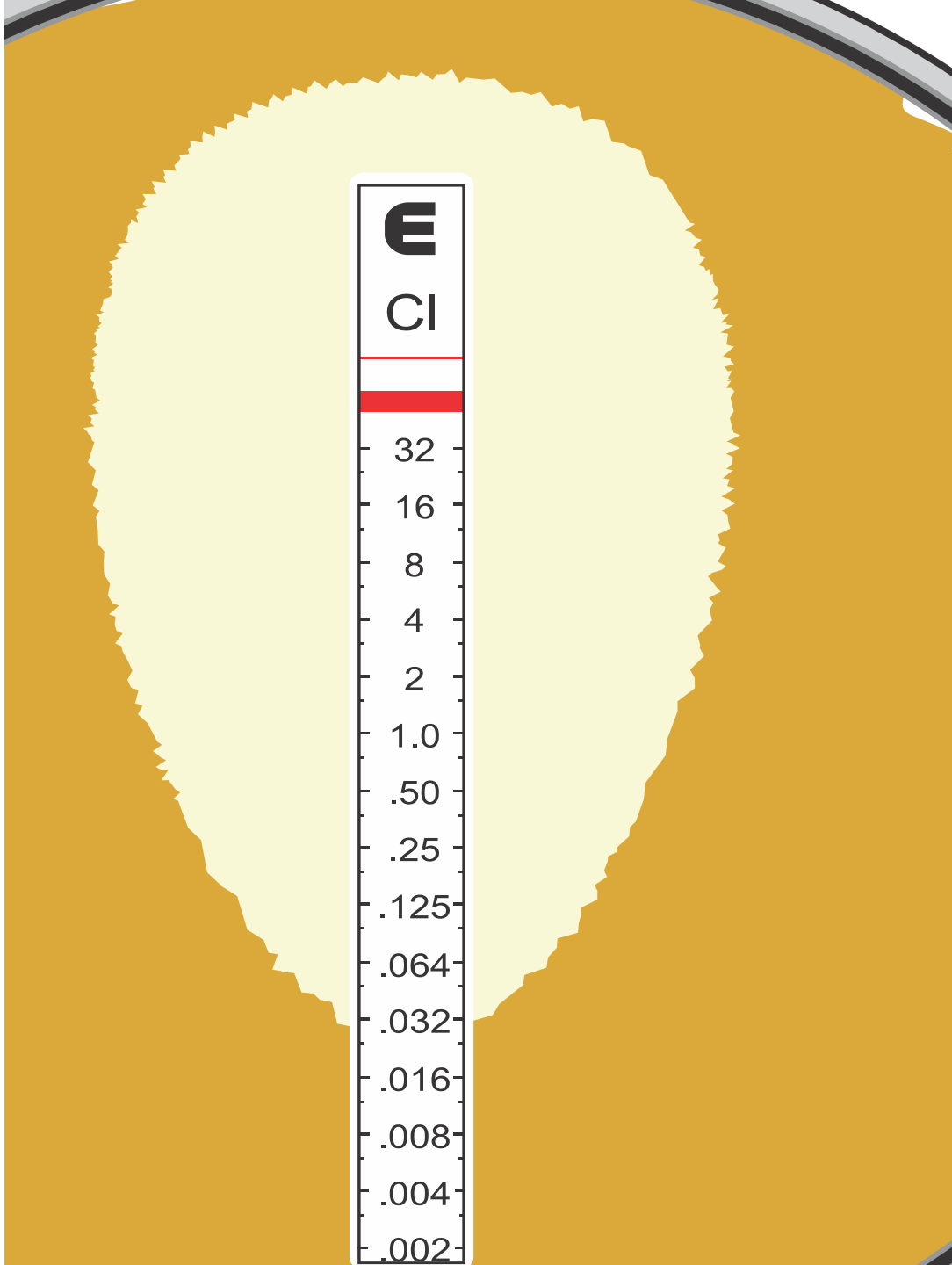
# 5. This test is used to:

