24th ECCMID Barcelona, Spain 10 – 13 May 2014
ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

In vitro models for the study of antibiotic PK/PD in biofilms

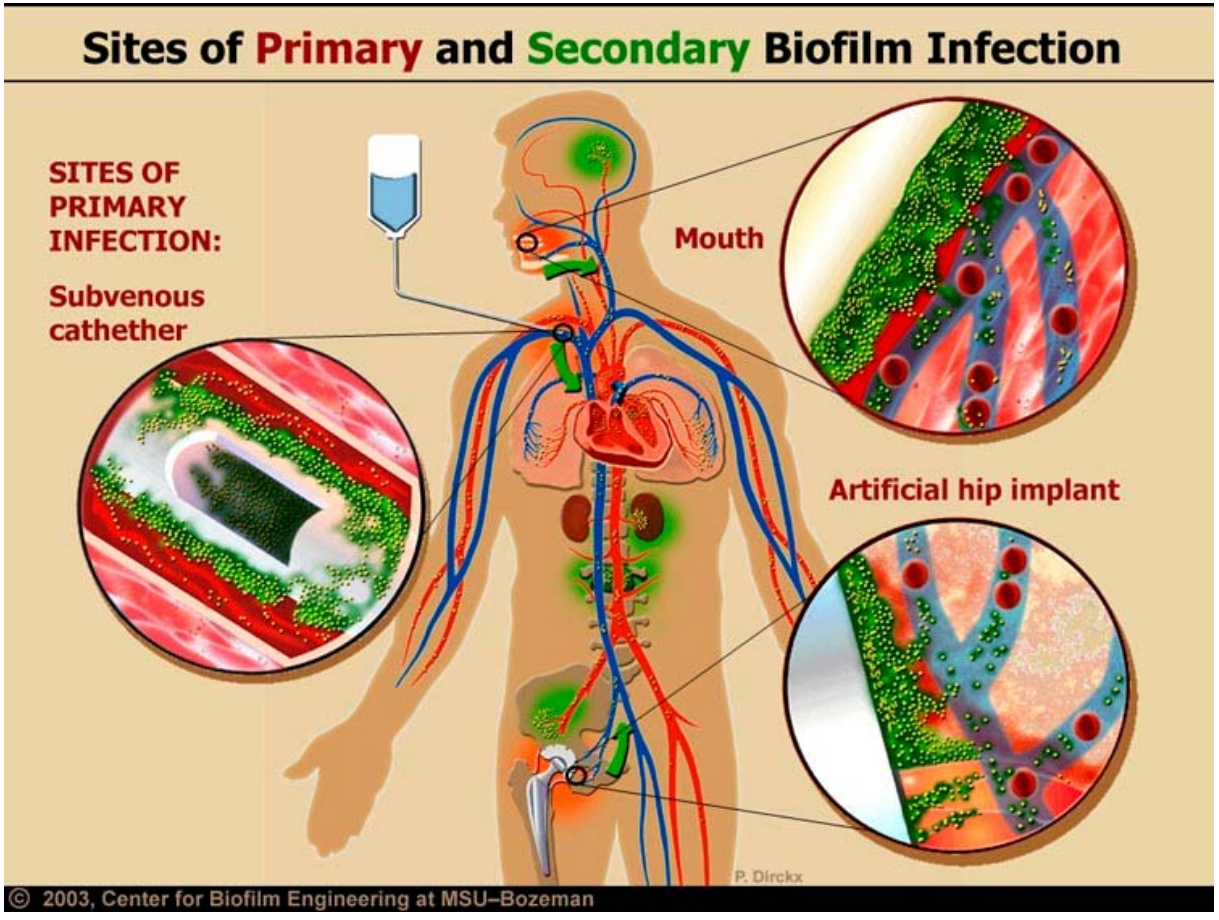
Françoise Van Bambeke, PharmD, PhD

Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute
Université catholique de Louvain,
Brussels, Belgium

<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*

Main pathogens in biofilm-related diseases

Major pathogens involved in biofilm-associated disease

Bacterial species	Biofilm infection
Escherichia coli	Acute and recurrent urinary tract infection, catheter-associated urinary tract infection, biliary tract infection
Pseudomonas aeruginosa	Cystic fibrosis lung infection, chronic wound infection, catheter-associated urinary tract infection, chronic rhinosinusitis, chronic otitis media, contact lens-related keratitis
Staphylococcus aureus	Chronic osteomyelitis, chronic rhinosinusitis, endocarditis, chronic otitis media, orthopaedic implants
Staphylococcus epidermidis	Central venous catheter, orthopaedic implants, chronic osteomyelitis
Streptococcus pneumoniae	Colonization of nasopharynx, chronic rhinosinositis, chronic otitis media, chronic obstructive pulmonary disease
Streptococcus pyogenes	Colonization of oral cavity and nasopharynx, recurrent tonsilitis

Römling & Balsalobre; J Intern Med. 2012; 272:541-61

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…

Studying antibiotic PK/PD against biofilms

Very complicated ?

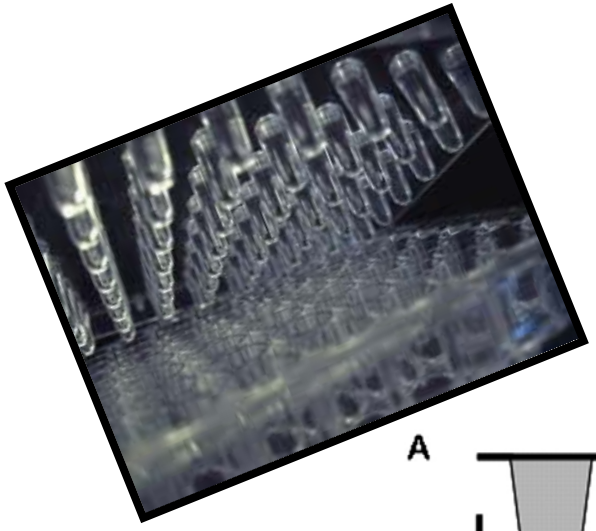
Very simple ?

