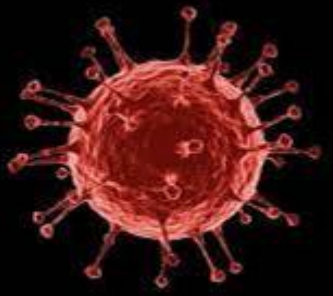
Mohammed Omer
Seminar presentation

Application of Colorimetric Assay for Antimicrobial Susceptibility Testing

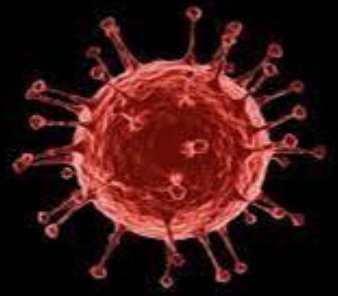
Learning outcomes



- In this seminar presentation you are going to be familiar with the following terms:-

  - What are antimicrobials?
  - What are antibacterial? And antibiotics?
  - What is Antibacterial resistance mechanisms?
  - Antibacterial research methods
  - The benefits of colorimetric method

Antimicrobial agent



Antimicrobials by definition are diverse group of natural, semi-synthetic or synthetic compound, which have effect on different classes of **Microbial growth**

- ❖ Antibacterial
- ❖ Antiviral
- ❖ Antifungal and
- ❖ Anti-parasitic drug

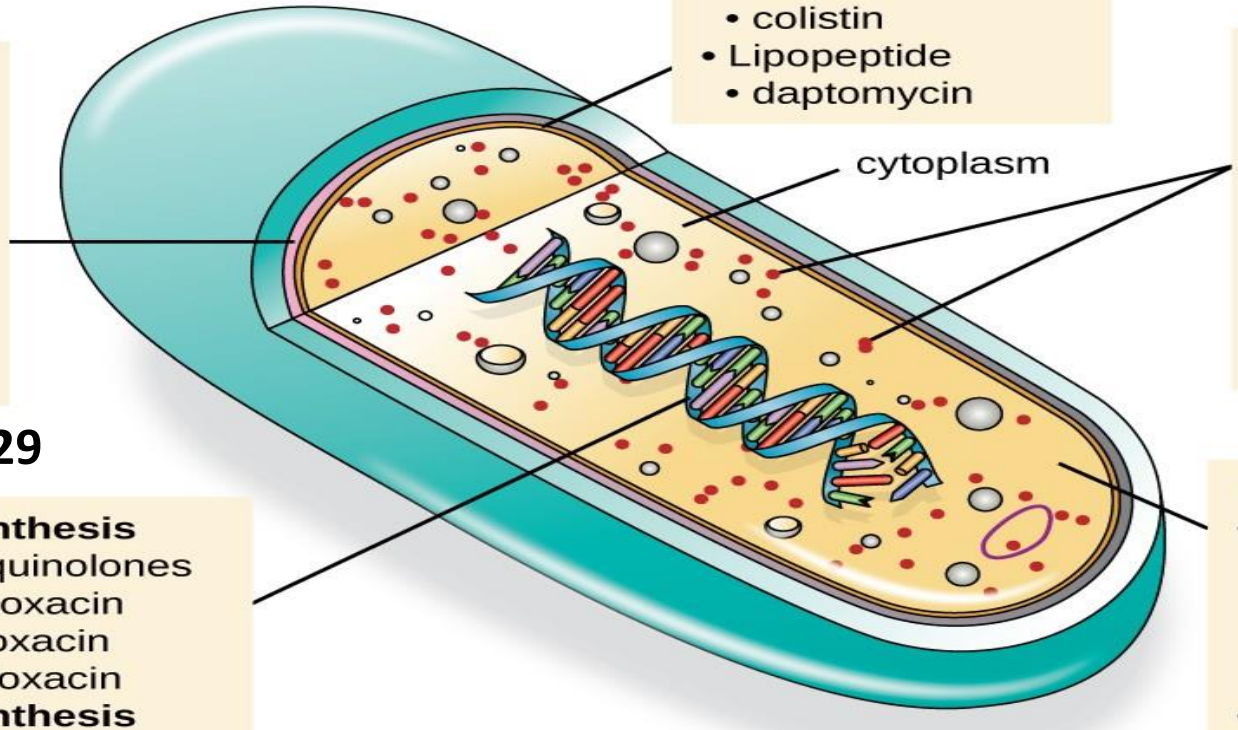
Cause killing microbes (with the **suffix-cidal**) or stop growing of microbes (with the **suffix- static**).

