Antiinfectives and biofilms interactions

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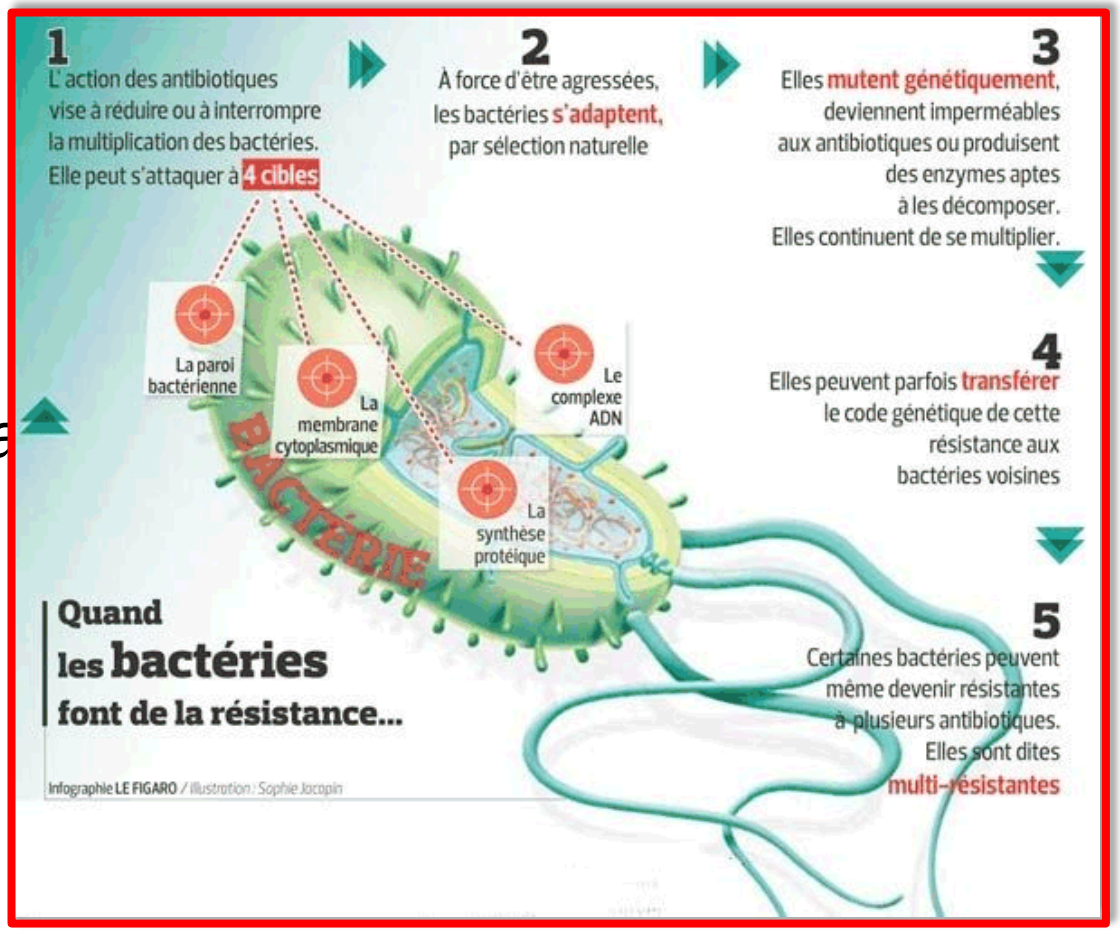
Microbiological difficulties and challenges in anti-infective therapy

- Resistance
- Persistence
- *Small Colony variants*
- Intracellular forms
- Biofilms

Difficultés et défis microbiologiques de l'antibiothérapie

- **Resistance**
- Persistence
- *Small Colony varia*
- Intracellular forms
- Biofilms



Difficultés et défis microbiologiques de l'antibiothérapie

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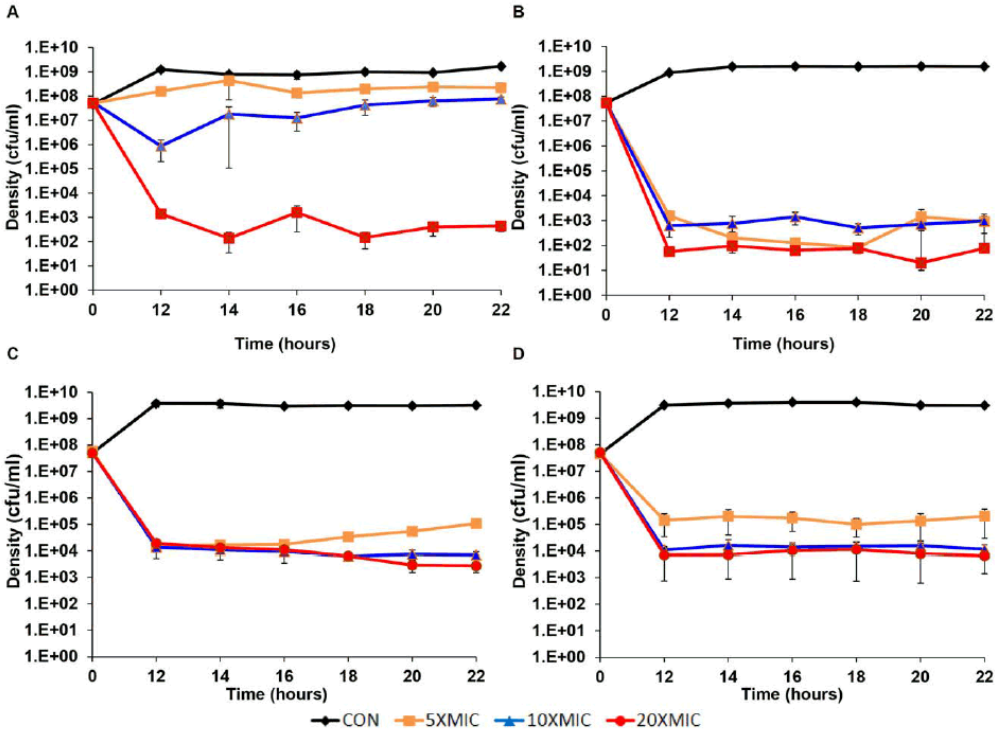


Figure 3. Longer-term time kill experiments. Changes in viable cell density, means and standard errors (bars), for three independent cultures of *S. aureus* each exposed to different concentrations (5× MIC, 10× MIC and 20× MIC) of four antibiotics: (A) Ciprofloxacin, (B) Gentamicin, (C) Oxacillin and (D) Vancomycin.
doi:10.1371/journal.pgen.1003123.g003

Johnson & Levin. PLoS Genet. 2013;9:e1003123. - PMID: 23300474;

Not all bacteria are dead !

Difficultés et défis microbiologiques de l'antibiothérapie

- Resistance
- Persistence
- ***Small Colony variants***
- Intracellular forms
- Biofilms

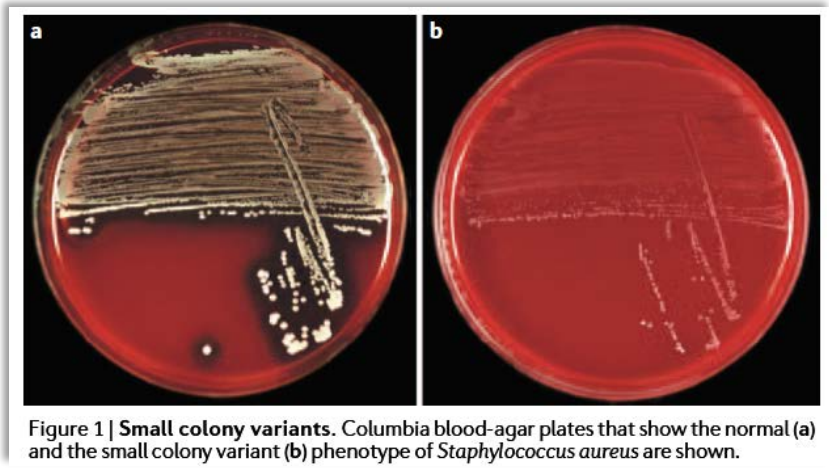


Figure 1 | **Small colony variants.** Columbia blood-agar plates that show the normal (**a**) and the small colony variant (**b**) phenotype of *Staphylococcus aureus* are shown.

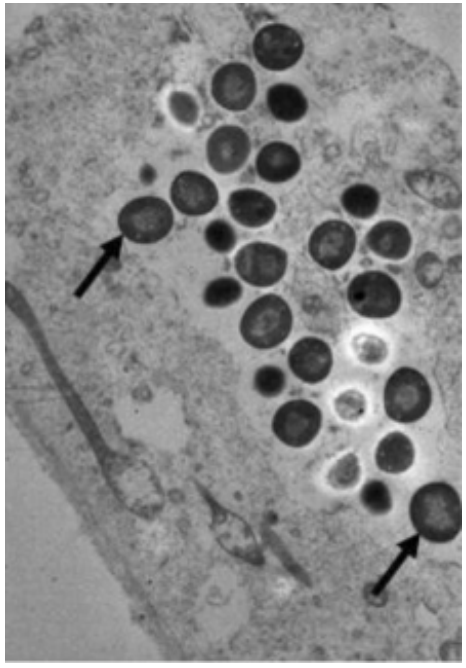
Proctor et al. Nat Rev Microbiol 2006;4:295–305 - PMID: 16541137

Eradication of 'small colony variants" requires prolonged antibiotic treatments often in multi-drug combinations

Difficultés et défis microbiologiques de l'antibiothérapie

- Resistance
- Persistence
- *Small Colony variants*
- **Intracellular forms**
- Biofilms

***S. aureus* in human osteoblasts**



Kalinka *et al.*, Int J Med Microbiol. 2014; 304:1038-49 - PMID: 25129555

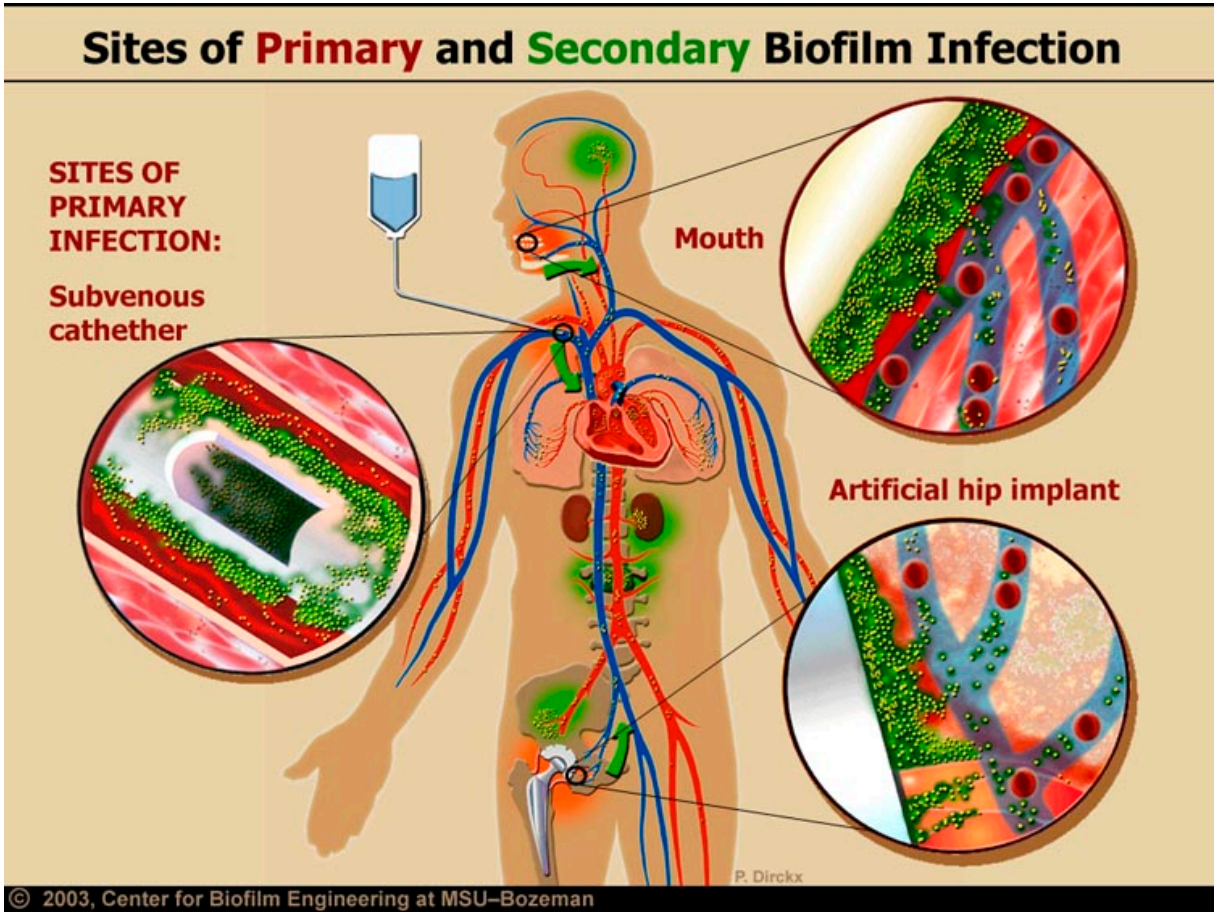
Recalcitrant to eradication….