Static models … for dynamic studies

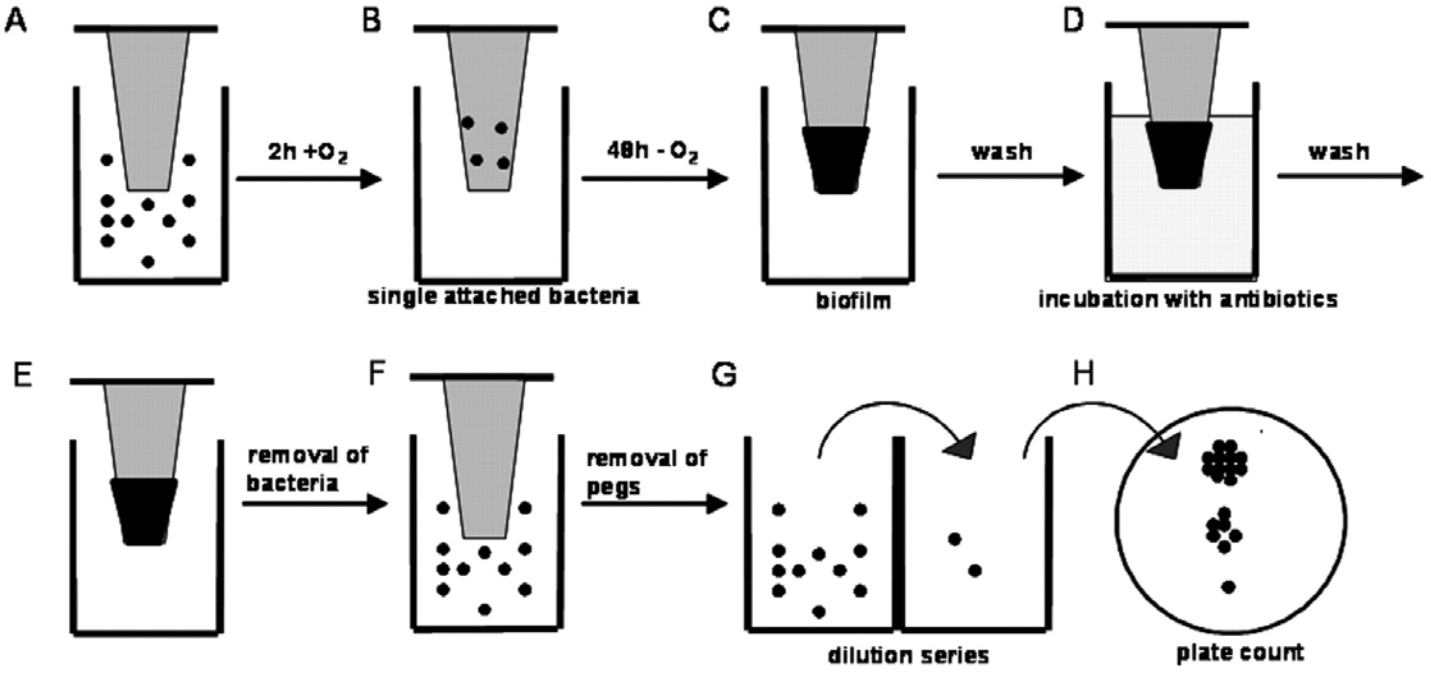
pegs

multiwell plates

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

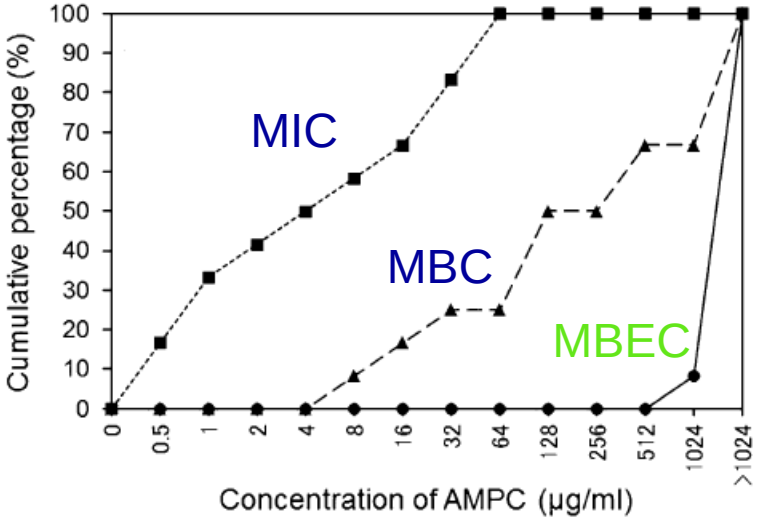
PD parameters: planktonic *vs*. biofilm cultures

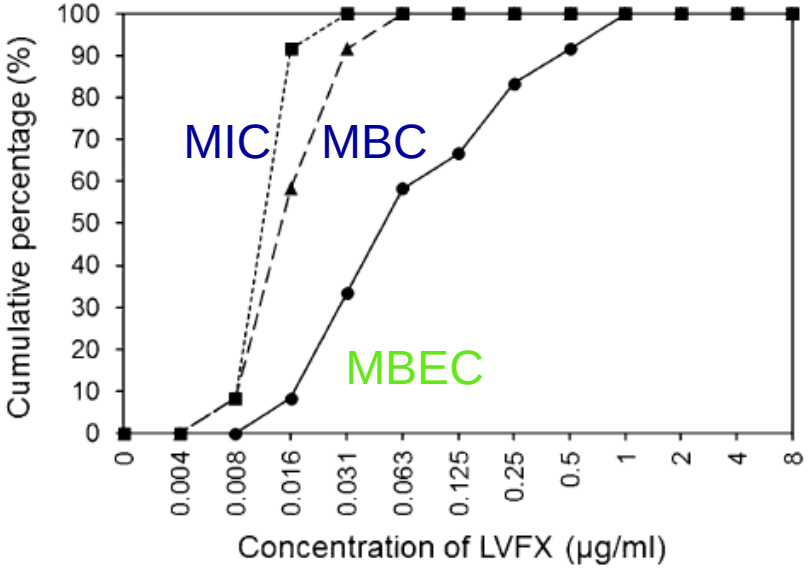
Parameter	Abbreviation	Definition
Minimal inhibitory concentration	MIC	The lowest concentration of an antibiotic that inhibits the visible growth of a planktonic culture after overnight incubation
Minimal biofilm inhibitory concentration	MBIC	The lowest concentrations of an antibiotic that resulted in 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 Apr 26

PD parameters: planktonic vs. biofilm cultures

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





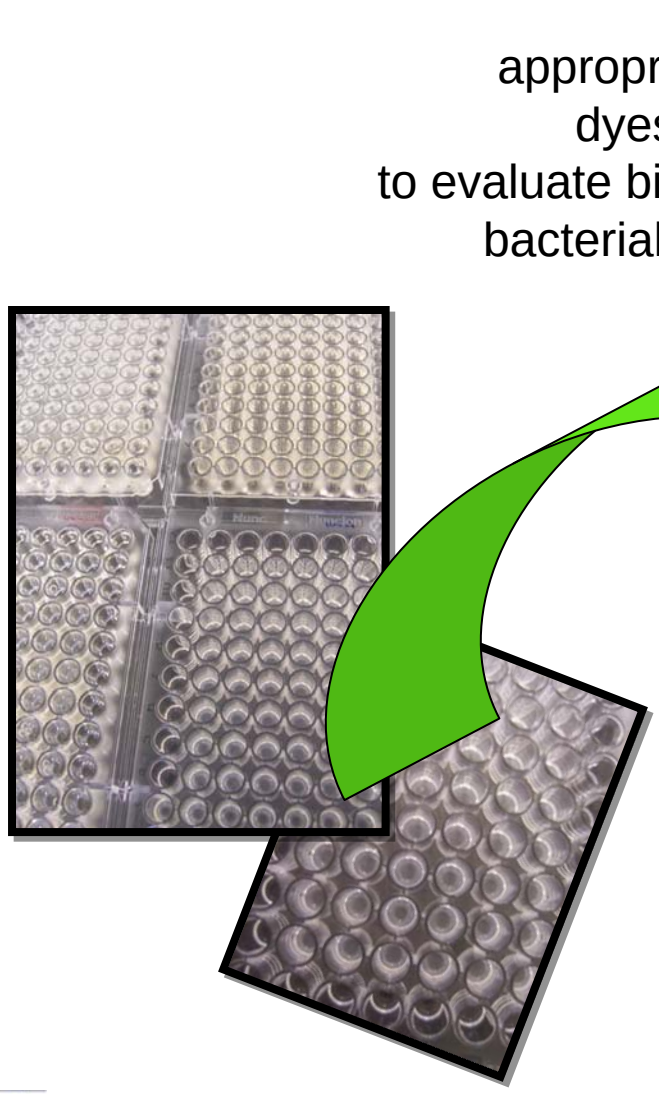
slowly bactericidal antibiotic:
MBEC >> MBC >>MIC

