

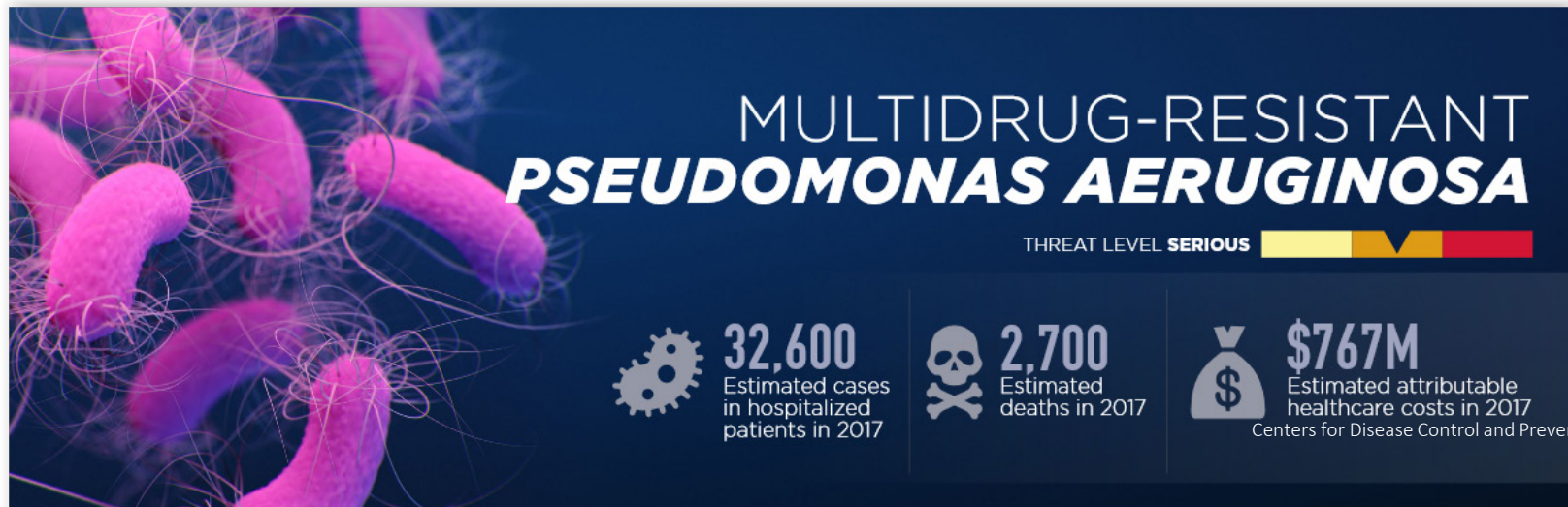
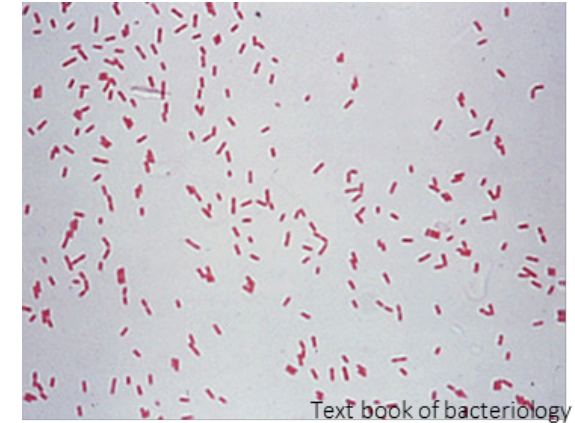
# Intracellular fate of *Pseudomonas aeruginosa*: Setting-up a bi-fluorescent model to follow its intracellular replication in the presence of antibiotics.

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FACM  
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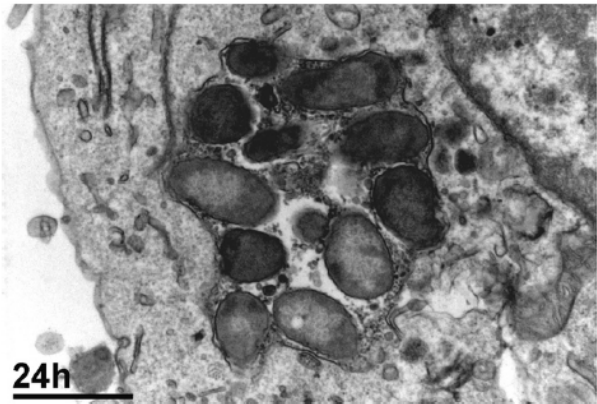
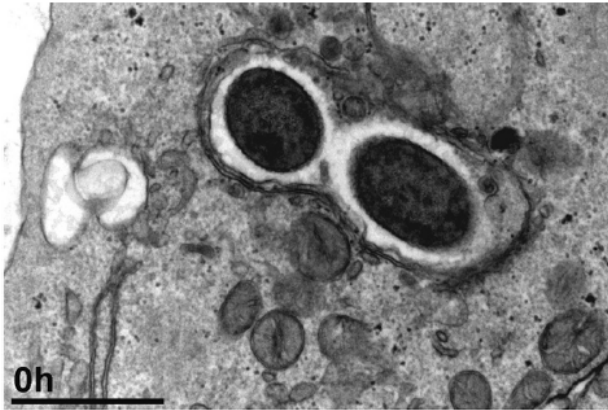
# What is *Pseudomonas aeruginosa* ?

- ❖ Gram-negative bacterium.
- ❖ Responsible for nosocomial infections with a high mortality rate in certain patients (immunocompromised, severe burns, cystic fibrosis, etc.).
- ❖ Symptoms: Multiple infections (wounds, blood, lungs, gastrointestinal...) leading to sepsis.
- ❖ Development of antibiotic resistance