A. Measure bacterial susceptibility to antibiotics
B. Identify the type of bacteria
C. Detect bacterial antigen



# 5. The MIC in this test is

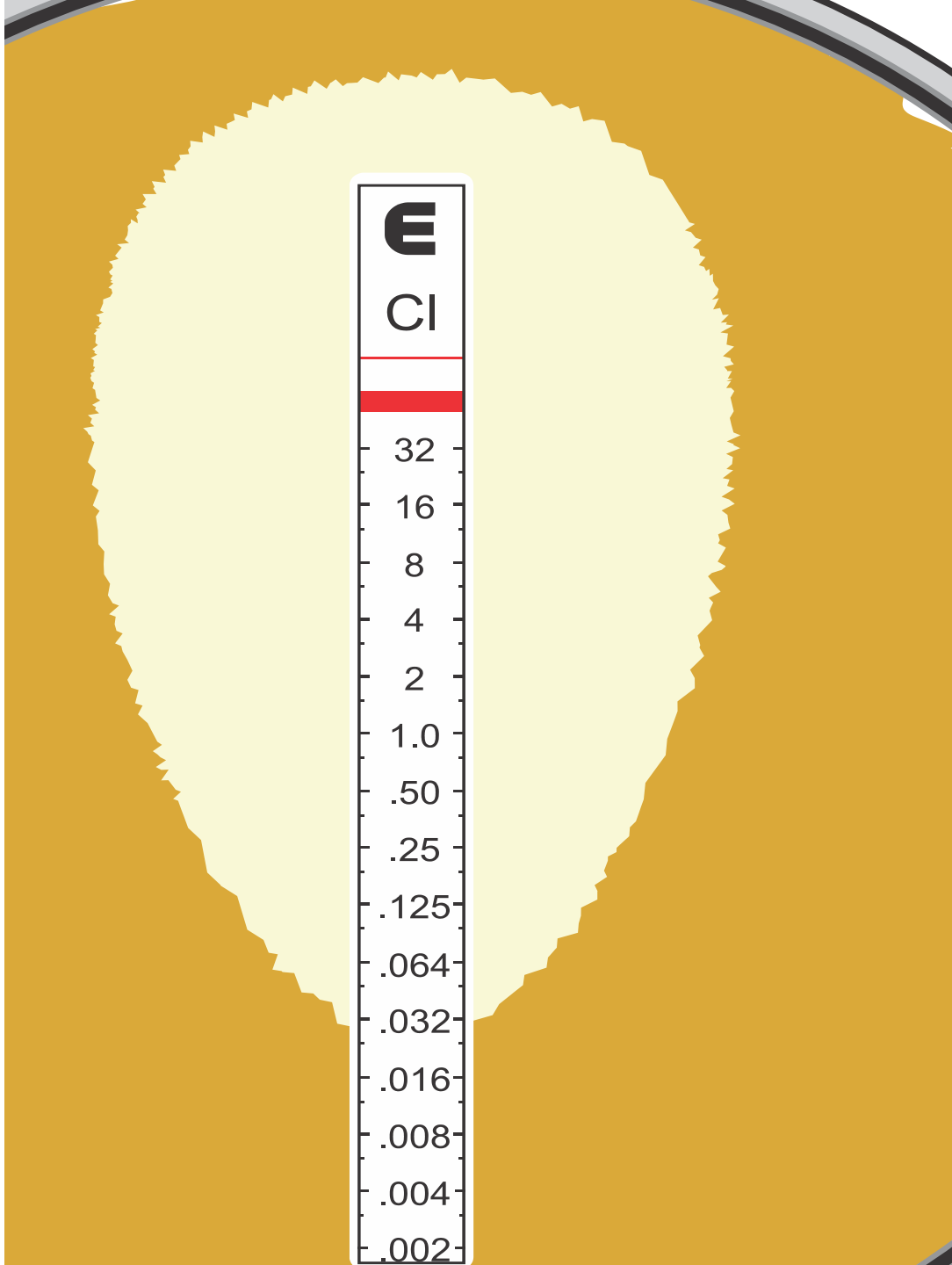
A. 0.002
B. 32
C. 0.032
D. 2



# 5. Higher MIC means

A. More resistance

