

Antibiotic Technology Laboratory

Lab 5: Combination action of Antibiotic

Combination action of antibiotics refers to the use of two or more antibiotics together to study or enhance their effects on microorganisms. This approach is commonly used in clinical practice and research to evaluate how multiple antimicrobial agents interact when treating a single infection.

In many cases, combination therapy is applied to improve treatment outcomes, broaden the antimicrobial spectrum, or prevent the development of resistance.

Advantages of Combination:

- 1- For urgent treatment to many infections and before completing the culture of bacteria.
- 2- To prevent or delay mutant strains.
- 3- To obtain synergism effect for antibiotics.
- 4- For treat unknown infection, or chronic infection especially vascular disease.
- 5- In the Mixed infection like septicemia.
- 6- To lead delay the mutation for any antibiotics in chronic infection so we use two antibiotics like tuberculosis.

Disadvantages

1. Increased Cost: Multiple drugs are expensive
2. Superinfections: Wiping out too much "good" flora can lead to *C. difficile* or yeast infections.
3. Increased Toxicity: Doubling the drugs often doubles the strain on the kidneys or liver.

Why Use Combinations? (Clinical Rationale)



While monotherapy is generally preferred to minimize side effects and cost, combinations are essential in specific scenarios:



Broadening the Spectrum

In “empiric therapy,” where the causative pathogen is unknown (e.g, sepsis), we use combinations to cover both **Gram-positive** and **Gram-negative** bacteria.

Preventing Resistance

For chronic infections like Tuberculosis (TB) or HIV, using **multiple** drugs ensures that if a mutant strain survives drug **A**, **drug B** will eliminate it.



Reducing Toxicity

By using two **drugs** that work together, we can sometimes lower the dose of a **highly toxic** drug (like **Amphotericin B**).

Synergy

The most critical **reason** – where the combined effect is significantly greater than the sum of their **individual** effects.



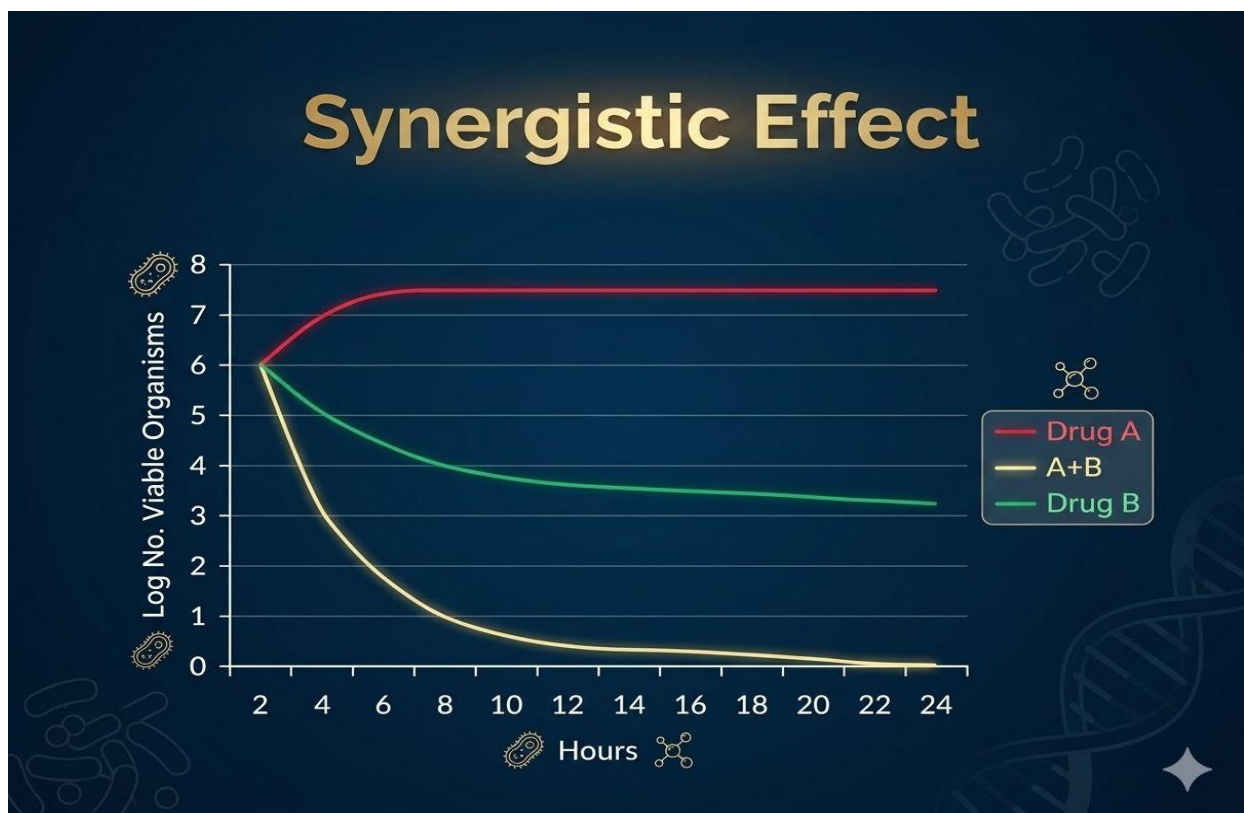
There are three primary ways antibiotics interact when combined:

- 1- **Synergistic effect** ($3 = 1+1$): The drugs enhance each other's activity; two drugs inhibit different steps in the same metabolic pathway. the activity of two drugs in combination is greater to the sum of their independent activity when studied separately.

Example: Trimethoprim + Sulfamethoxazole. Sulfonamides block the first step of folic acid synthesis, and Trimethoprim blocks the second .Facilitating Entry: One drug breaks down the bacterial defenses so the second can enter.

Example: Penicillin + Aminoglycosides. Penicillin inhibits cell wall synthesis, creating "holes" that allow aminoglycosides to enter the cell and reach the ribosomes.

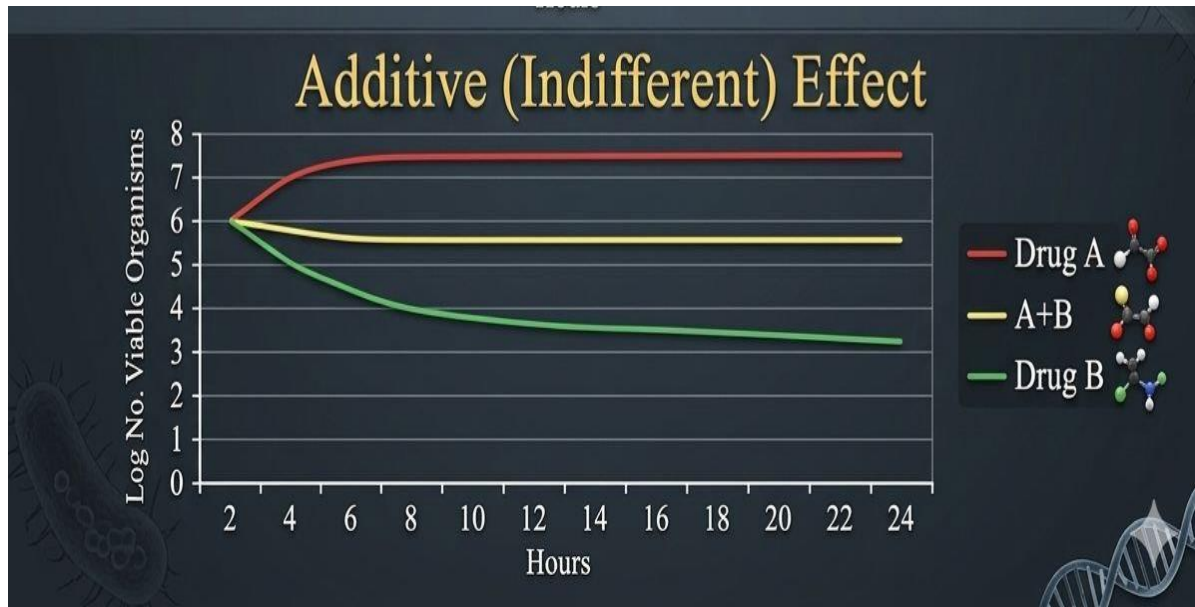
Against: Aminoglycoside+ Penicillins against *Niesseria gonorrhoeae*.