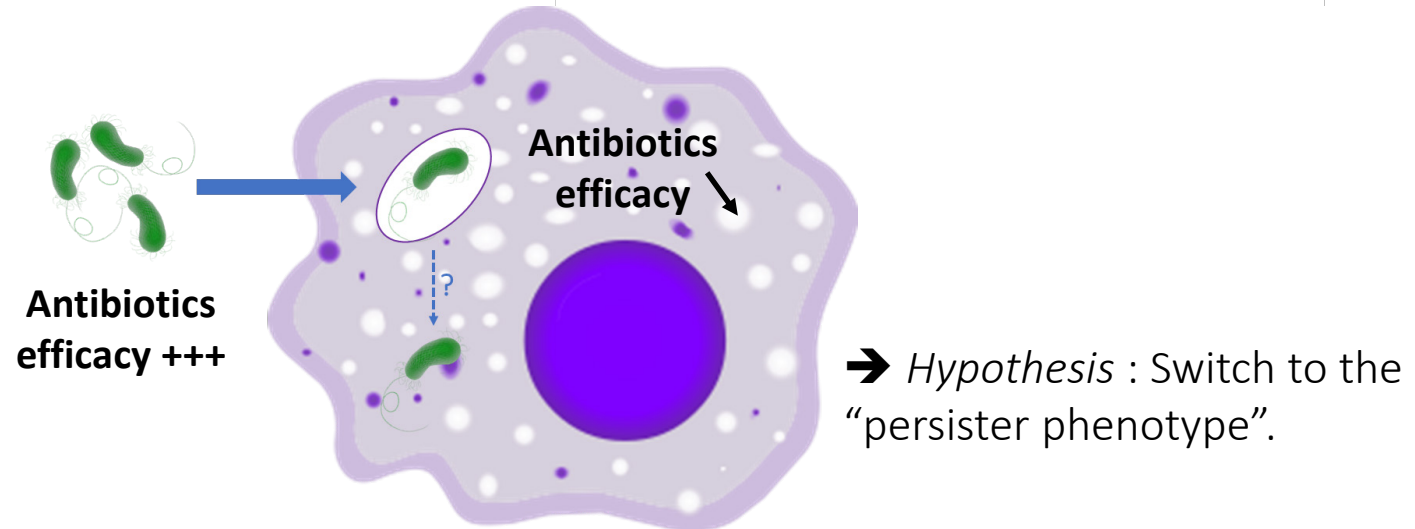
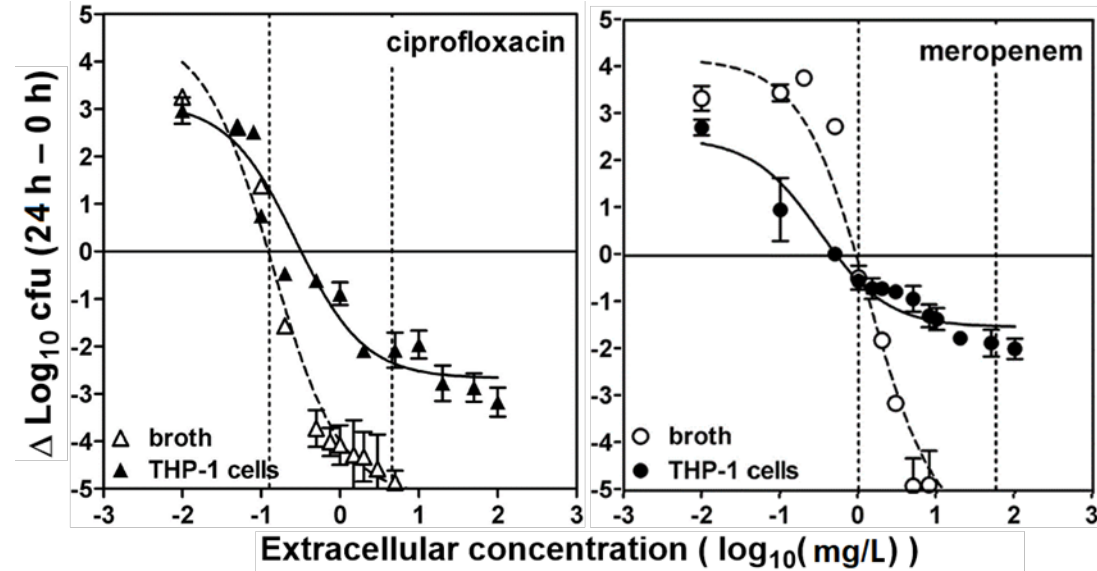


# Intracellular lifestyle → Reduced antibiotic efficacy

Intracellular PAO1 in THP-1 cells 0h and 24h post infection



Concentration-response curve of PAO1 exposed to antibiotics



\* Adapted from Buyck *et al.*, Pharmacodynamic Evaluation of the Intracellular Activity of Antibiotics towards *Pseudomonas aeruginosa* PAO1 in a Model of THP-1 Human Monocytes. *Antimicrobial Agents and Chemotherapy* Vol 57 p.2310-2318 (2013)

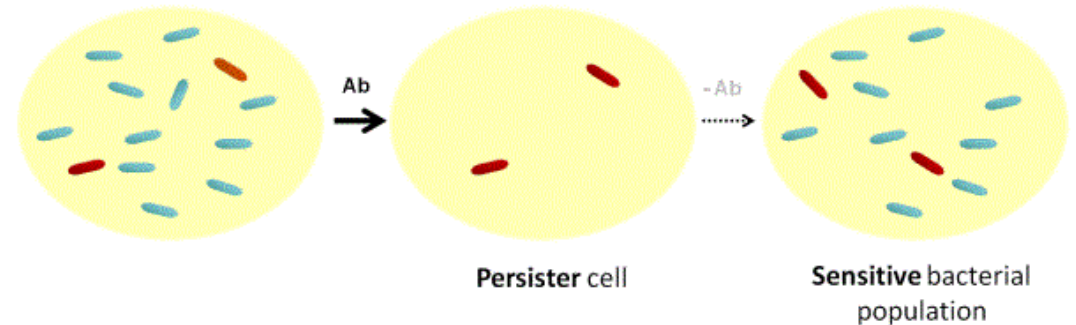
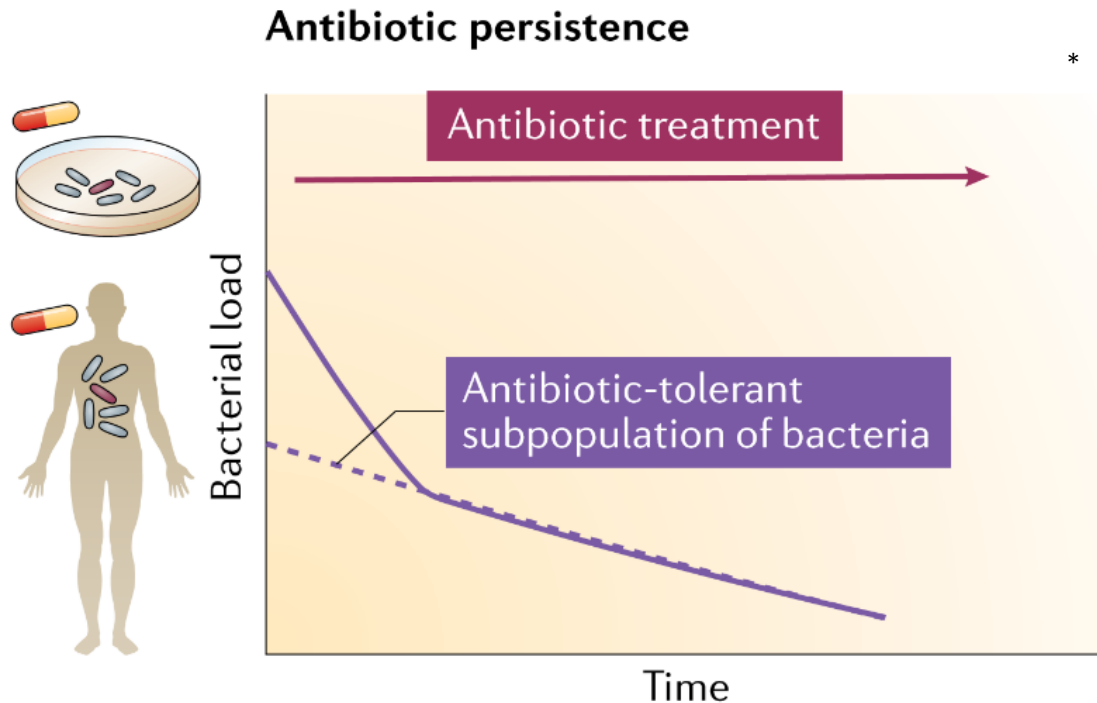
# Persister phenotype