(Willey et al., 2020, p. 187-206; L, Paul et al., 2018, p.431-458)

Antibacterial drug targets

Cell wall
- β-lactams
  - penicillins
  - cephalosporins
  - monobactams
  - carbapenems
- Glycopeptides
  - vancomycin
- Bacitracin

Plasma membrane
- Polymyxins
  - polymyxin B
  - colistin
- Lipopeptide
  - daptomycin



Ribosomes
- 30S subunit
  - aminoglycosides
  - tetracyclines
- 50S subunit
  - macrolides
  - lincosamides
  - chloramphenicol
  - oxazolidinones

1920- 1929

DNA synthesis
- Fluoroquinolones
  - ciprofloxacin
  - levofloxacin
  - moxifloxacin

RNA synthesis
- Rifamycins
  - rifampin

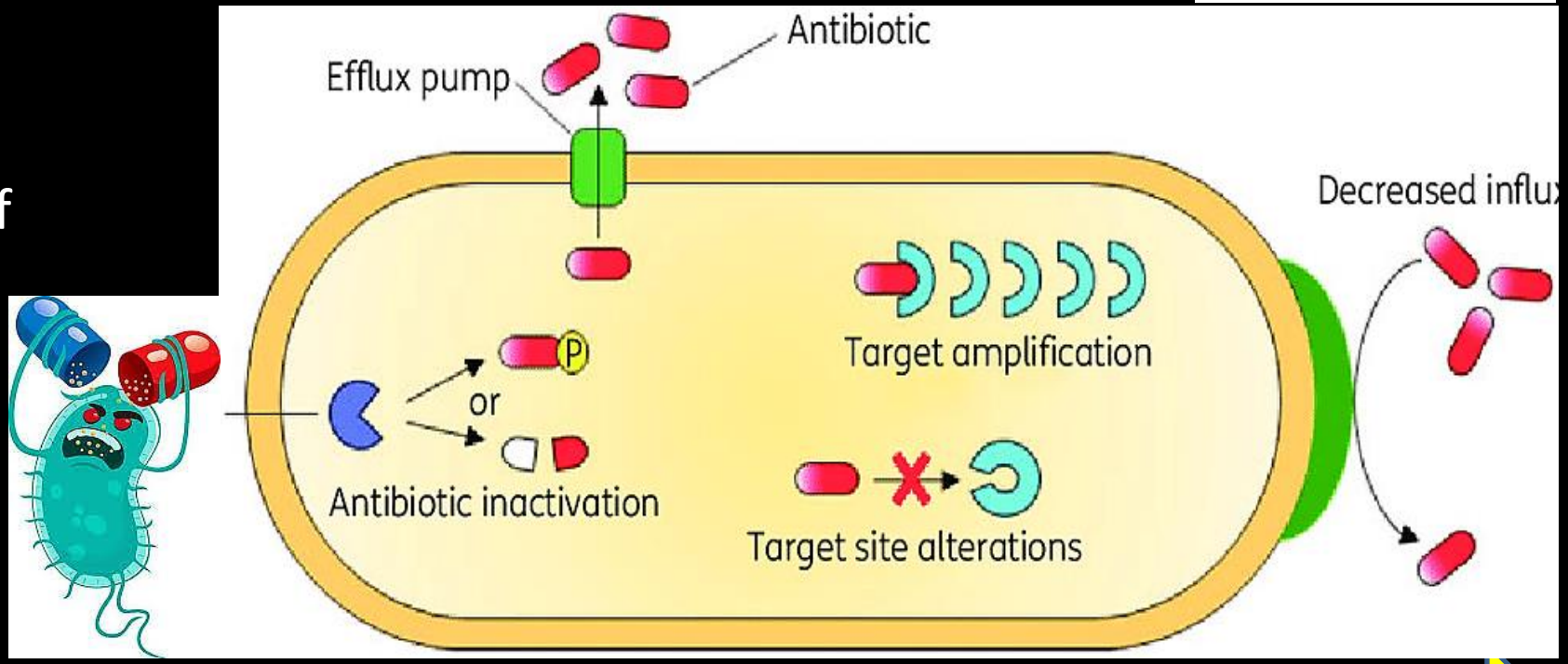
Metabolic pathways
- Folic acid synthesis
  - sulfonamides
  - sulfones
  - trimethoprim
- Mycolic acid synthesis
  - izoniazid

(MADIGAN et al., 2021, p.930-940)

Antibacterial resistance mechanisms

Commonly Bacteria can get resistant against the antibacterial through:-

- Alteration of the specific antibacterial targets
- Deactivation and changing of antibacterial structure
- Cutting the antibacterial drug
- Efflux pump system