Difficultés et défis microbiologiques de l'antibiothérapie

- Résistance
- Persistance
- *Small Colony variants*
- Formes intracellulaires
- **Biofilms**

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

ELSEVIER

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,*]

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2003, p. 4043–4048 Vol. 41, No. 9

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…

In vitro static models

pegs

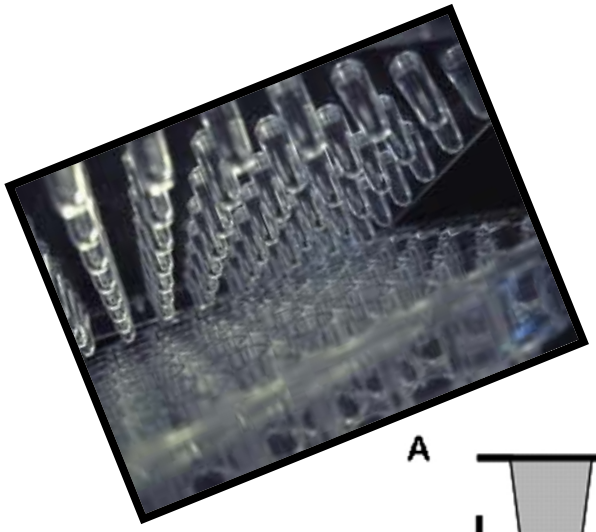
multiwell plates

Antibiotic activity: planktonic vs. biofilm cultures

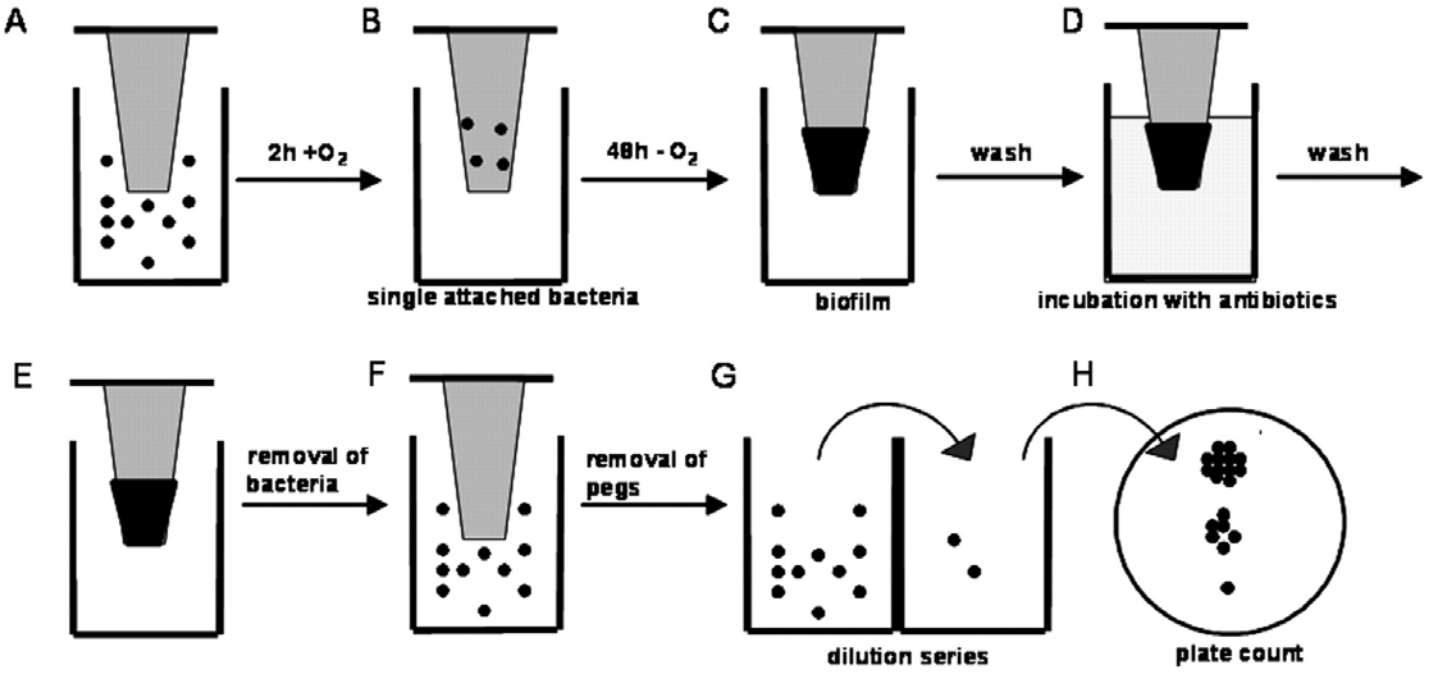
Parameter	Abbreviation	Definition
Minimal inhibitory concentration	MIC	The lowest concentration of an antibiotic that inhibits the visible growth of a planktonic culture after overnight incubation
Minimal biofilm inhibitory concentration	MBIC	The lowest concentrations of an antibiotic that resulted in 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

Static models: Calgary Biofilm Device



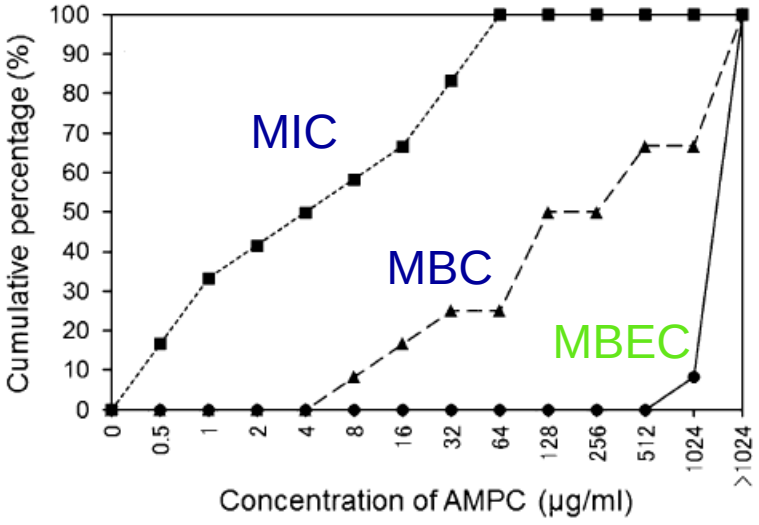
Determination of
Minimal Biofilm Eradication Concentration
(MBEC)

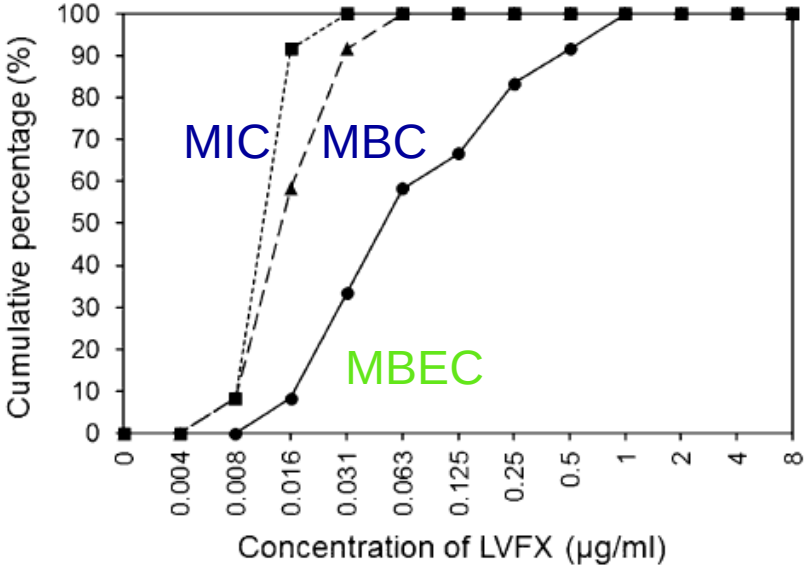


Ceri et al, J. Clin. Microbiol. 1999; 37:1771-6; Herrmann et al, J Infect Dis. 2010;202:1585-92

Comparing antibiotic activity: planktonic / biofilm cultures

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





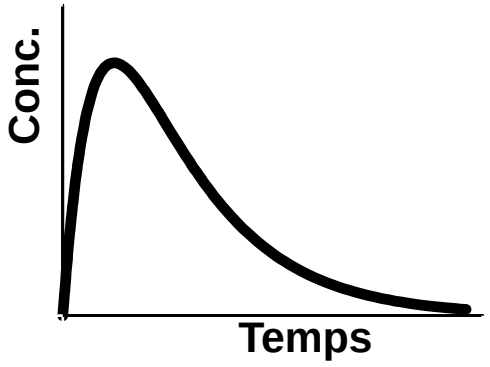
Activity against biofilm << activity against planktonic bacteria

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

PK/PD studies: the principles

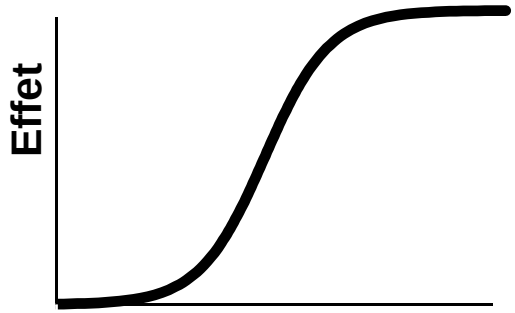
Pharmacokinetics

conc. vs time



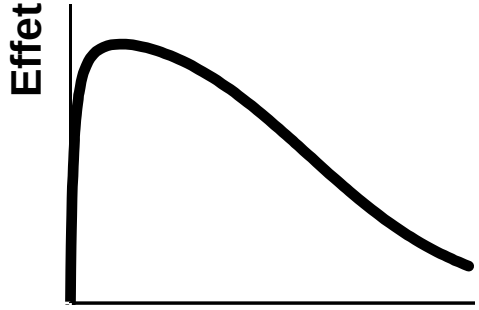
Pharmacodynamics

conc. vs effect



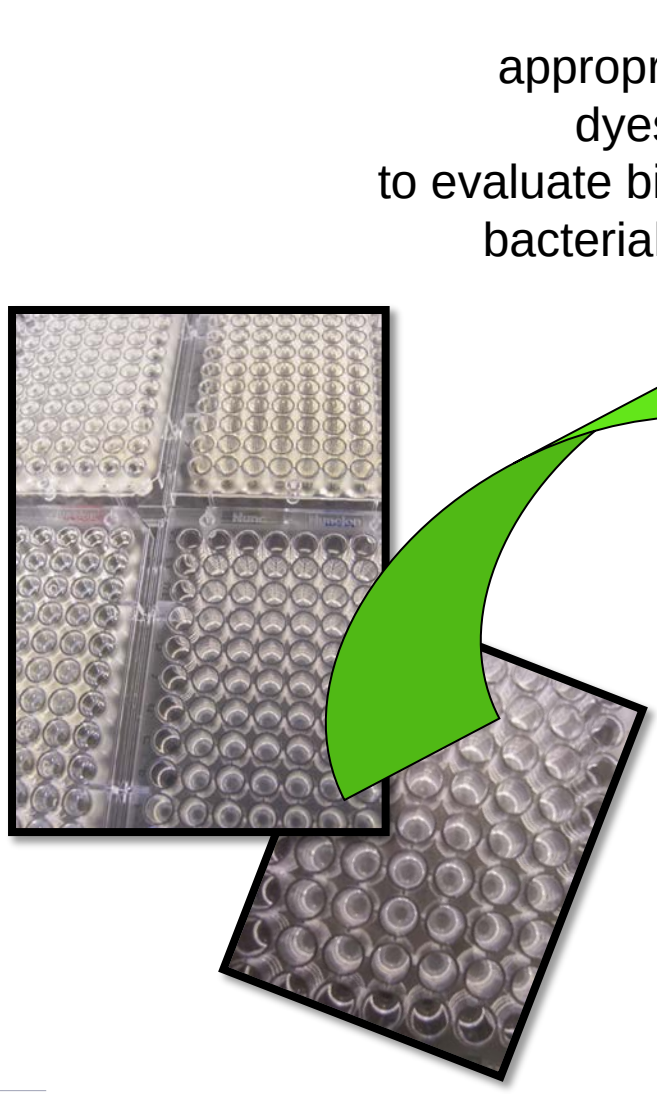
PK/PD

effect vs time



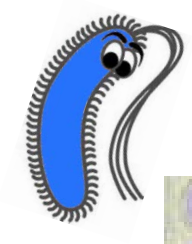
Static models: 96-well polystyrene plates

appropriate
dyes
to evaluate biomass or
bacterial load

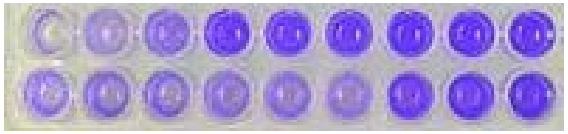


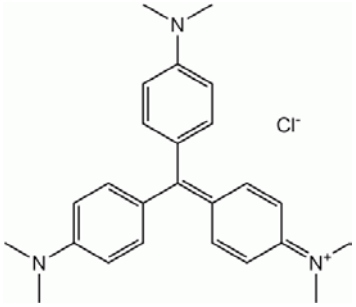
Quantifying biomass and metabolic activity in biofilms

biofilm mass



crystal violet

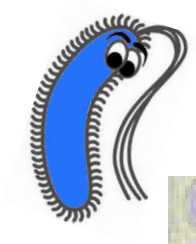




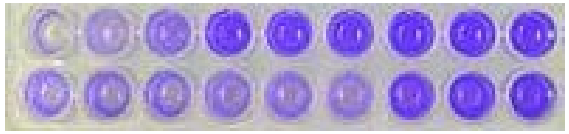
Christensen et al, Infect. Immun. 1982; 37:318–26

