Pharmacodynamic evaluation of the intracellular activity of tobramycin, doripenem, levofloxacin, and colistin towards *Pseudomonas aeruginosa* (PAO1) after phagocytosis by human THP-1 macrophages.

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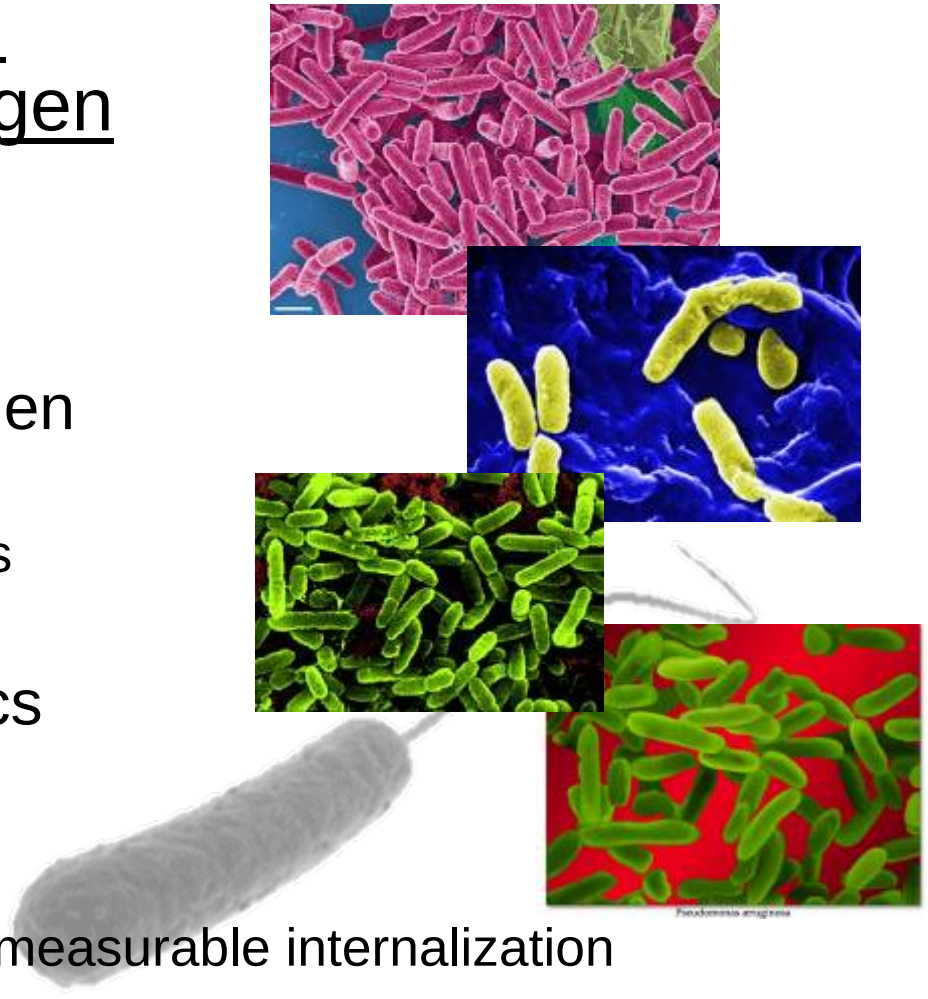
Introduction (1/2)

- *Pseudomonas aeruginosa,* an important human pathogen
  - Gram-negative bacillus
  - Opportunistic human pathogen
    - respiratory system infections
    - chronic infection in CF patients
  - (multi)resistance to antibiotics
  - Intracellular survival
    - ~ 50% of strains demonstrate measurable internalization

    (Engel, 2003)