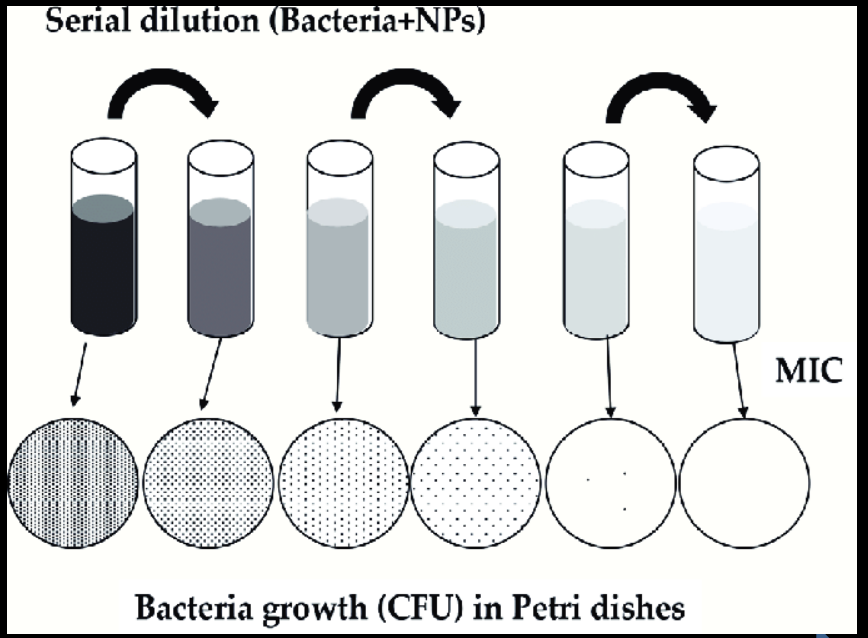


(L, Paul et al., 2018, p.431-458)

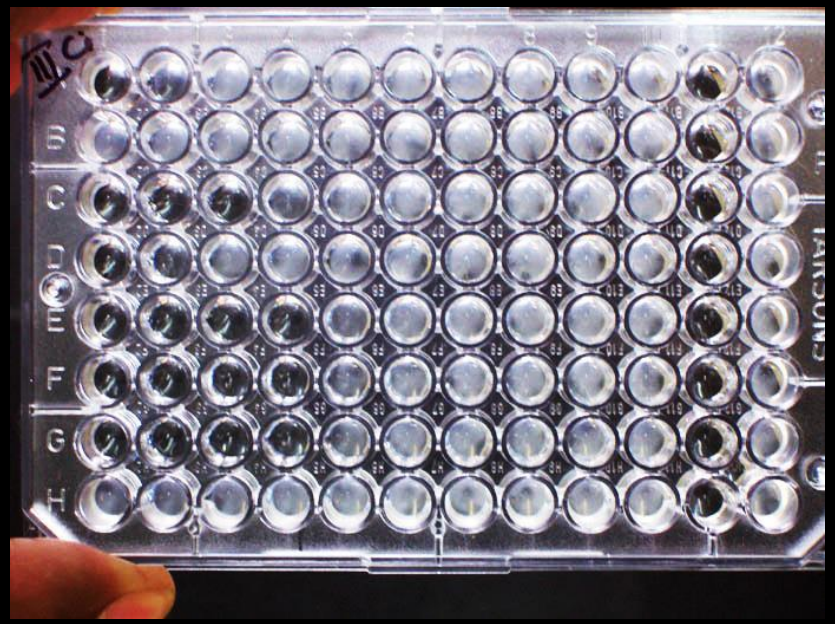
Antimicrobial research methods

- Antimicrobial research methods

	Antimicrobial Susceptibility Techniques	Sub-methods
1	Diffusion method	- Disk diffusion method - Well diffusion method
2	Dilution method	- Broth Macrodiluion - Broth Microdilution - Agar dilution method



