In vitro models for the study of the activity of anti-infective agents against biofilms

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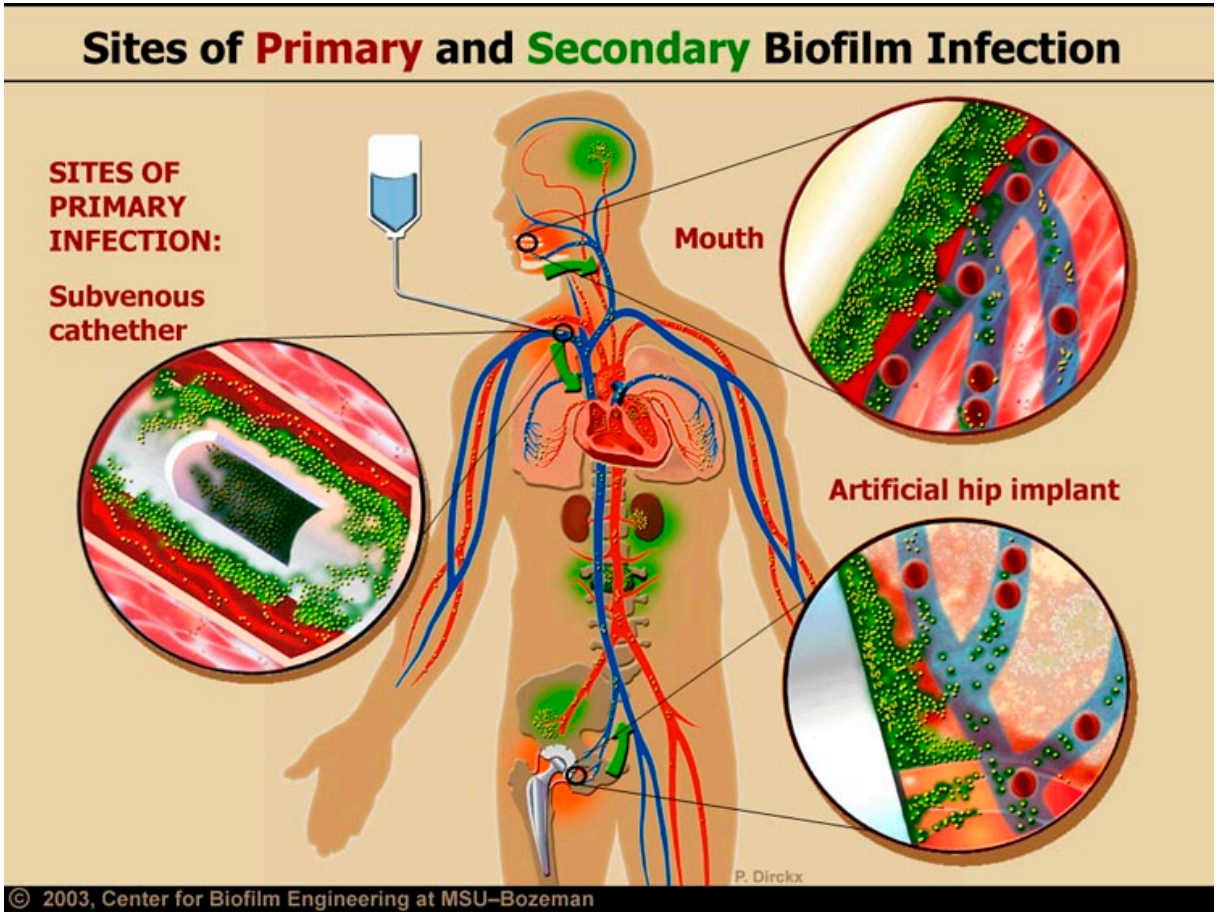
<www.facm.ucl.ac.be>

Biofilms in human infections

Biofilms are associated to 65[a]-80[b] % of human infections and can colonize virtually all organs …



ear
nose
throat
mouth & teeth
eye
lung
heart
kidney
gall bladder
pancreas
nervous system
skin
bone
***
implanted medical devices

[a]*CDC 1999;* [b]*Lewis et al, Nat Rev Microbiol. 2007; 5:48-56*

Antibiotics and biofilms in clinical practice

Curr Opin Otolaryngol Head Neck Surg. 2013 Nov 22. [Epub ahead of print]

When and how should we treat biofilms in chronic sinusitis?

Jain R, Douglas R.

Journal of Endodontics

Volume 39, Issue 5, May 2013, Pages 712–718

Case Report/Clinical Techniques

Exuberant Biofilm Infection in a Lateral Canal as the Cause of Short-term Endodontic Treatment Failure: Report of a Case

Domenico Ricucci, MD, DDS*, Simona Loghin, DDS*, José F. Siqueira Jr., DDS, MSc, PhD†

AAC Journals.ASM.org March 2013 Volume 57 Number 3 Antimicrobial Agents and Chemotherapy p. 1447–1454

Reduced Vancomycin Susceptibility in an *In Vitro* Catheter-Related Biofilm Model Correlates with Poor Therapeutic Outcomes in Experimental Endocarditis Due to Methicillin-Resistant *Staphylococcus aureus*

Wessam Abdelhady,[a] Arnold S. Bayer,[a,b] Kati Seidl,[c] Cynthia C. Nast,[b,d] Megan R. Kiedrowski,[e] Alexander R. Horswill,[e] Michael R. Yeaman,[a,b] Yan Q. Xiong[a,b]

Int J Artif Organs 2011; 34(9): 737-751

REVIEW

Antibiotic-induced biofilm formation

Jeffrey B. Kaplan

Contents lists available at ScienceDirect

Microbial Pathogenesis

Microbial Pathogenesis 51 (2011) 58–68

journal homepage: www.elsevier.com/locate/micpath

Biofilm formation or internalization into epithelial cells enable *Streptococcus pyogenes* to evade antibiotic eradication in patients with pharyngitis

Taiji Ogawa[a,e], Yutaka Terao[a], Hisashi Okuni[b], Keiko Ninomiya[c], Hiroshi Sakata[d], Yoshinobu Maeda[e], Shigetada Kawabata[a,*]

Vol. 41, No. 9

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2003, p. 4043–4048

0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.9.4043–4048.2003

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Biofilm Formation by Group A Streptococci: Is There a Relationship with Treatment Failure?

Joslyn Conley,[1] Merle E. Olson,[2] Linda S. Cook,[1] Howard Ceri,[3] Van Phan,[3] and H. Dele Davies[1,2,4]*

Pathog Dis. 2013 Nov;69(2):142-8. doi: 10.1111/2049-632X.12100. Epub 2013 Oct 7.

The presence of antibiotic-resistant nosocomial pathogens in endotracheal tube biofilms and corresponding surveillance cultures.

Vandecandelaere I, Matthijs N, Nelis HJ, Depuydt P, Coenye T.

→ Treatment failure is not rare…

How to find a solution ?

→ Appropriate models…

In vitro static models

pegs

multiwell plates

Antibiotic activity: planktonic vs. biofilm cultures

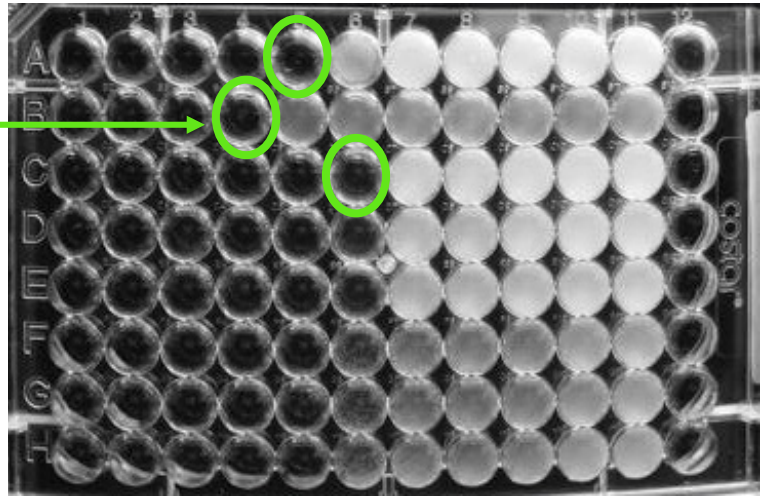
Parameter	Abbreviation	Definition
Minimal inhibitory concentration	MIC	The lowest concentration of an antibiotic that inhibits the visible growth of a planktonic culture after overnight incubation
Minimal biofilm inhibitory concentration	MBIC	The lowest concentrations of an antibiotic that resulted in an OD650 difference at or below 10% (1 Log difference in growth after 6 h of incubation) of the mean of two positive control well readings.
Minimal bactericidal concentration	MBC	The lowest concentration of an antibiotic producing a 99.9% CFUs reduction of the initial inoculum of a planktonic culture.
Biofilm bactericidal concentration	BBC	The lowest concentration of an antibiotic producing a 99.9% reduction of the CFUs recovered from a biofilm culture compared to growth control.
Minimal biofilm eradication concentration	MBEC	The lowest concentration of an antibiotic that prevents visible growth in the recovery medium used to collect biofilm cells.
Biofilm prevention concentration	BPC	Same as MBIC but bacterial inoculation and antibiotic exposure occur simultaneously.

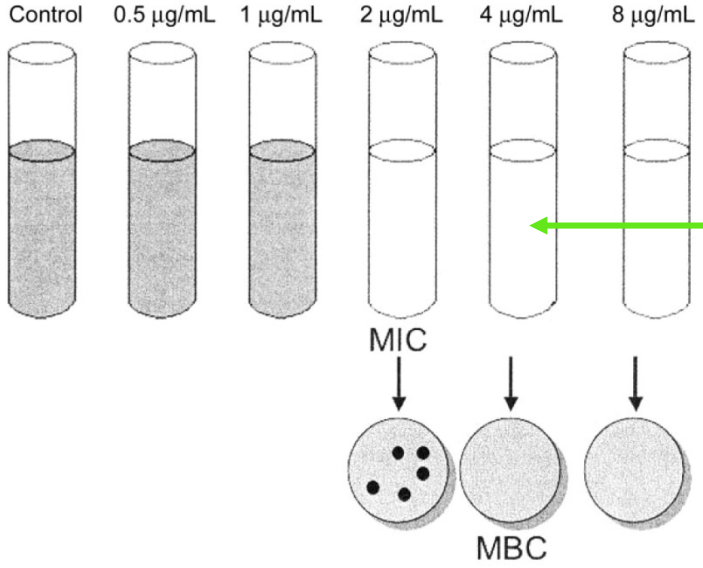
Macià et al, Clin Microbiol Infect. 2014; 20(10):981-9

Determining antibiotic activity against planktonic bacteria

antibiotic conc.

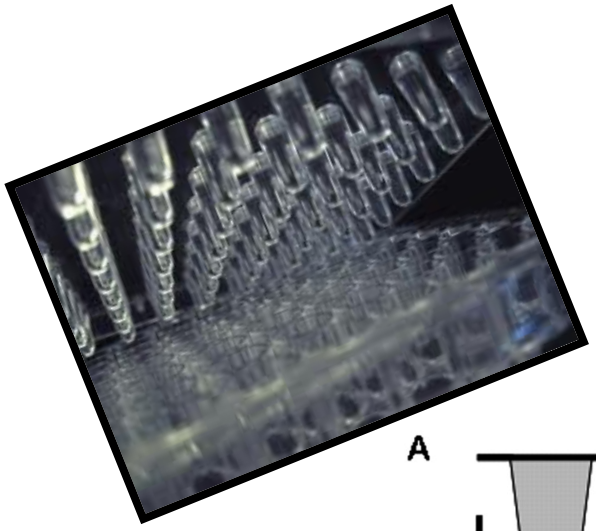
MIC
(minimal inhibitory concentration)



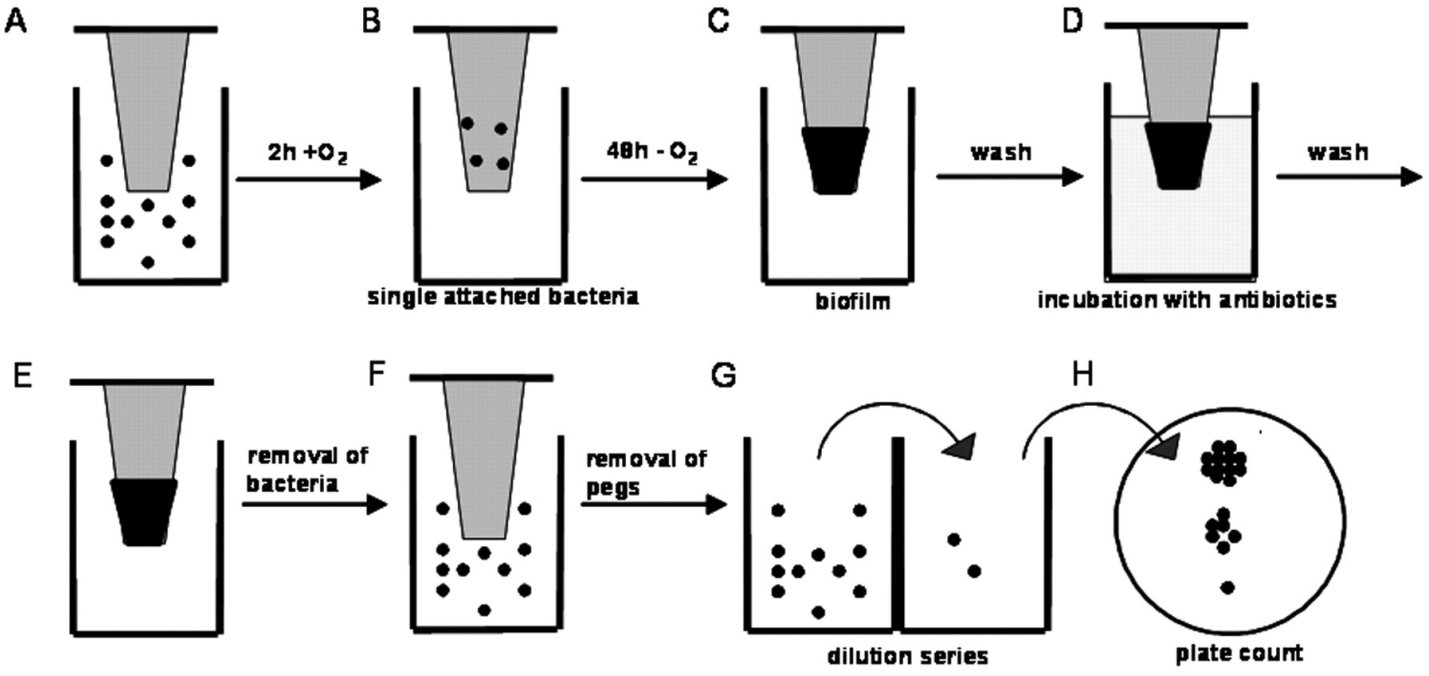


MBC
(minimal bactericidal concentration)