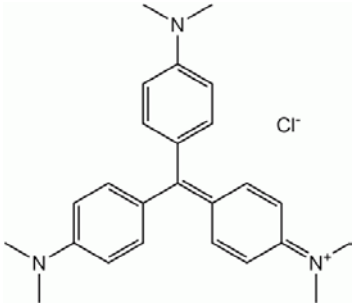
Quantifying biomass and metabolic activity in biofilms

biofilm mass



crystal violet



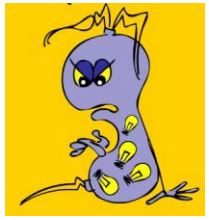


Christensen et al, Infect. Immun. 1982; 37:318–26

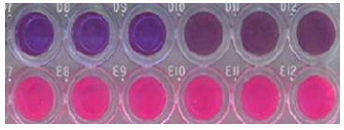
Gram(+) bacteria

metabolic activity

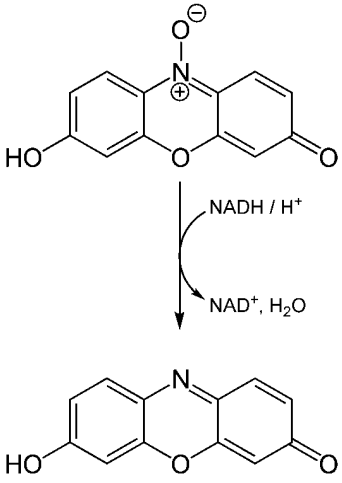
Gram(-) bacteria



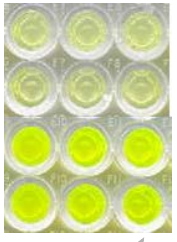
resazurin



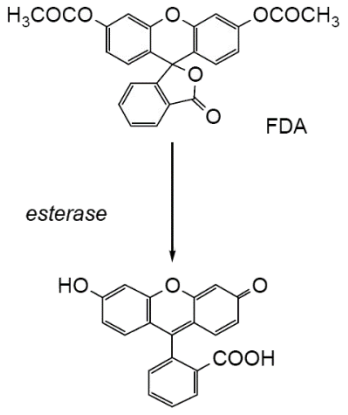
resorufin



fluorescein diacetate



fluorescein

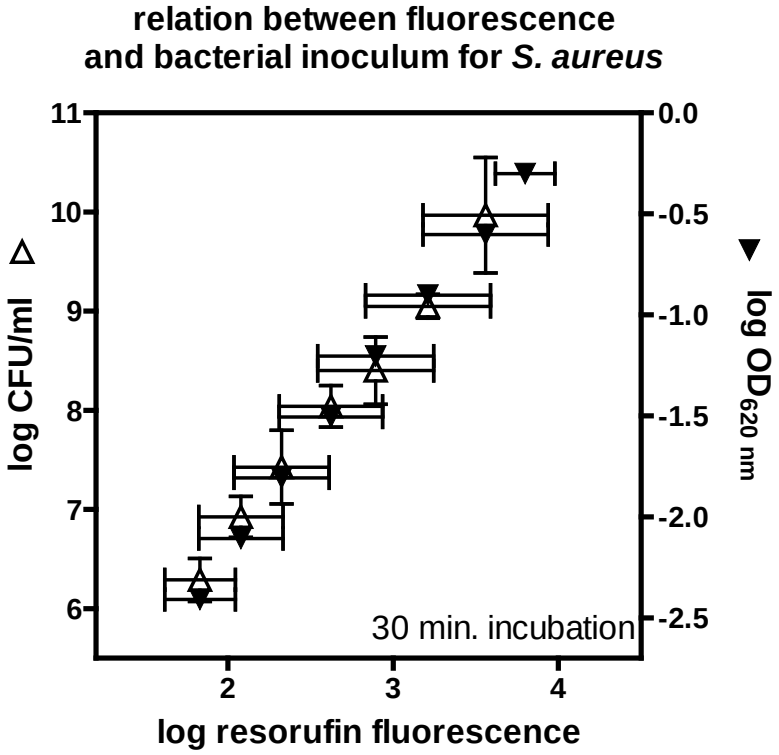


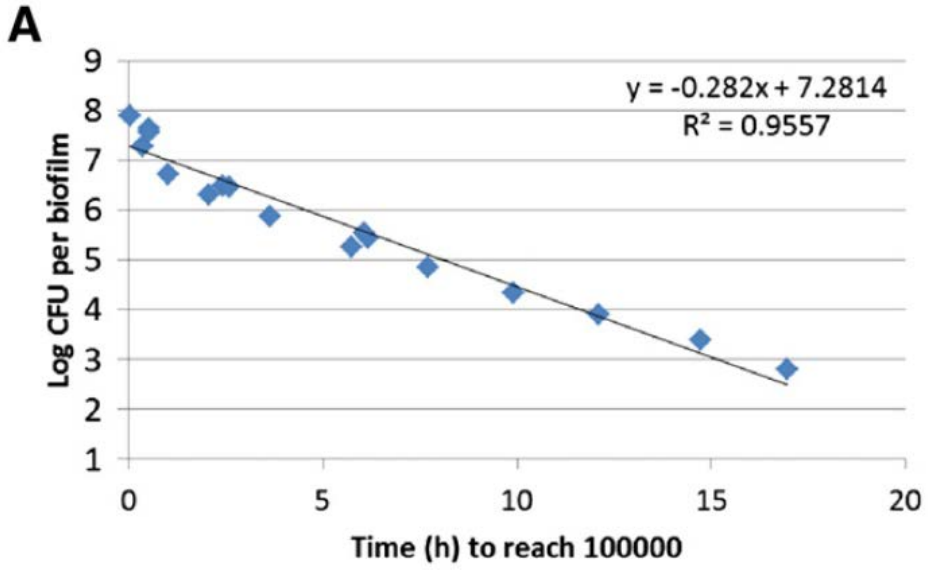
Tote et al, 2008; Lett. Appl. Microbiol. 46:249–254

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

CFU counting *vs.* RF fluorescence

An example for *S. aureus*



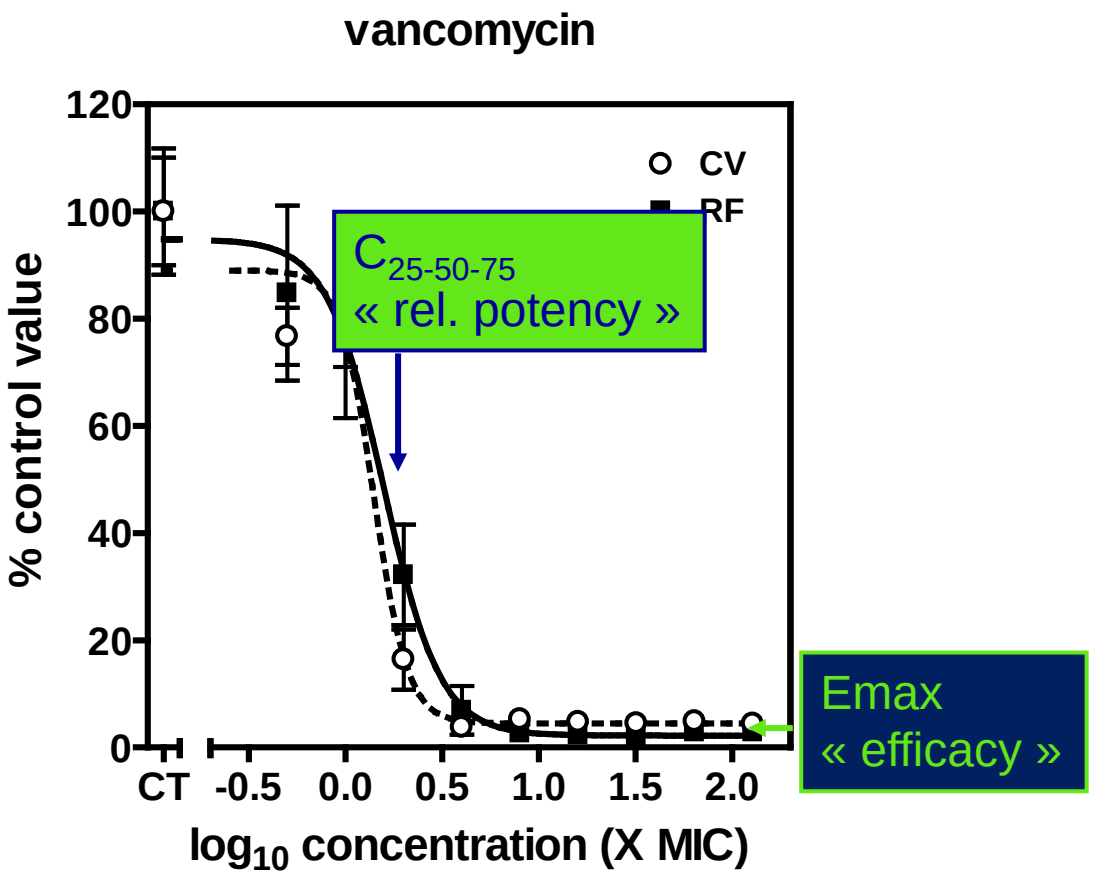


CFU & RF signal proportional

sensitivity depending on incubation time

Pharmacodynamic model for antibiotic activity

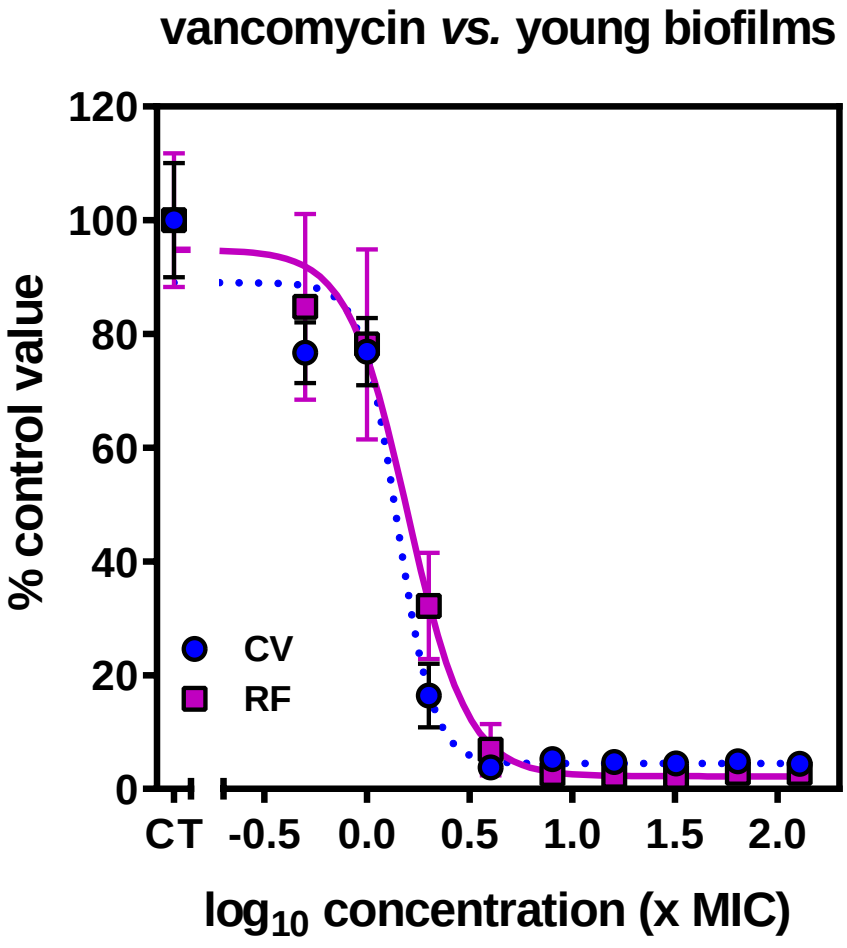
An example with young biofilm of *S. aureus*