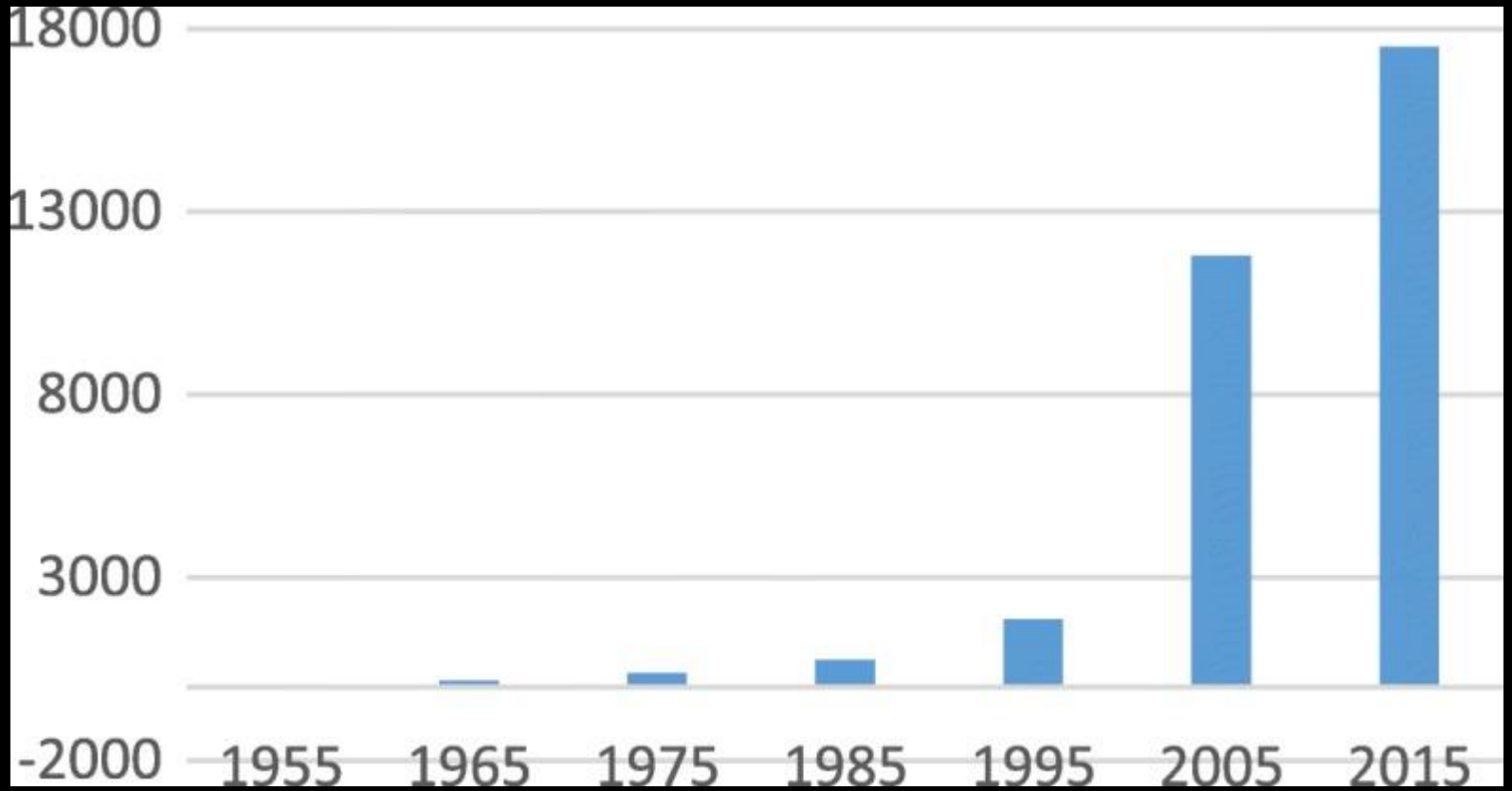
Antimicrobial research methods



Antimicrobial research methods

(Eloff J. N. , 2019) study.

Why most of them didn't success ?