Static models: Calgary Biofilm Device



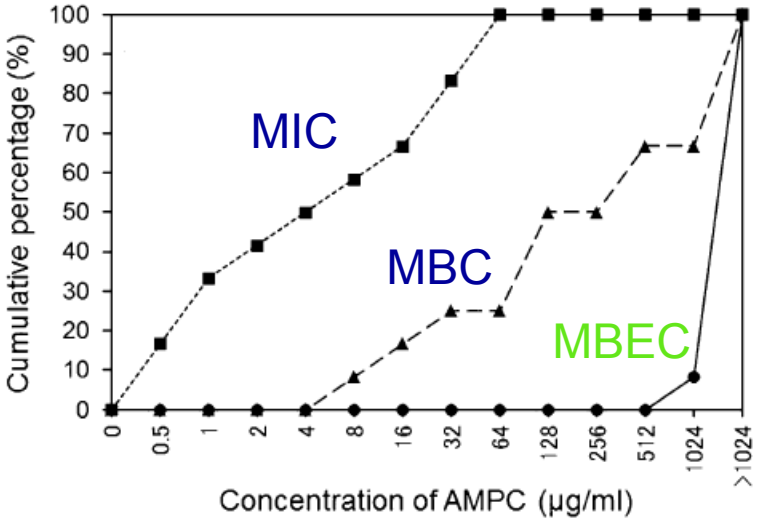
Determination of
Minimal Biofilm Eradication Concentration
(MBEC)

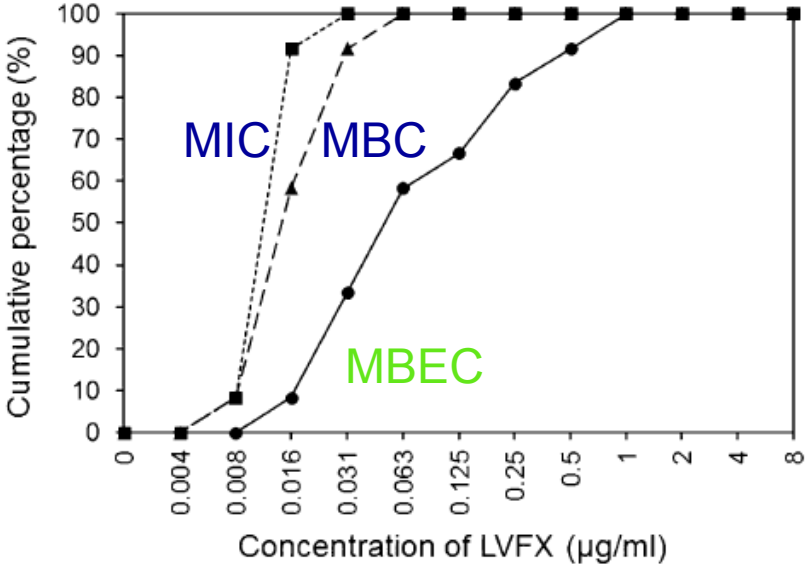


Ceri et al, J. Clin. Microbiol. 1999; 37:1771-6; Herrmann et al, J Infect Dis. 2010;202:1585-92

Comparing antibiotic activity: planktonic / biofilm cultures

Ampicillin and levofloxacin vs. *H. influenzae* from middle ear fluid

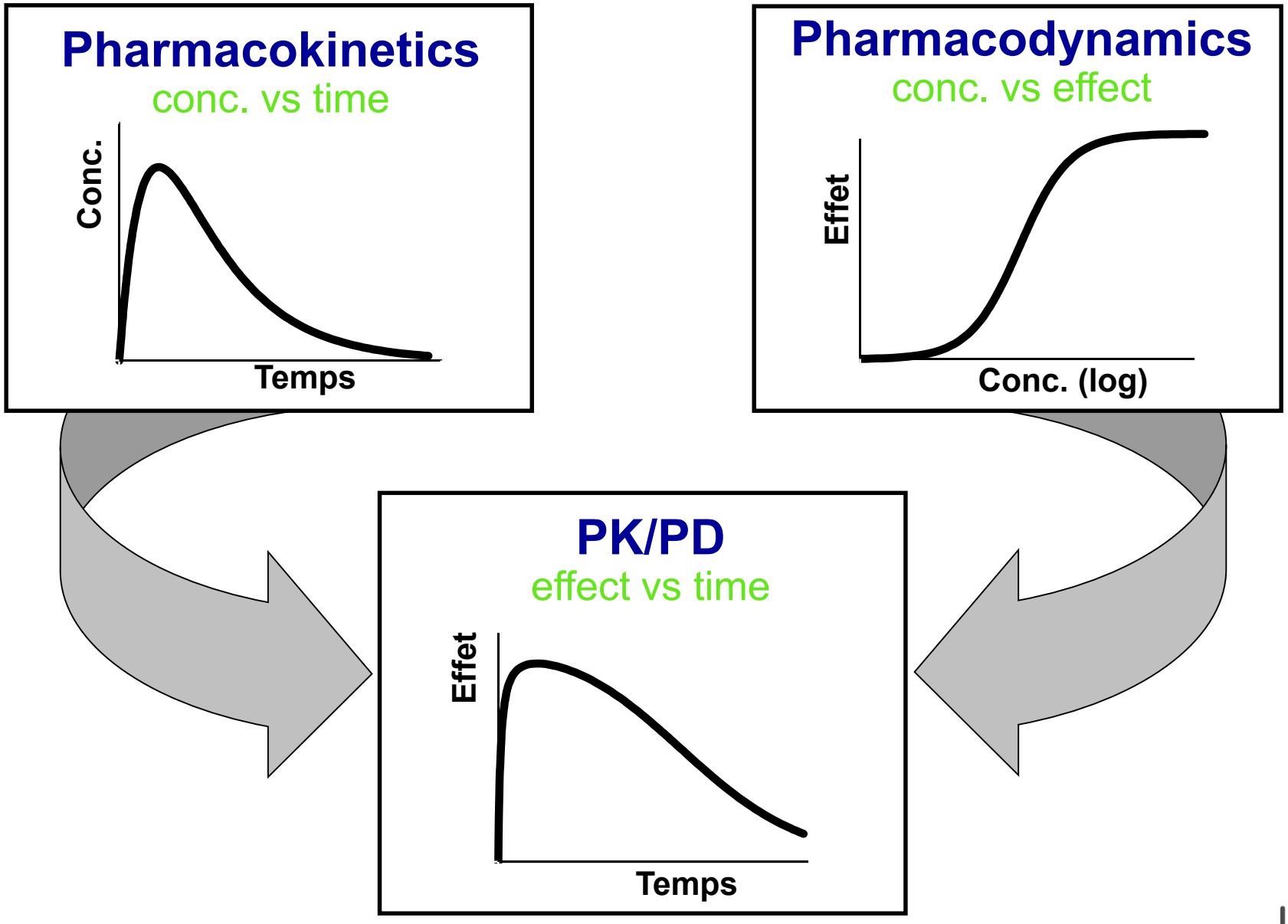




Activity against biofilm << activity against planktonic bacteria

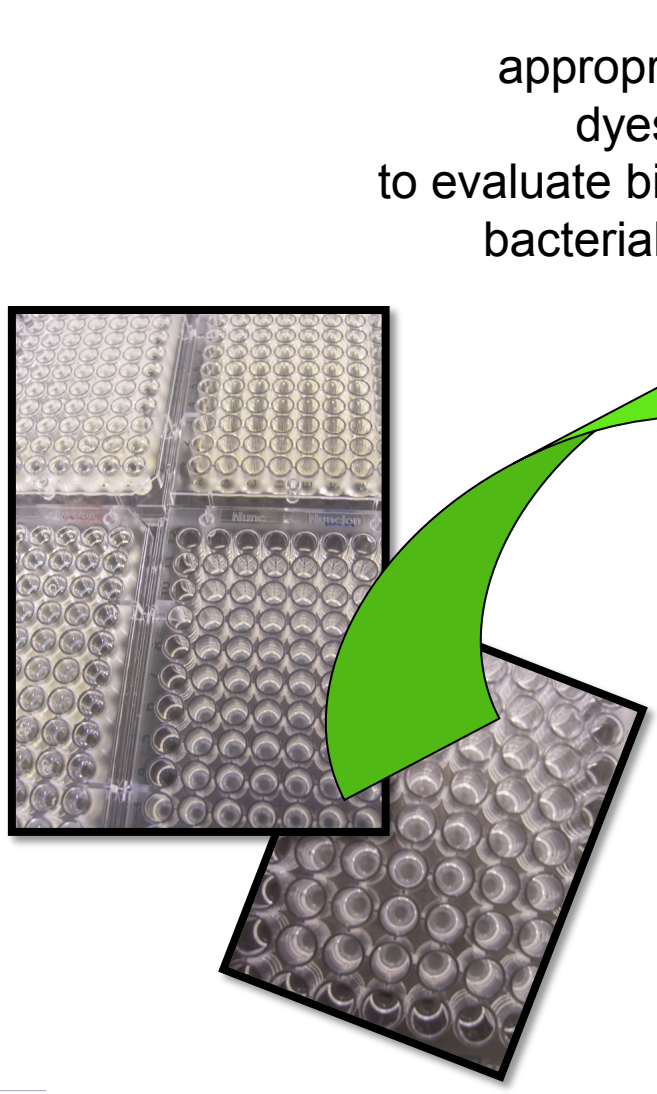
Takei et al, J Infect Chemother 2013; 19:504–9

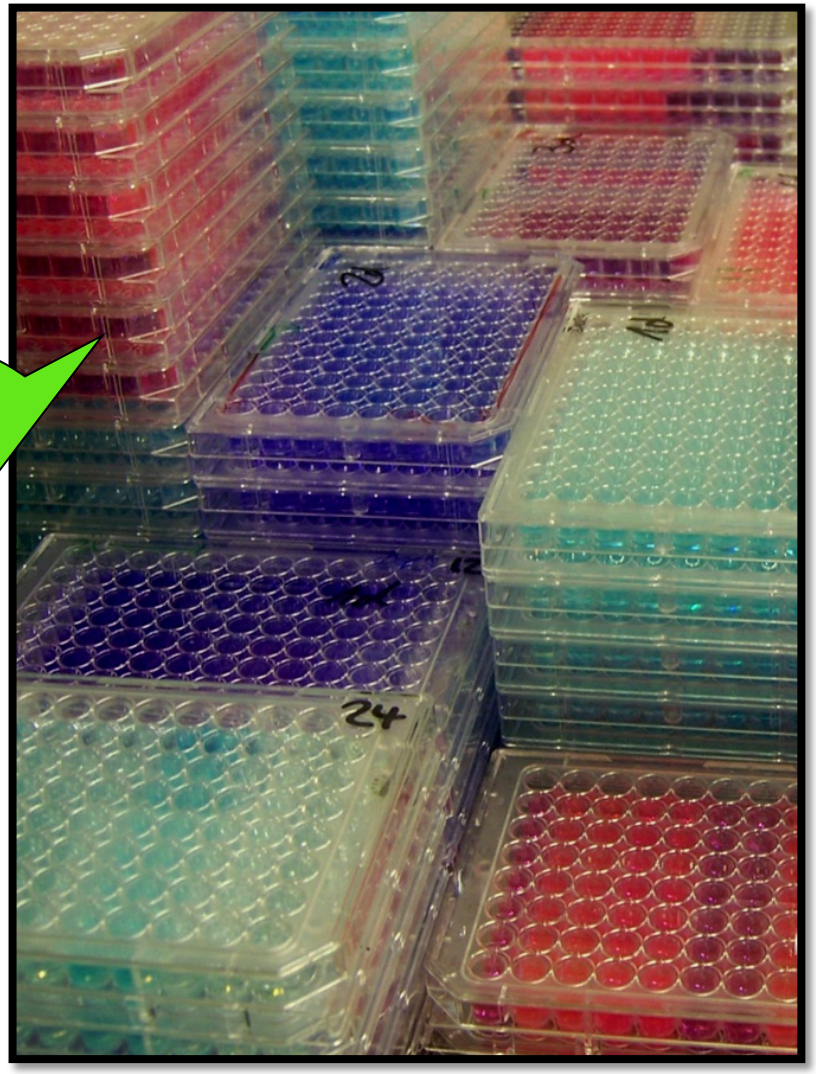
PK/PD studies: the principles



Static models: 96-well polystyrene plates

appropriate
dyes
to evaluate biomass or
bacterial load

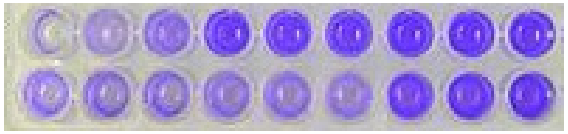


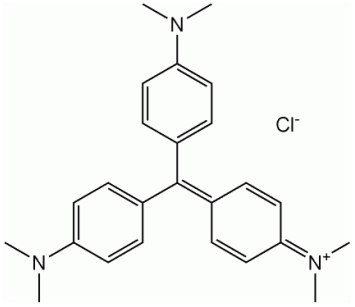


Quantifying biomass and metabolic activity in biofilms

biofilm mass

crystal violet



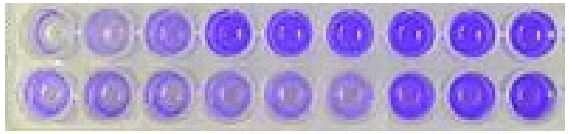


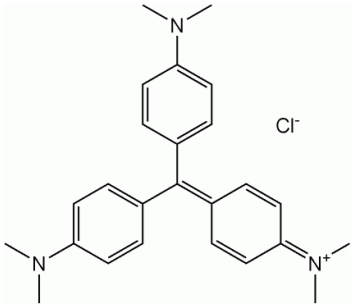
Christensen et al, Infect. Immun. 1982; 37:318–26