Bacteria are said to be “persister to antibiotics” when they have the following characteristics:

- Biphasic mortality curve in the presence of antibiotics.
- Survival at bactericidal concentrations of antibiotics.
- Low impact of antibiotic concentration (if  $\gg \gg$  MIC) on persistence level.
- No replication in the presence of antibiotics.
- Phenotype reversible on withdrawal of the antibiotic.

➔ CFU analysis

➔ Following intracellular bacterial replication at single-cell level



\*Balaban *et al.*, Definitions and guidelines for research on antibiotic persistence. Nature Reviews Microbiology Vol 17 p.441-448 (2019)

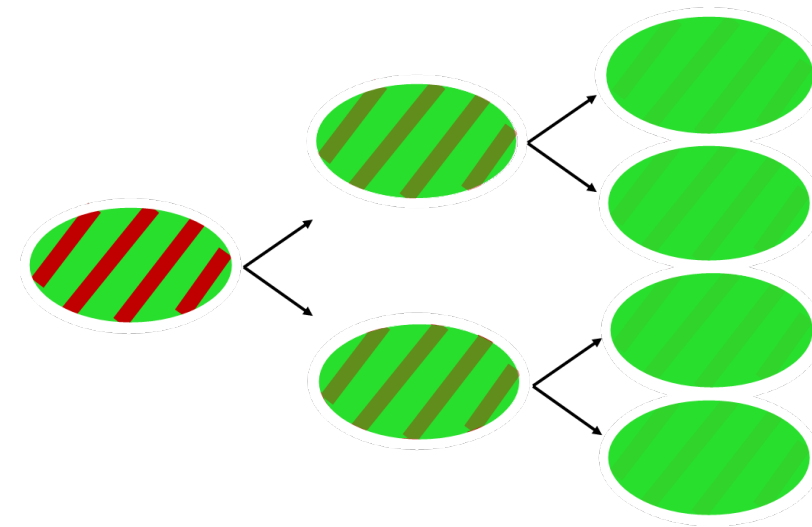
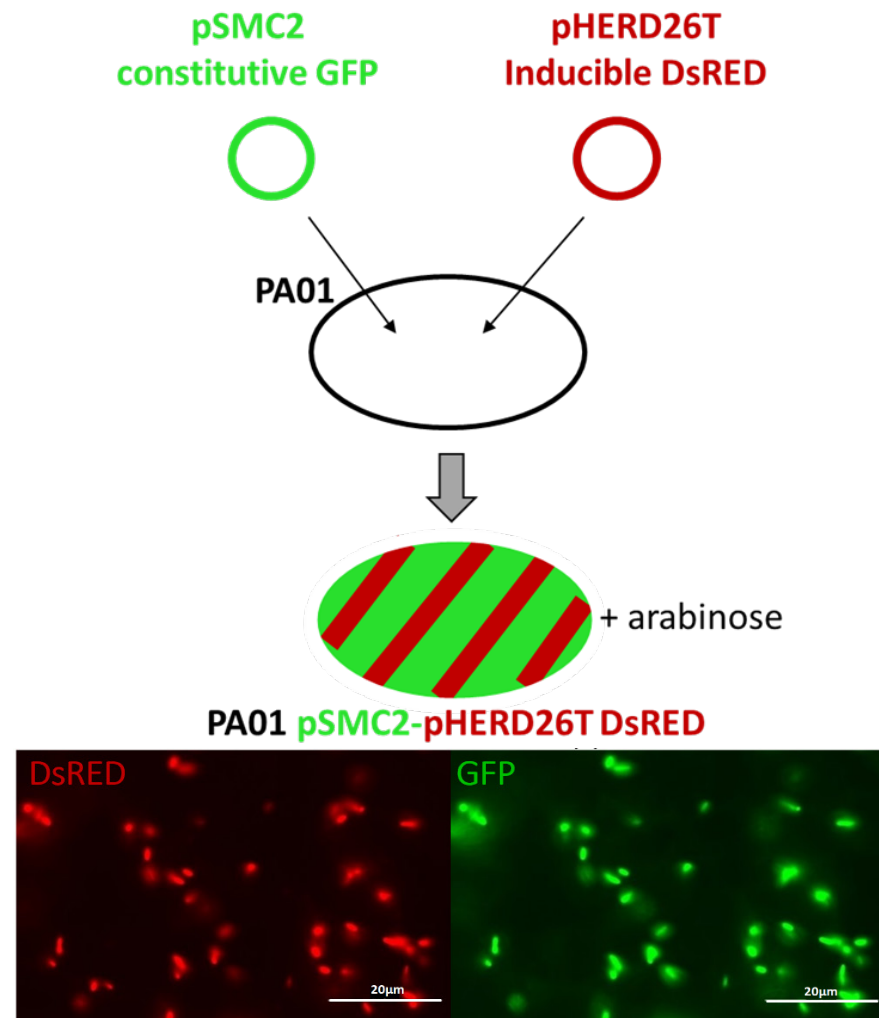
\*\*Conlon B. *Staphylococcus aureus* chronic and relapsing infections: Evidence of a role for persister cells. Bioessays Vol 36 issue 10 (2014)

# Objectives & Method

→ Develop a tool to follow in real time and at the single cell level the intracellular replication of *P. aeruginosa* order to examine whether antibiotics can select persister subpopulations.