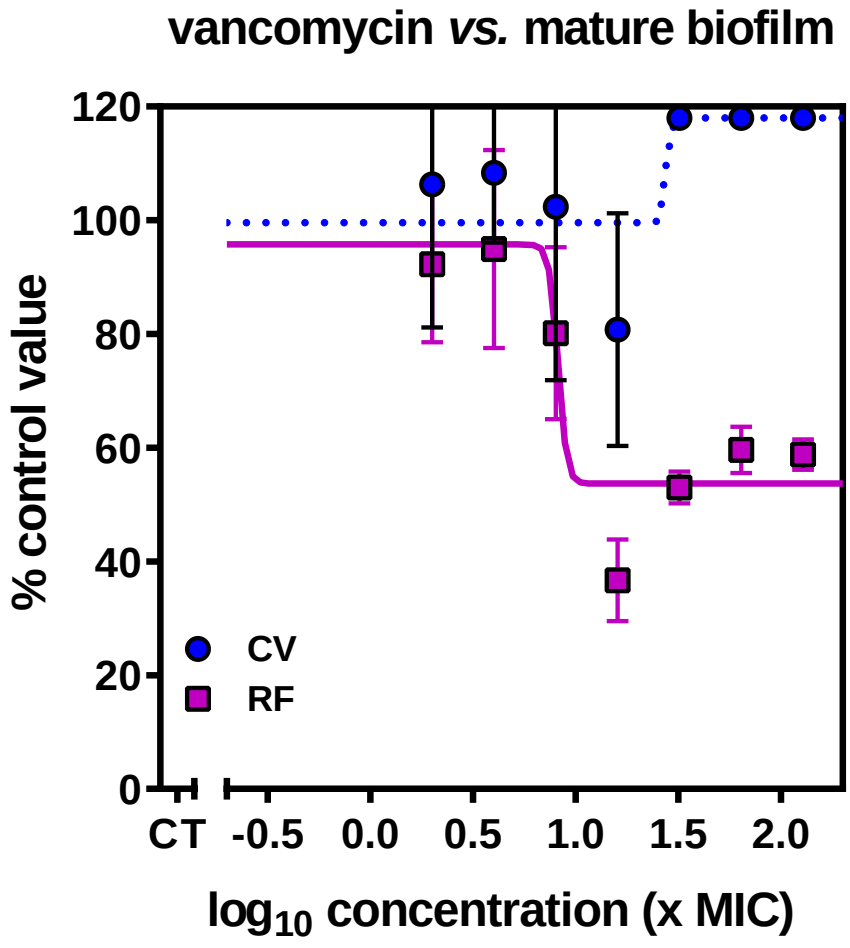


Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37

Pharmacodynamic model for antibiotic activity

Comparison between young and mature biofilms

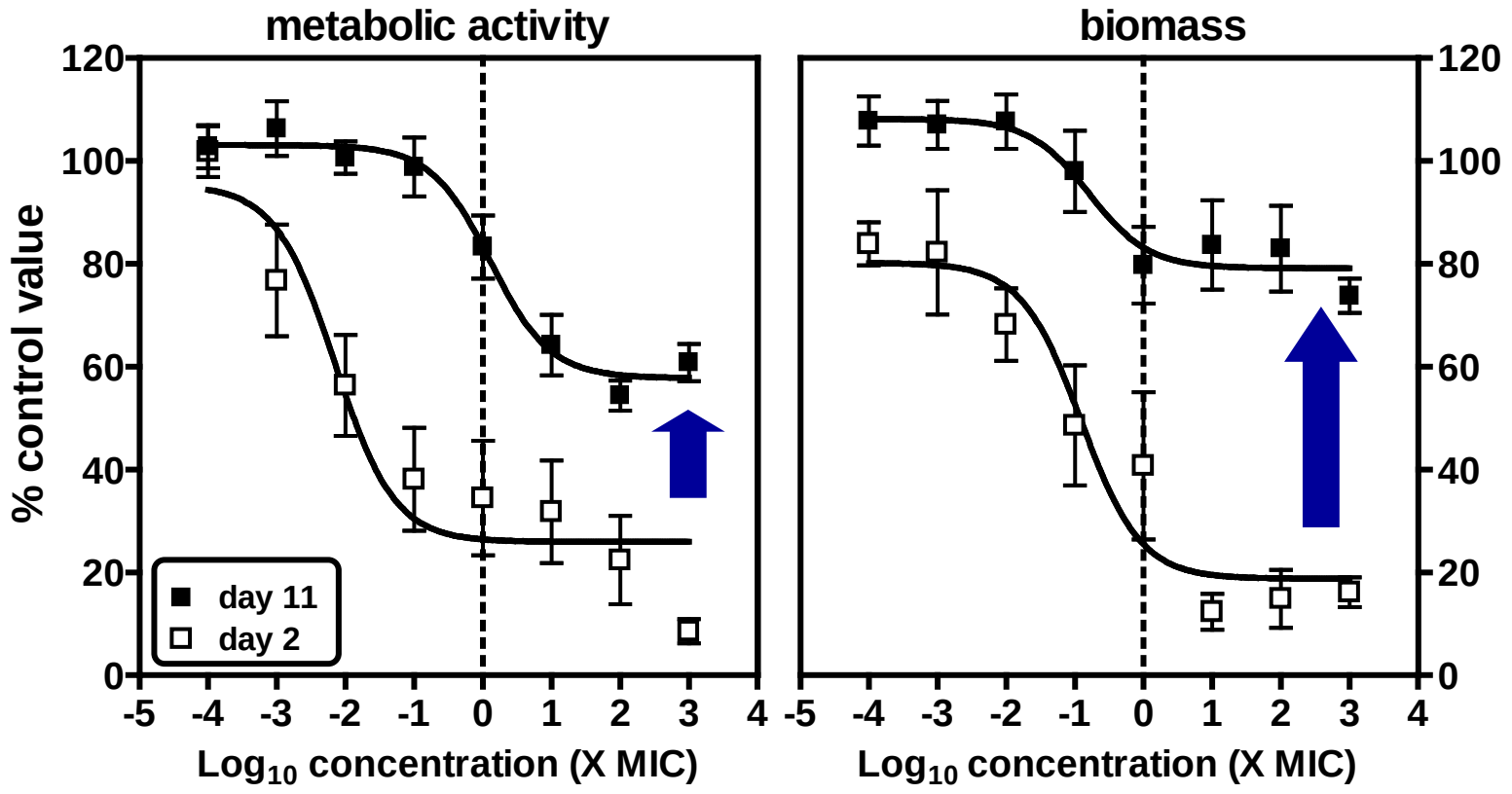




Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37

S. pneumoniae biofilms - influence of maturity

moxifloxacin

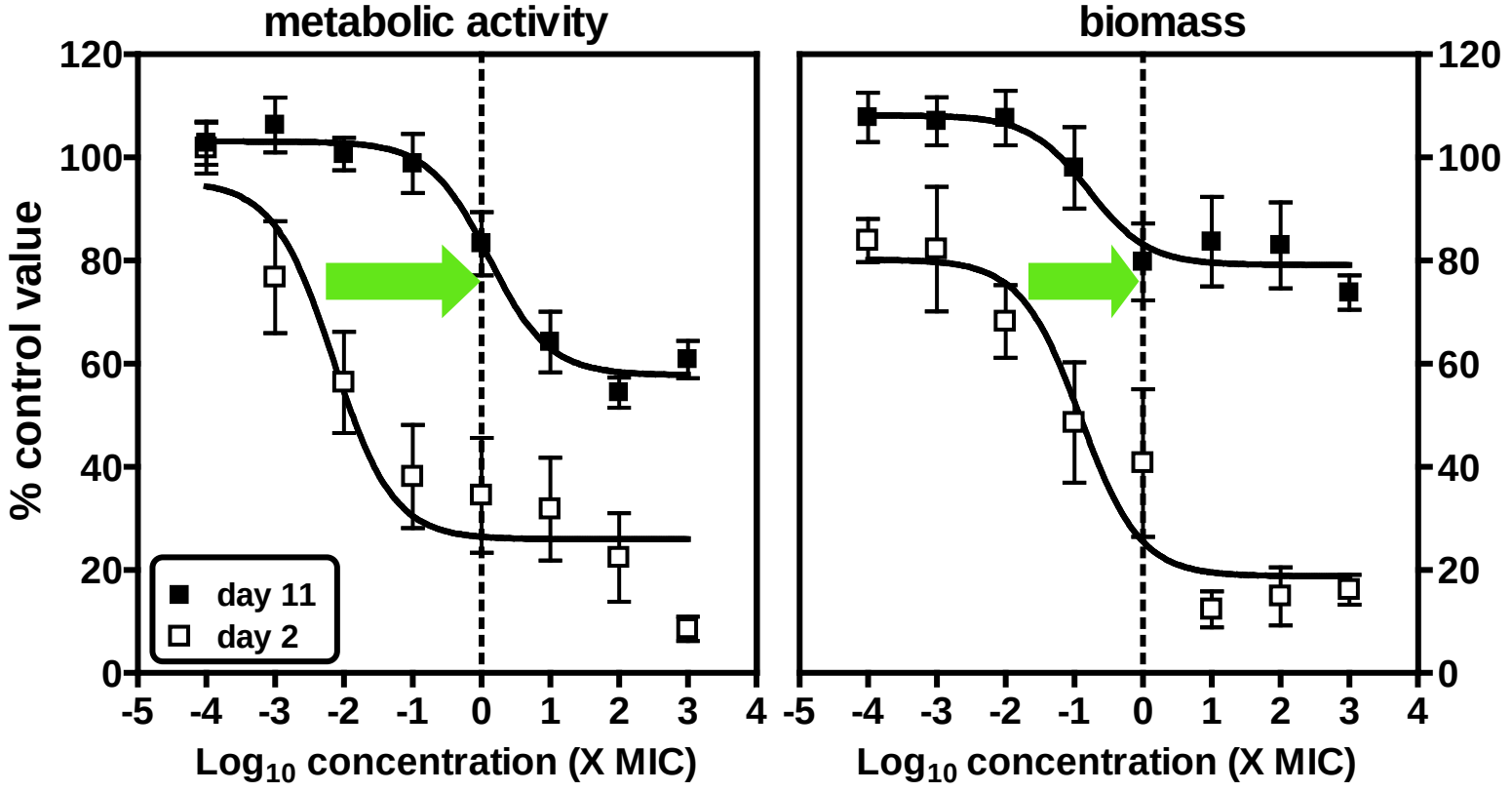


maximal efficacy ↘ with maturity

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

S. pneumoniae biofilms - influence of maturity

moxifloxacin

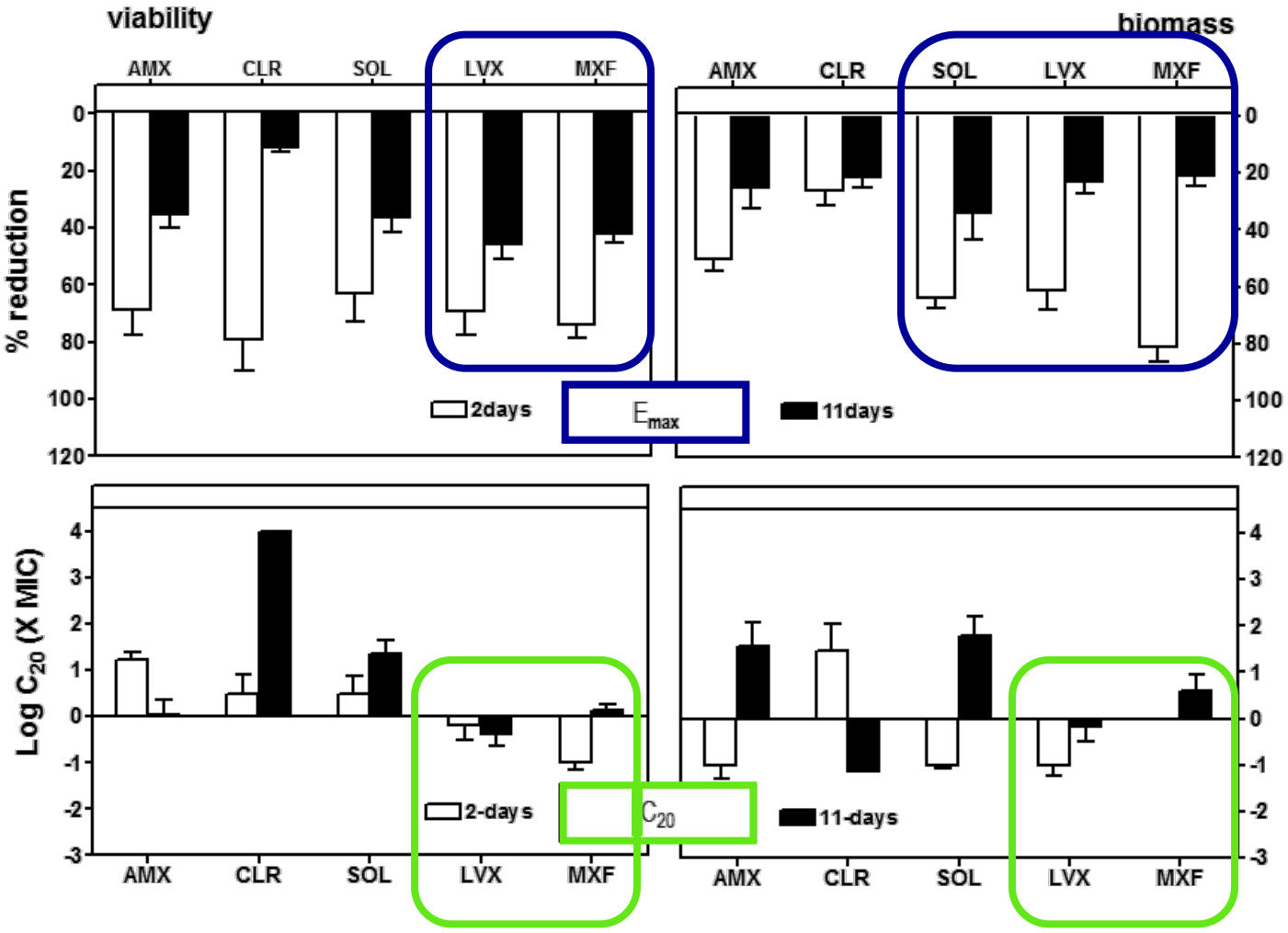


relative potency ⇘ with maturity

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

Comparison of PD parameters for different drugs

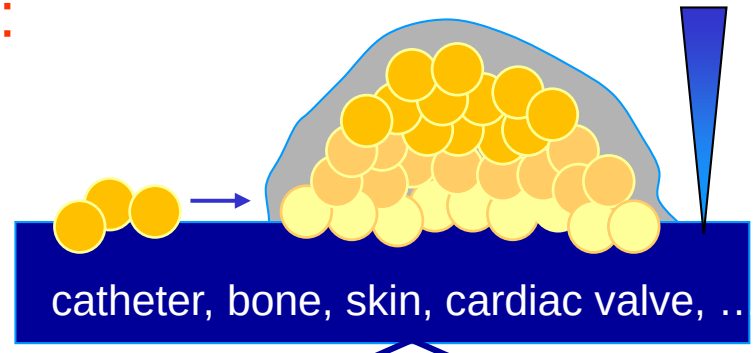
S. pneumoniae



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

How to explain this “apparent” resistance or tolerance?