Quantifying biomass and metabolic activity in biofilms

biofilm mass

crystal violet



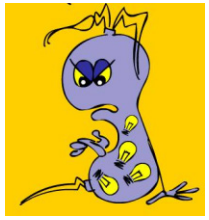


Christensen et al, Infect. Immun. 1982; 37:318–26

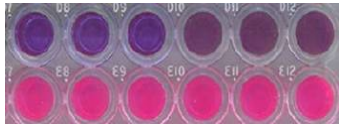
Gram(+) bacteria

metabolic activity

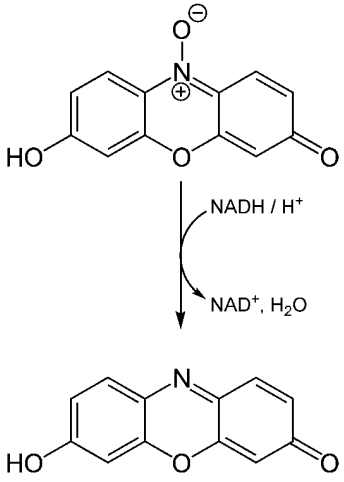
Gram(-) bacteria



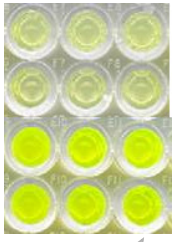
resazurin



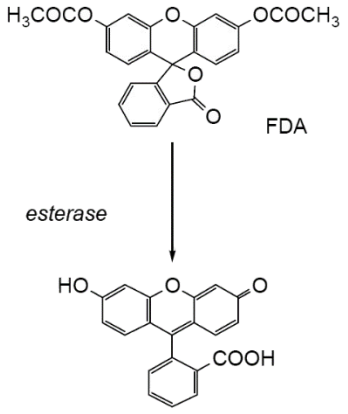
resorufin



fluorescein diacetate



fluorescein

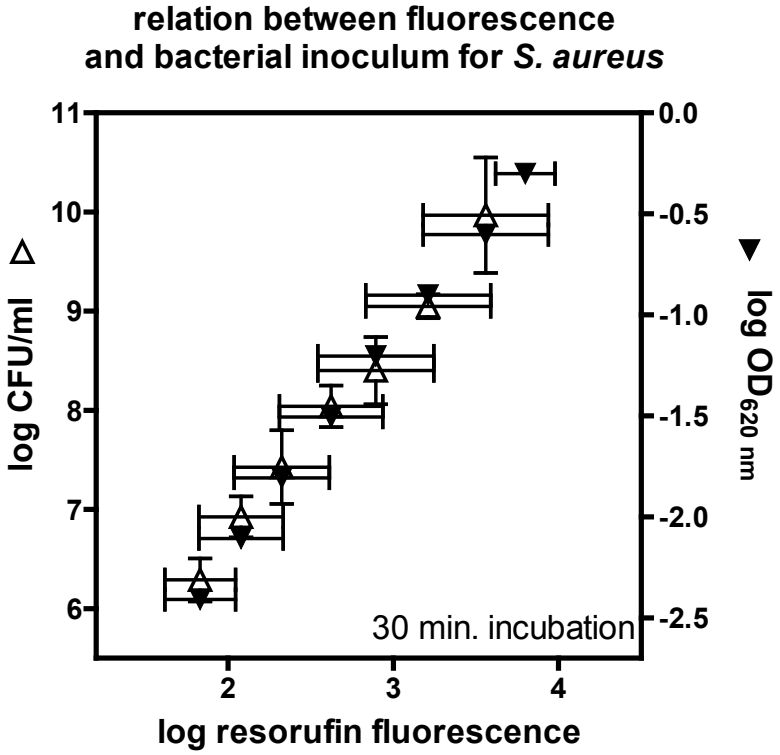


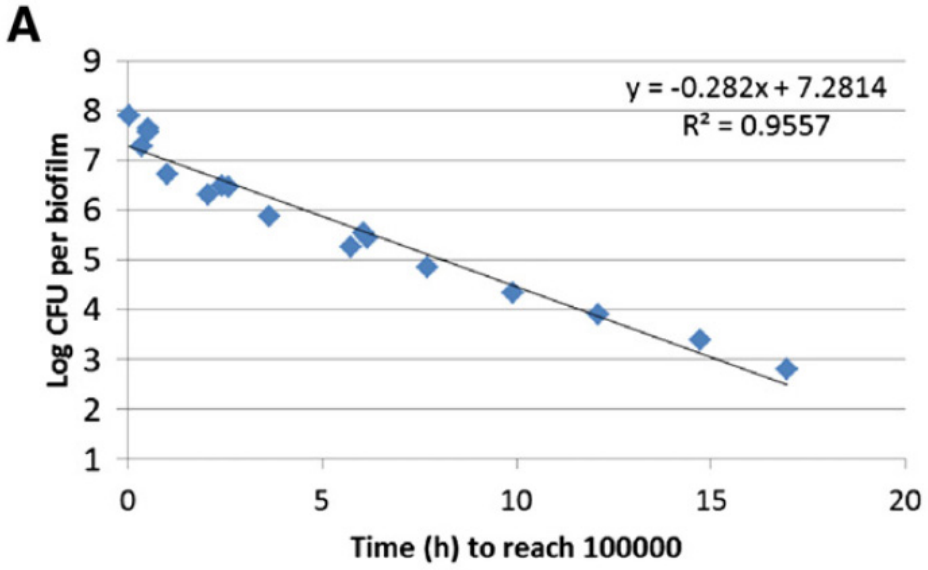
Tote et al, 2008; Lett. Appl. Microbiol. 46:249–254

Wanandy et al, J Microbiol Methods 2005;60:21-30

CFU counting *vs.* RF fluorescence

An example for *S. aureus*





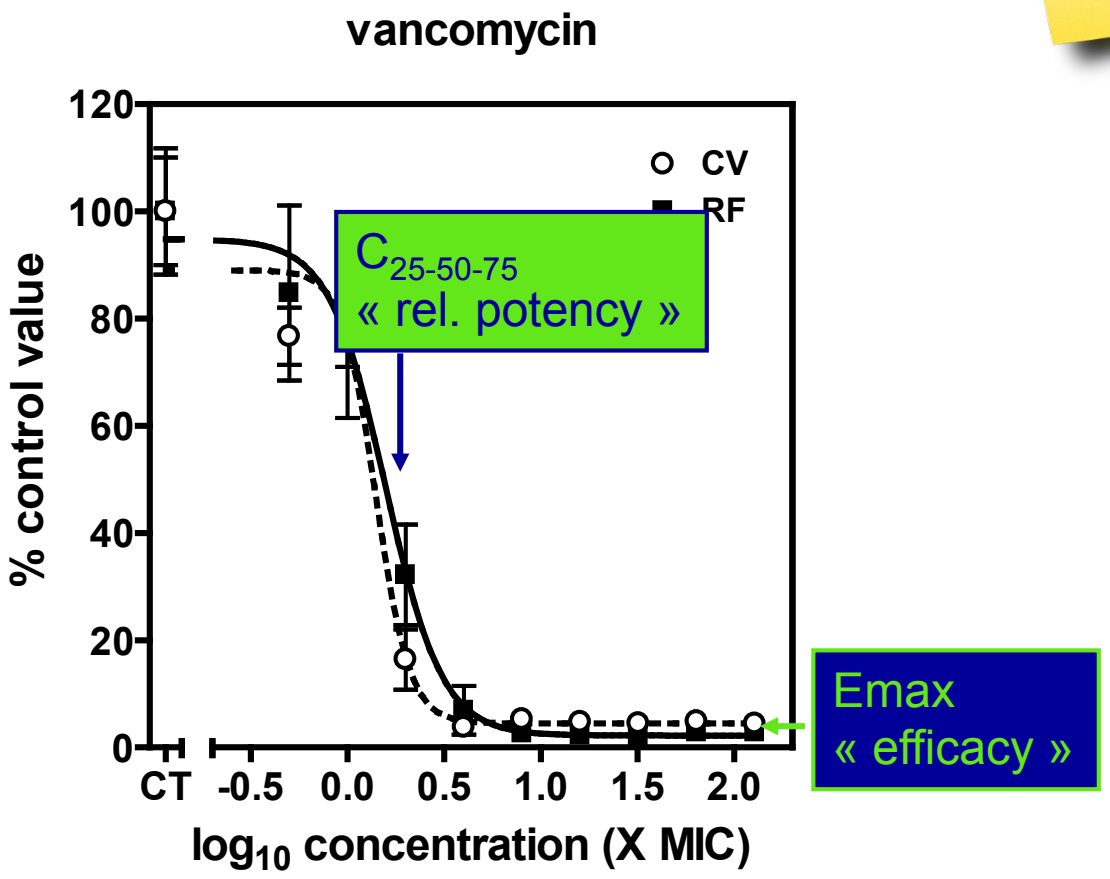
CFU & RF signal proportional

sensitivity depending on incubation time

Pharmacodynamic model for antibiotic activity

An example with young biofilm of *S. aureus*

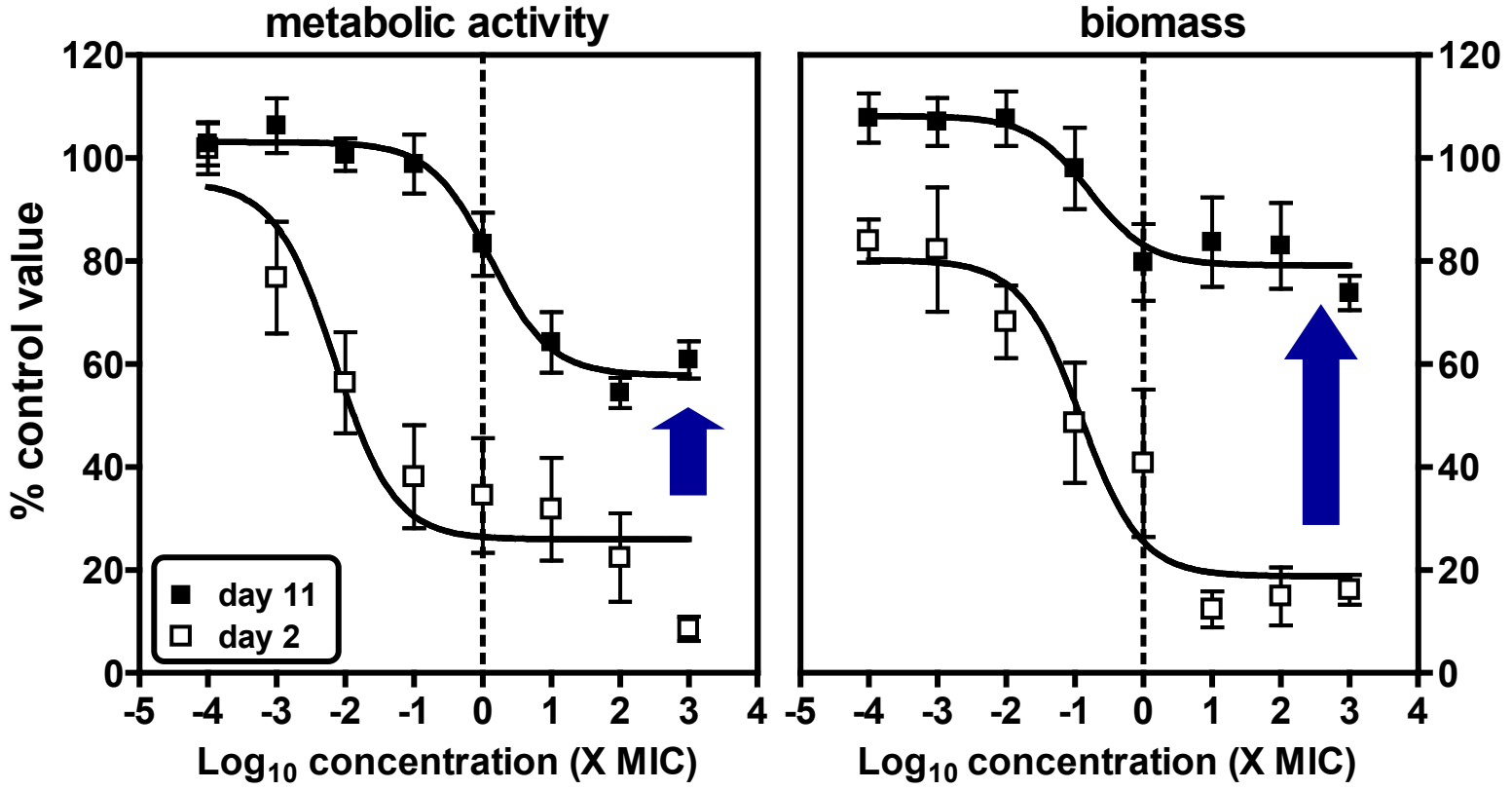
See also poster by Diaz Iglesias *et al.*



Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37

S. pneumoniae biofilms - influence of maturity

moxifloxacin

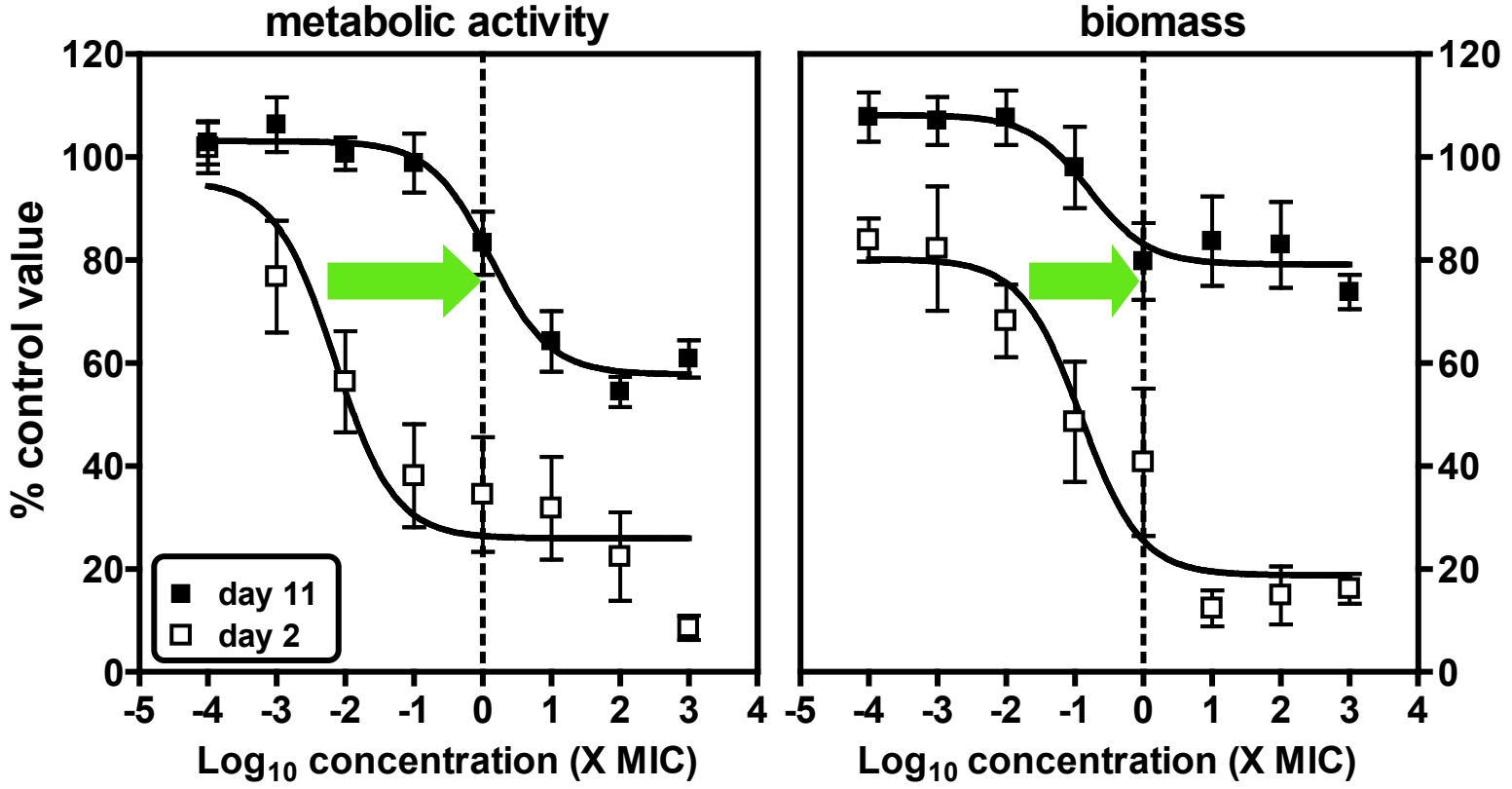


maximal efficacy ⇘ with maturity

Vandevelde et al, Antimicrob Ag Chemother. 2014; 58:1348-58

S. pneumoniae biofilms - influence of maturity

moxifloxacin

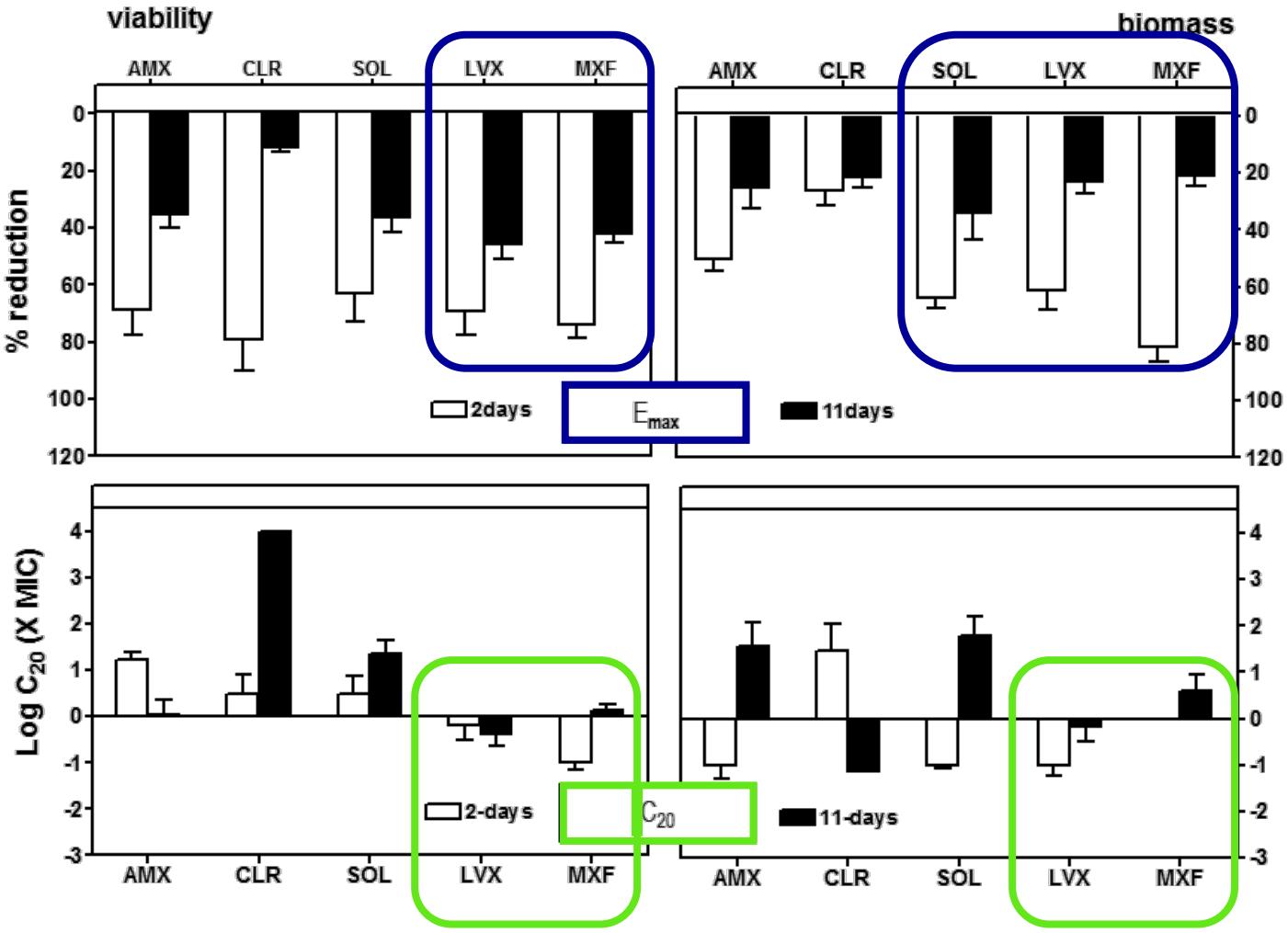


relative potency ⇘ with maturity

Vandevelde et al, Antimicrob Ag Chemother. 2014; 58:1348-58

Comparison of PD parameters for different drugs

S. pneumoniae

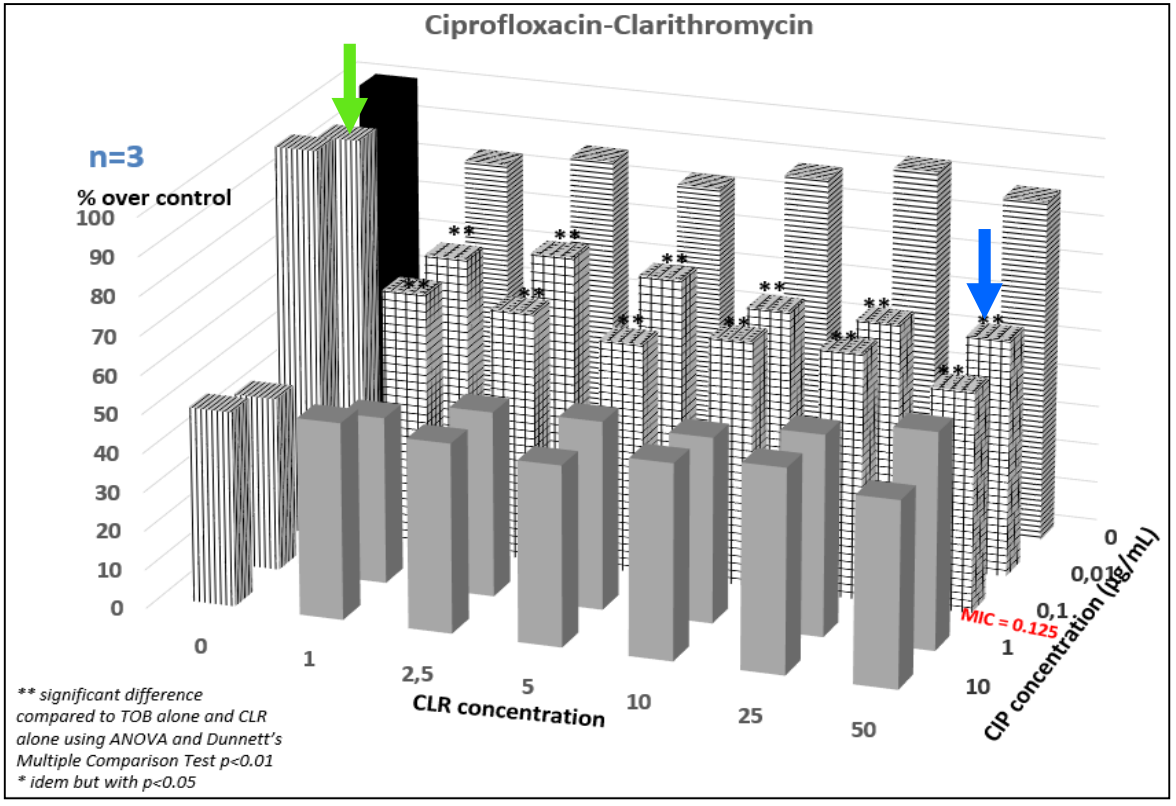


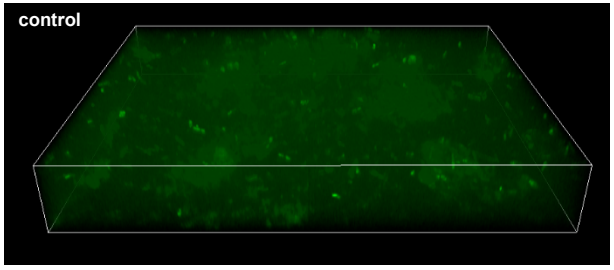
Vandevelde et al, Antimicrob Ag Chemother. 2014; 58:1348-58

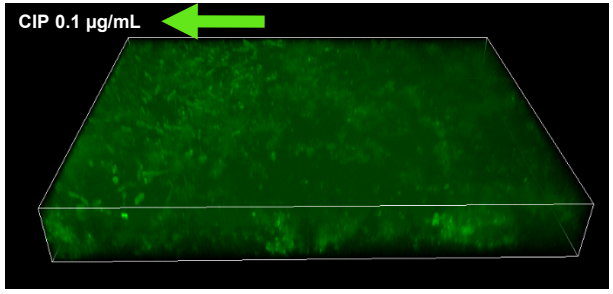
Study of drug combinations

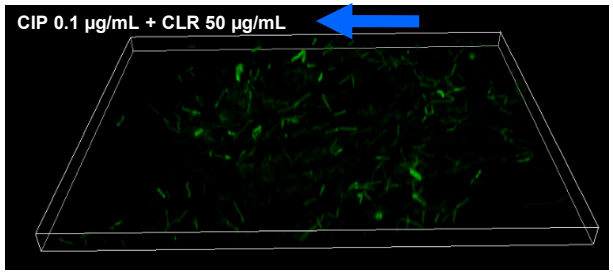
See also poster by Mustafa et al.

P. aeruginosa exposed to ciprofloxacin + clarithromycin







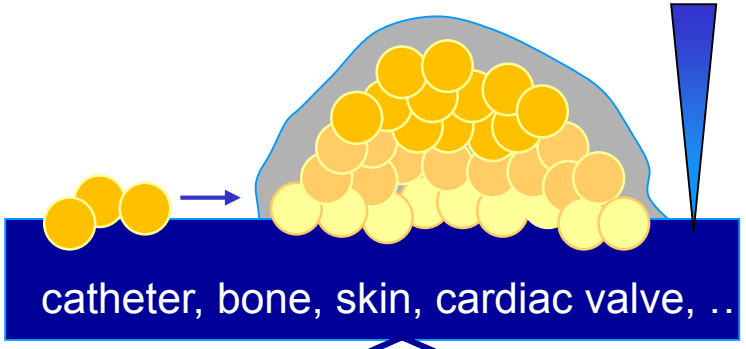


Macrolides are synergistic with fluoroquinolones on preformed biofilms

How to explain this “apparent” resistance or tolerance?

→importance of PK/PD parameters

PK/PD parameters in biofilms

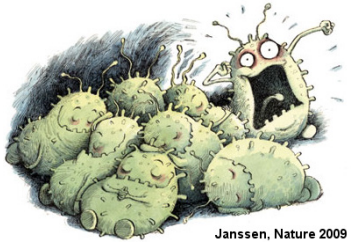


pharmacokinetics

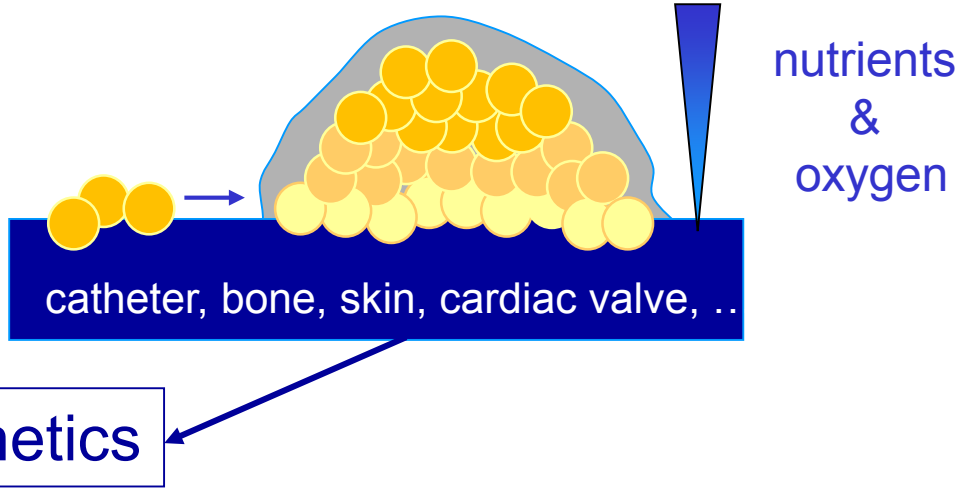
- diffusibility through the matrix
- bioavailability within the biofilm
- access to bacteria
- efflux out of bacteria

pharmacodynamics

- bacterial responsiveness (metabolic activity of bacteria)
- antibiotic expression of activity (local environment [O_2, pH, ..])