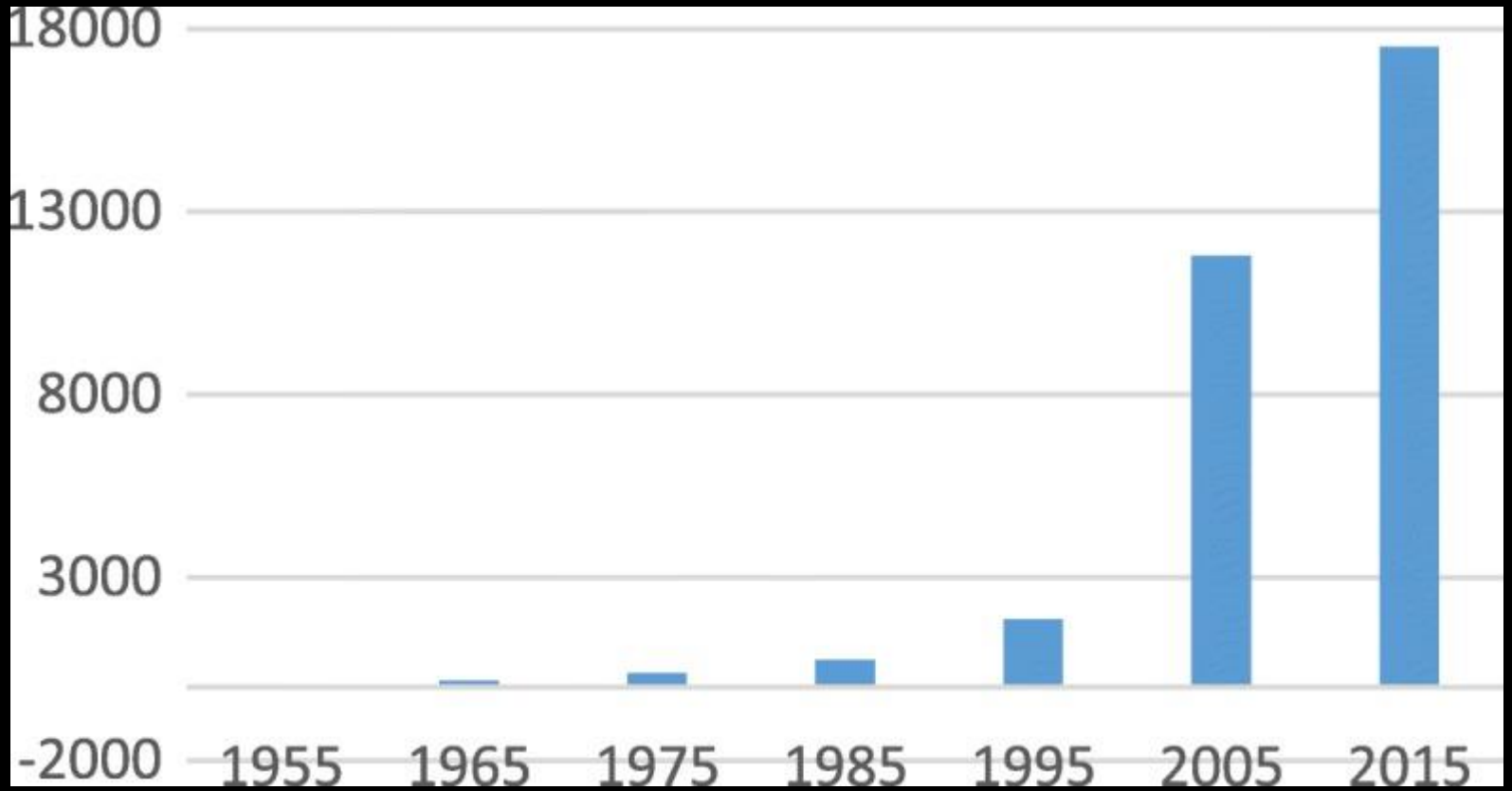
Eloff J. N. (2019). Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results. BMC complementary and alternative medicine, 19(1), 106. https://doi.org/10.1186/s12906-019-2519-3

Antimicrobial research methods

(Eloff J. N. , 2019) study.

Because

- Wrong method
- Wrong plant



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Antimicrobial research methods

➢ Reason for not using traditional method

1. Most of the plant extracts have non-polar compounds and Oils
2. They precipitate in dilution method
3. They have color, which avoid detection

(Eloff J. N. , 2019) study.