- 2- **Additive (indifferent) effect** ($1+1=2$): The combined effect is equal to the sum of the individual effects the activity of two drugs in combination is equal to the sum (or a partial sum) of their independent activity when studied separately. The combined effect is exactly what you'd expect from adding them together. This is

common when using two drugs with the same mechanism of action to increase the total "kill rate" without increasing the dose of a single agent to toxic levels.

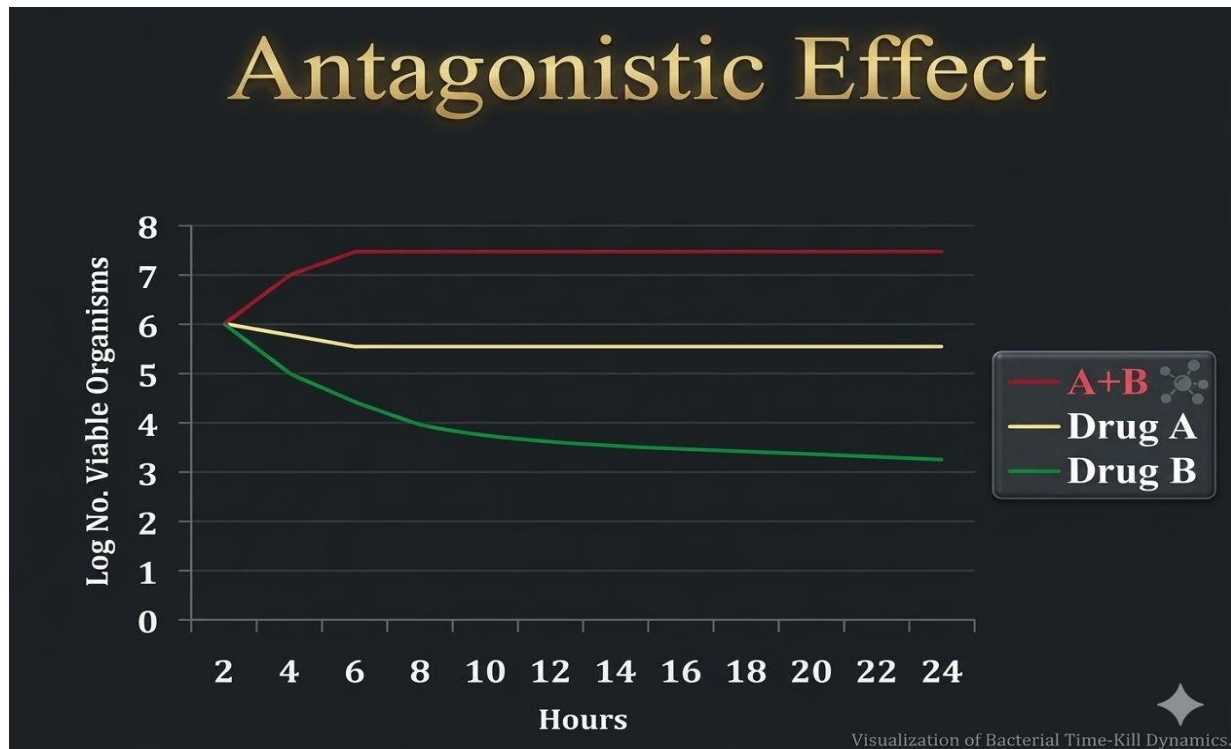
against *Staph. aureu* Gentamicin + Linezolid



3- Antagonistic effect ($1+1=0.5$): One antibiotic reduces or interferes with the activity of the other, the activity of two drugs in combination is less to the sum (or a partial sum) of their independent activity when studied separately. This is the danger zone. One drug interferes with the action of the other. The Classic Conflict: Combining a Bacteriostatic drug (which stops growth) with a Bactericidal drug (which kills actively growing cells).

Example: Tetracycline + Penicillin. Penicillin needs the bacteria to be actively building cell walls to kill them. Tetracycline stops growth, making the bacteria "dormant" and effectively protecting them from the Penicillin.

Against Ciprofloxacin + Gentamicin against *Proteus mirabilis*.



So, antibiotic combination therapy is an important strategy in treating serious infections, multidrug-resistant organisms, and mixed microbial infections, while also helping to reduce the likelihood of resistance development.

Activity of Antibiotic Combinations

The activity of combined antibiotics depends on the **individual characteristics of each drug**, particularly whether they are:

1. **Bactericidal antibiotics** – agents that **kill bacteria directly**.
2. **Bacteriostatic antibiotics** – agents that **inhibit bacterial growth without killing the bacteria**.