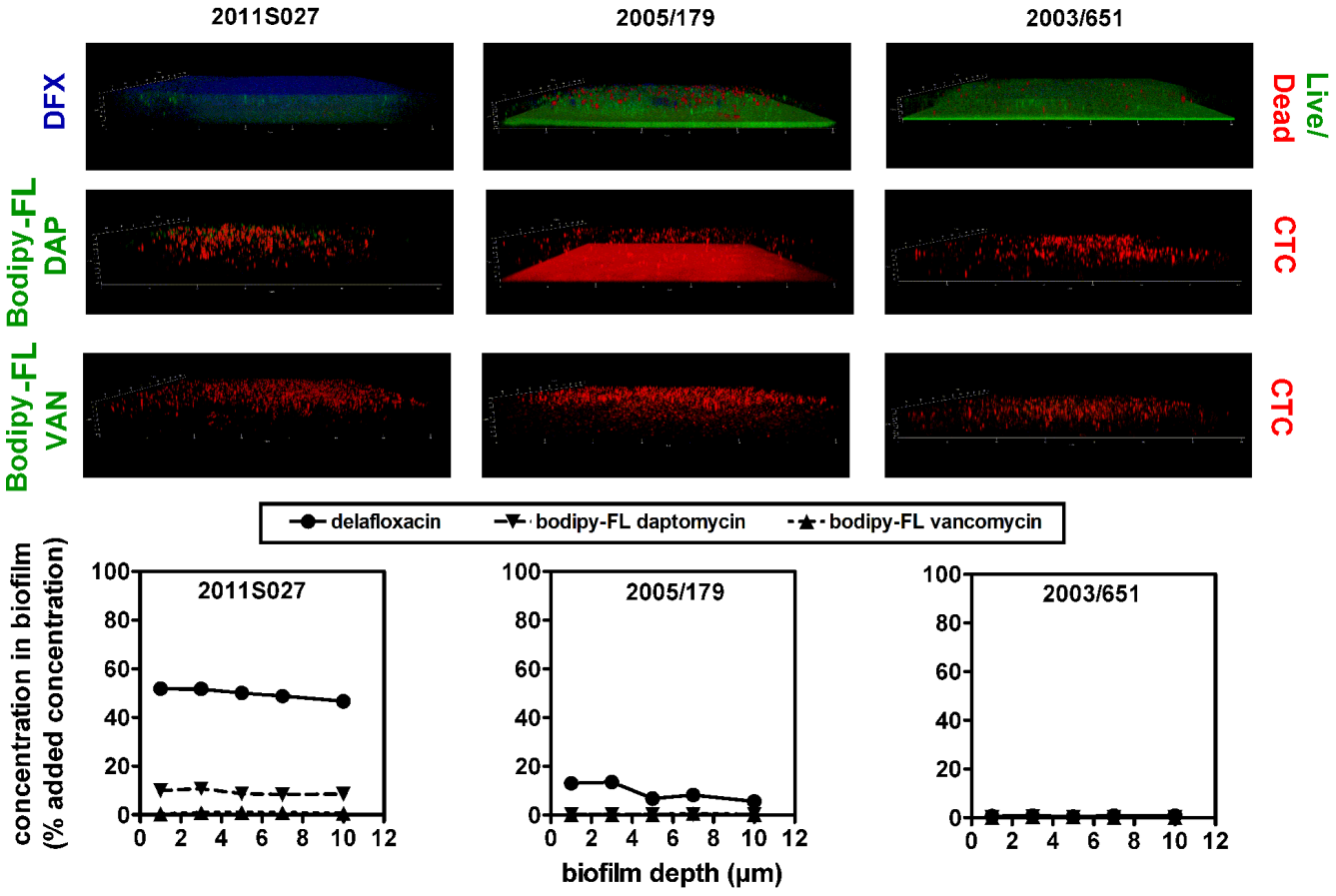
PK/PD parameters in biofilms

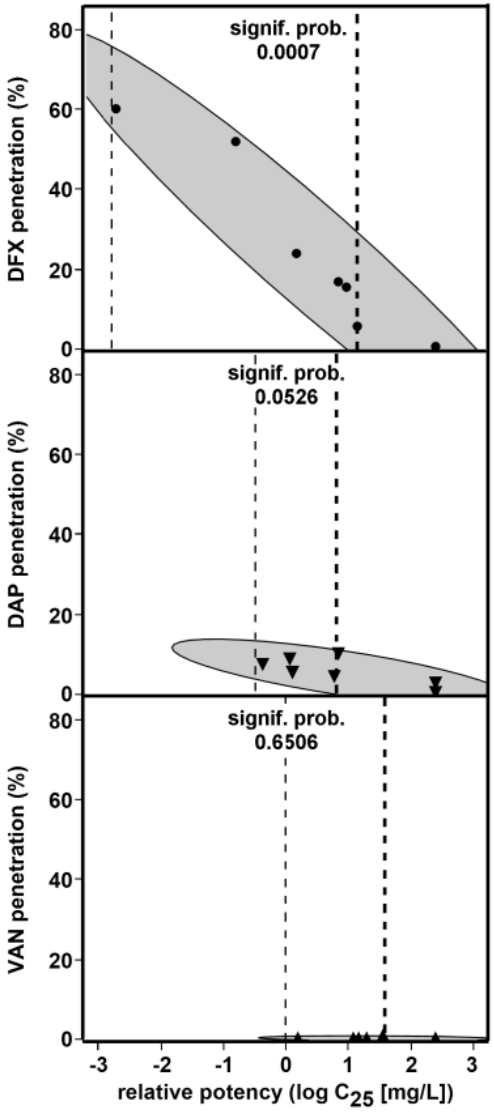


- diffusibility through the matrix
- bioavailability within the biofilm
- access to bacteria
- efflux out of bacteria

Importance of antibiotic concentration inside biofilms for activity

S. aureus biofilms





Activity in biofilm is correlated to antibiotic penetration

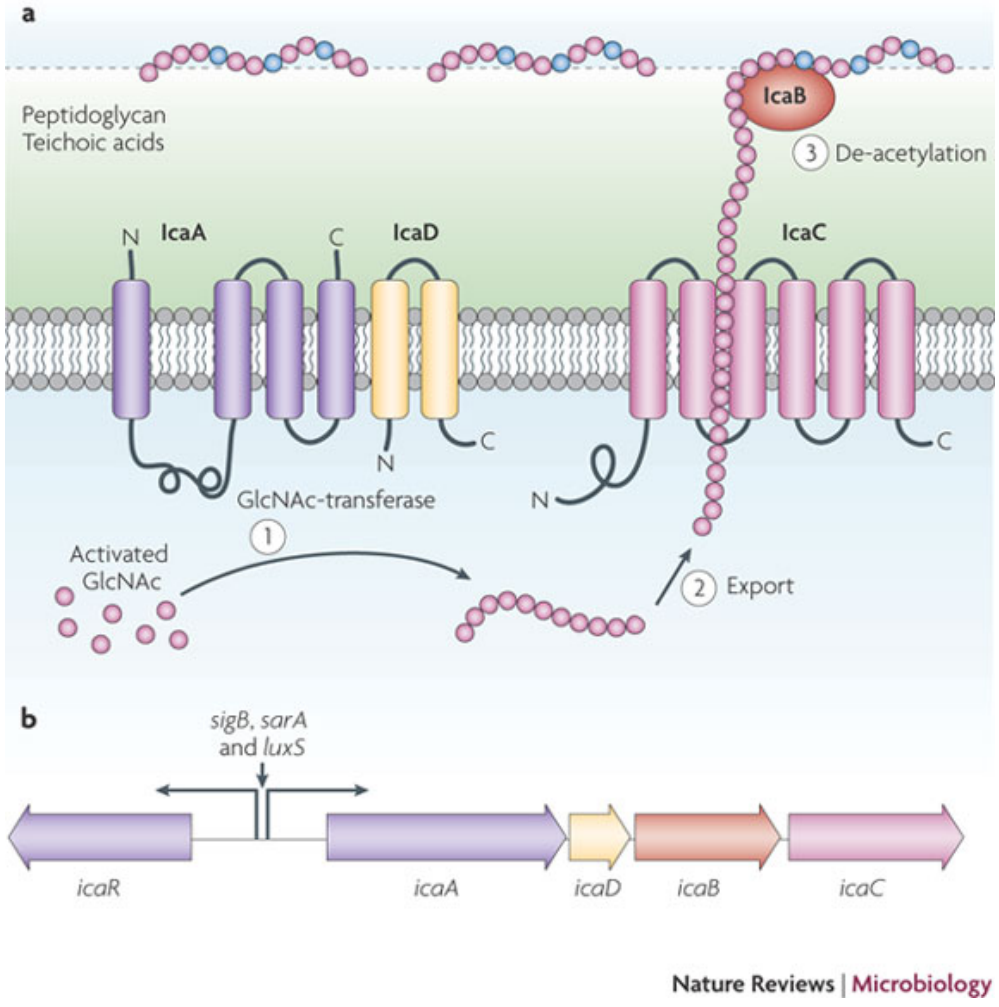
Siala et al, Antimicrob. Ag. Chemother. 2014, 58: 6385–6397.

How to help antibiotic to reach their target ?

→disruption of the matrix

IacA and polysaccharide synthesis in *S. aureus*

Ica A is involved in N-acetylglucosamine homopolymer synthesis

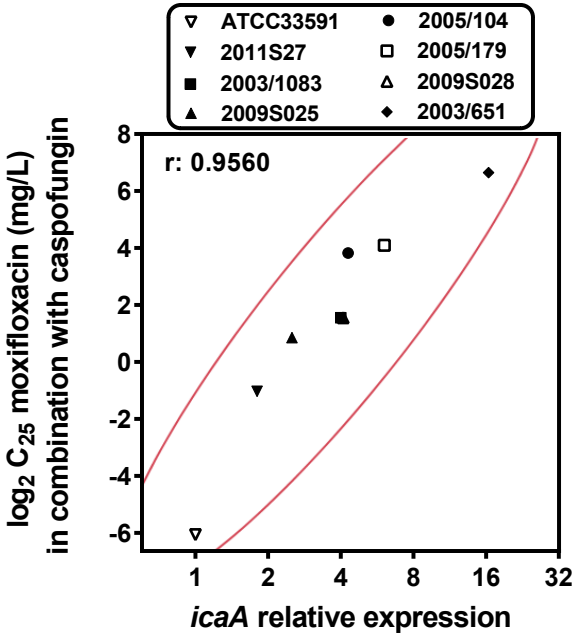


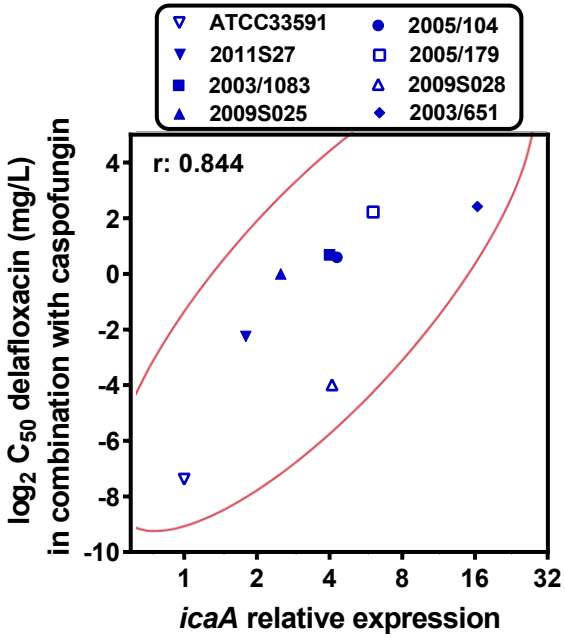
Otto et al., Nat. Rev. Microbiol. 2009; 7:555-67

Importance of *icaA* expression and PNAG abundance for antibiotic activity in biofilms

S. aureus biofilms

strain	*icaA* expression
ATCC33591	1
2011S027	1.8 ± 0.5*
2003/1083	4.0 ± 0.6 *
2009S025	2.5 ± 0.5*
2005/104	4.2 ± 0.4*
2005/179	6.0 ± 0.9*
2009S028	4.1 ± 0.2*
2003/651	16.3 ± 0.7*

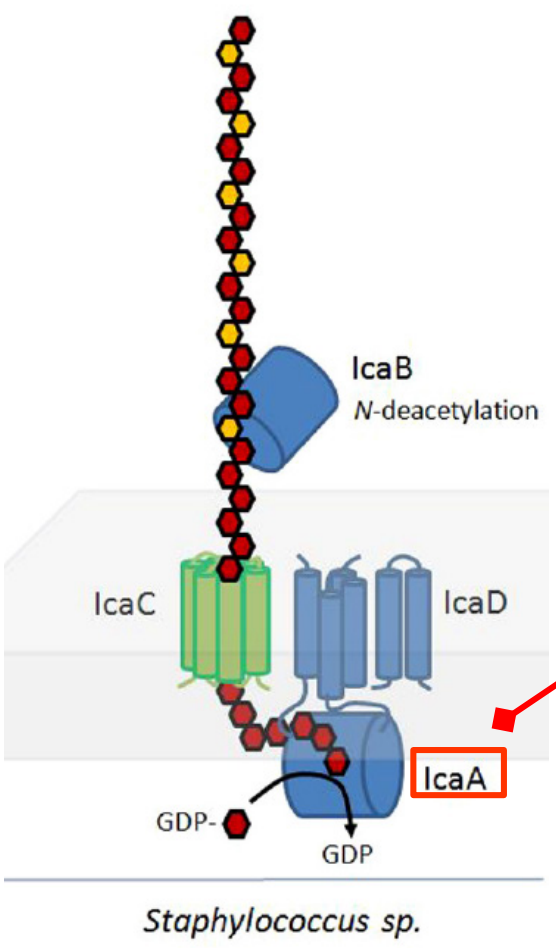


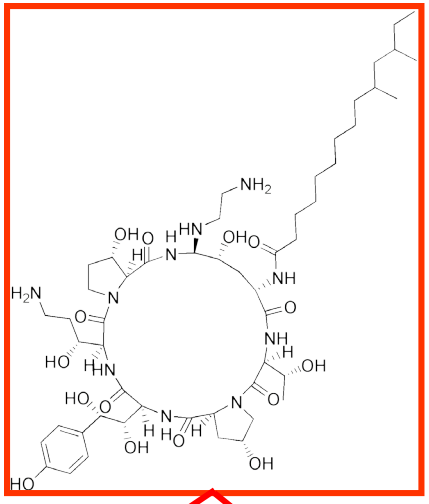


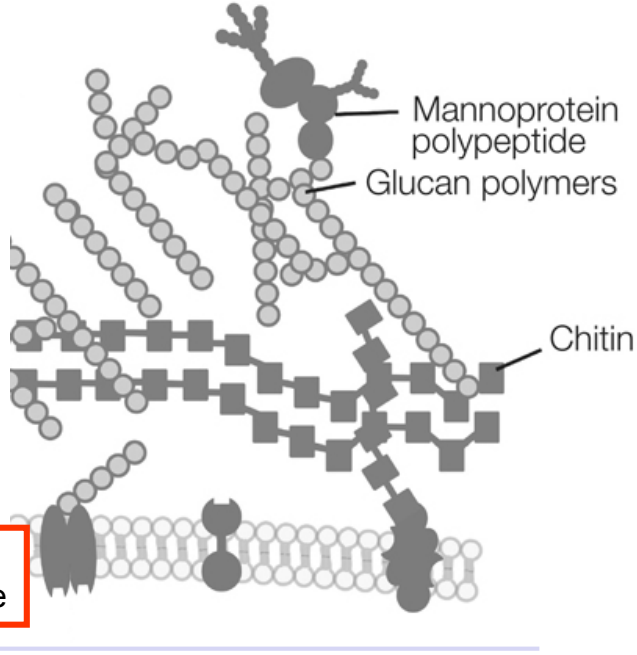
Fluoroquinolone activity in biofilm is inversely correlated with *icaA* expression