rapidly bactericidal antibiotic:
MBEC > MBC ~ MIC

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

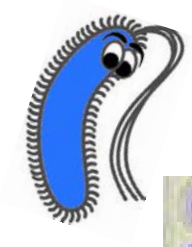
Static models: 96-well polystyrene plates

appropriate
dyes
to evaluate biomass or
bacterial load

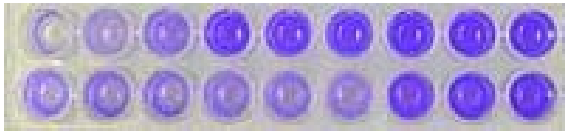


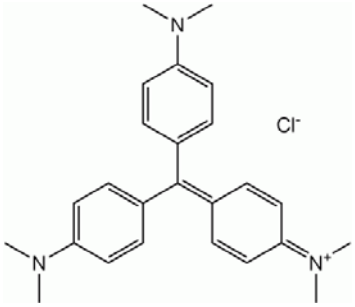
Quantifying biomass and metabolic activity in biofilms

biofilm mass



crystal violet

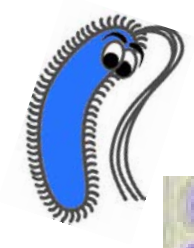




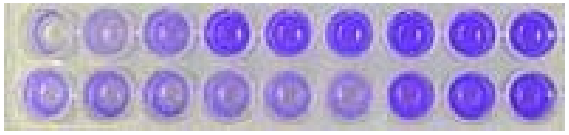
Christensen et al, Infect. Immun. 1982; 37:318–26

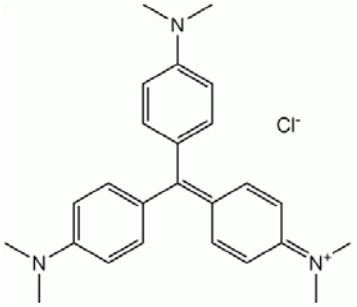
Quantifying biomass and metabolic activity in biofilms