PK/PD in biofilms:



nutrients
&
oxygen

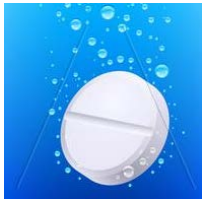
pharmacokinetics

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

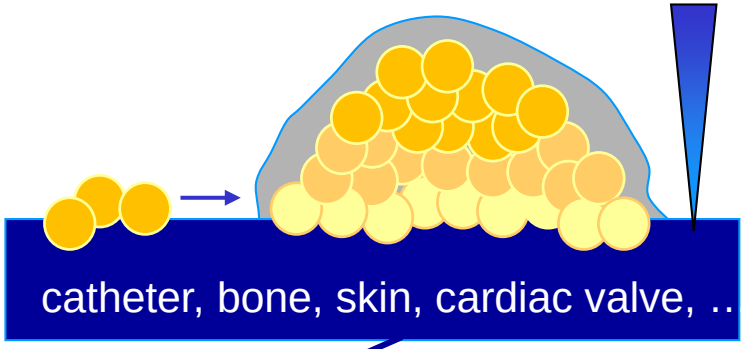
pharmacodynamics

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


Janssen, Nature 2009



PK/PD parameters in biofilms

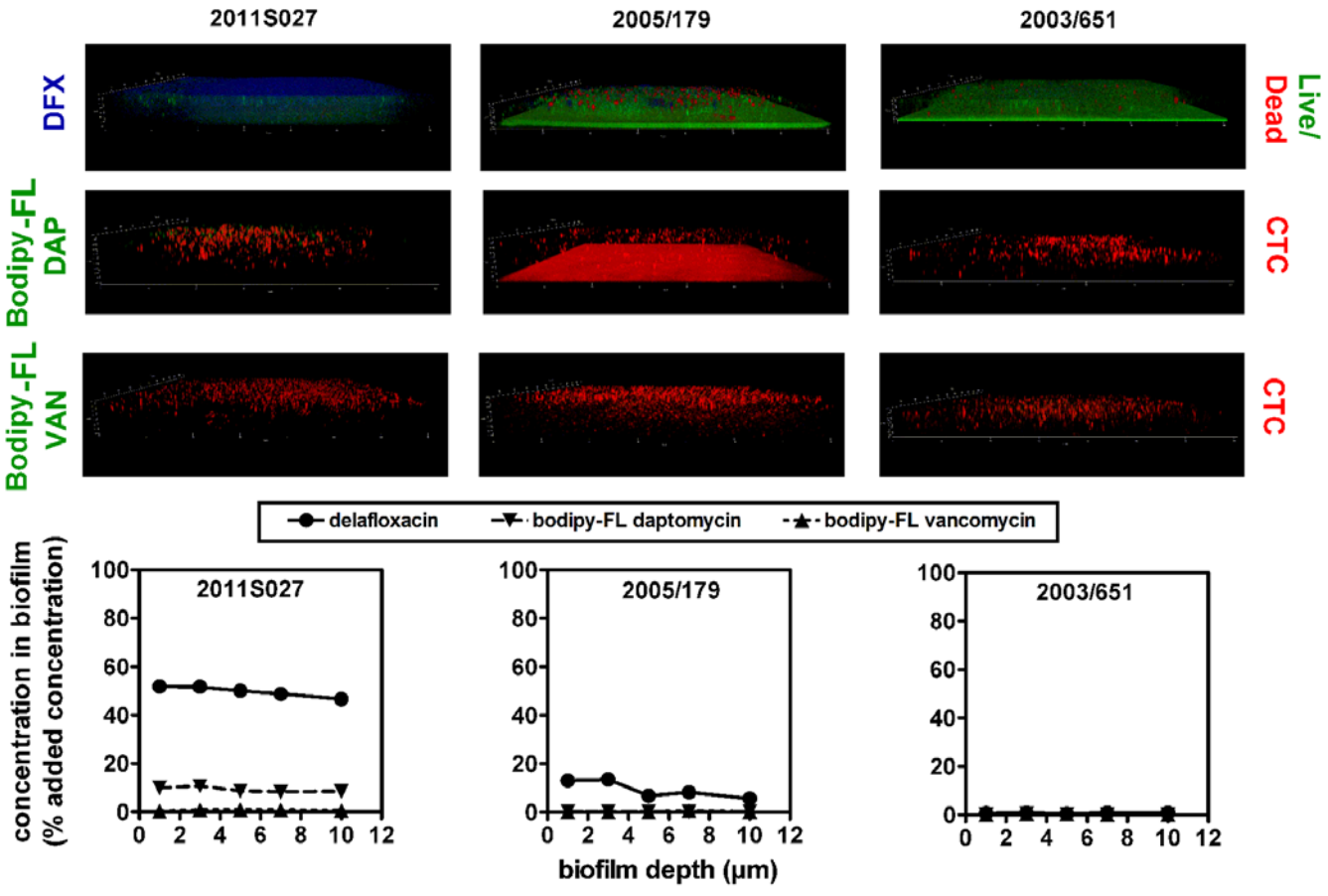


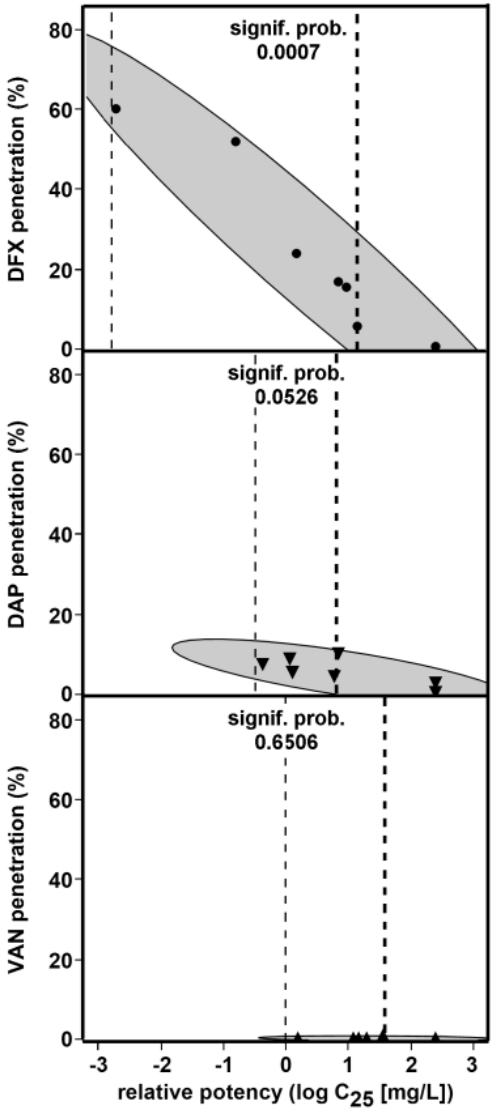
pharmacokinetics

- 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



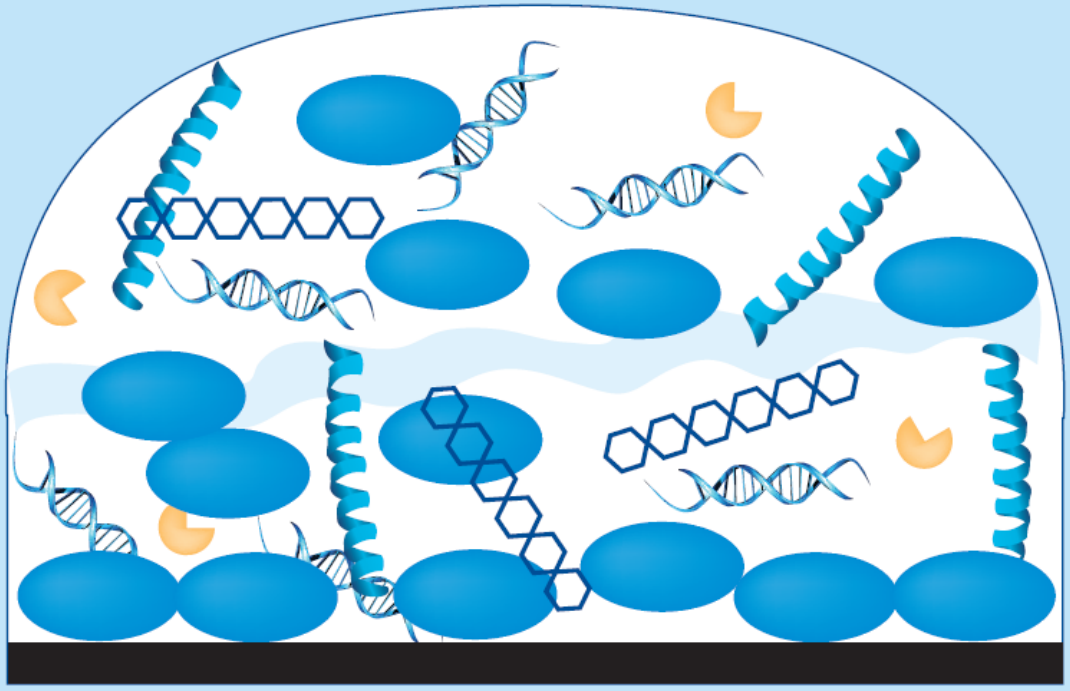


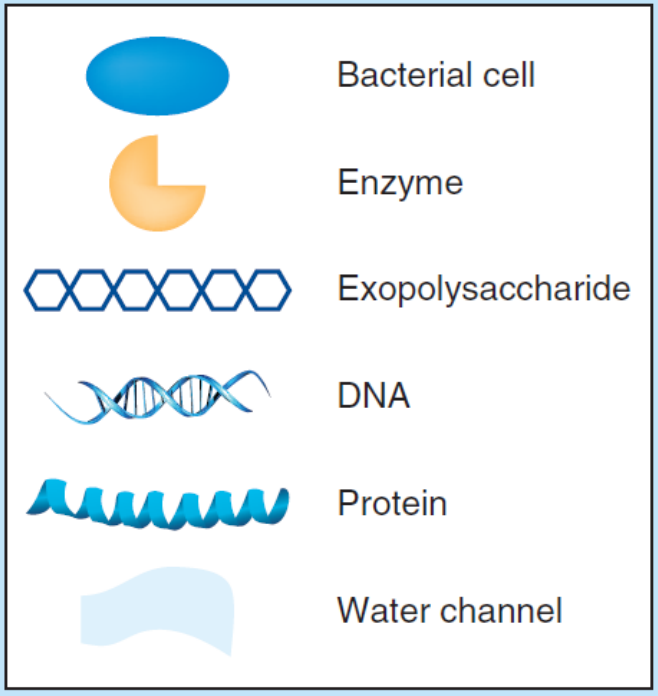
Activity in biofilm is correlated to antibiotic penetration

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

How to help antibiotic to reach their target ?