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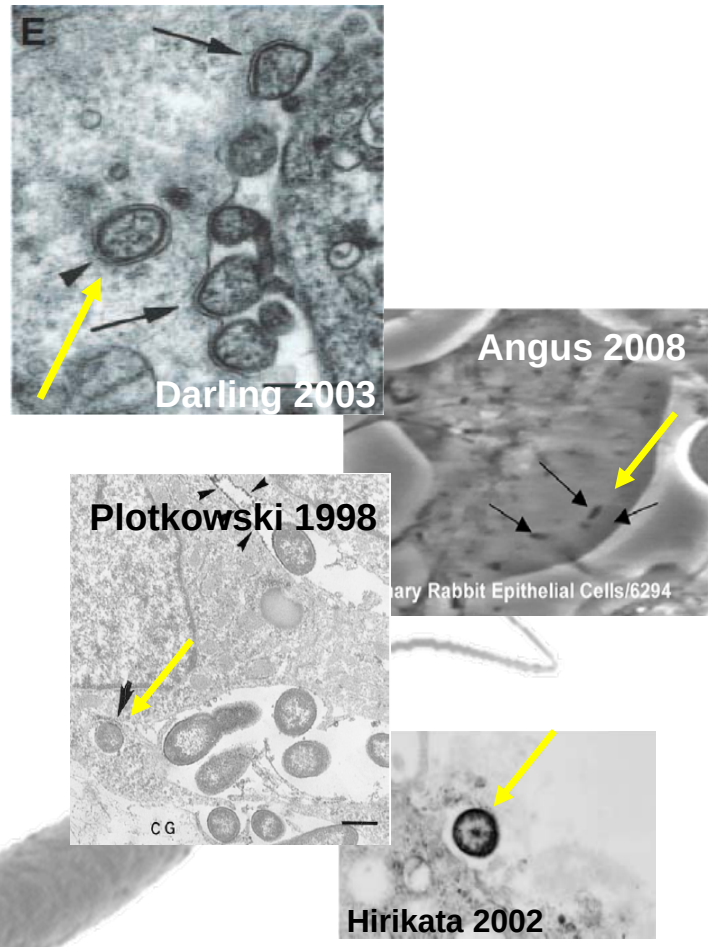
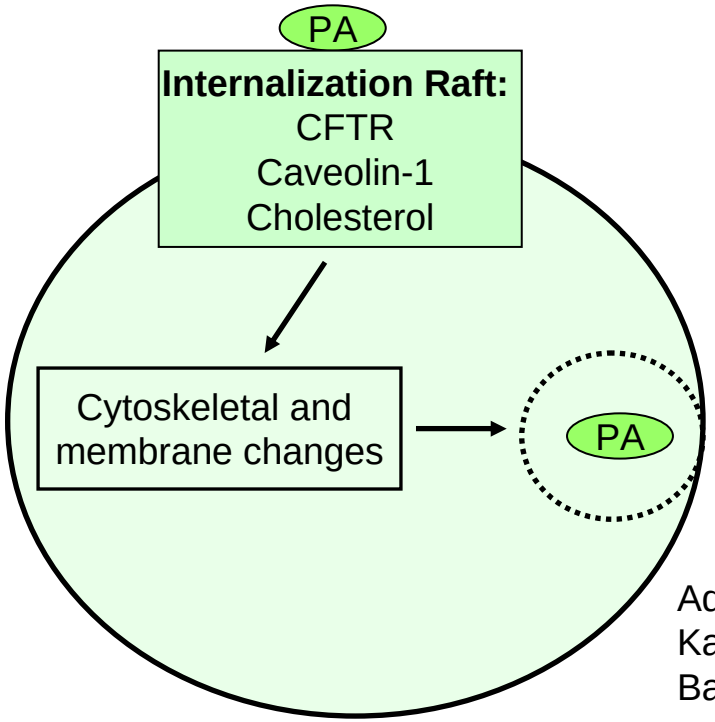


Introduction (2/2)

- Current view of the internalization pathway

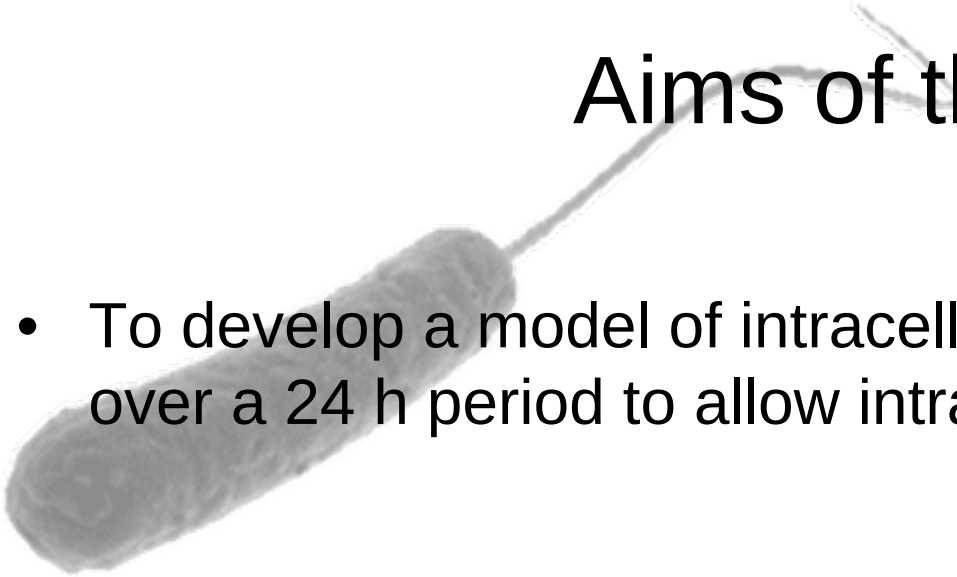


Adapted from:
Kannan et al., 2008
Bajmoczi et al., 2009

- Potential role for intracellular reservoir ?