biofilm mass



crystal violet



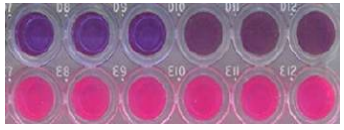


Christensen et al, Infect. Immun. 1982; 37:318–26

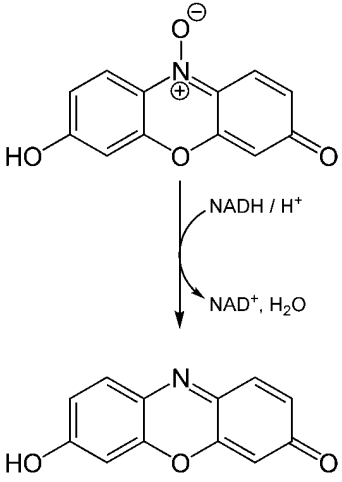
metabolic activity

Gram(+) bacteria

resazurin

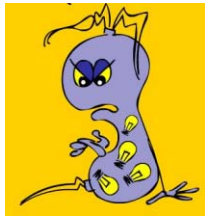


resorufin

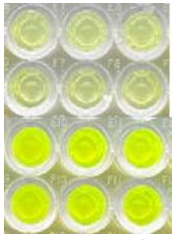


Tote et al, 2008; Lett. Appl. Microbiol. 46:249–254

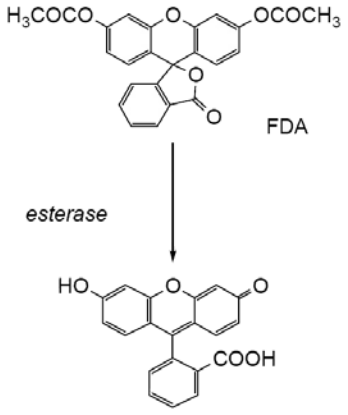
Gram(-) bacteria



fluorescein diacetate



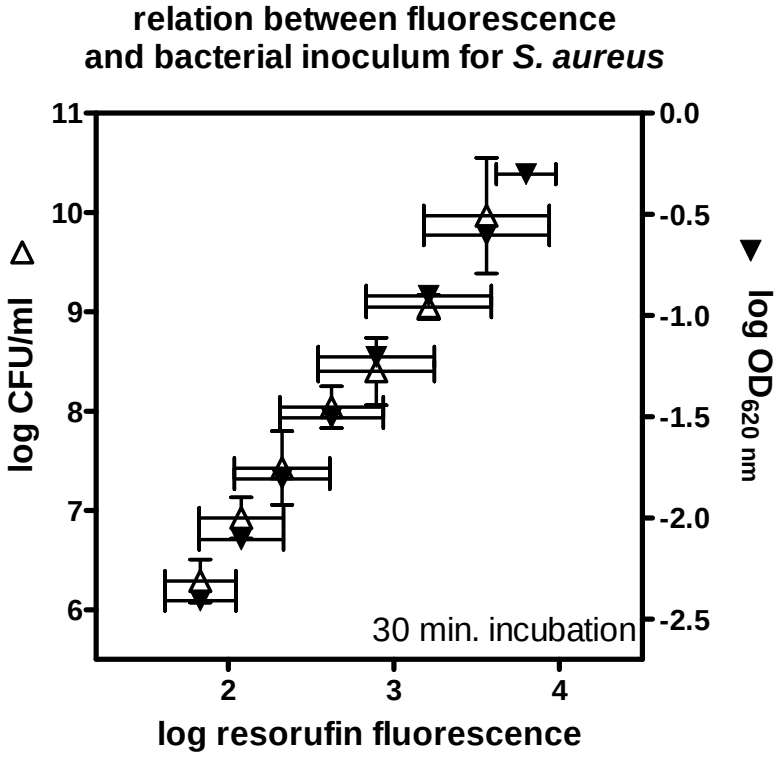
fluorescein

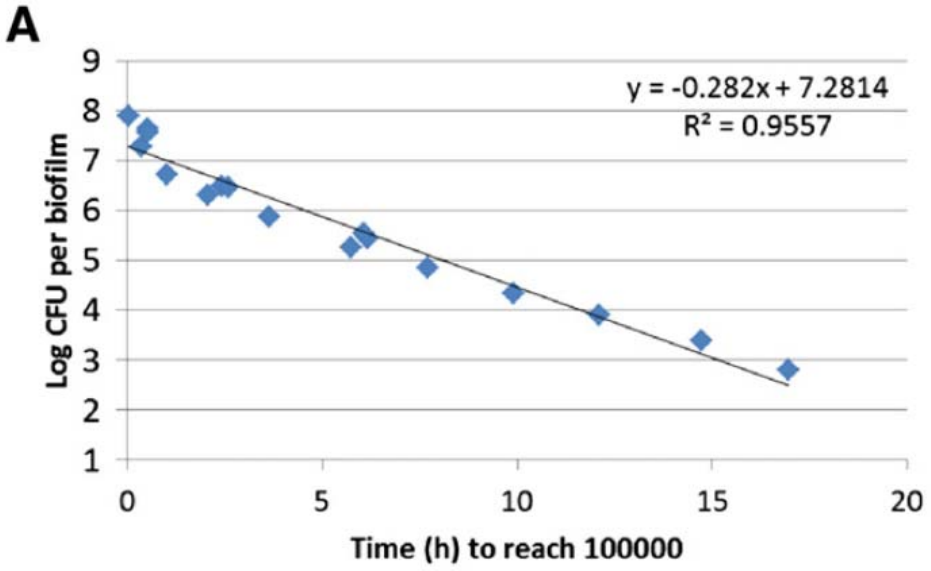


Wanandy et al, J Microbiol Methods 2005;60:21-30

CFU counting *vs.* RF fluorescence

An example for *S. aureus*





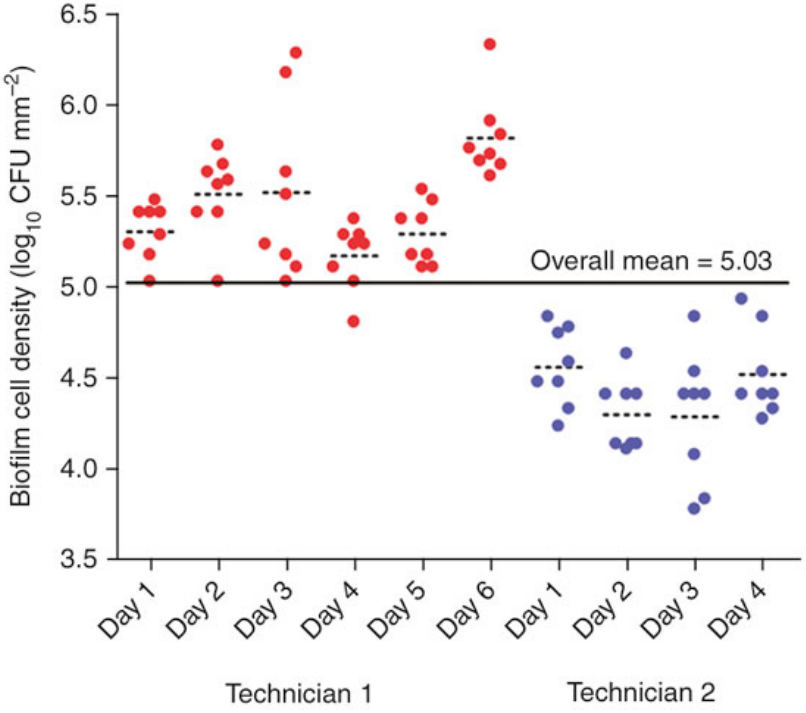
CFU & RF signal proportional

sensitivity depending on incubation time

Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37; Van den Driessche et al, J.Micr. Meth. 2014; 98:31–4