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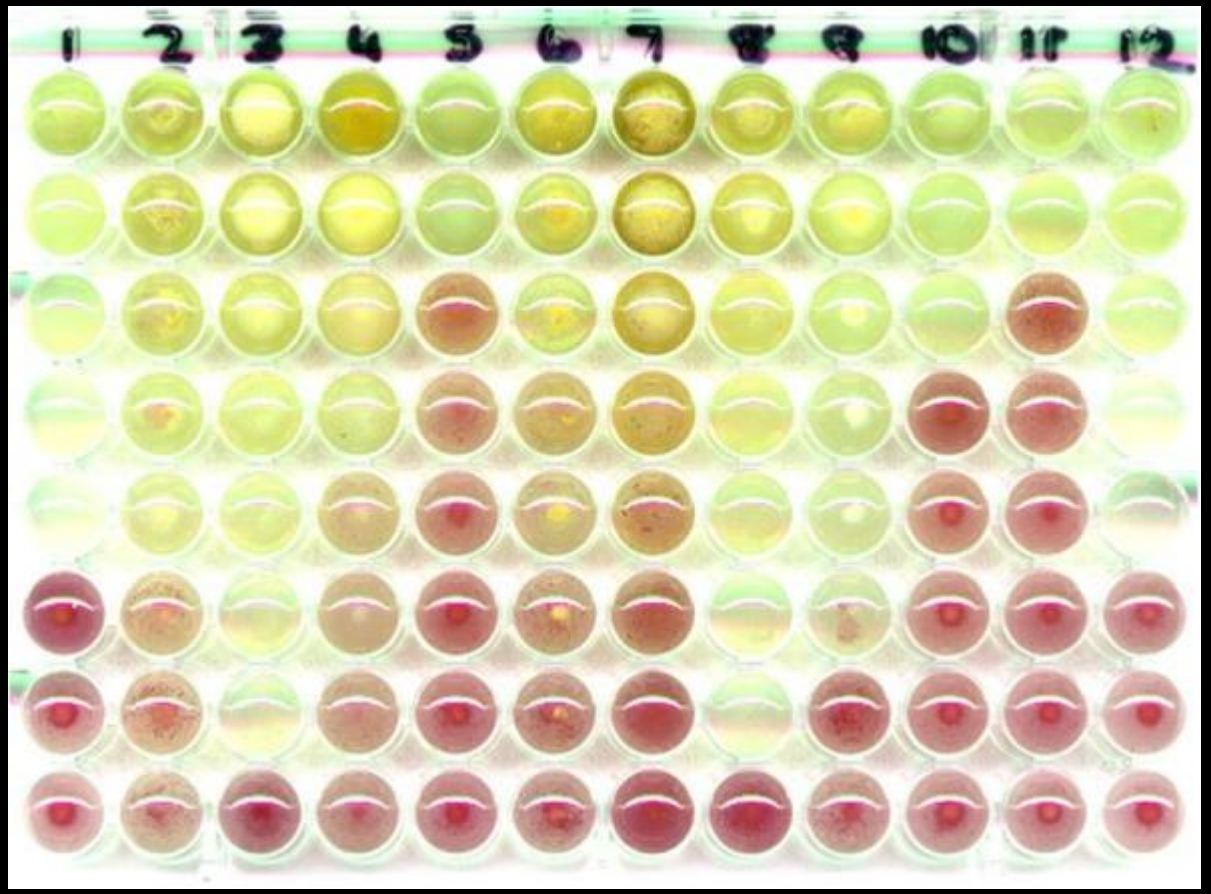
Antimicrobial research methods

➢ Solutions & Recommendation

- ✓ Using stain (dye) to detect bacterial viability.
- ✓ Examples (Tetrazolium salts)

Note: 1830 times in Google Scholar

(Eloff J. N. , 2019) study.



MTT and INT

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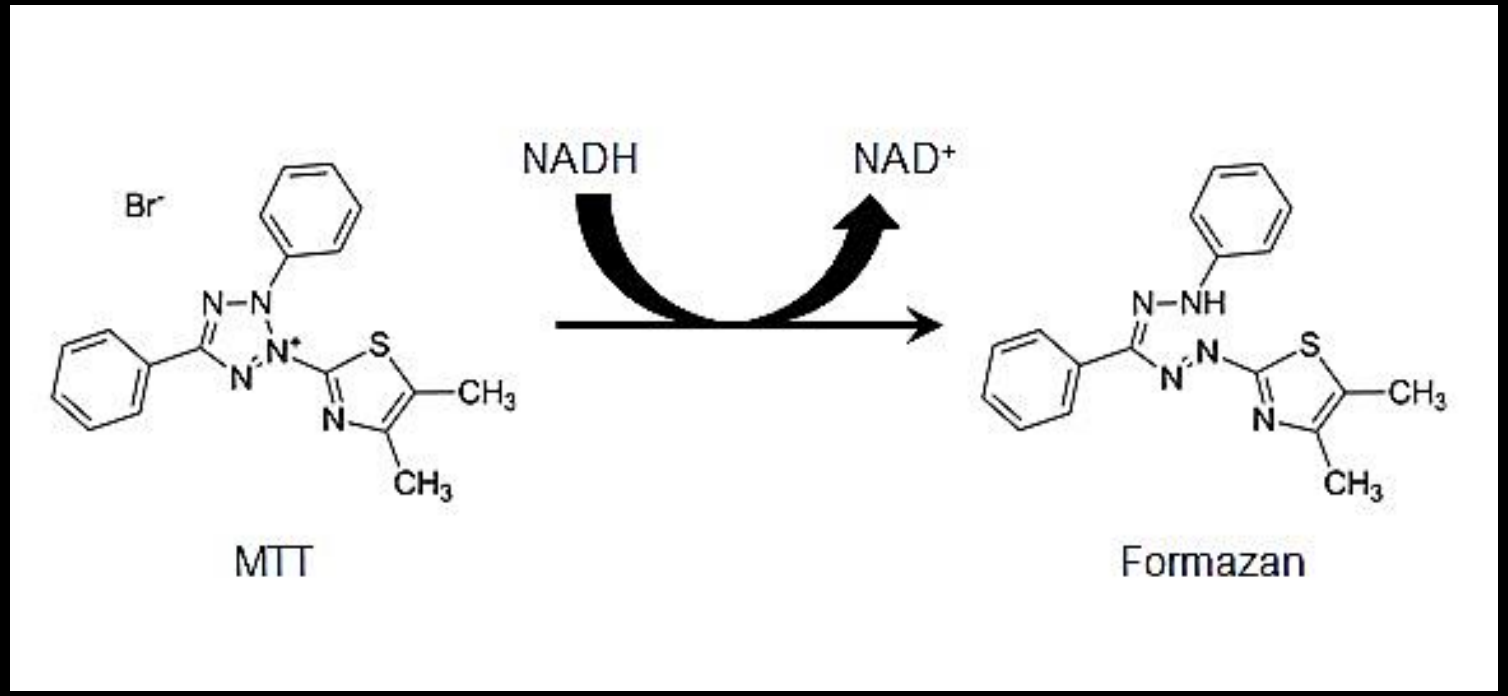
Tetrazolium mechanisms