How ?

## 1. Obtention of a bi-fluorescent strain



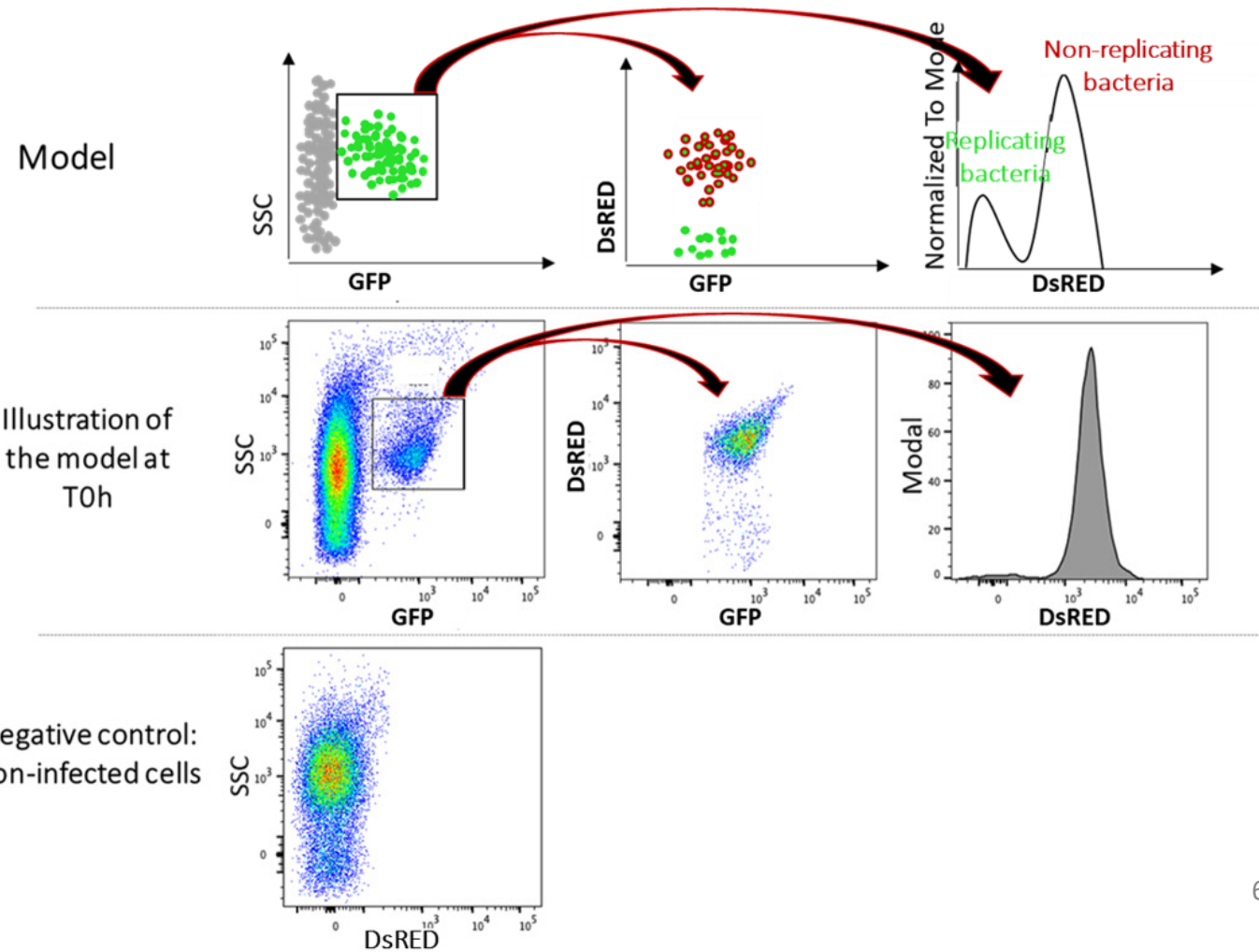
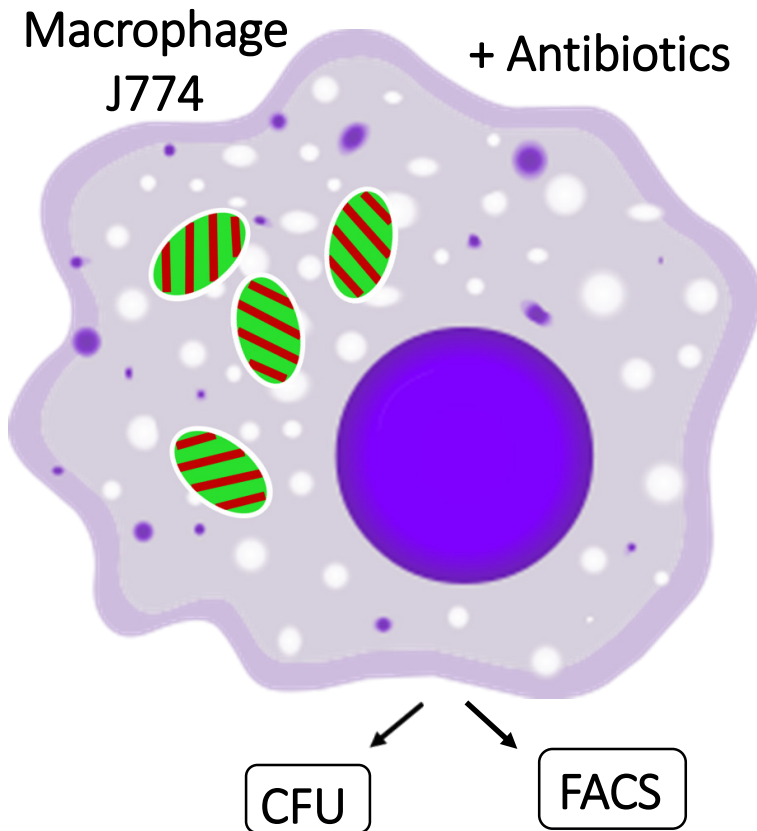
Constitutive fluorescence (**GFP**)  
+  
Inducible fluorescence (**DsRED**)