The interaction between antibiotics may sometimes lead to **antagonism**, where one drug interferes with the action of another.

Example: The use of **Penicillin** in combination with **Tetracycline** for infections caused by **Streptococcus pneumoniae** in cases of **Meningitis** resulted in an **increase in mortality rate from 22% to 79%**.

This occurred because **tetracycline (bacteriostatic)** inhibited bacterial growth, which reduced the effectiveness of **penicillin (bactericidal)**, since penicillin requires **actively growing bacteria** to exert its lethal effect.

Therefore, this combination produced an **antagonistic interaction**, where one antibiotic **inhibited the activity of the other**, leading to poorer clinical outcomes.

Guidelines for Using Antibiotic Combinations

When using combinations of antibiotics, several important principles should be considered:

1. Evaluate the effectiveness of single therapy

The effectiveness of a **single antibiotic** should first be determined.

- If one antibiotic effectively eliminates the causative organism, **combination therapy is not necessary**.
- However, if the infection involves **resistant strains or mixed pathogens**, the use of **antibiotic combinations may be required**.

2. Determine the MIC of each antibiotic

Before combining antibiotics, the **Minimum Inhibitory Concentration (MIC)** of each drug should be determined separately.

- In experimental studies, antibiotics are often used at **sub-MIC concentrations** (concentrations below the MIC) to evaluate their **combined effects**.

3. Consider the type of antimicrobial action

The interaction between antibiotics depends on whether they are **bactericidal** or **bacteriostatic**.

The combination should be selected carefully because some combinations may produce **synergistic effects**, while others may result in **antagonism**.

Types of Antibiotic Combinations

1. Combined bacteriostatic action (Bacteriostatic + Bacteriostatic)

An example is the combination of **Trimethoprim** and **Sulfamethoxazole**.

- Trimethoprim inhibits the enzyme **dihydrofolate reductase**, which is involved in **folic acid synthesis**.
- Sulfonamides inhibit **para-aminobenzoic acid (PABA)** utilization in the same metabolic pathway.

Because both drugs inhibit **sequential steps in folic acid synthesis**, the combination is effective against organisms such as **Proteus mirabilis**.

2. Combined bactericidal action (Bactericidal + Bactericidal)

An example is the combination of **Amikacin** and **Piperacillin**.

- Piperacillin acts on the **bacterial cell wall**.
- Amikacin inhibits **protein synthesis** by binding to the bacterial ribosome.

This combination may be used in severe infections such as **Endocarditis**, **Meningitis**, and infections caused by **Pseudomonas aeruginosa**.

3. Combined bacteriostatic and bactericidal action (Bacteriostatic + Bactericidal)

In some cases, combining a **bactericidal antibiotic** with a **bacteriostatic antibiotic** may lead to **antagonism**.

For example, combining **Penicillin** (bactericidal) with **Chloramphenicol** or **Erythromycin** (bacteriostatic).

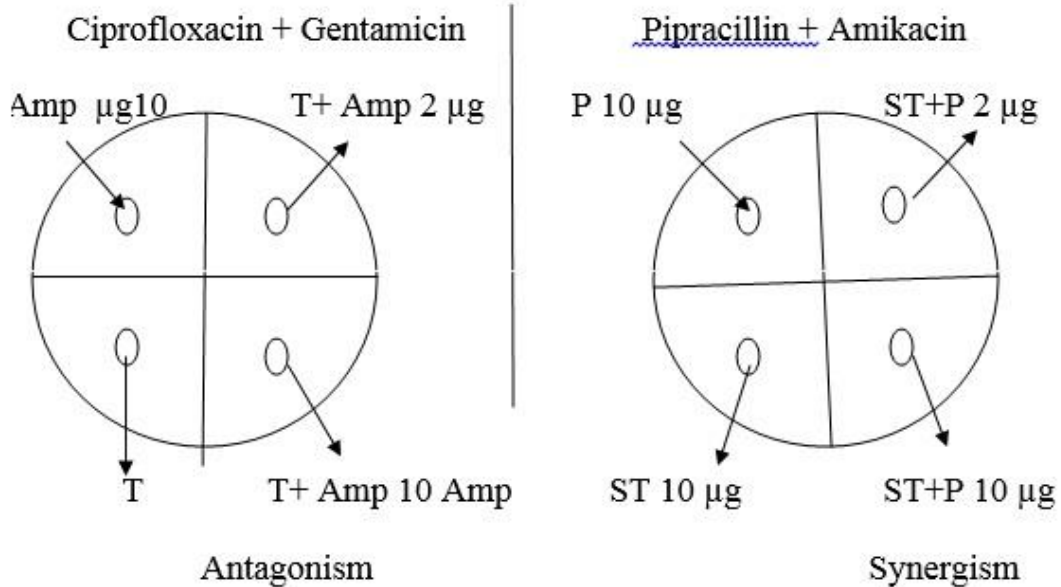
- Penicillin acts on the **cell wall** and requires **actively dividing bacteria**.
- Bacteriostatic antibiotics inhibit **protein synthesis**, stopping bacterial growth.