  May constitute a source for chronic infection

Aims of the study



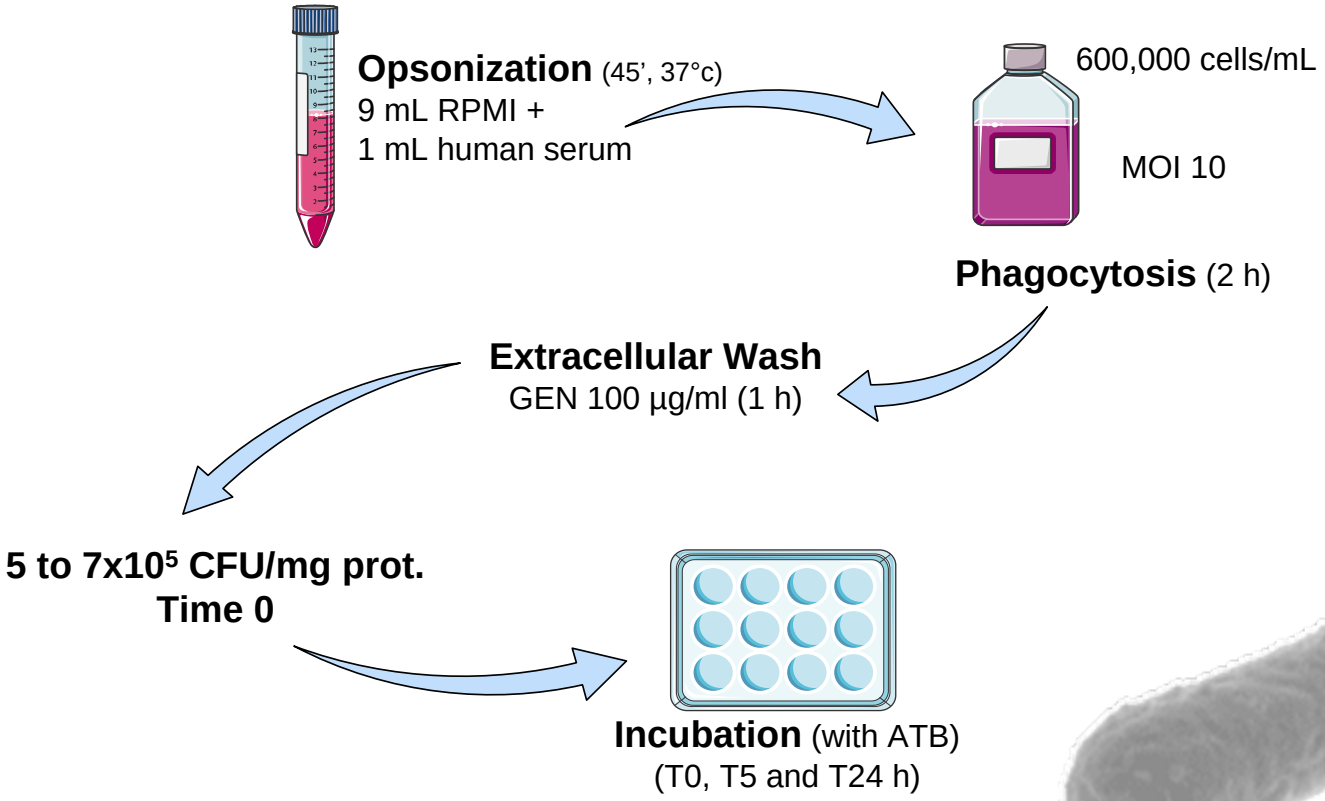
- To develop a model of intracellular infection by *P. aeruginosa* over a 24 h period to allow intracellular growth

- To study in this model the activity of antibiotics representative of the main classes currently used in the clinics

- To compare pertinent pharmacological descriptors of antibiotic activity (maximal efficacy, relative potency) against both extracellular and intracellular forms of *P. aeruginosa*