# Objectives & Method

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How ? 2. Intracellular infection

3. Flow cytometry analysis

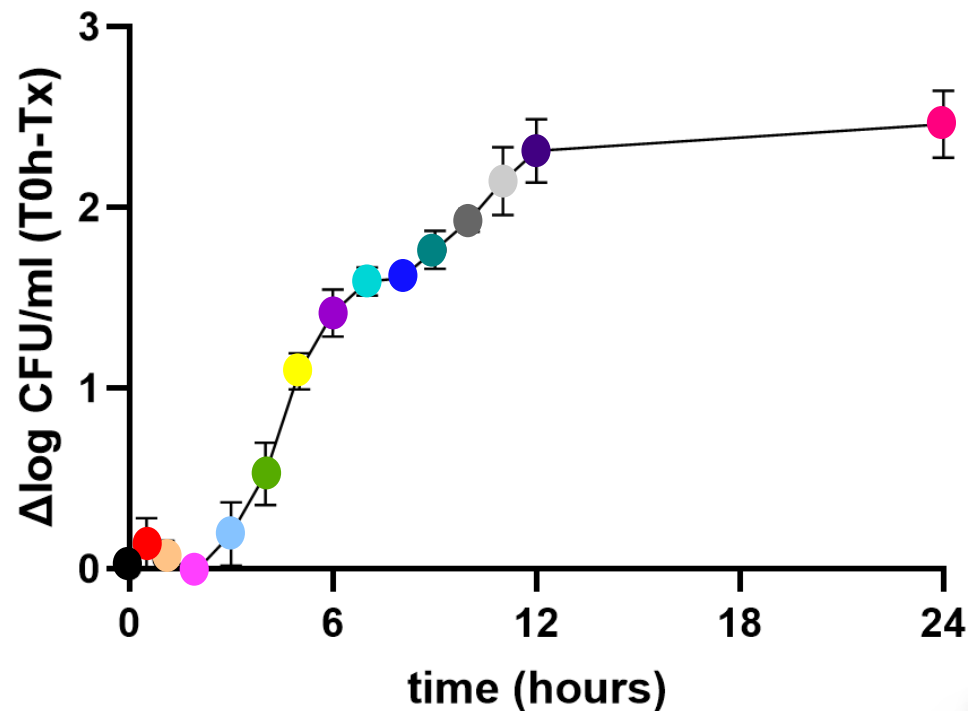
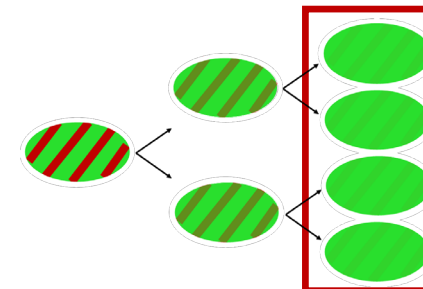