Disrupt the biofilm matrix …

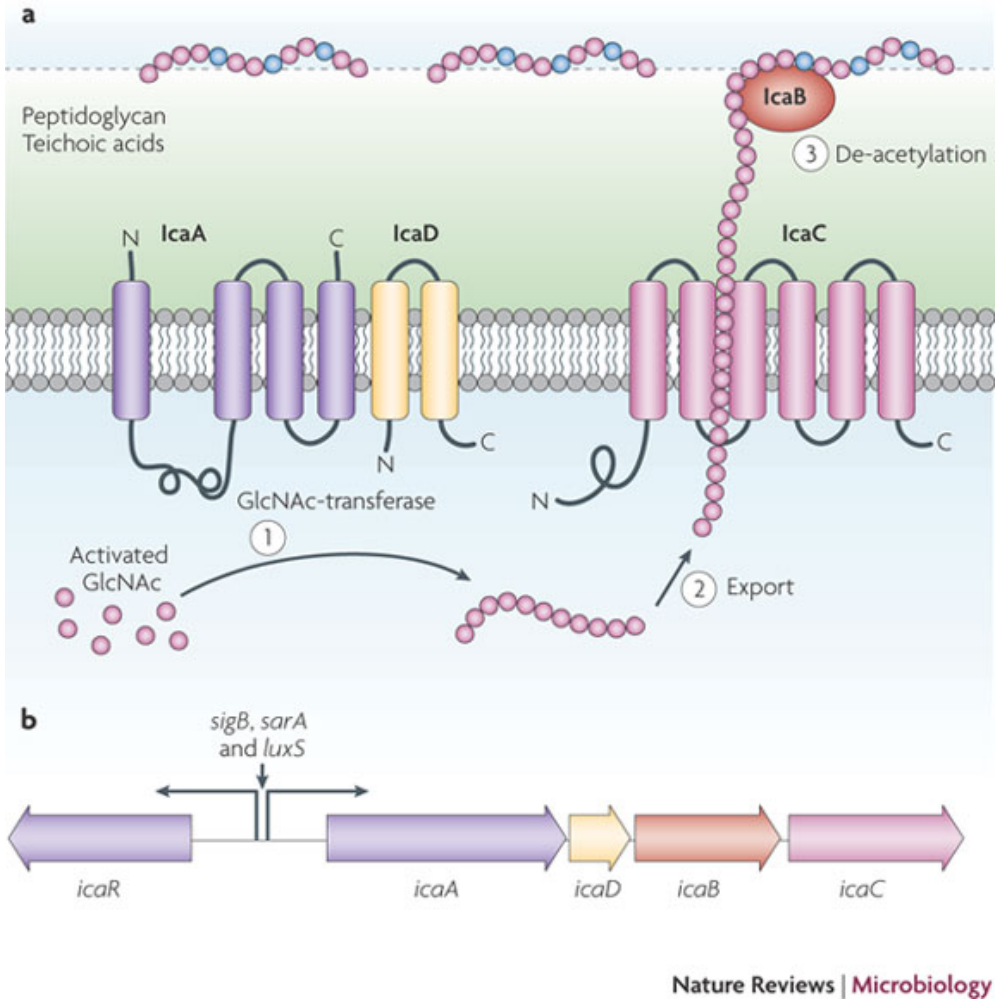




Rabin et al., Future Med. Chem. 2015; 7:493–512

IacA and polysaccharide synthesis in *S. aureus*

Ica A is involved in N-acetylglucosamine homopolymer synthesis

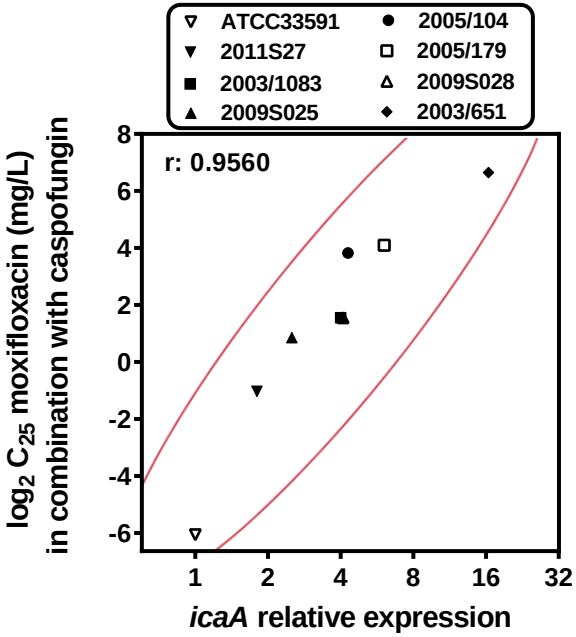


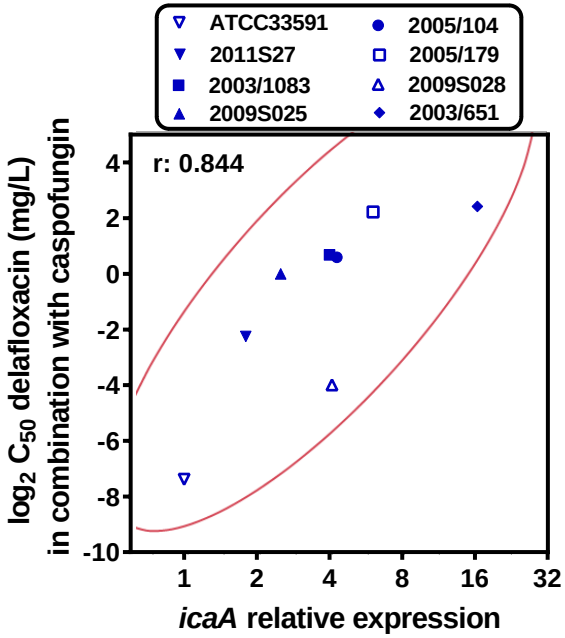
Otto et al., Nat. Rev. Microbiol. 2009; 7:555-67

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*

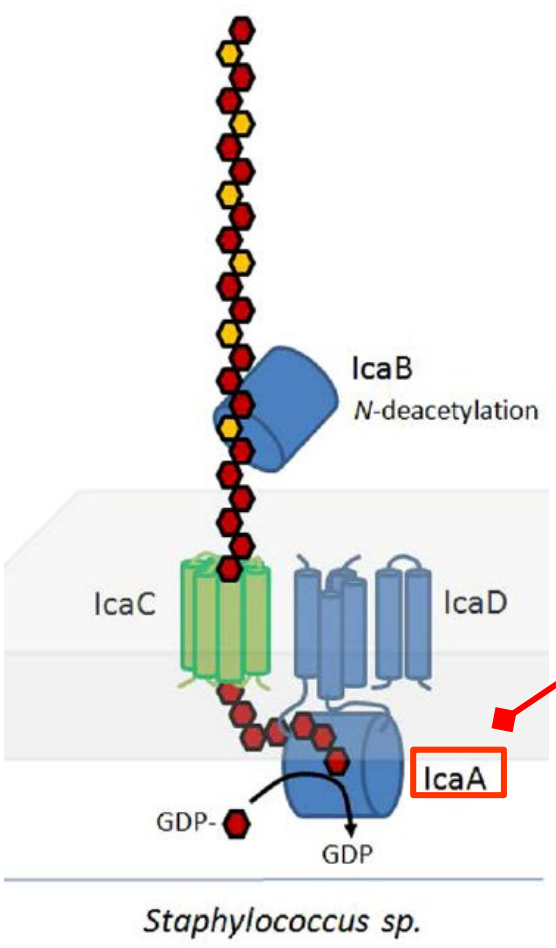


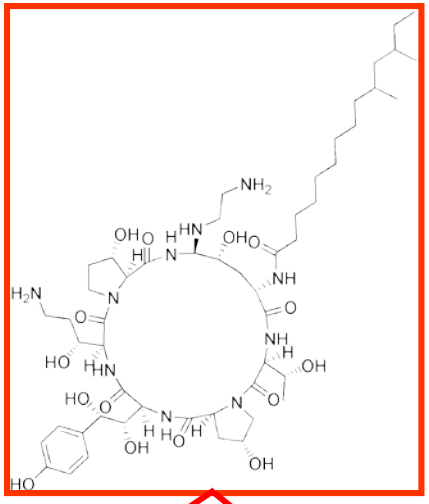


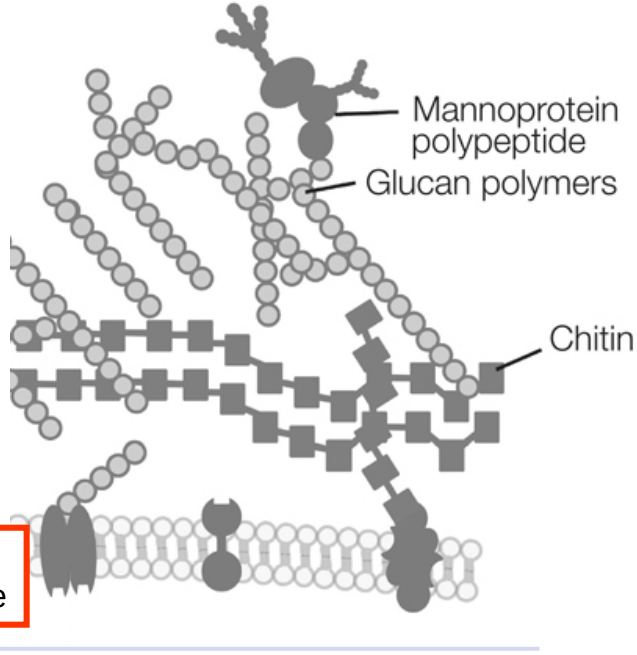
Fluoroquinolone activity in biofilm is inversely correlated with *icaA* expression

*Siala et al, Nature Communications 2016; 7:*13286

The antifungal caspofungin as an inhibitor of polysaccharide synthesis







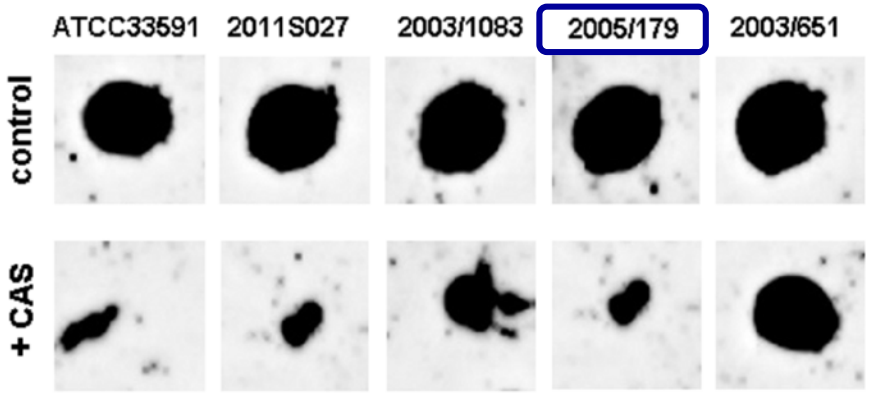
Atkin et al, FEBS Lett. 2014;588:1869-72

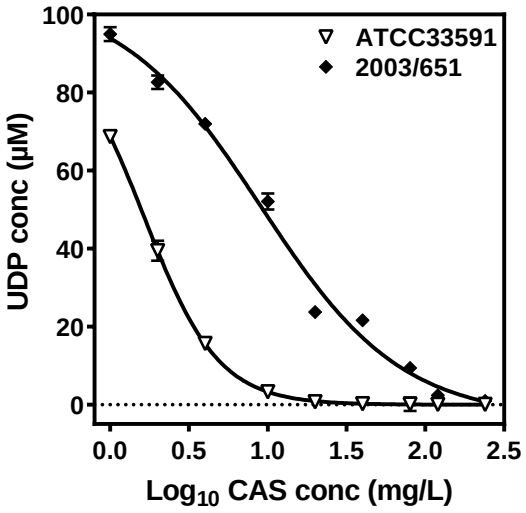
Adapted from Arnold, Kucer's 6the edition

*Siala et al, Nature Communications 2016; 7:*13286

Inhibition of IcaA by caspofungin increases fluoroquinolone penetration in biofilms