Distinguishing between static and cidal effect

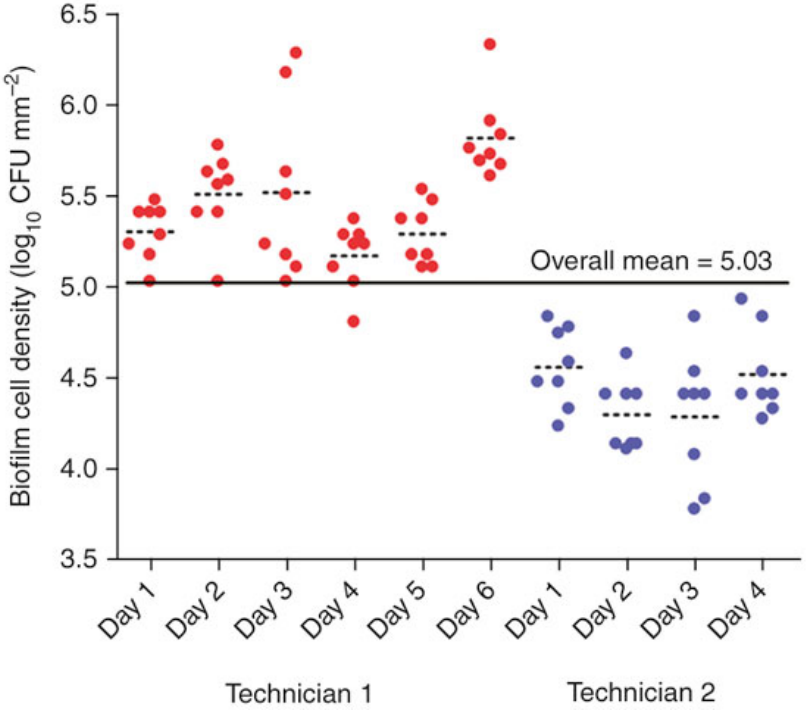
CFU counting



Hardrson et al, Nature Protocols 2010; 5:1236-54

Distinguishing between static and cidal effect

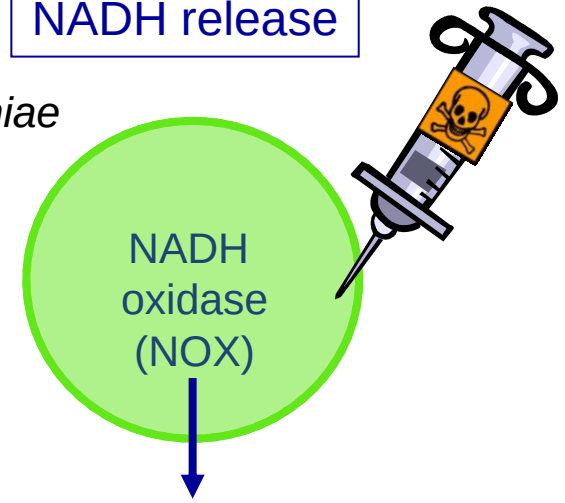
CFU counting



Hardrson et al, Nature Protocols 2010; 5:1236-54

NADH release

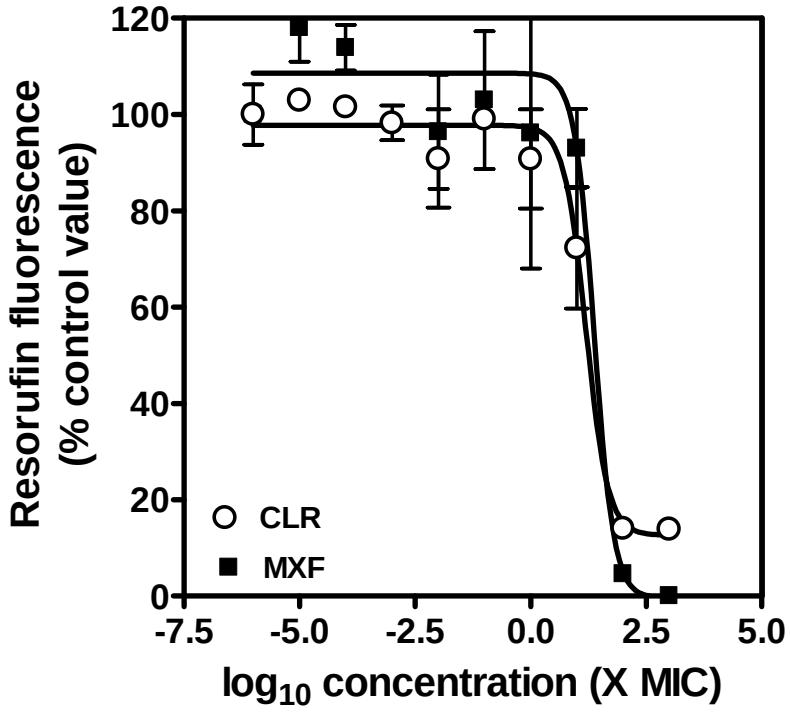
S. pneumoniae

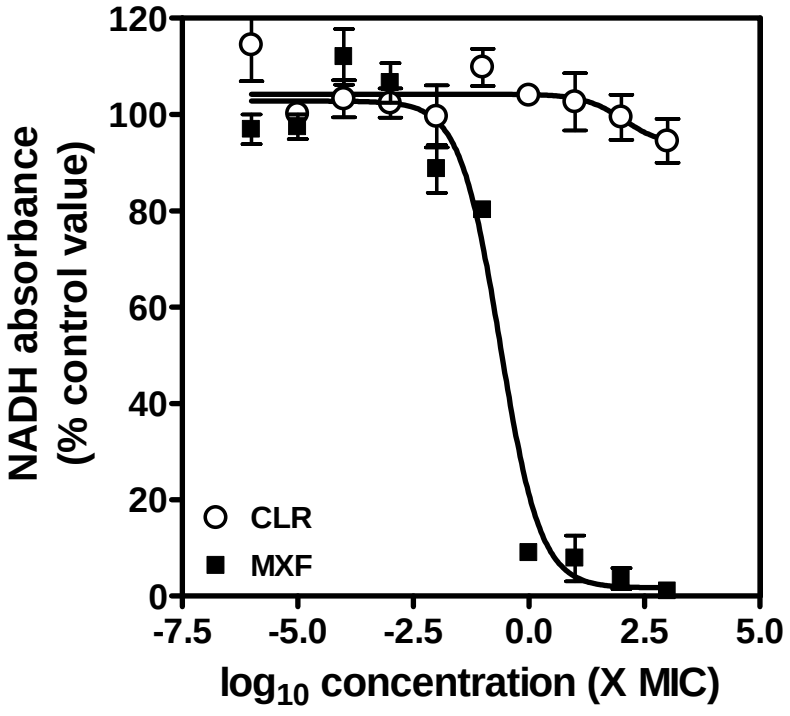


Yu et al, Microbiology. 2001;147(Pt 2):431-8

RF fluorescence *vs.* NADH oxidase

An example for *S. pneumoniae*





residual living bacteria

dead bacteria only

Vandevelde et al, ECCMID 2014; eP288

PK/PD studies: a few exemples