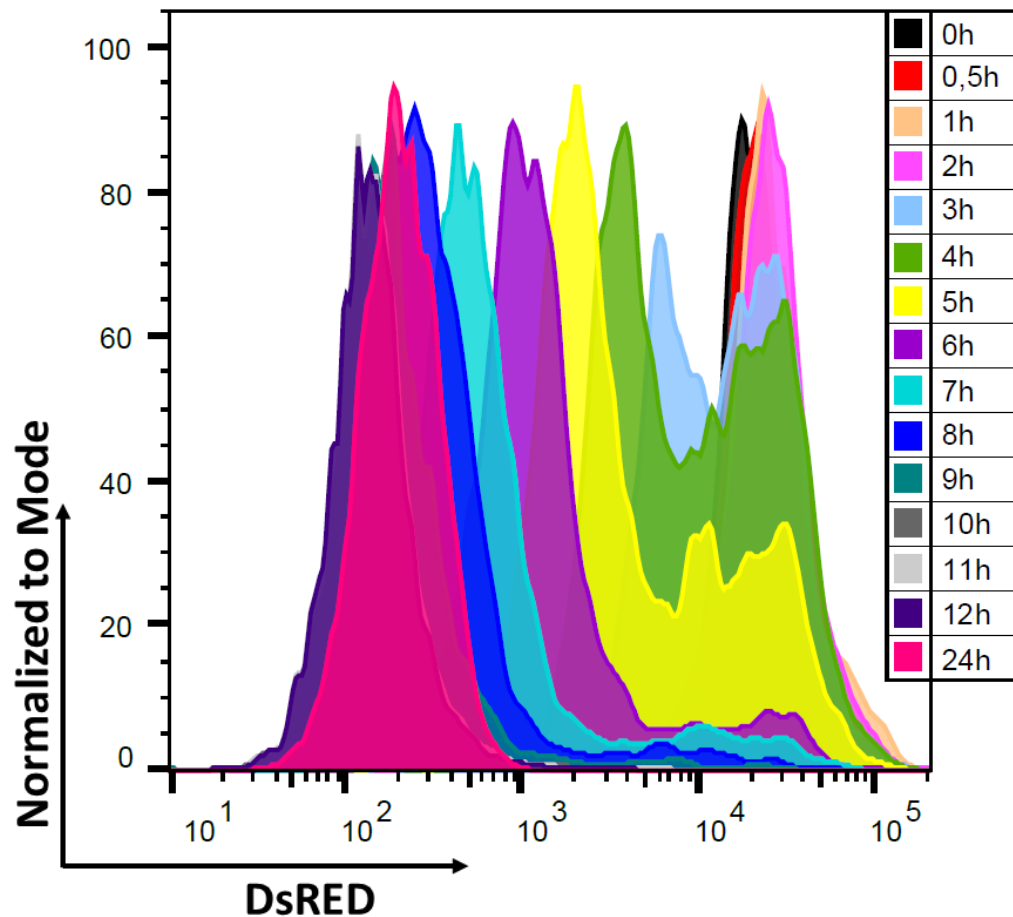
# Results

*Without antibiotics*

→ Fluorescence dilution over time



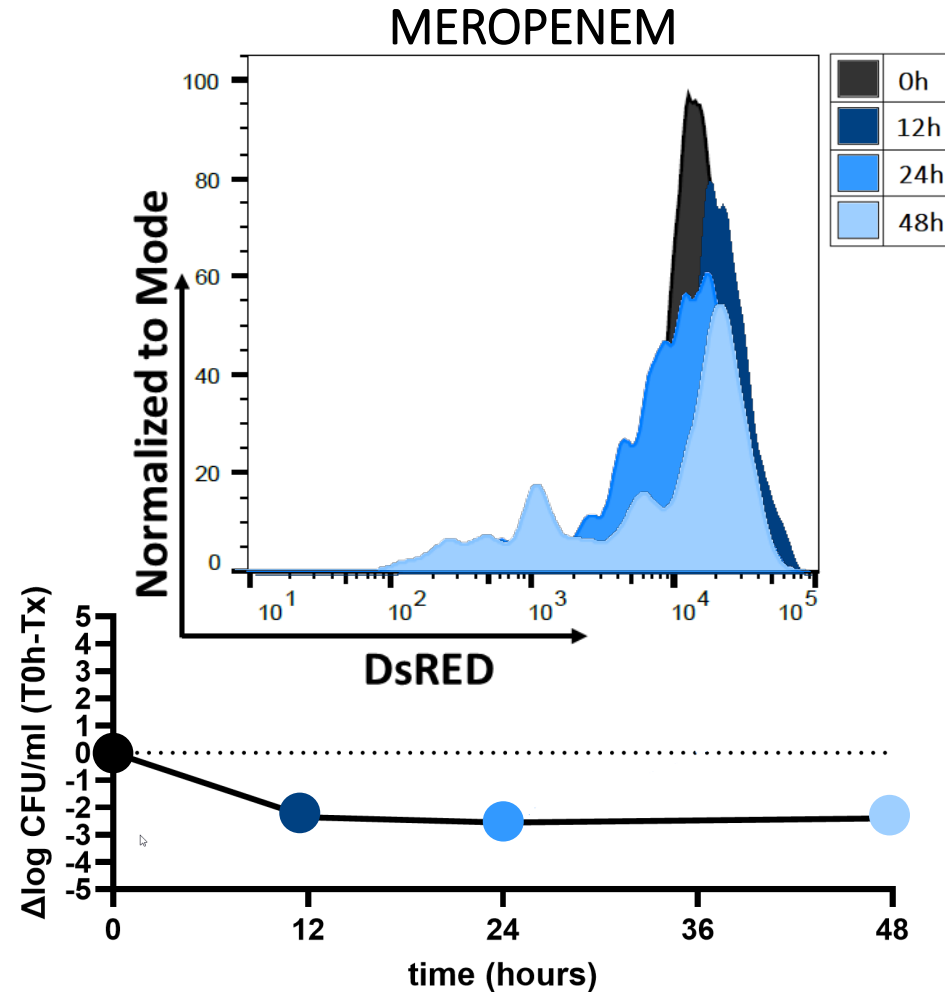
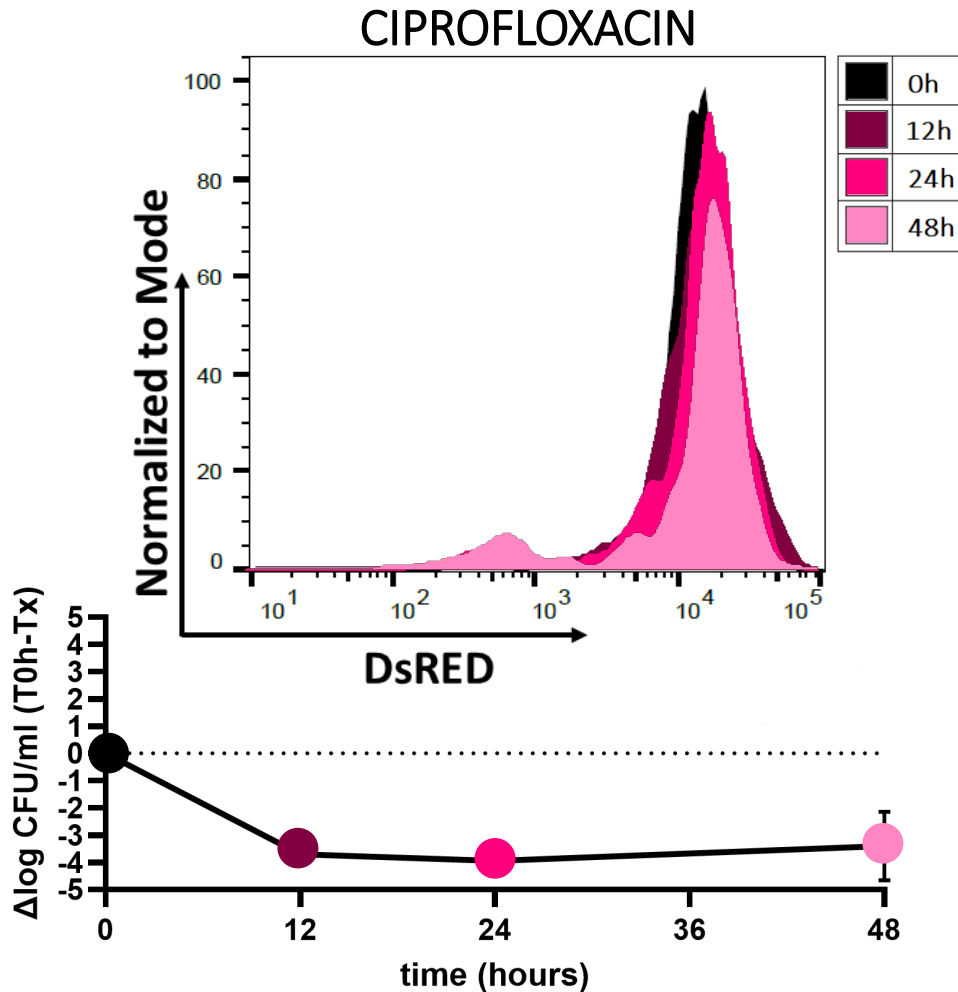
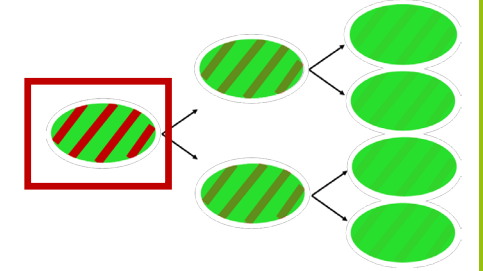
→ Mean replication time: 1h17



# Results

With 100x MIC of Ciprofloxacin (12,5µg/ml) and Meropenem (25µg/ml).