B. More susceptibility

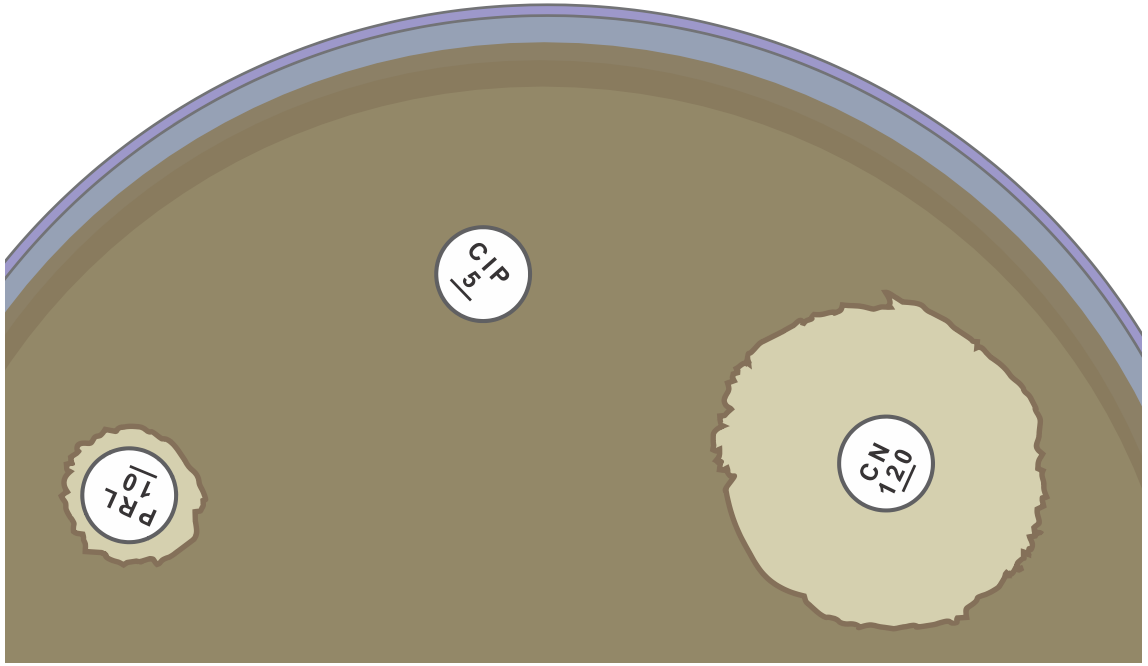


# 6. Mention 4 mechanisms of antibiotic resistance:

1. ……
2. ……
3. ……
4. ……

# 7. This test is used to:

A. Measure bacterial susceptibility to antibiotics
B. Identify the type of bacteria