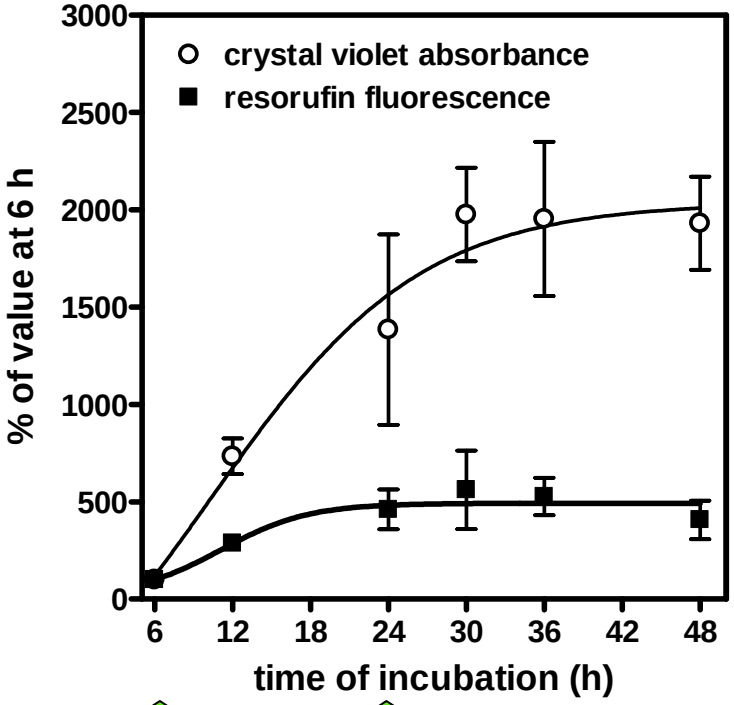
Cooncentration effects

Time effects

S. aureus & S. pneumoniae models

Kinetics of biofilm formation

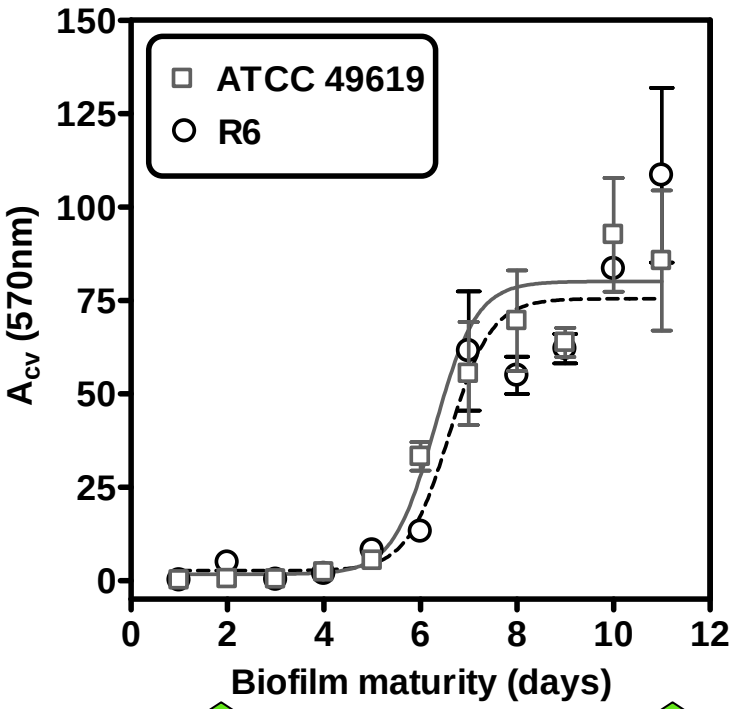
S. aureus



young biofilm

mature biofilm

S. pneumoniae

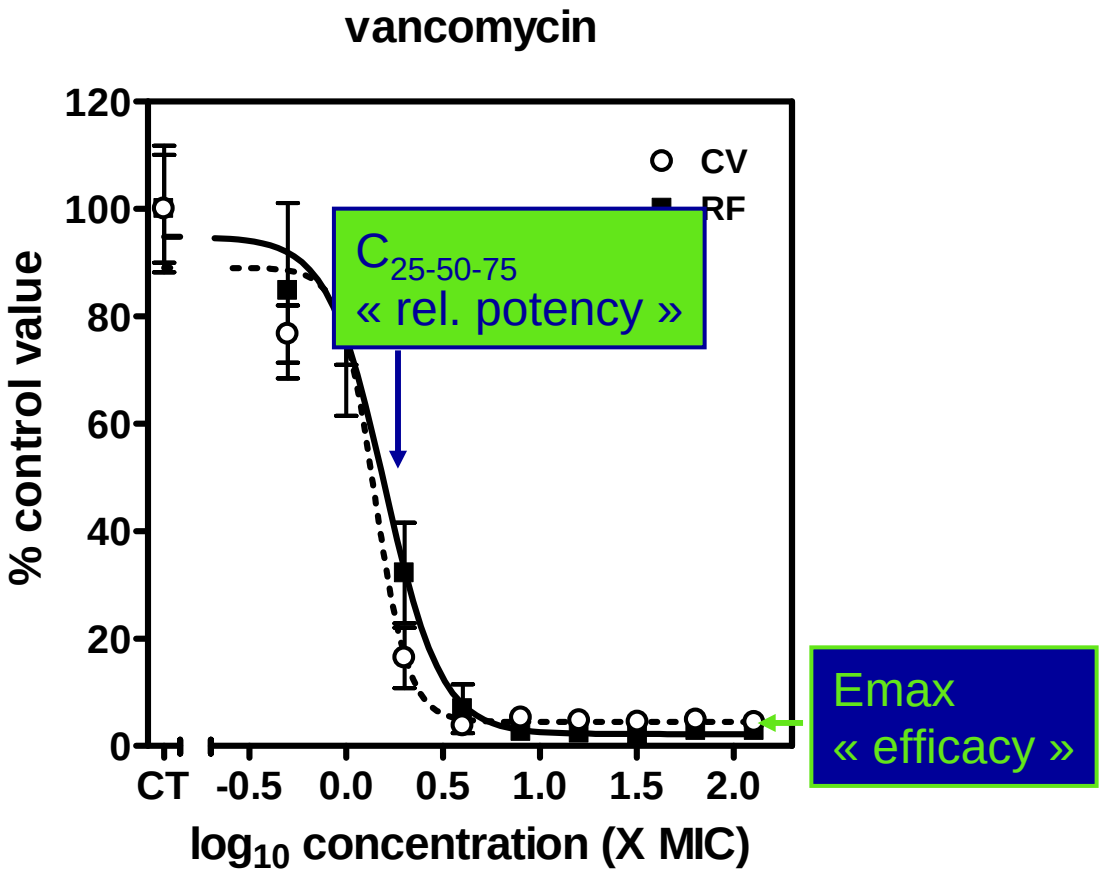


young biofilm

mature biofilm

Pharmacodynamic model for antibiotic activity

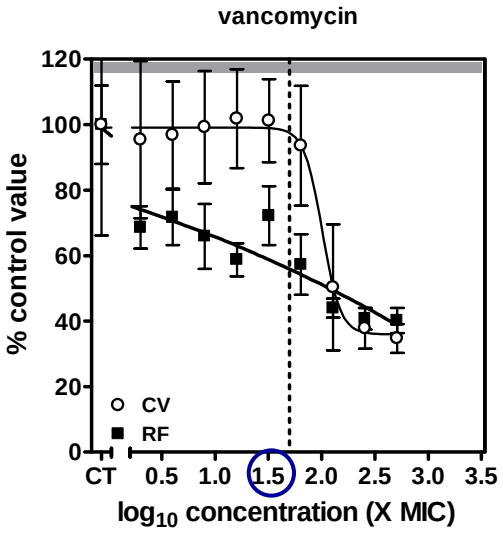
An example with young biofilm of *S. aureus*

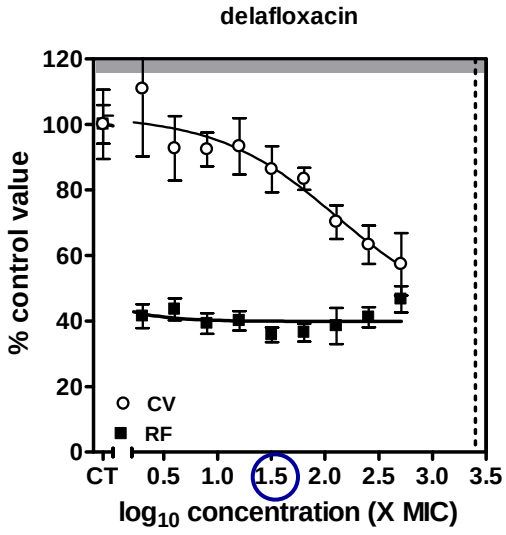


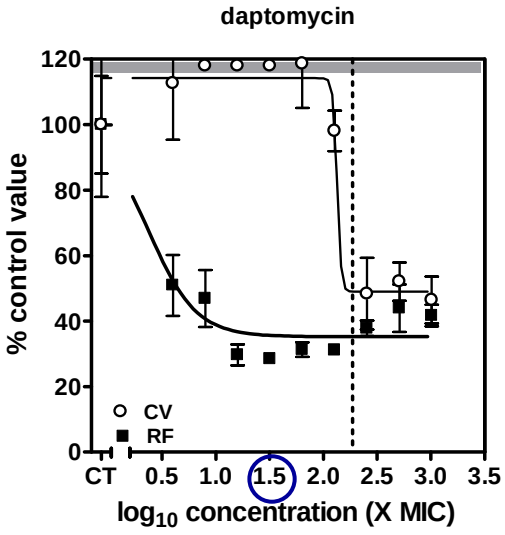
Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37

S. aureus mature biofilms: comparison of drugs

ATCC33591 (MRSA)