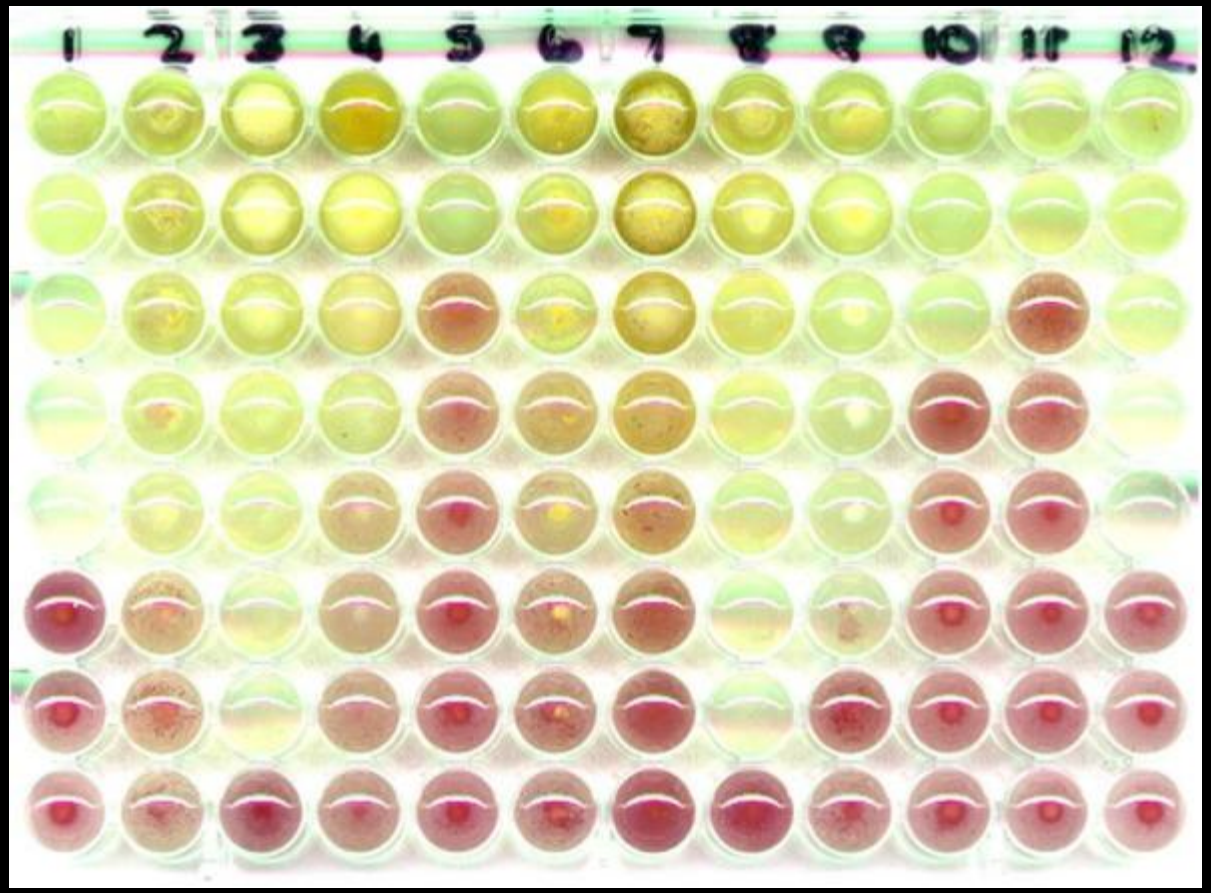
- **MTT** or (**Methyl thiazolyl diphenyl-tetrazolium bromide**)
- INT Or (p-iodonitrotetrazolium violet)