C. Detect bacterial antigen

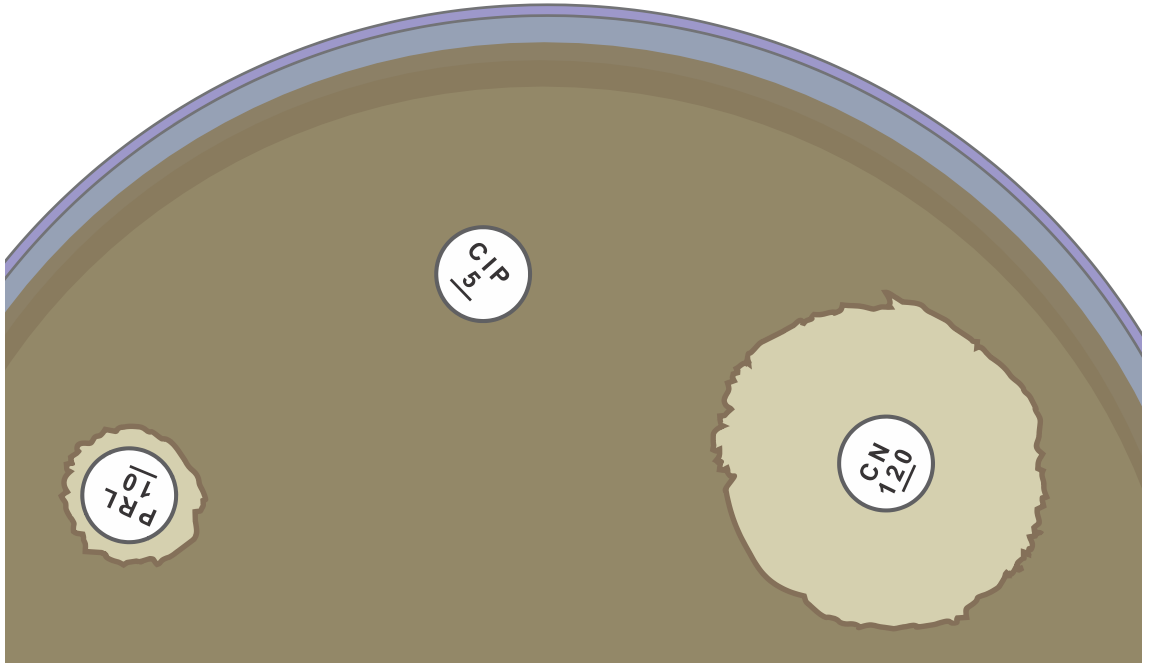


# 7. The name of this test

A. Disc diffusion Method

B. E test

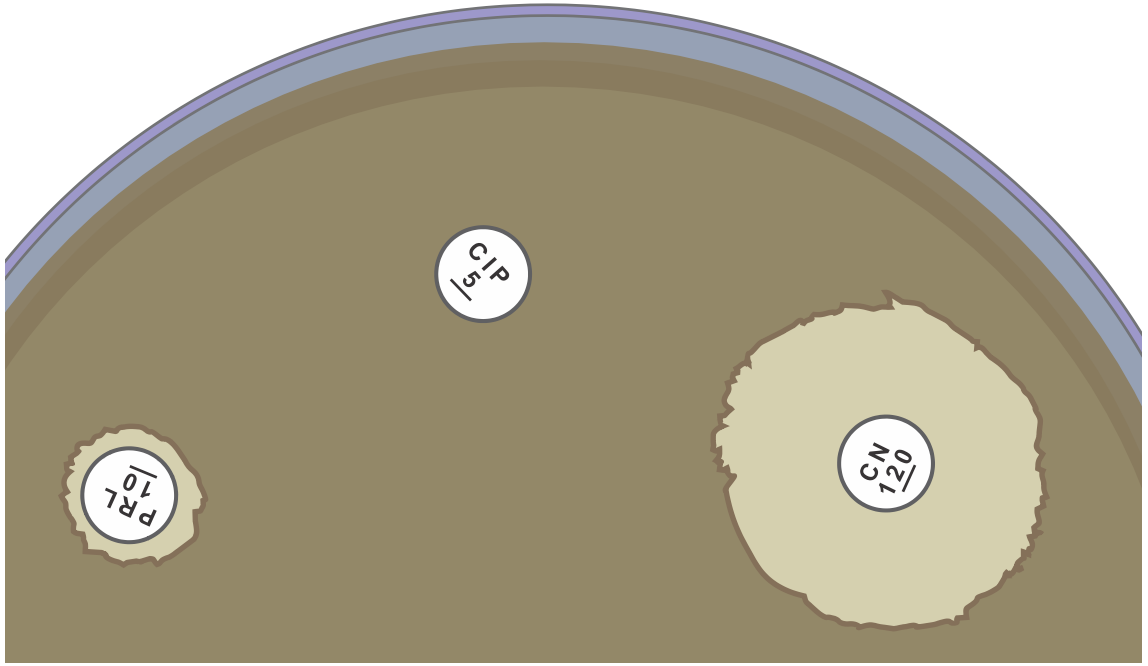
C. Broth dilution Method



# 7. The increase in diameter means:

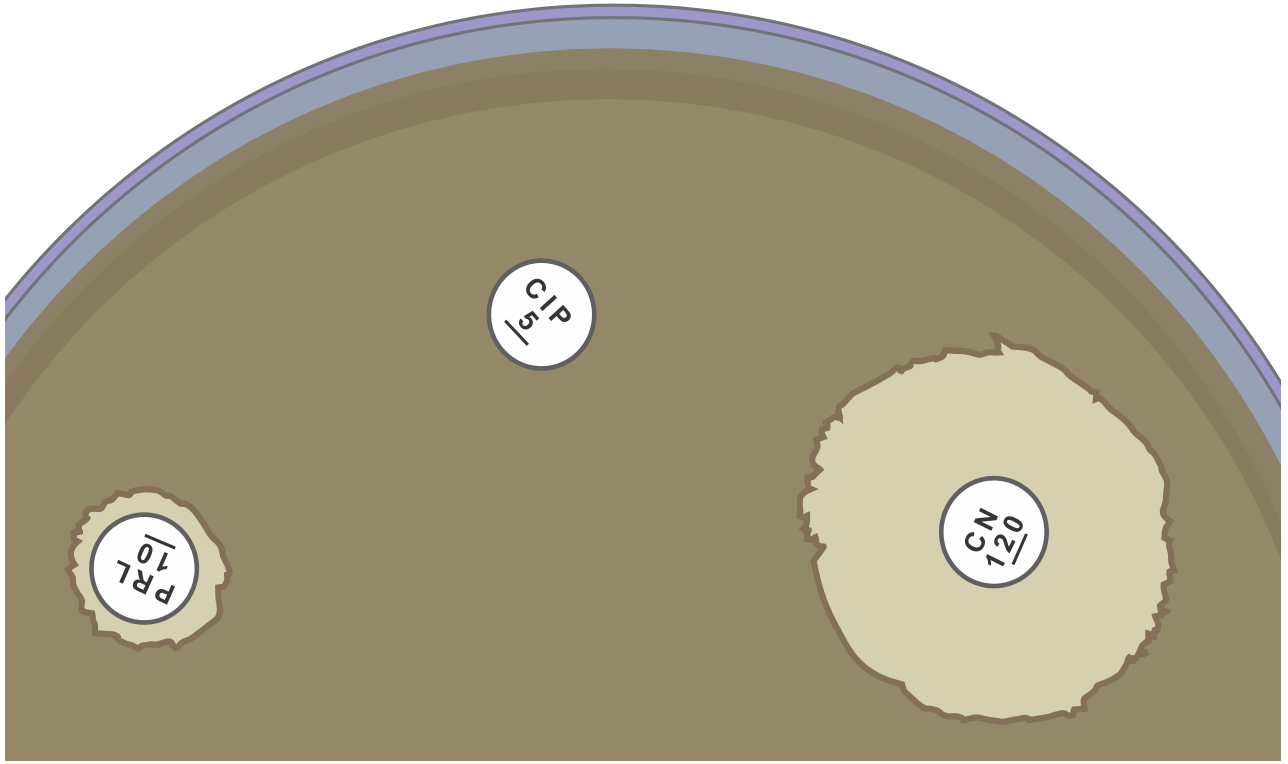
A. More resistance

B. More susceptibility



# 7 Apparently, Which is most effective antibiotic:

A. Ciprofloxacin
B. Piperacillin
C. Gentamycin




**CIP = ciprofloxacin**

**PRL = piperacillin**

CN = gentamycin