Siala et al, in the press

The antifungal caspofungin as an inhibitor of polysaccharide synthesis







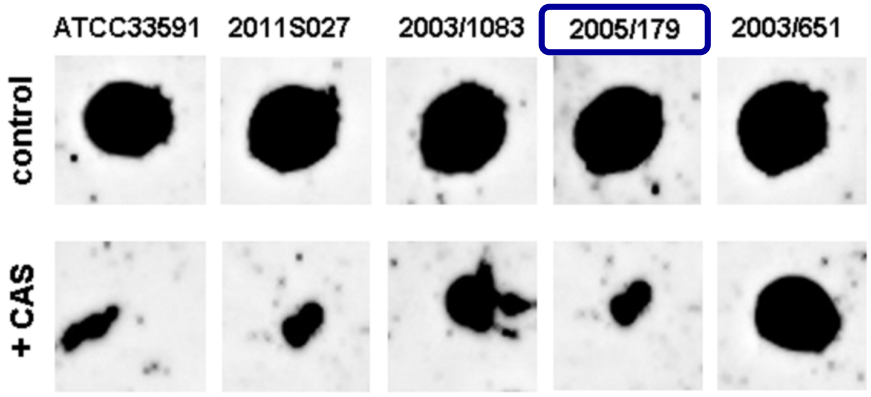
Atkin et al, FEBS Lett. 2014;588:1869-72

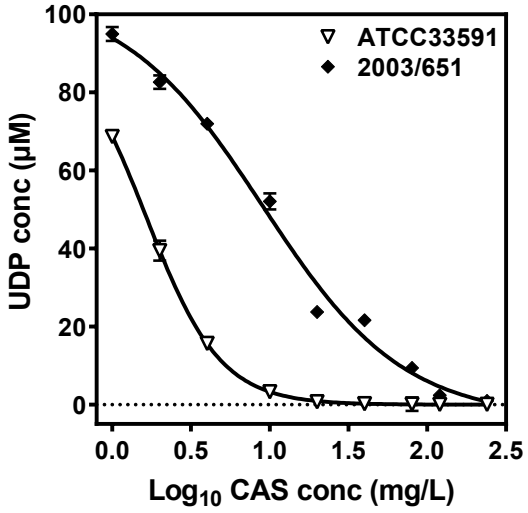
Adapted from Arnold, Kucer's 6the edition

Siala et al, in the press

Inhibition of IcaA by caspofungin increases fluoroquinolone penetration in biofilms

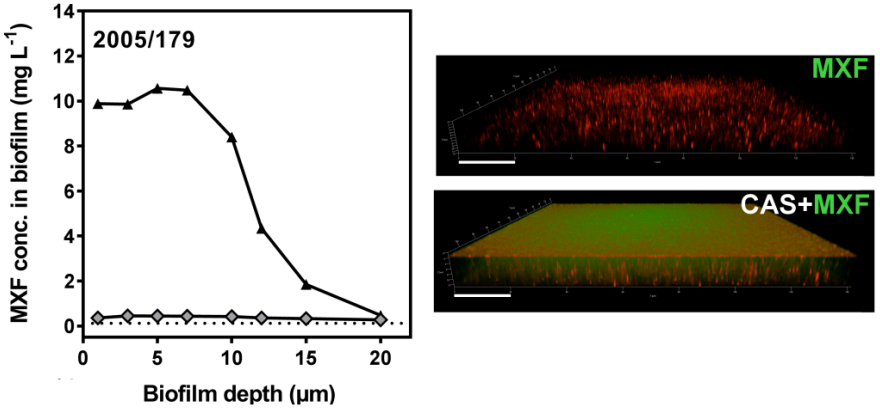
S. aureus biofilms

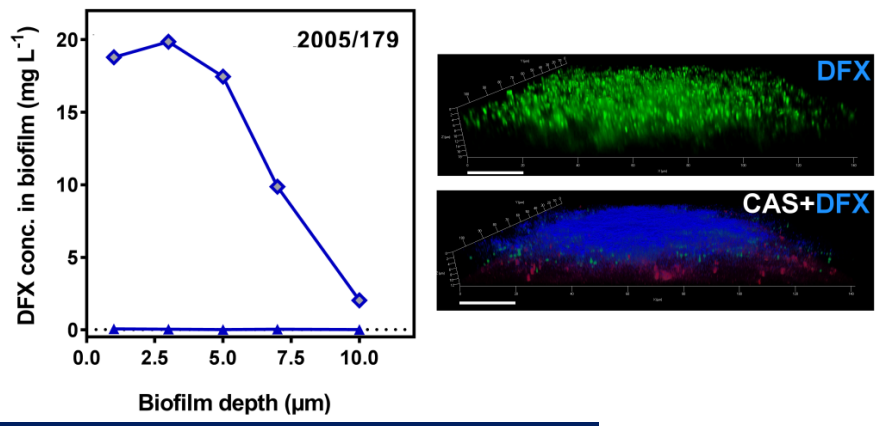




CAS reduces PNAG in the matrix

CAS inhibits IcaA activity



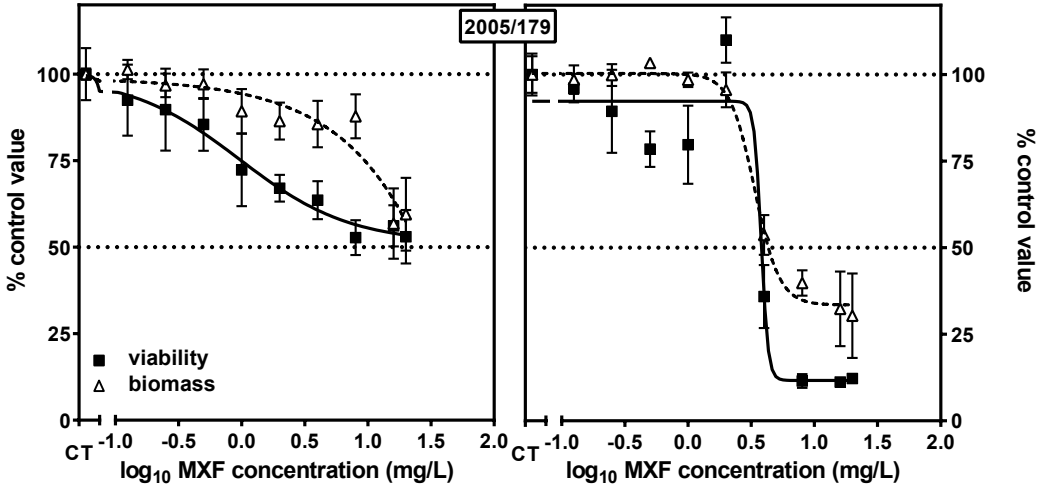


CAS increases fluoroquinolone concentration in biofilms

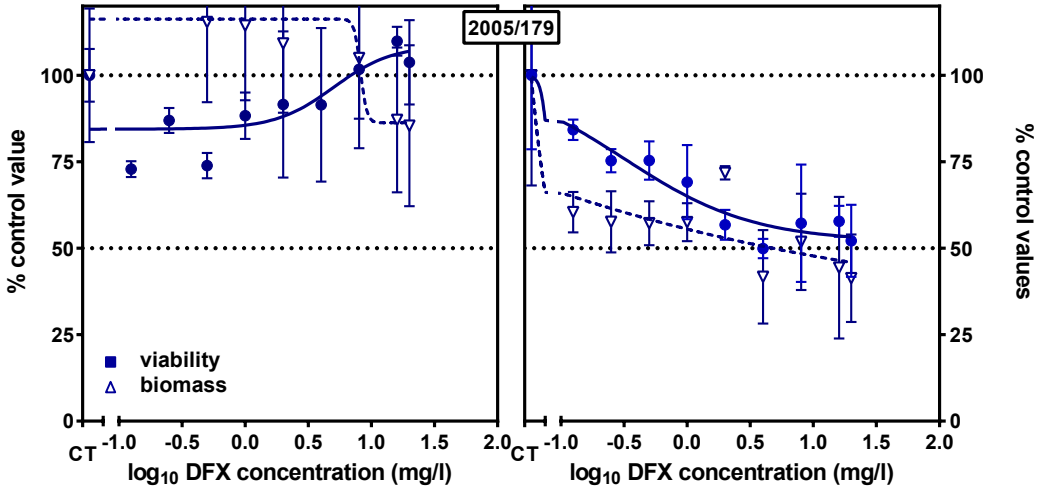
Siala et al, in the press

Inhibition of IcaA by caspofungin increases fluoroquinolone efficacy in biofilms

S. aureus biofilms



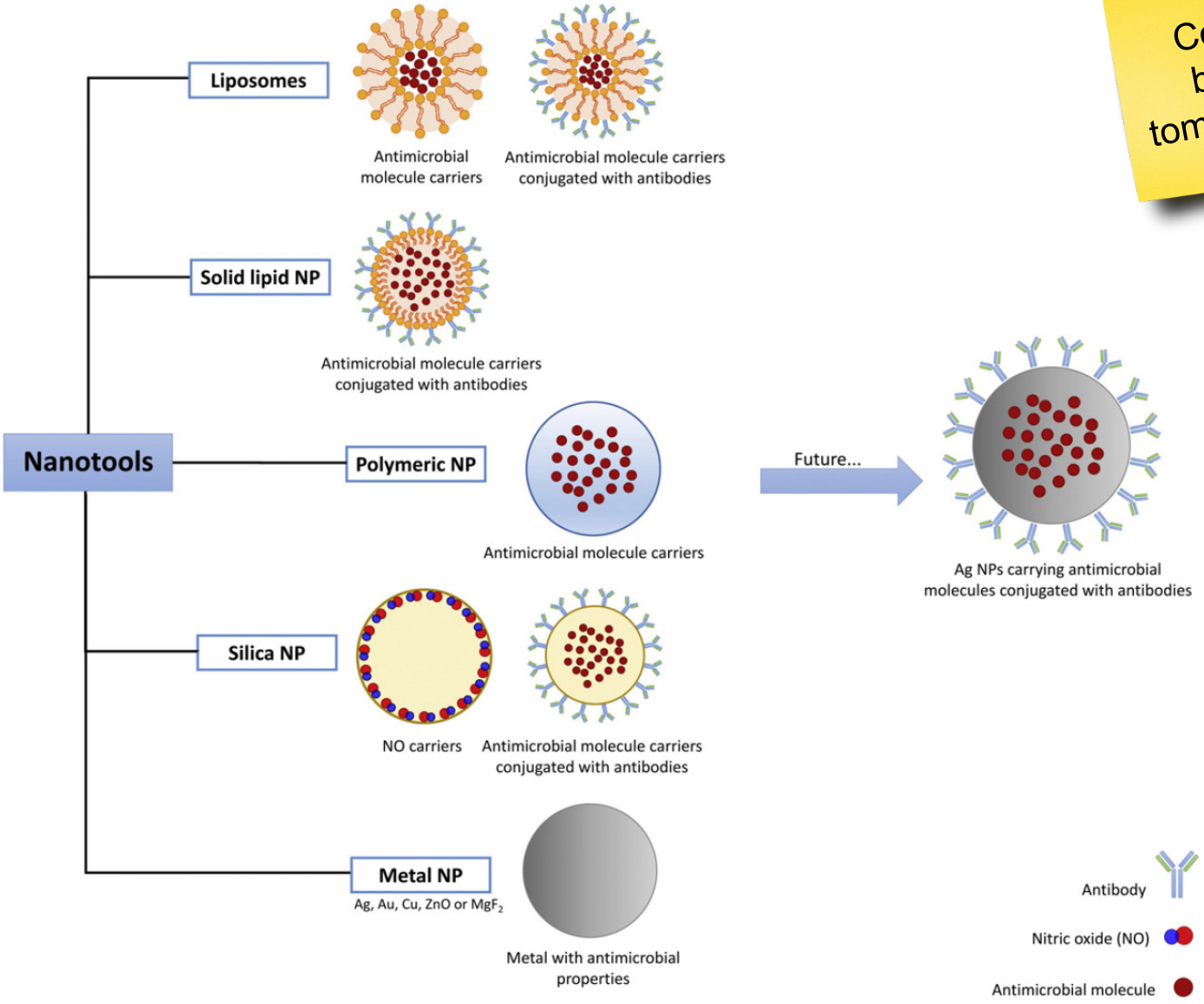
Fluoroquinolone-CAS combinations are highly active !