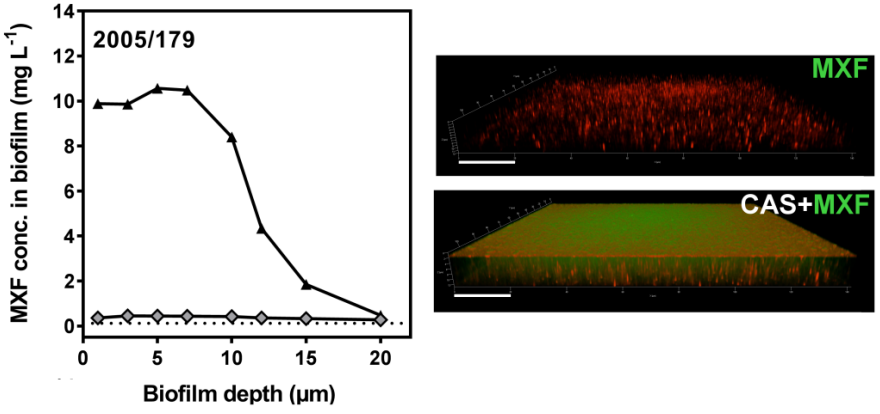
S. aureus biofilms

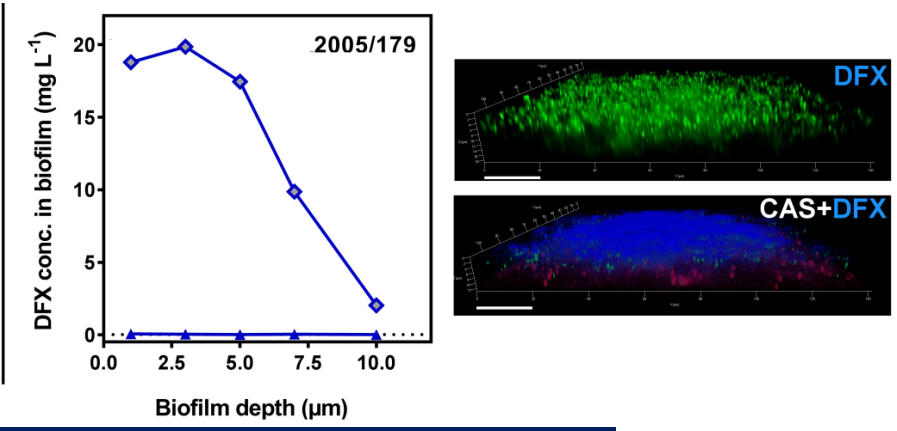




CAS reduces PNAG in the matrix

CAS inhibits IcaA activity



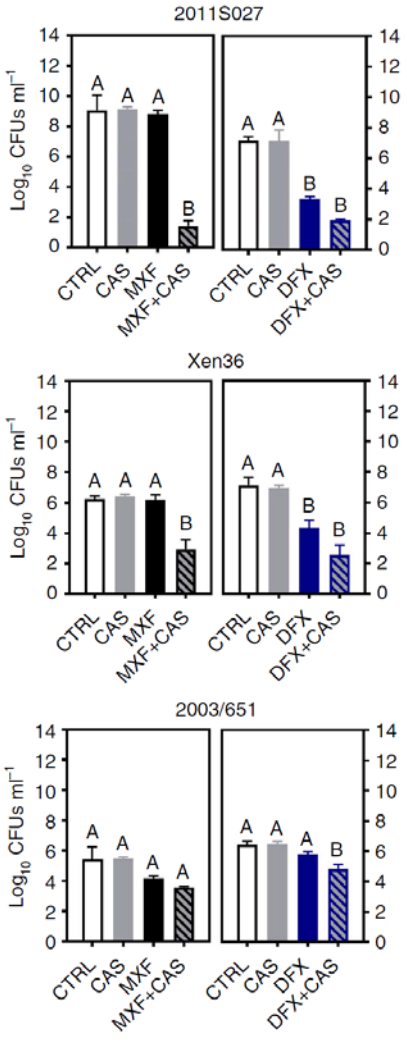


CAS increases fluoroquinolone concentration in biofilms

*Siala et al, Nature Communications 2016; 7:*13286

Caspofungin increases fluoroquinolone activity *in vitro* and *in vivo*

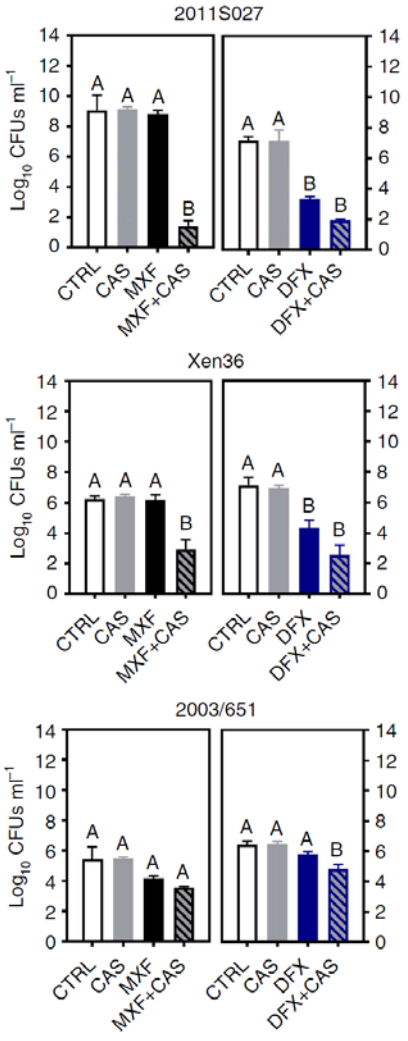
Catheters in vitro



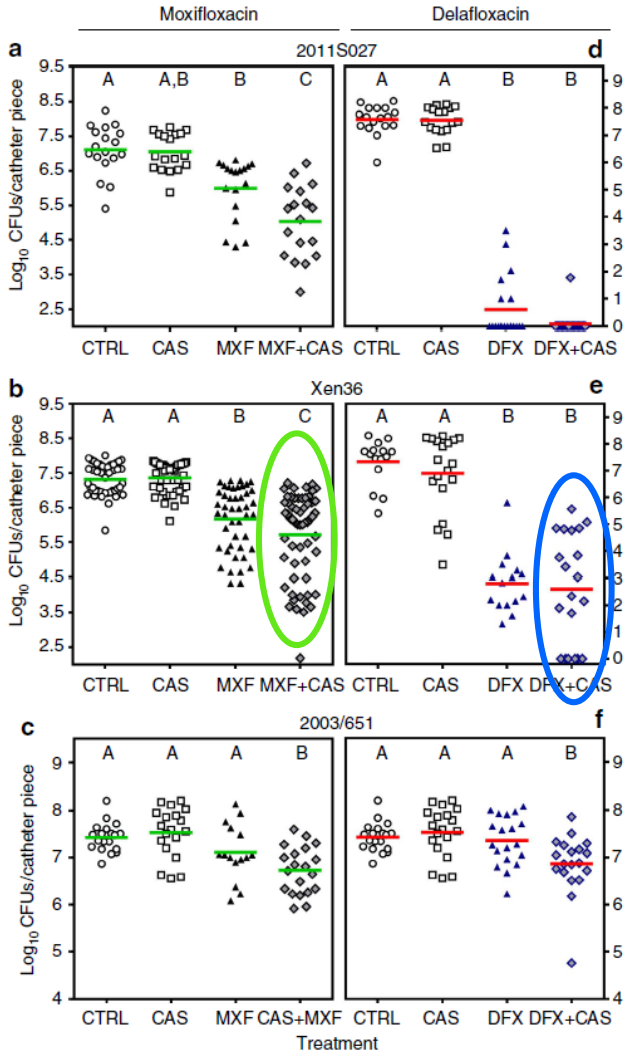
*Siala et al, Nature Communications 2016; 7:*13286

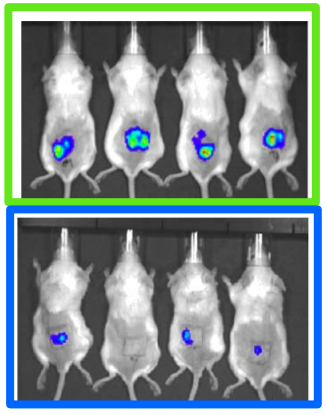
Caspofungin increases fluoroquinolone activity *in vitro* and *in vivo*

Catheters in vitro



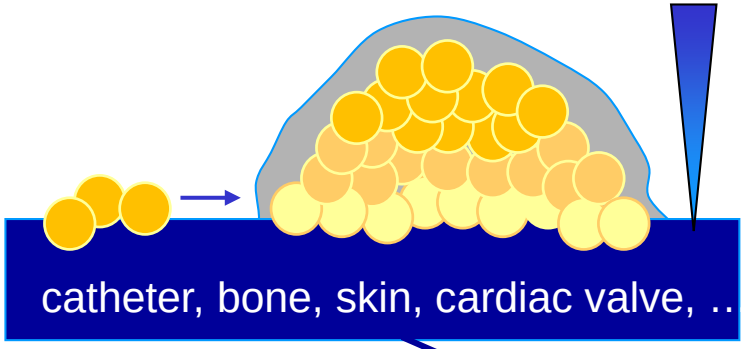
Catheters in vivo





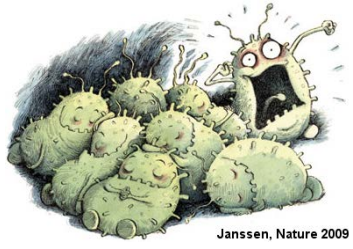
*Siala et al, Nature Communications 2016; 7:*13286

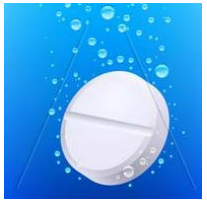
PK/PD parameters in biofilms



pharmacodynamics

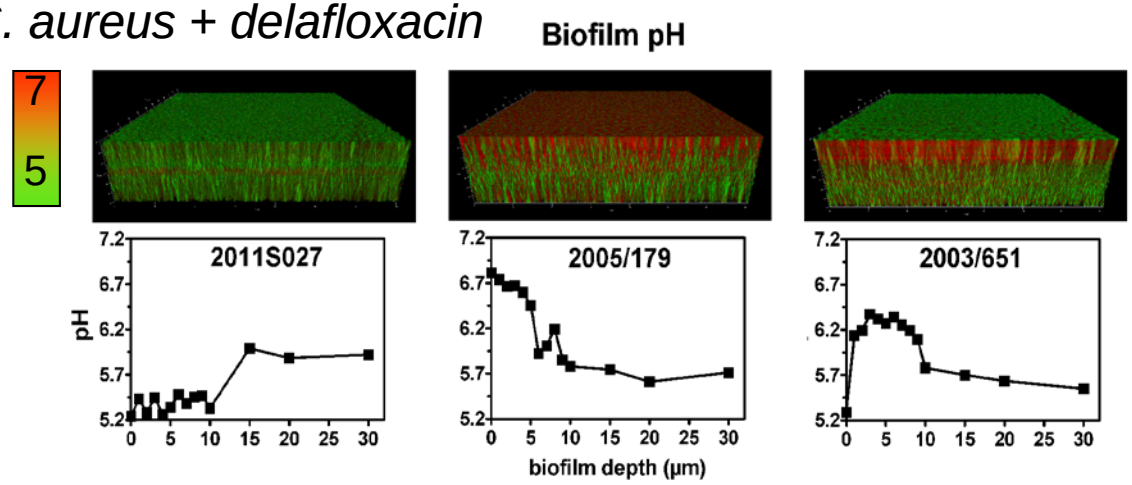
- bacterial responsiveness (metabolic activity of bacteria)
- antibiotic expression of activity (local environment [O_2, pH, ..])



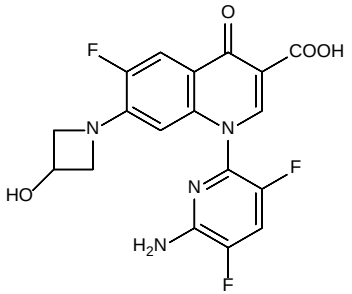


Environmental pH

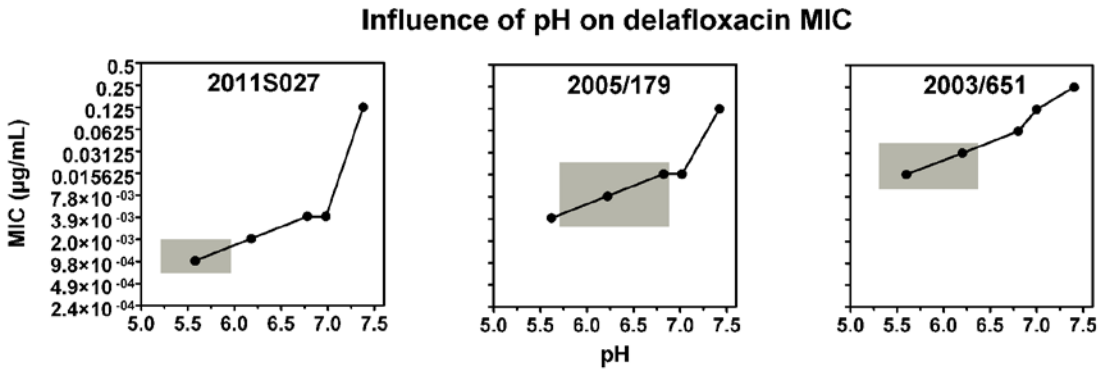
S. aureus + delafloxacin



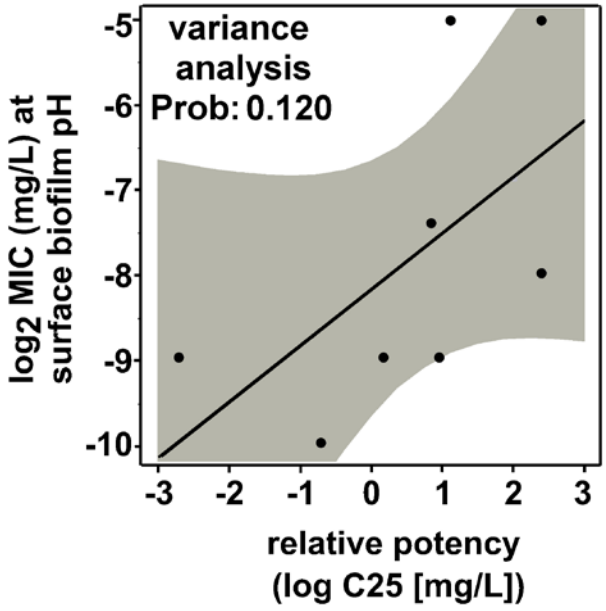
* Labelling with Seminaphthorhodafluor-4F 5-(and-6) carboxylic acid (C-SNARF-4)



delafloxacin



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



Biofilm pH may influence antibiotic intrinsic activity

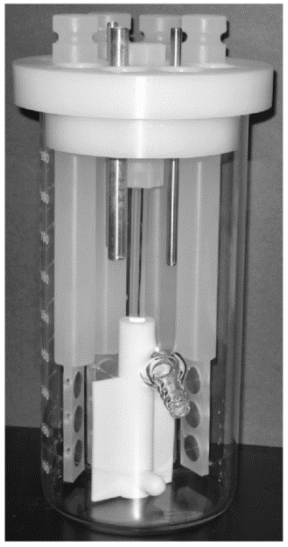
Siala et al, Antimicrob. Ag. Chemother. 2014, 58: 6385–6397.