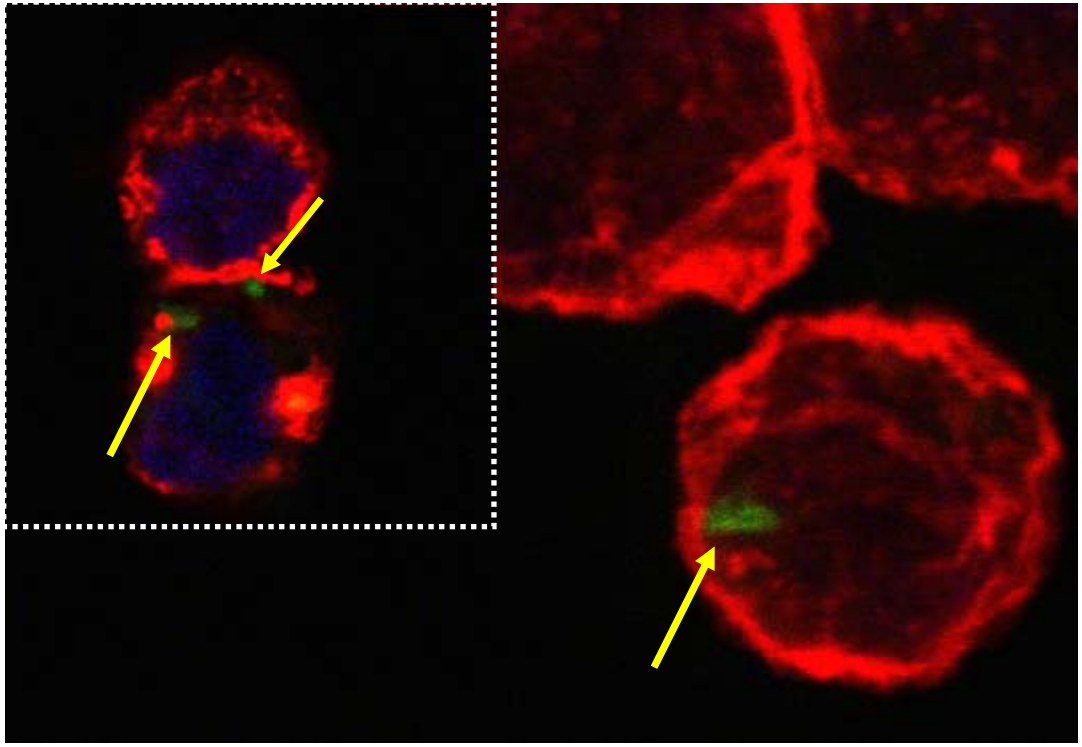
Experimental procedure

- Model:
  - THP-1 cells: Human acute monocytic leukemia cell line
  - PAO1 strain



Setting-up the model

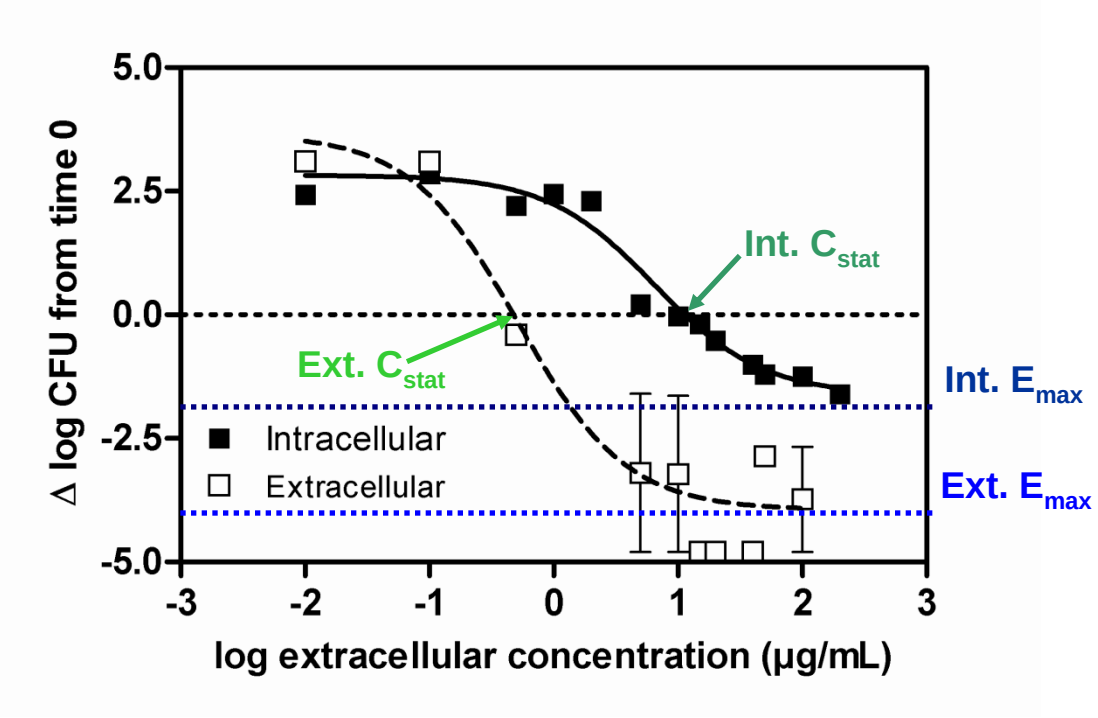
- Intracellular localisation of PA:
  - Confocal imaging