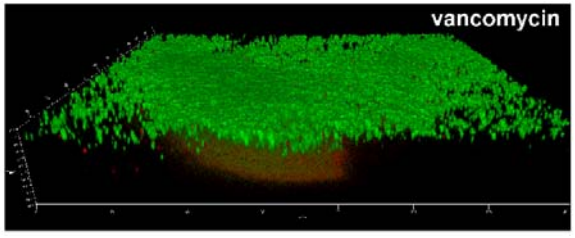


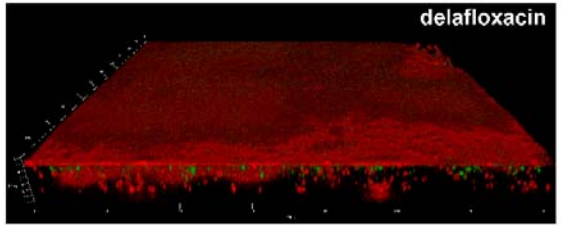


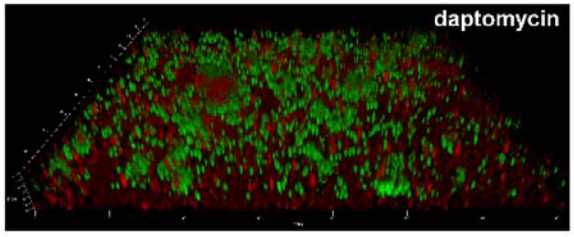


life

dead





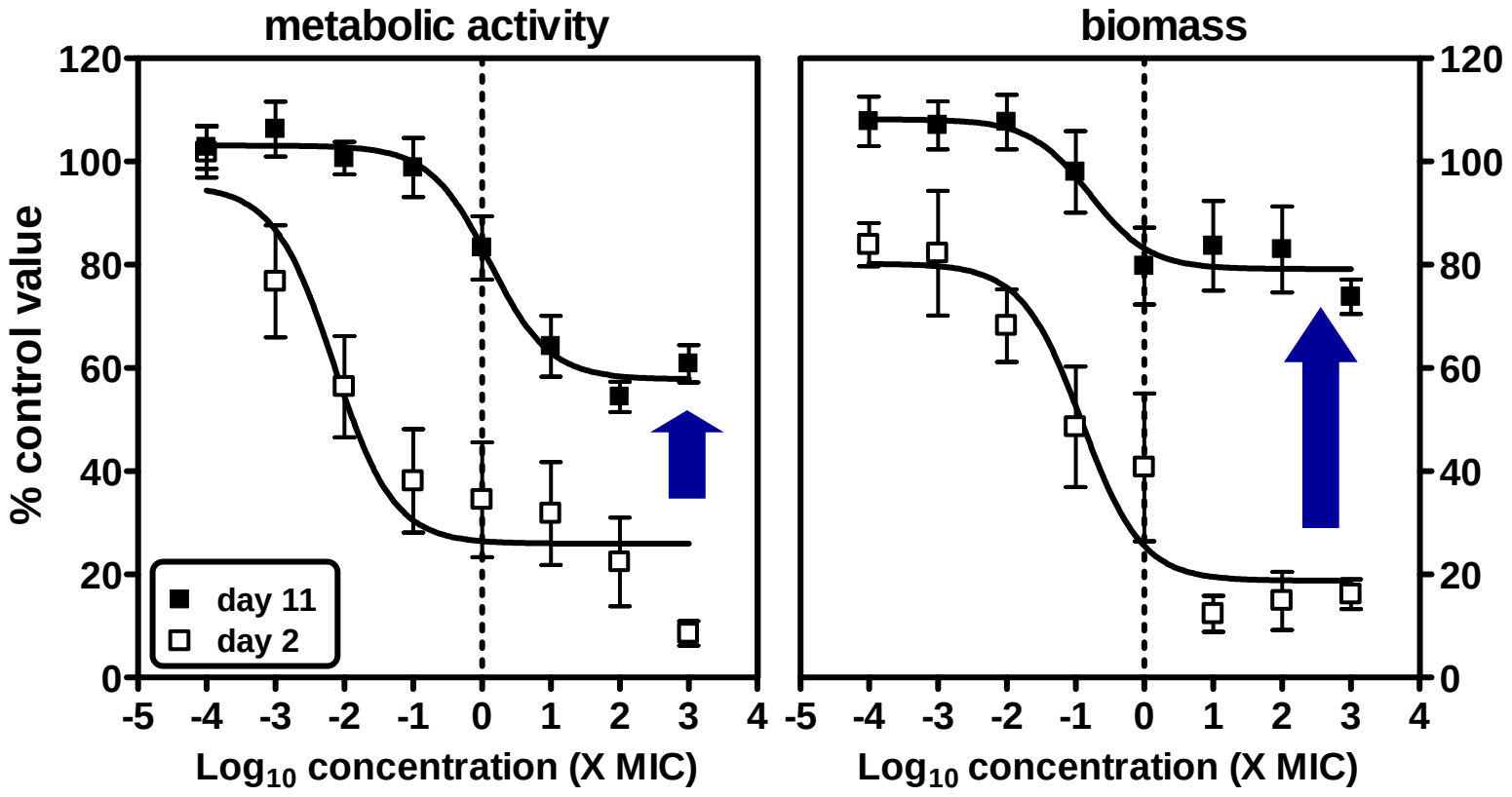


- more active on viability than on matrix
- huge difference among drugs

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

S. pneumoniae biofilms - influence of maturity

moxifloxacin

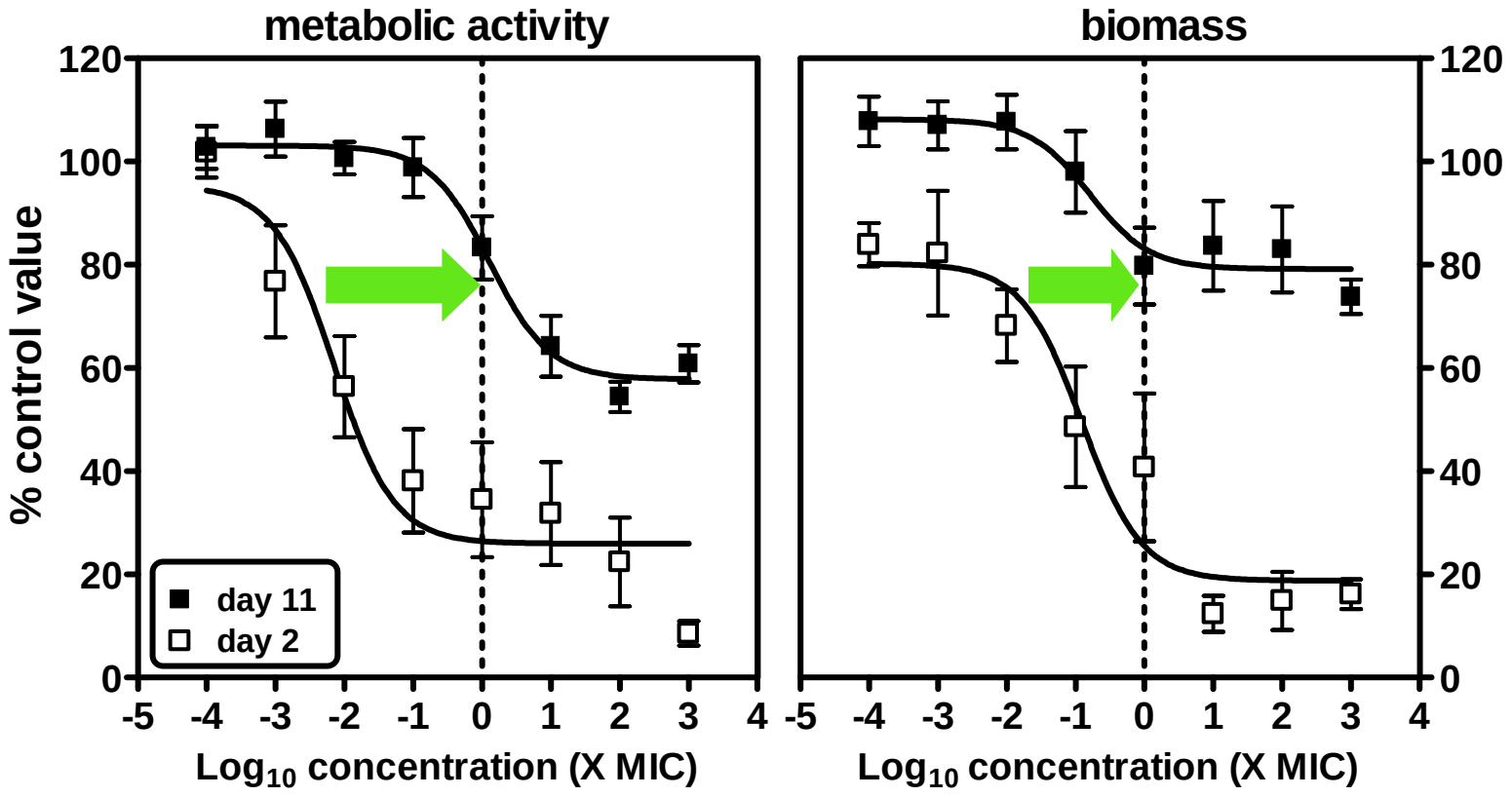


maximal efficacy ⇘ with maturity

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

S. pneumoniae biofilms - influence of maturity

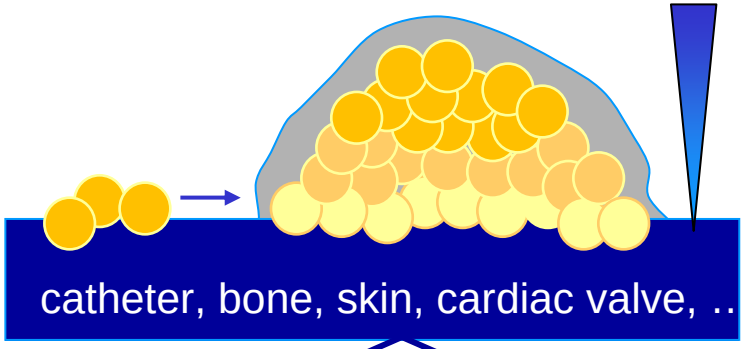
moxifloxacin