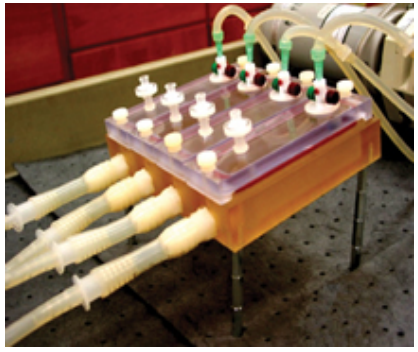
In vitro dynamic models

permanent fluid stirring

unidirectional flow replacement

constant conditions

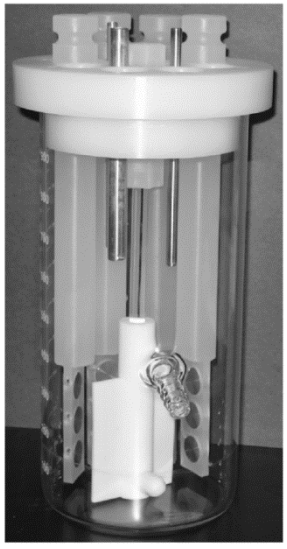


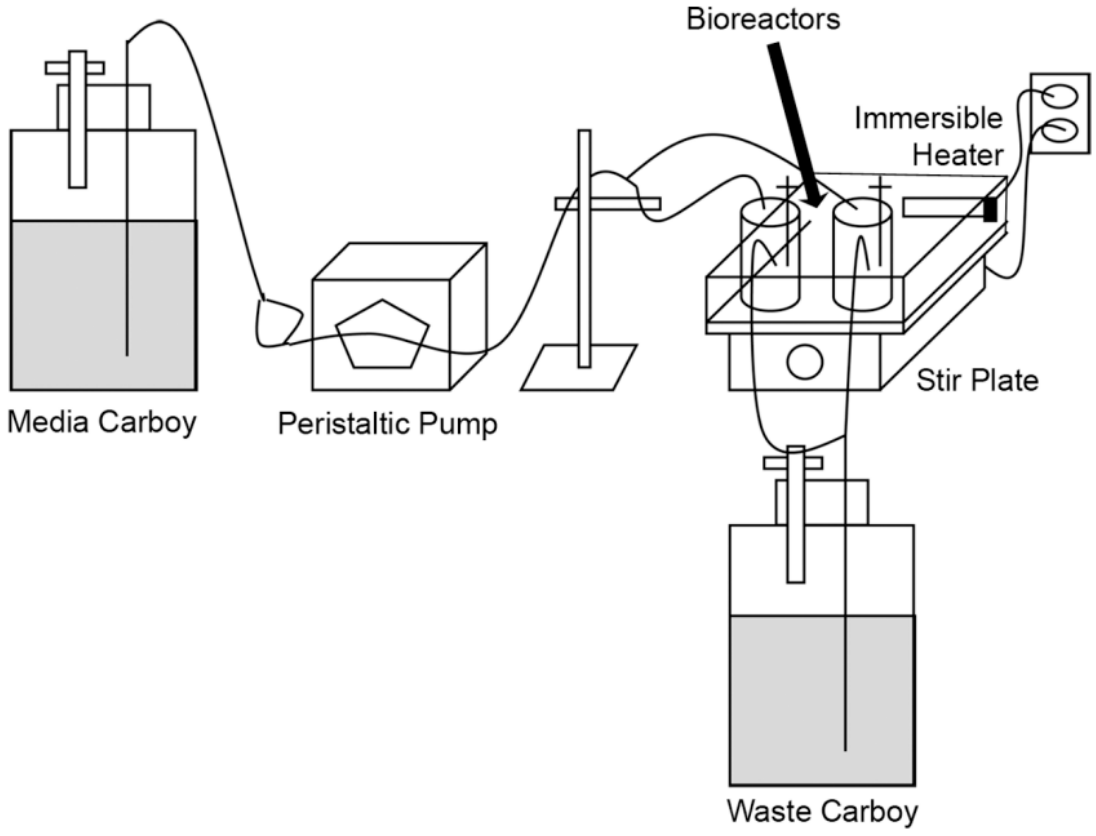


Dynamic models: bioreactors

CDC reactor:

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



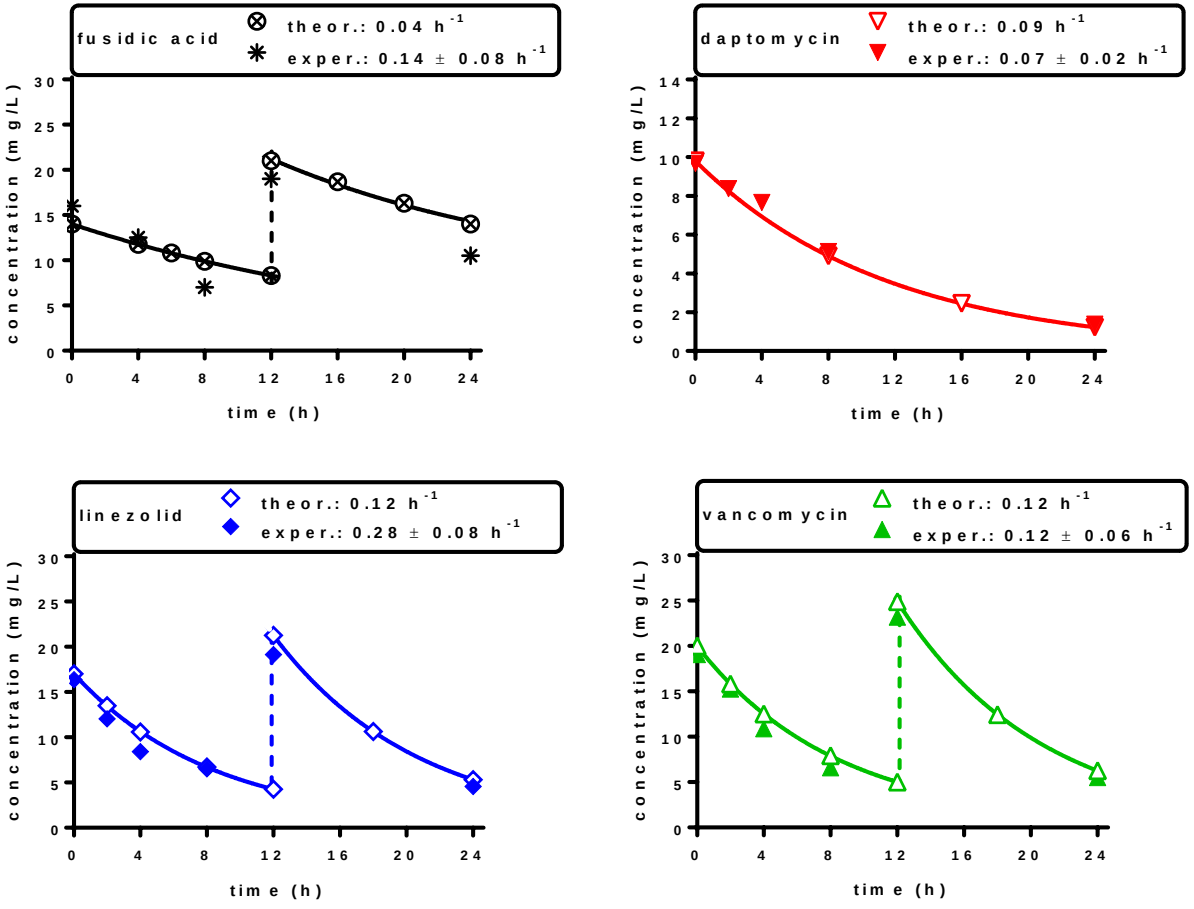


Stewart et al, PLoS One 2012;7(11):e50560

Dynamic models: bioreactors

CDC reactor:

Mimicking the pharmacokinetic profile of selected antistaphylococcal agents



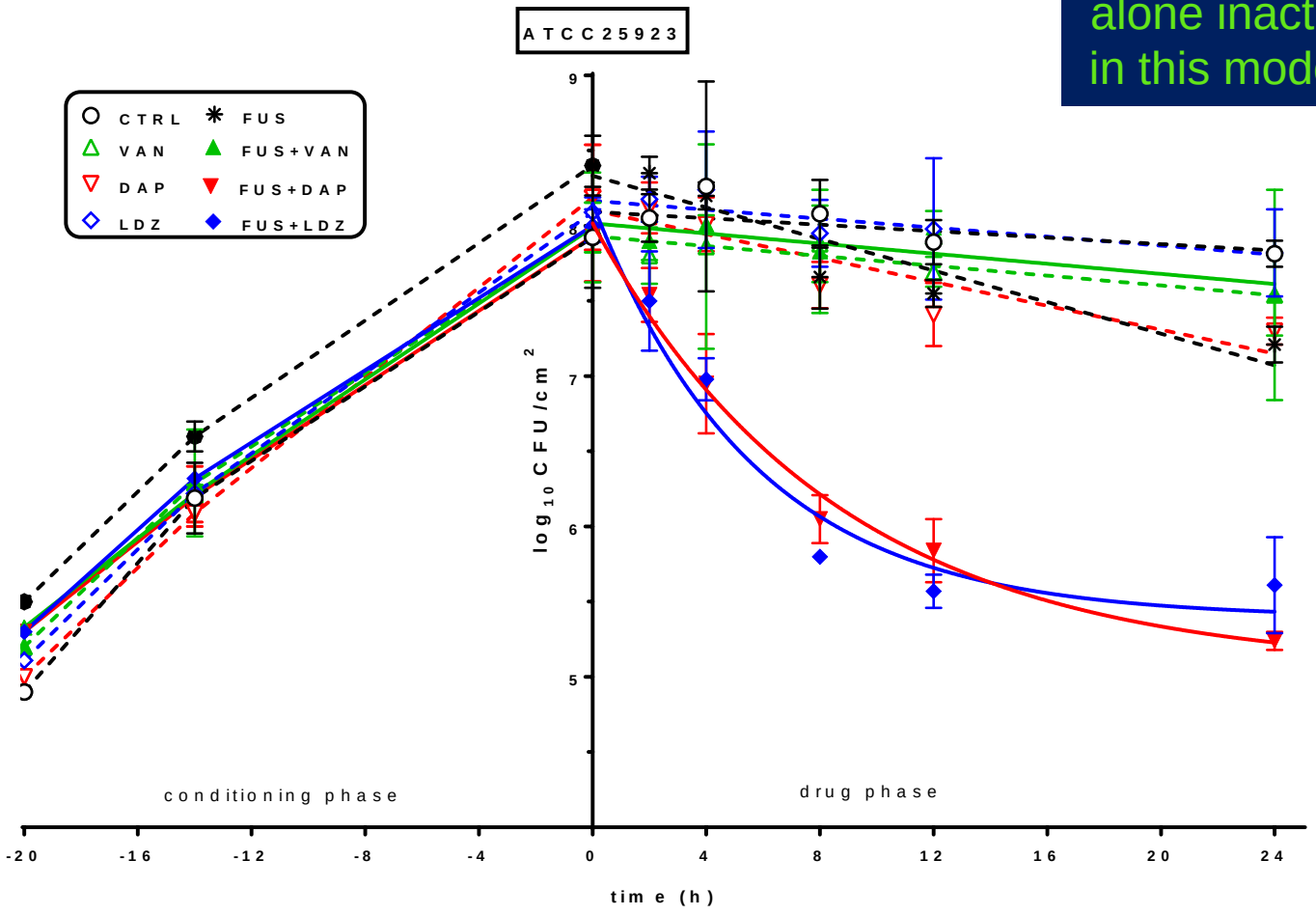
Siala et al, Antimicrob. Agents Cheomther. in press

Dynamic models: bioreactors

CDC reactor:

Activity of antibiotics alone or in combination

Antibiotics alone inactive in this model !



Siala et al, submitted

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

- Many methods to evaluate biomass / bacterial survival
  - no real consensus on the best options
- Many models to grow biofilms *in vitro*
  - comparison between studies difficult
  - more relevant model ?
- 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

Pros and Cons on biofilms models …

Standardized biofilm models **more predictive** than planktonic cultures viz.

→ Antibiotic activity against sessile bacteria

→ Resistance and tolerance mechanisms in biofilms

Possible applications

→ Drug/device registration

→ Drug/device comparisons

→ Support to move forward with a clinical trial

→ Basic biology of biofilms

Data interpretation with **caution** due to *in vitro/in vivo* differences in biofilm biology

Modulation of drug activity by PK/PD parameters, host (e.g. immune response, host tissue) and environmental factors (e.g. oxygen, nutrients)

Not predictive of clinical success due to differences in

→ Underlying biofilm biology

→ Environment (flow, shear stress)

→ Matrix composition

→ Interplay with host

PK/PD issues limiting applicability

→ Access of drugs to biofilms in deep tissues

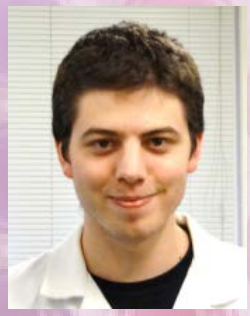
→ Effective antibiotic concentration not achievable

→ Drug tolerant phenotypes

Coenye et al, Clin Microbiol Infect. 2018. pii: S1198-743X(18)30052-1. doi: 10.1016/j.cmi.2018.01.003.

Acknowledgments

Hariri Mustafa



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