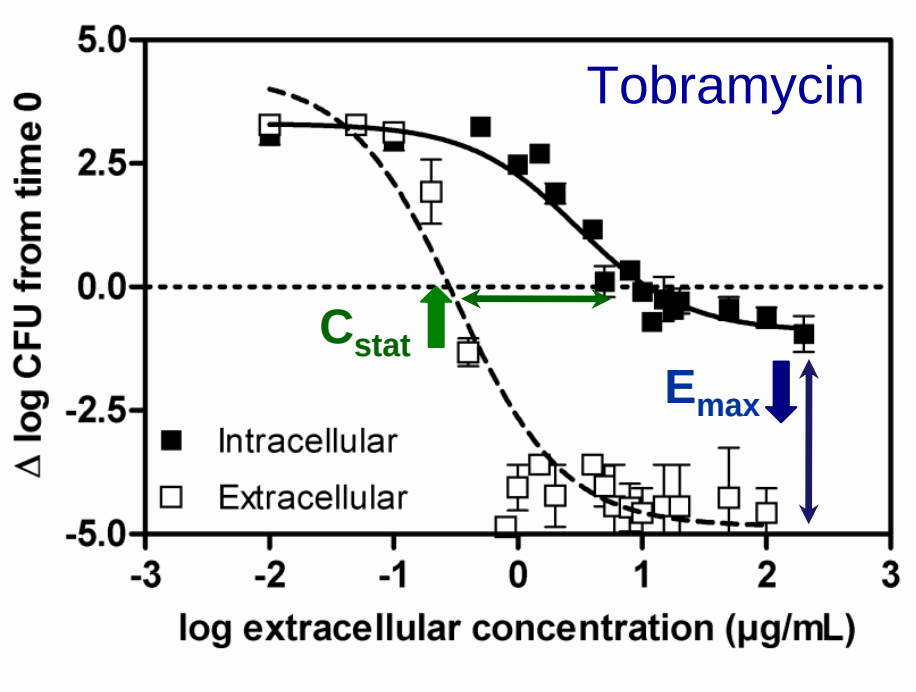
Blue: **nucleus** staining by TO-PRO 3,
Red: **actin** staining by Rhodamin-phalloïdin,
Green: ***Pseudomonas*** specific staining by a FITC-labeled antibody