Siala et al, ICAAC 2014

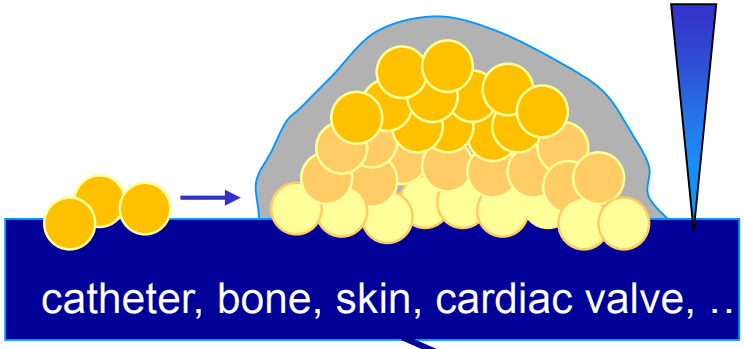
Improving drug delivery thanks to vectors

Come back tomorrow…



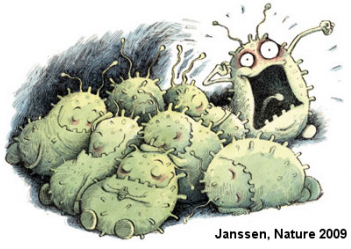
Ribeiro et al. , Pharmacology & Therapeutics 2016; 160:133–144

PK/PD parameters in biofilms



pharmacodynamics

- bacterial responsiveness (metabolic activity of bacteria)
- antibiotic expression of activity (local environment [O_2, pH, ..])

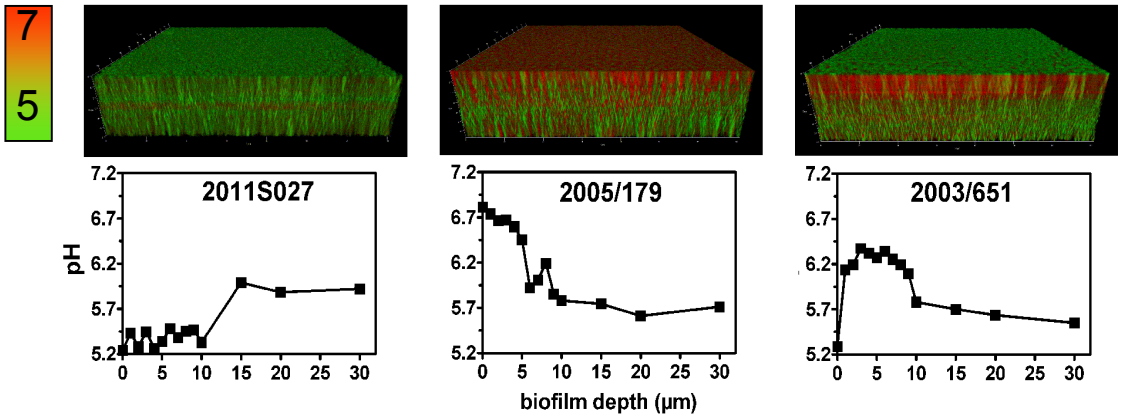

Janssen, Nature 2009

Why do bacteria feel well and antibiotics feel bad in biofilm ?

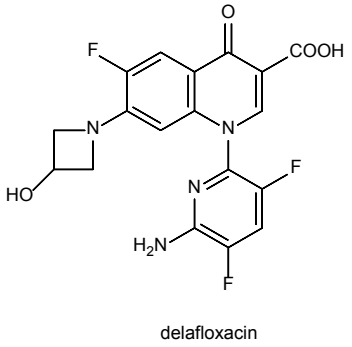
→environment suboptimal (≠ broth)

Environmental pH

S. aureus + delafloxacin

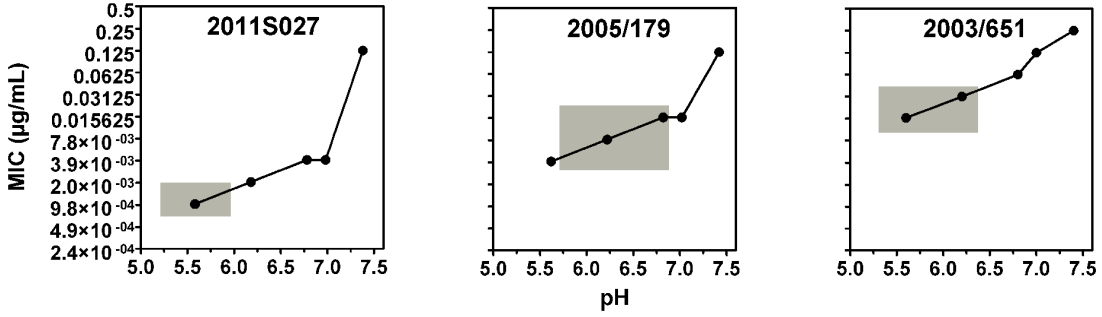


* Labelling with Seminaphthorhodafluor-4F 5-(and-6) carboxylic acid (C-SNARF-4)

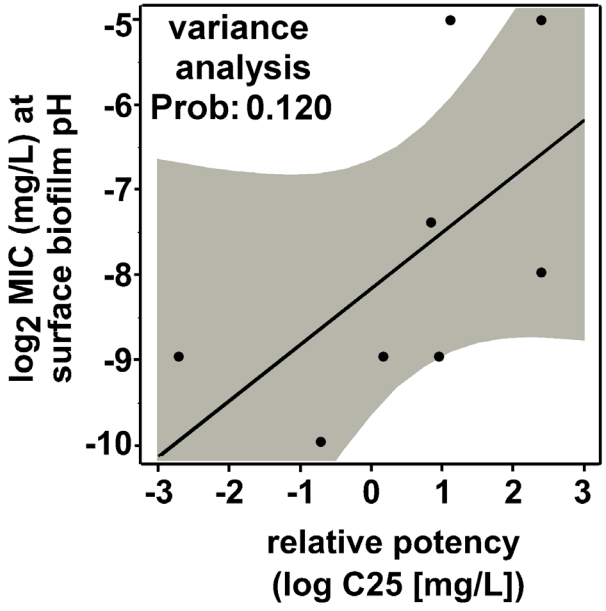


delafloxacin

Influence of pH on delafloxacin MIC



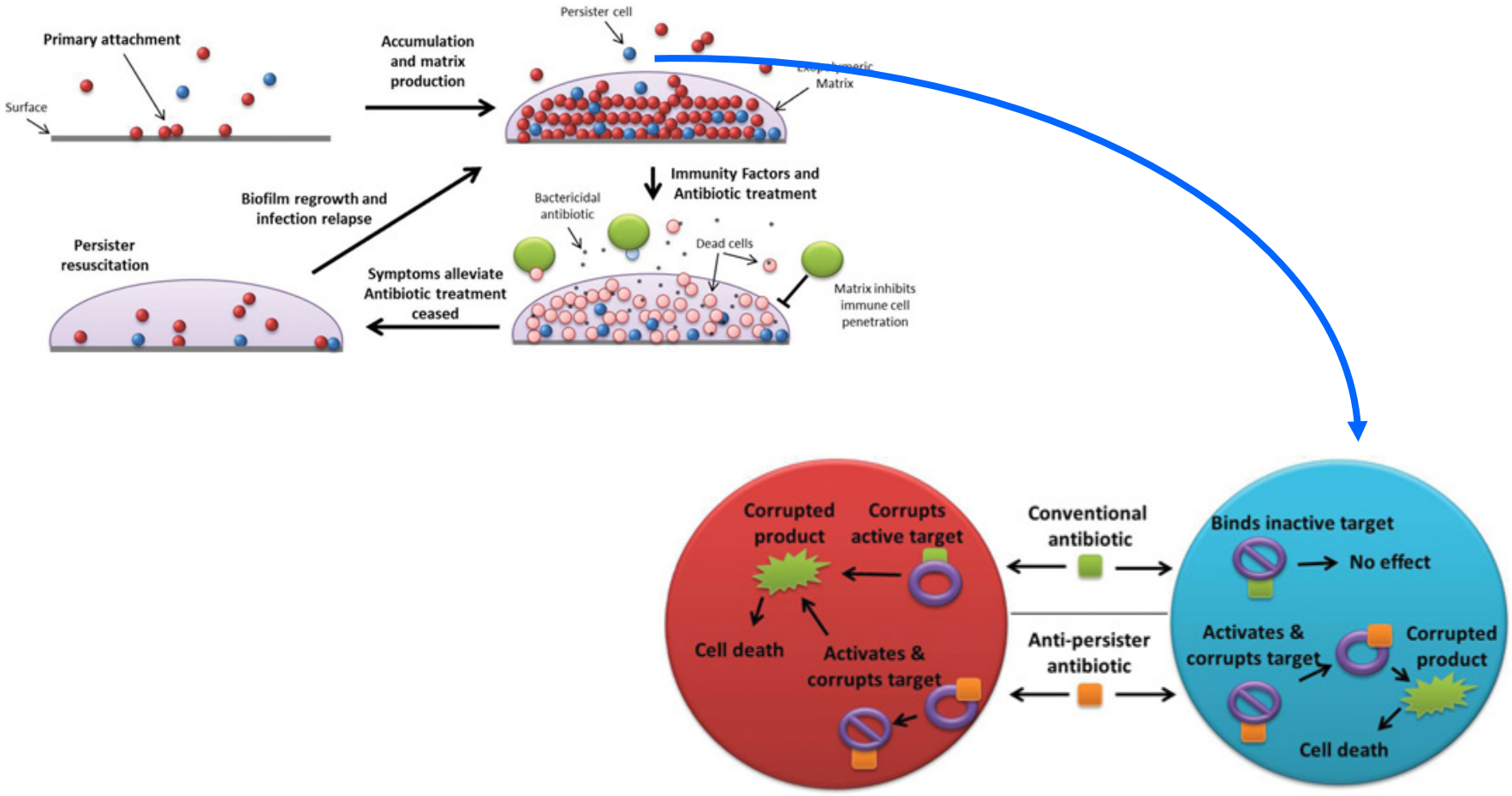
Correlation between delafloxacin relative potency and MIC at the pH of the surface of the biofilm



Biofilm pH may influence antibiotic intrinsic activity

Siala et al, Antimicrob. Ag. Chemother. 2014, 58: 6385–6397.

Specific phenotypes in biofilms: persisters



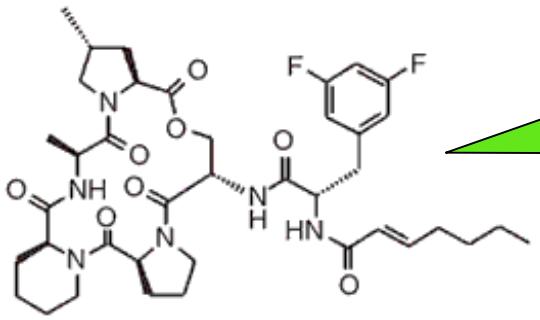
Coulon, Bioassays 2014; 36: 991–6

How to help antibiotics waking up bacteria ?

→anti-persister compounds

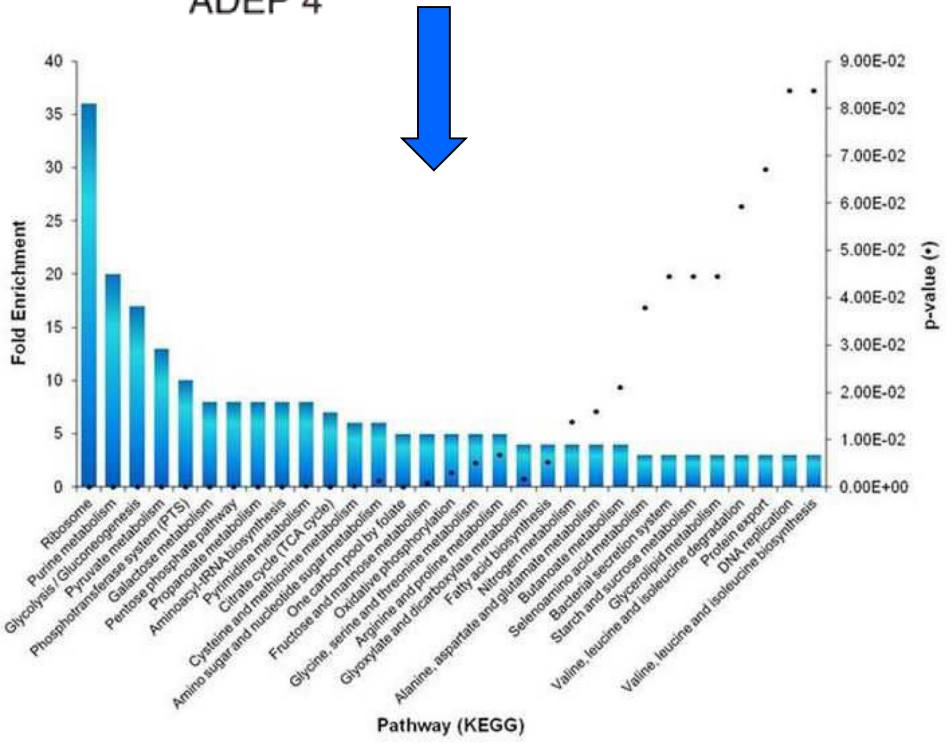
Antipersisters + antibiotics

S. aureus + ADEP4 + antibiotics

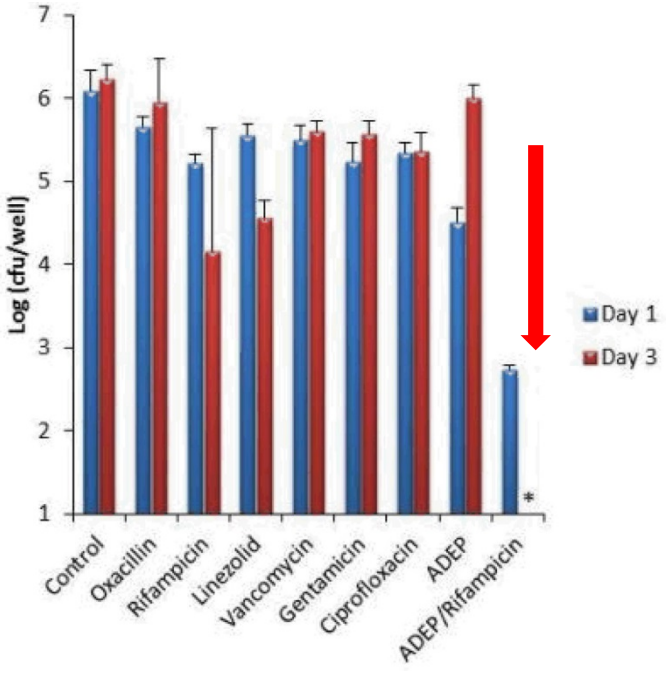


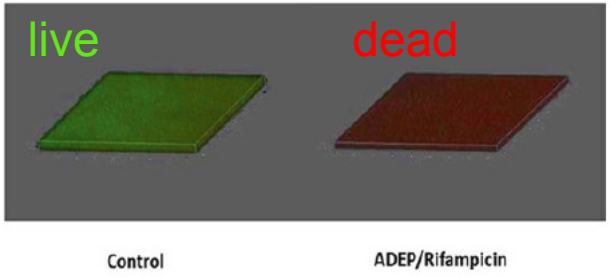
ADEP 4

targets ClpP, core unit of a major bacterial protease complex.