They have been used to determine the bacterial viability



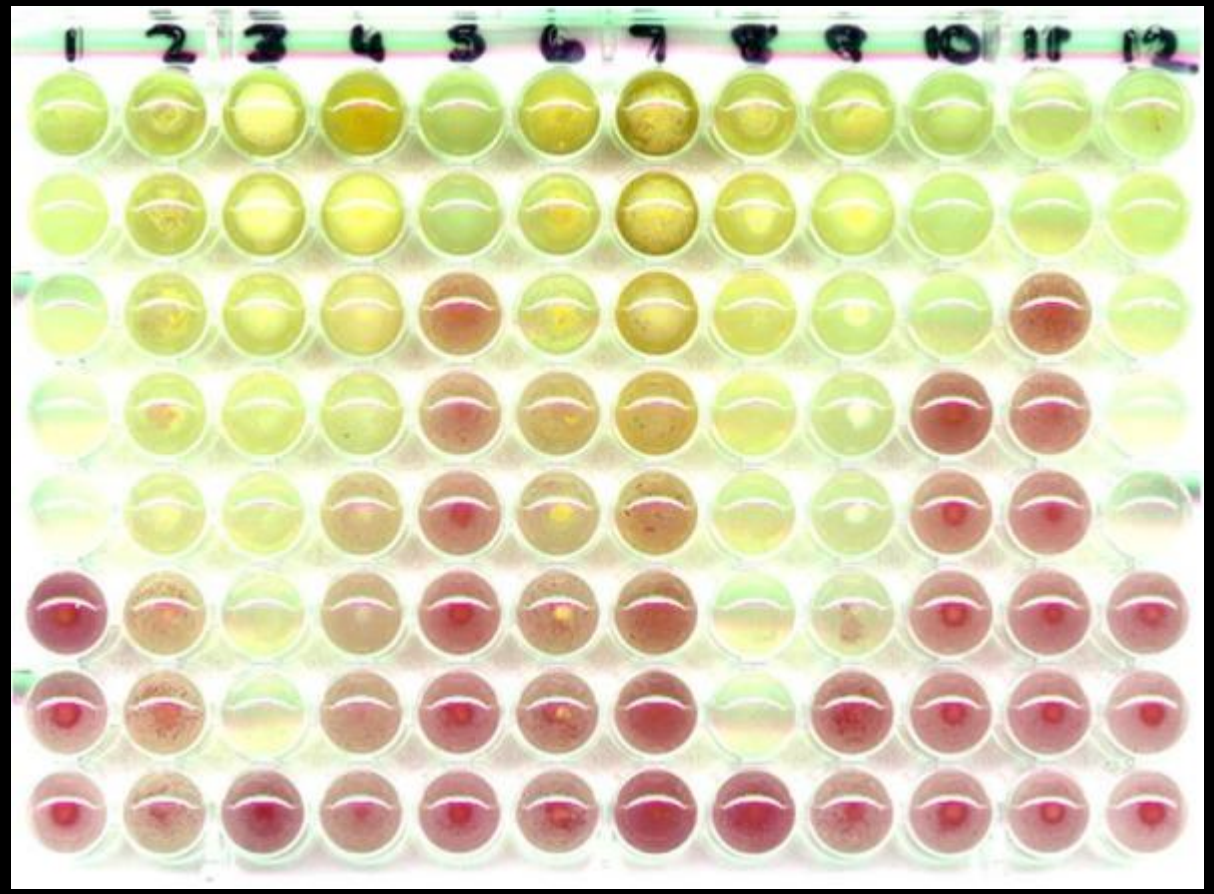
Example

- An example of the color developments in serially diluted plant extracts incubated and treated with INT is provided in



Conclusion and summary

- Antibacterial researches are **urgent needs**.
- **Diffusion and dilution** are traditional common techniques, but they have limits.
- MICs determined by using **serial microdilution with INT** as indicator of growth are realistic modern way of research.



Acknowledgement

THANK YOU