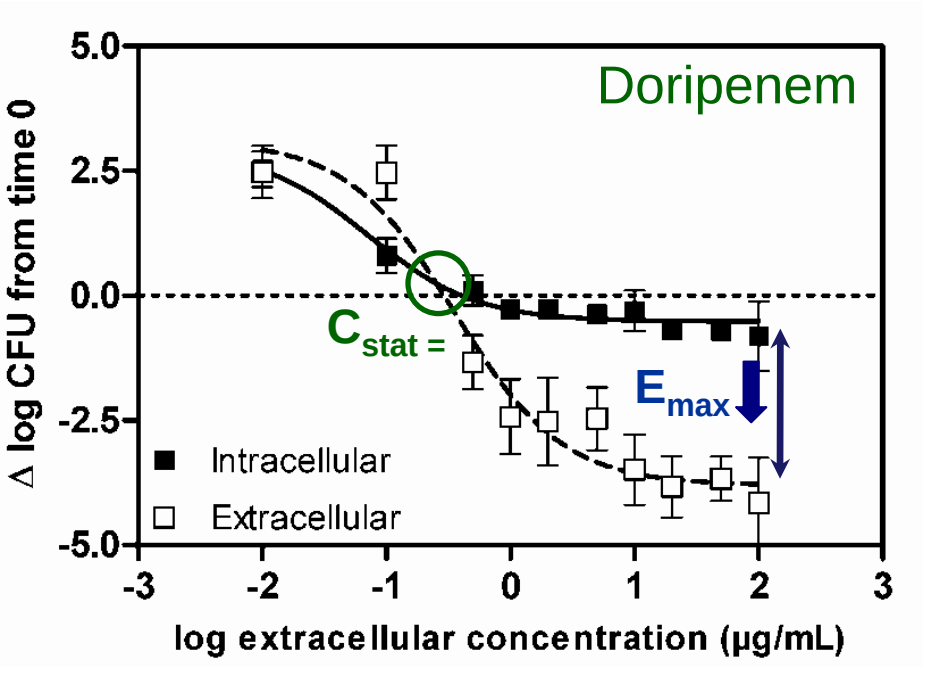
Results

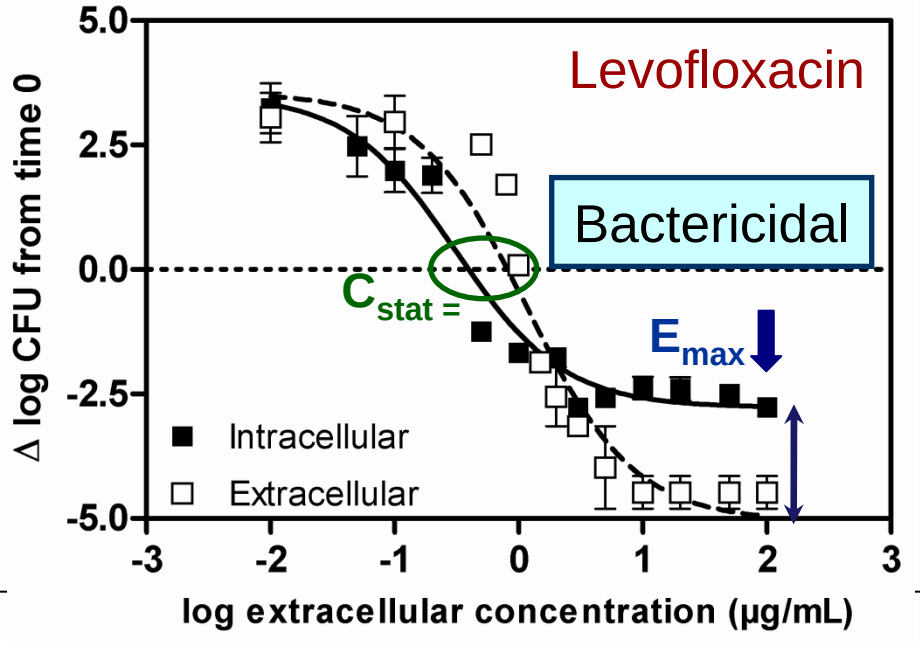
- Definition of pharmacodynamic parameters:
  - Gentamicin as an example

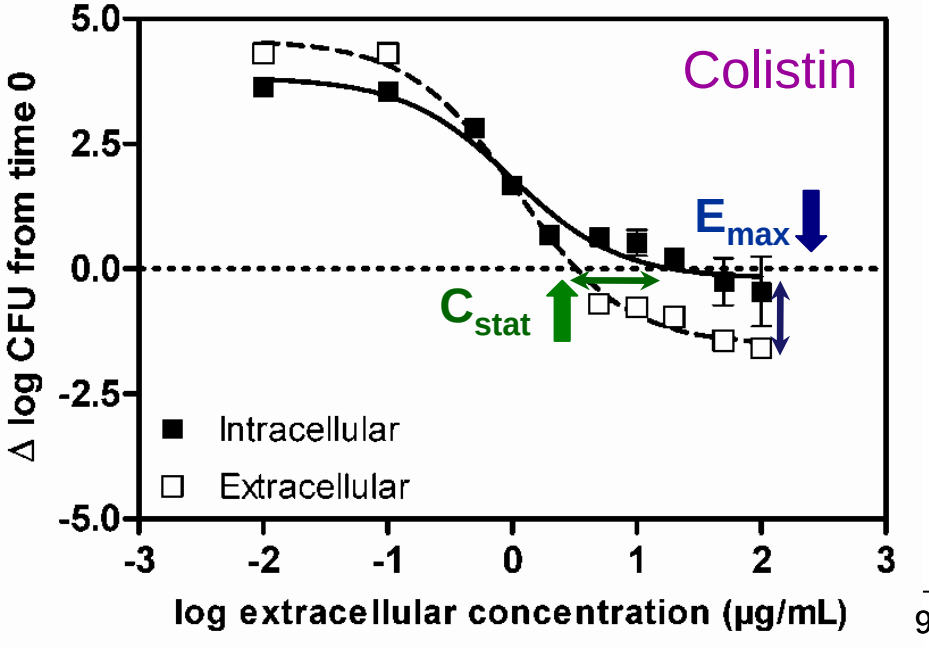


	MIC	E_{max}	C_{static}(xMIC)
Extracellular	0.5	**-3.95** ± 0.41	**0.96**
Intracellular	-	**-1.62** ± 0.19	**22.95**