→ Absence of replication of main population suggested by the not diluted fluorescence of main peak

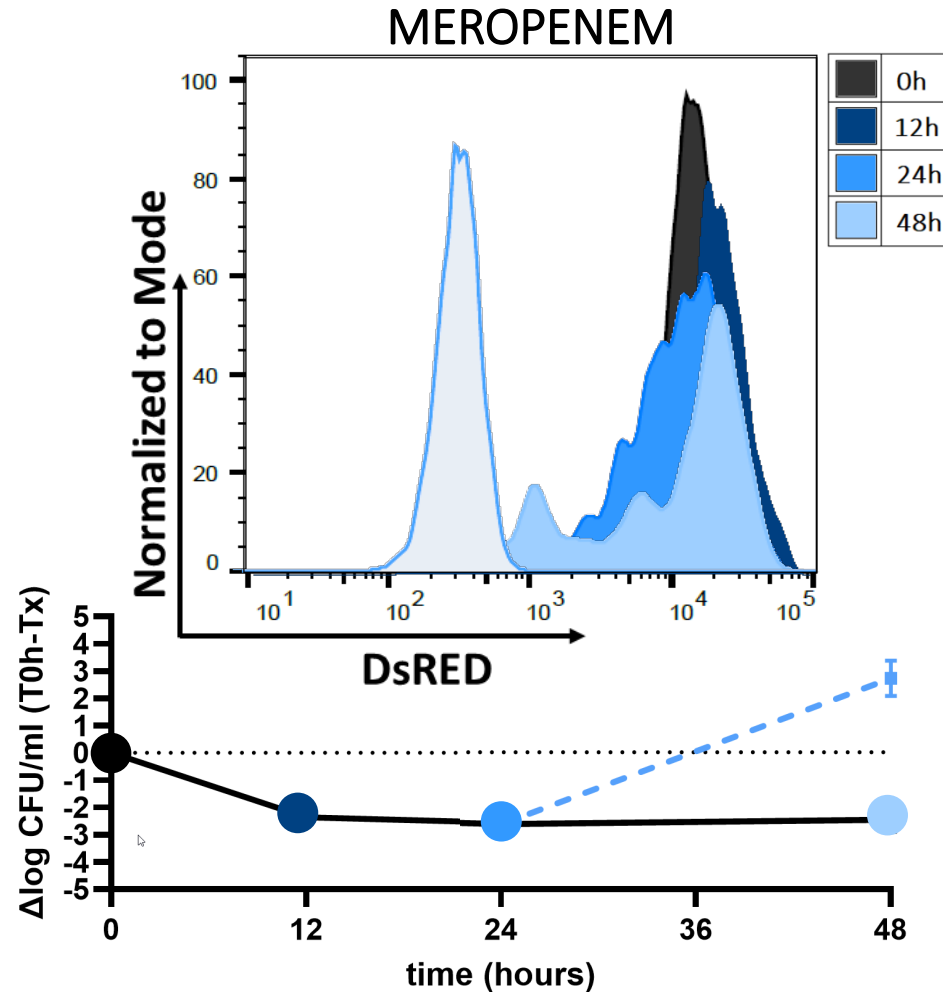
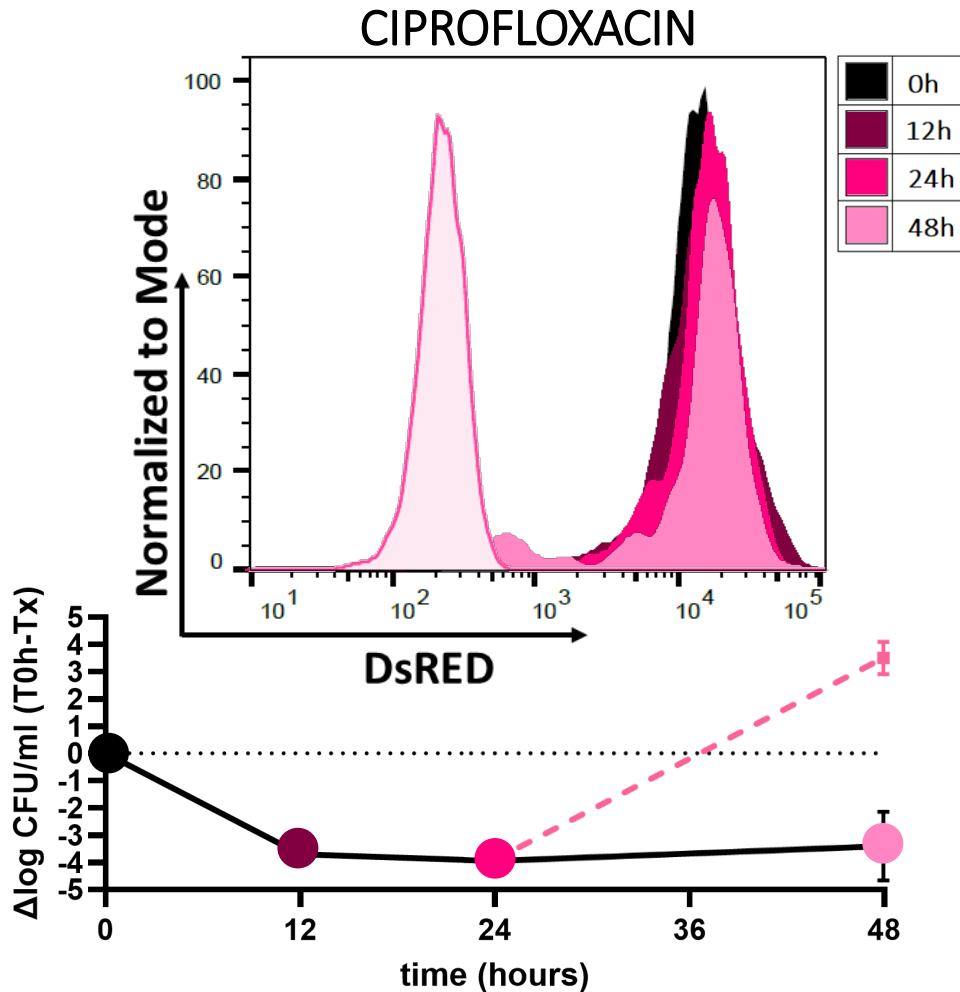
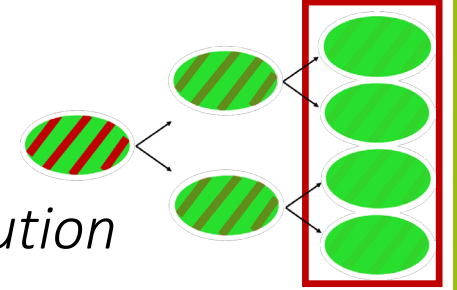