As a result, the bacteriostatic drug may **reduce the effectiveness of the bactericidal drug**, leading to **antagonistic interaction**.

Procedure:

- 1- Prepare serial dilution of antibiotics, which required in test and get the require concentration which if we take 0.1ml (100 µl) from it this volume will be carried the same concentration which we needed.
- 2- Cultured MHA plates with required bacteria by using lawn streaking.

- 3- Making wells in MHA plates by using cork borer and loaded by require concentration.
- 4- Incubate the plates at 37°C for 24 hours, and read the results.



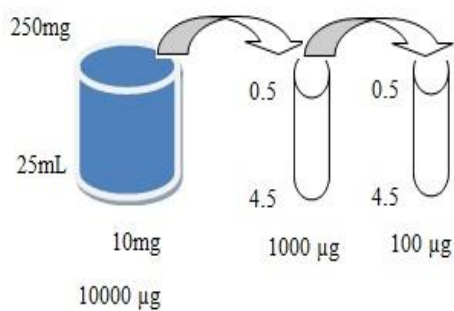
Penicillin and Streptomycin↓

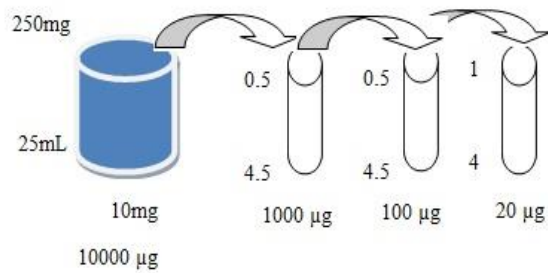
Penicillin or Ampicillin (10 µg, 2 µg)

$$10 = n * 100 \quad (1)$$

$$N = 0.1 \text{ mg/mL}$$

$$0.1 * 1000 = 100 \text{ µg/mL}$$





$$100 \times n = 2 \quad (2)$$

$$N = 0.02 \text{ mg/mL}$$

$$0.02 \times 1000 = 20 \text{ µg/mL}$$

Streptomycin (10 µg)

$$100 \times n = 10$$

$$N = 0.1 \text{ mg/mL}$$

$$0.1 \times 1000 = 100 \text{ µg/mL}$$

Ticarcillin + Amikacin against *staph.* G+ ve

Important Notes:

Synergy → beneficial interaction

Additive → neutral interaction

Antagonism → harmful interaction

Some drug combinations can reduce effectiveness, increase toxicity, or even become life-threatening. Always check for interactions before prescribing, dispensing, or taking medications. Patient safety comes first.

Drugs You Must Never Mix

 **Clopidogrel + Omeprazole** → Antiplatelet effect (CYP2C19 inhibition)

 **Statins (Simvastatin) + Clarithromycin** → Rhabdomyolysis

 **Metformin + IV Contrast** → Lactic acidosis

 **Theophylline + Ciprofloxacin** → Theophylline toxicity

 **Digoxin + Verapamil** → Digoxin toxicity

 **Isotretinoin + Tetracyclines** → Pseudotumor cerebri

 **Levodopa + Pyridoxine (Vit B₆)** → ↓ Levodopa effect (peripheral metabolism)

 **Lithium + Thiazide diuretics** → Lithium toxicity

 **Methotrexate + NSAIDs** → Bone marrow suppression

 **Warfarin + Metronidazole** → ↑ INR → Bleeding