Tobramycin
Δ log CFU from time 0
5.0
2.5
0.0
-2.5
-5.0
-3
-2
-1
0
1
2
3
log extracellular concentration (µg/mL)
C stat
E max
Intracellular
Extracellular

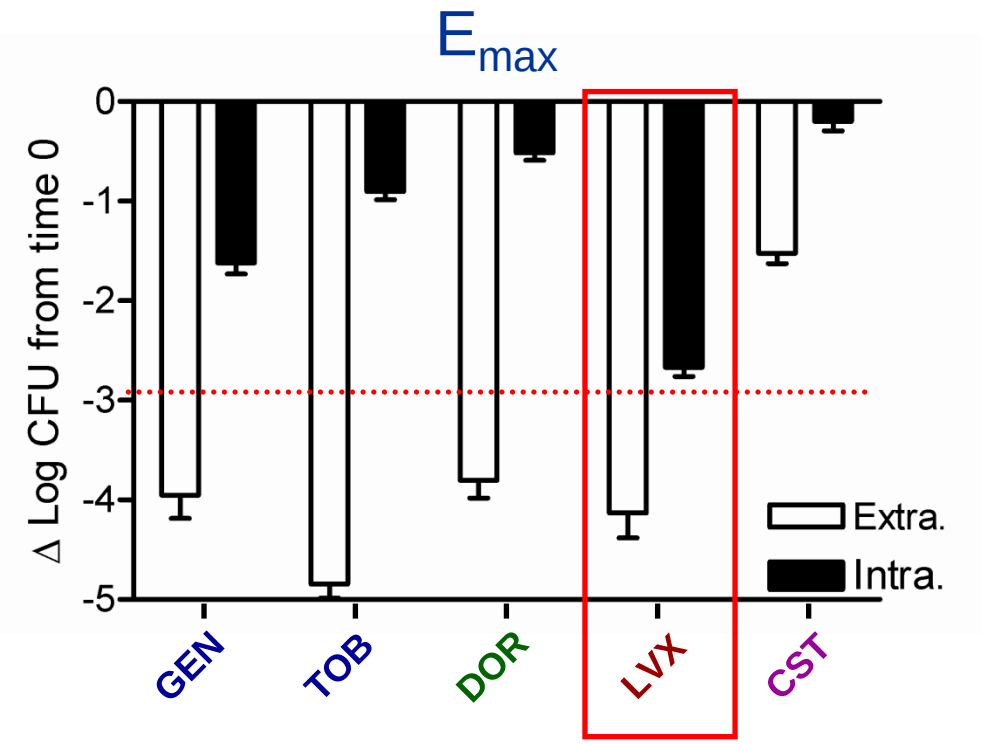

Doripenem
Δ log CFU from time 0
5.0
2.5
0.0
-2.5
-5.0
-3
-2
-1
0
1
2
3
log extracellular concentration (µg/mL)
C stat =
E max
Intracellular
Extracellular