# Results

*With 100x MIC of Ciprofloxacin (12,5µg/ml) and Meropenem (25µg/ml)  
+ Antibiotics removal*

→ Reversible phenotype suggested by the come back of fluorescence dilution



# Conclusion

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➔ Following intracellular bacterial replication at single-cell level

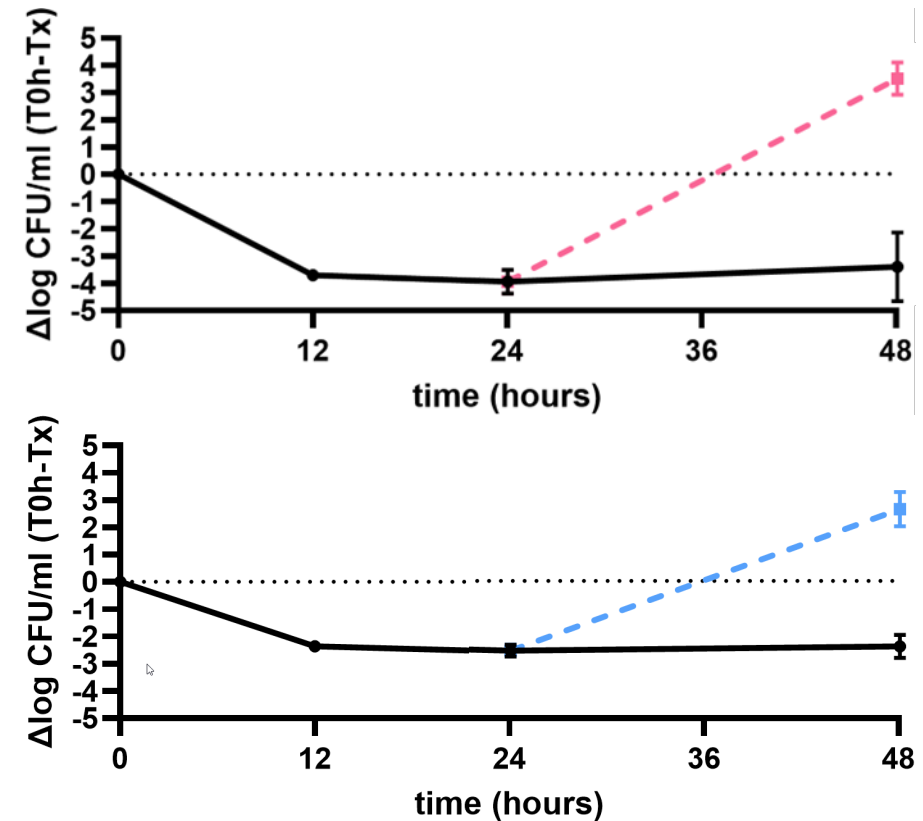
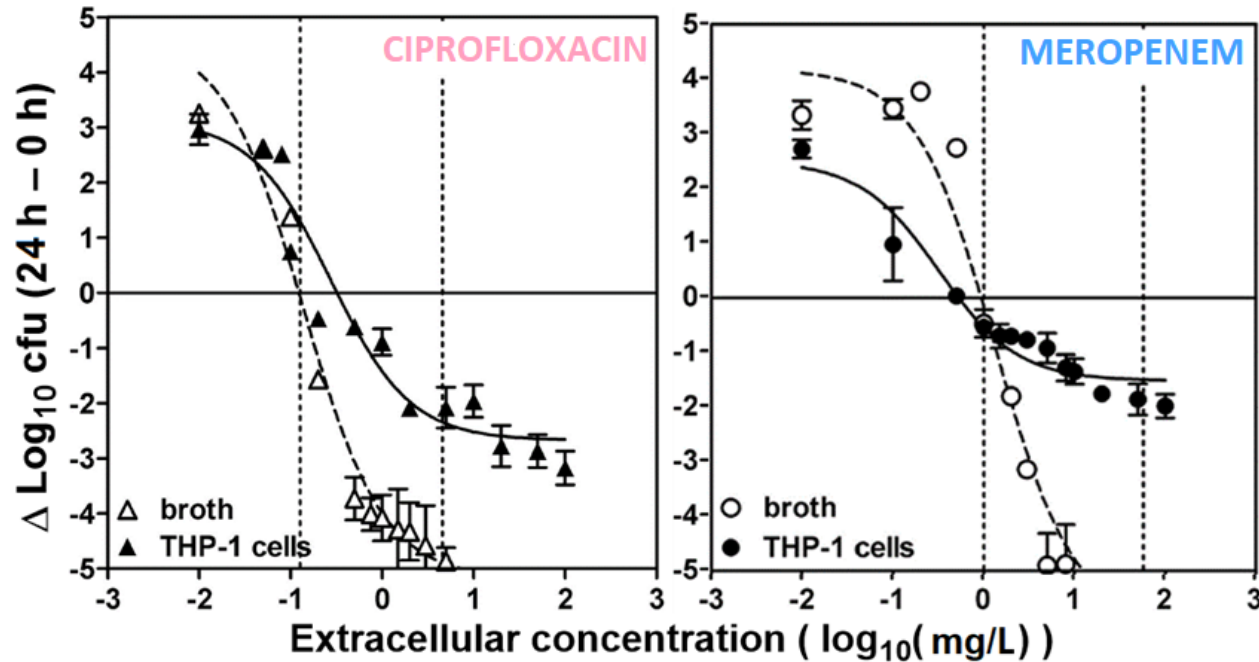
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- Biphasic mortality curve in the presence of antibiotics. → **CFU analysis**
- Survival at bactericidal concentrations of antibiotics.
- Low impact of antibiotic concentration (if  $\gg \gg$  MIC) on persistence level.
- No replication in the presence of antibiotics.
- Phenotype reversible on withdrawal of the antibiotic.



*Lot of thanks...*

The FACM team



*Thanks for your attention!*