Anti-persisters allow antibiotics to eradicate biofilms





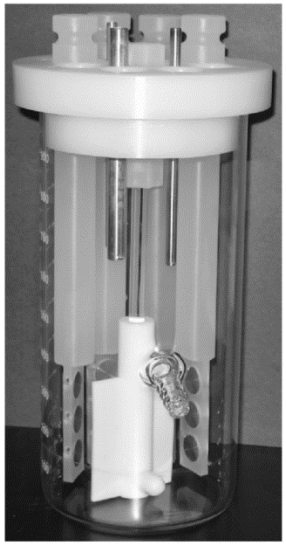
Coulon et al, Nature 2013; 503: 365–70

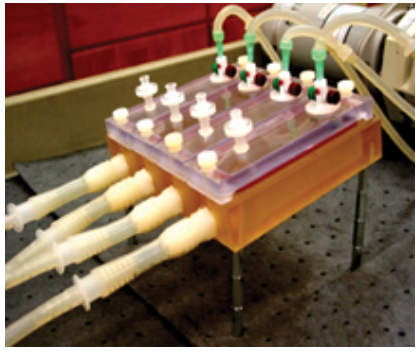
In vitro dynamic models

permanent fluid stirring

unidirectional flow replacement

constant conditions





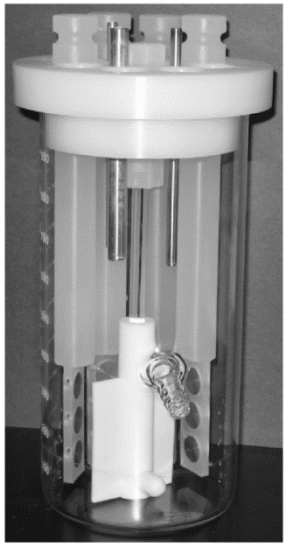
What does constant flux to the story ?

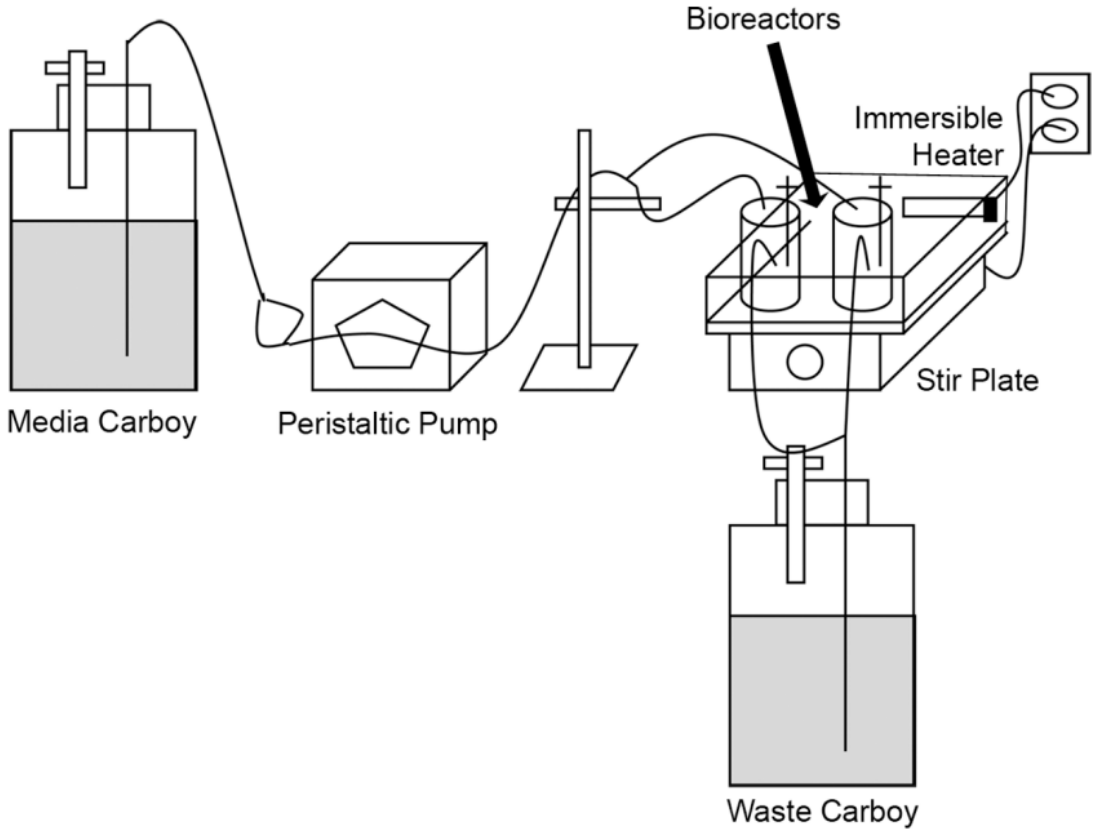
→ closer from in vivo situation

Dynamic models: bioreactors

CDC reactor:

- constant mixing by stirring
  → kinetic experiments with change in medium composition over time
- high shear stress



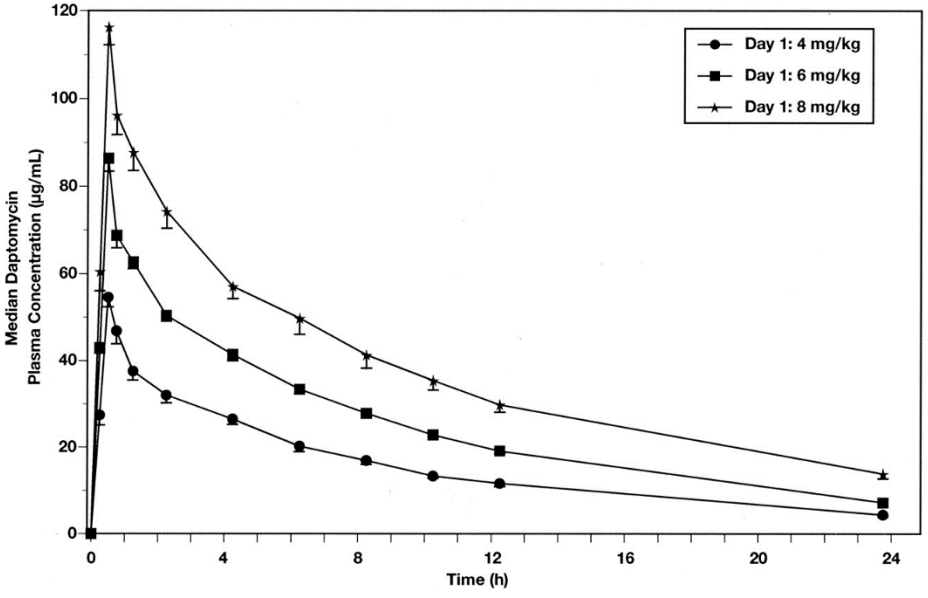


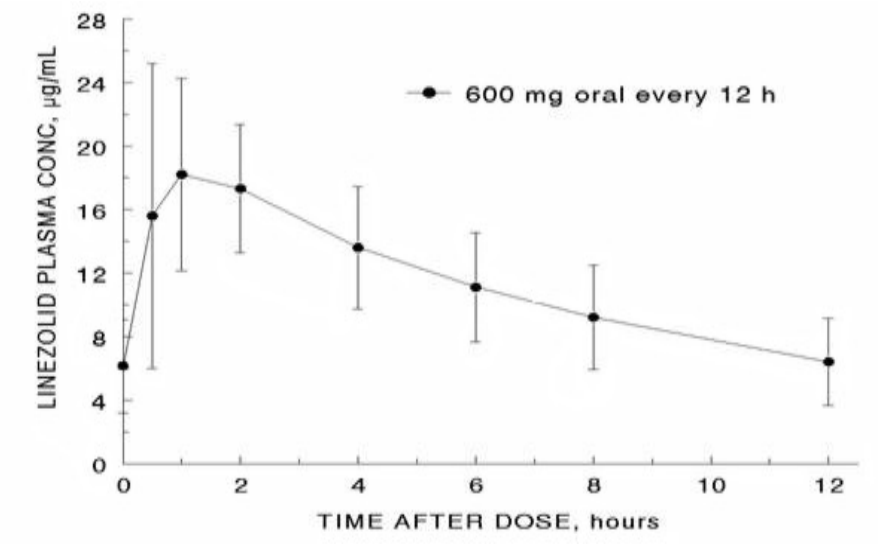
Stewart et al, PLoS One 2012;7(11):e50560

Study of antibiotic activity - mimicking human exposure

S. aureus biofilms

Simulated regimens:
DAP (10 mg/kg once daily) / LZD (600 mg twice daily)





human pharmacokinetic profile

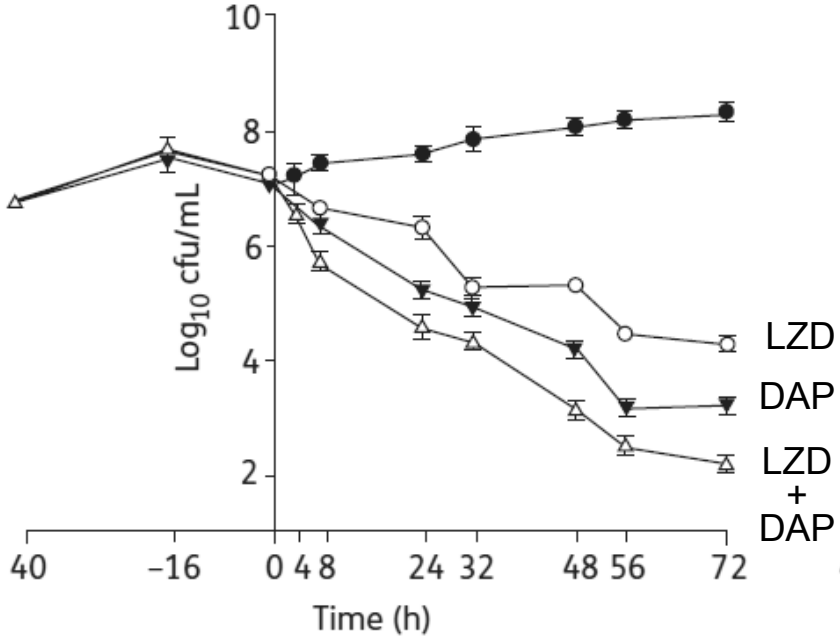
Study of antibiotic activity - mimicking human exposure

S. aureus biofilms

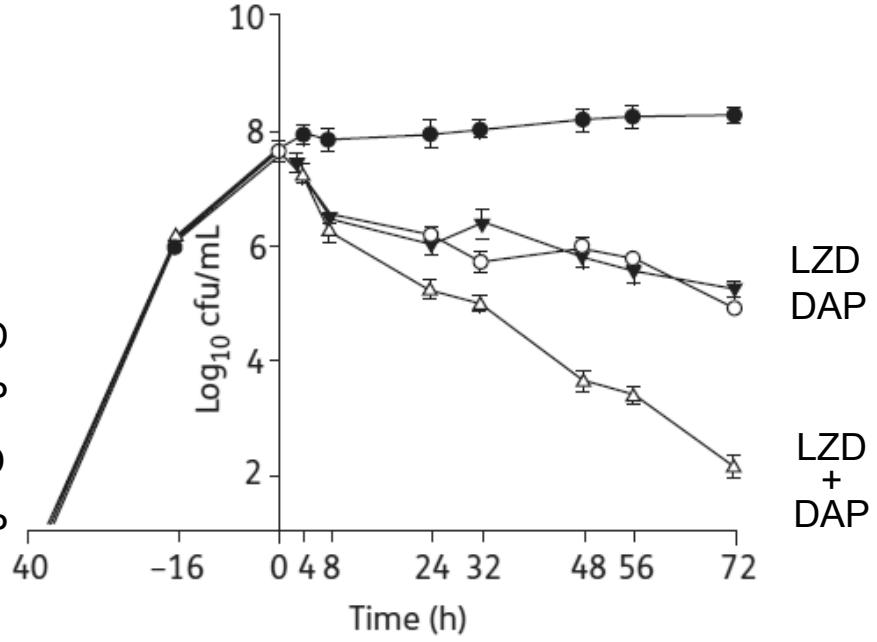
See also poster by Siala et al.

Simulated regimens:
DAP (10 mg/kg once daily) / LZD (600 mg twice daily)

Planktonic cultures



Biofilm (CDC reactor)



Combination more useful against biofilm than planktonic bacteria

Parra-Ruiz et al, J Antimicrob Chemother 2012; 67:2682–5

Conclusion: PK/PD in biofilms: what did we learn ?

Painting of the establishment of the State University of Ghent in 1817
when the city was under Dutch rule

Conclusion: PK/PD in biofilms: what did we learn ?

- Many methods to evaluate biomass / bacterial survival
  - no real consensus on the best options

Looking forward to PRO/CON debate !

- Many models to grow biofilms *in vitro*
  - comparison between studies difficult
  - more relevant model ?

many models presented here !

- Antibiotic activity on biofilms <<< planktonic bacteria
  - no or limited effect on the matrix
  - determining PK parameters: diffusion / bioavailability
  - determining PD parameters: expression of activity / bacterial responsiveness

Perspectives: PK/PD in biofilms: where do we go ?

PK/PD in biofilms: Where do we go ?

Increasing antibiotic activity against biofilms

much more to be learned during this meeting !

PK

→ dispersing the matrix
- matrix constituants ?
- nanovectors to improve delivery

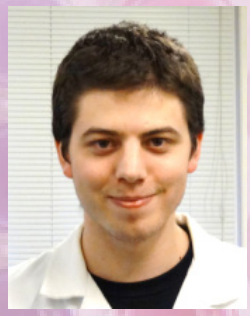
PD

→ modifying environmental factors
- metabolism within biofilm ?
- factors affecting antibiotic activity ?

→ modifying bacterial metabolism
- reversion from persister phenotype

Acknowledgments

Hariri Mustafa



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