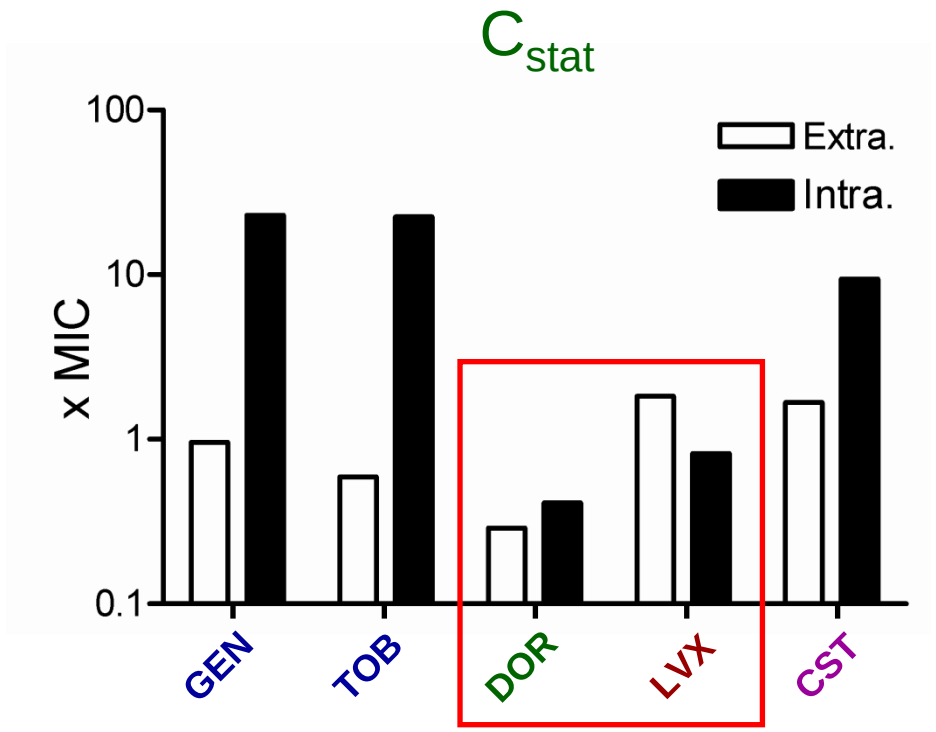

Levofloxacin
Bactericidal
Δ log CFU from time 0
5.0
2.5
0.0
-2.5
-5.0
-3
-2
-1
0
1
2
3
log extracellular concentration (µg/mL)
C stat =
E max
Intracellular
Extracellular


Colistin
Δ log CFU from time 0
5.0
2.5
0.0
-2.5
-5.0
-3
-2
-1
0
1
2
3
log extracellular concentration (µg/mL)
E max
C stat
Intracellular
Extracellular

Summary

- Intracellular *Pseudomonas aeruginosa*

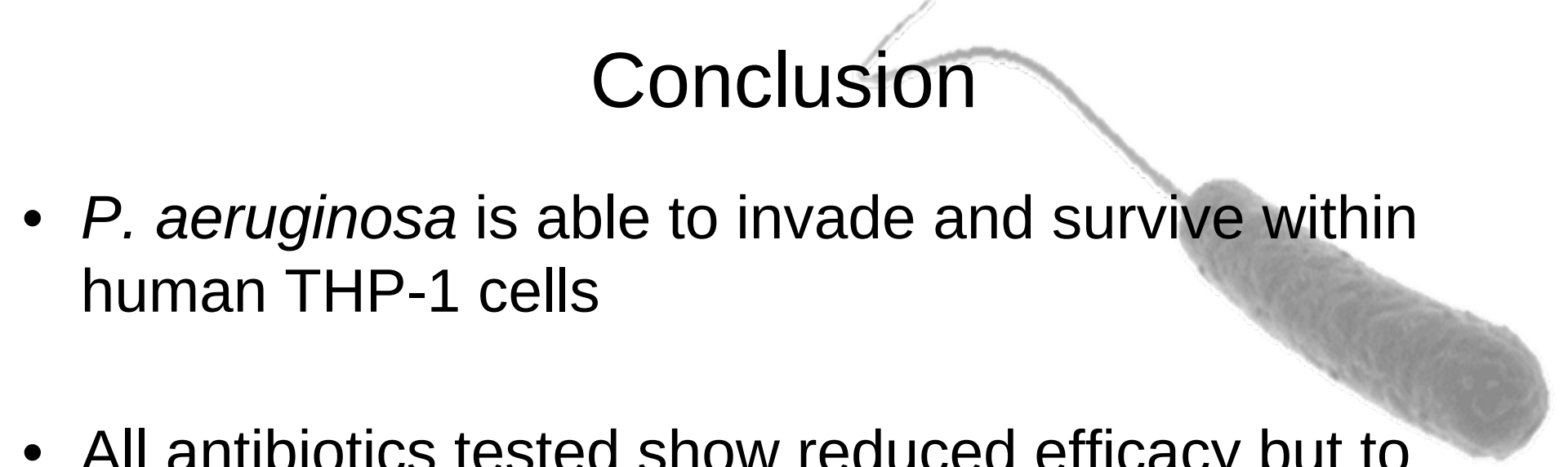




✓ Emax ⬇ for all antibiotics

✓ Cstat ⬆ for GEN, TOB and CST; ~ for DOR, LVX

Conclusion



- *P. aeruginosa* is able to invade and survive within human THP-1 cells
- All antibiotics tested show reduced efficacy but to different extents
- This lower activity may contribute to persistence or recurrence of infection.
- Fluoroquinolones seem of interest for further investigation

Acknowledgments

- *Financial support:*

- *FACM Team:*

Acknowledgments