relative potency ⇘ with maturity

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

PK/PD parameters in biofilms

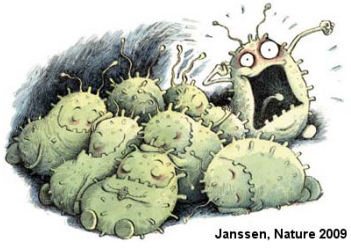


pharmacokinetics

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

pharmacodynamics

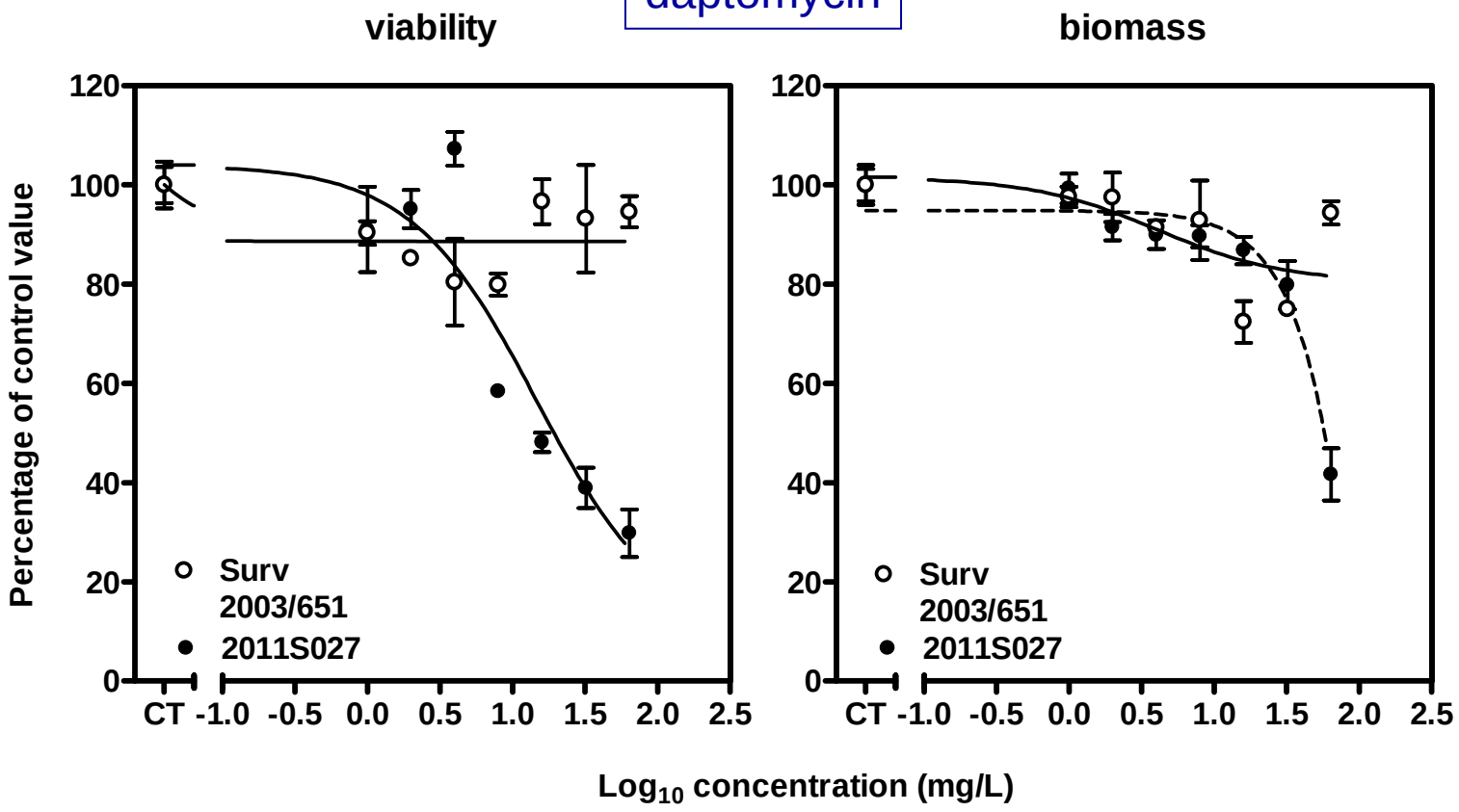
- bacterial responsiveness (metabolic activity of bacteria)
- antibiotic expression of activity (local environment [O_2, pH, ..])



Parameters affecting antibiotic activity in biofilms

2 clinical isolates of *S. aureus*

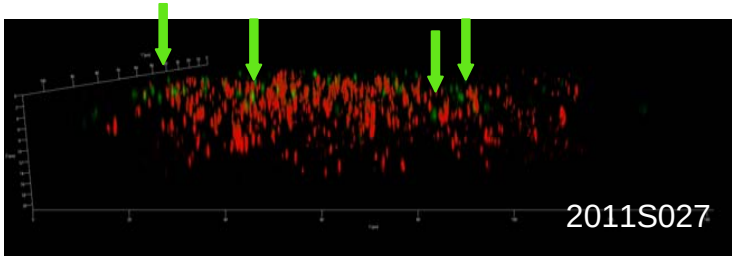
daptomycin

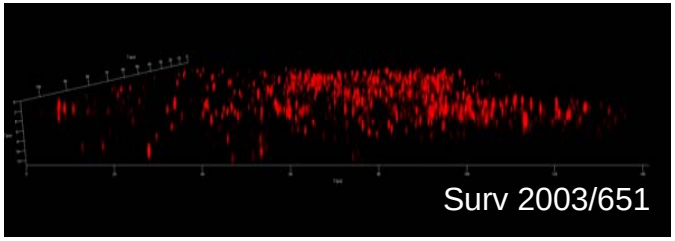


What makes the difference ?

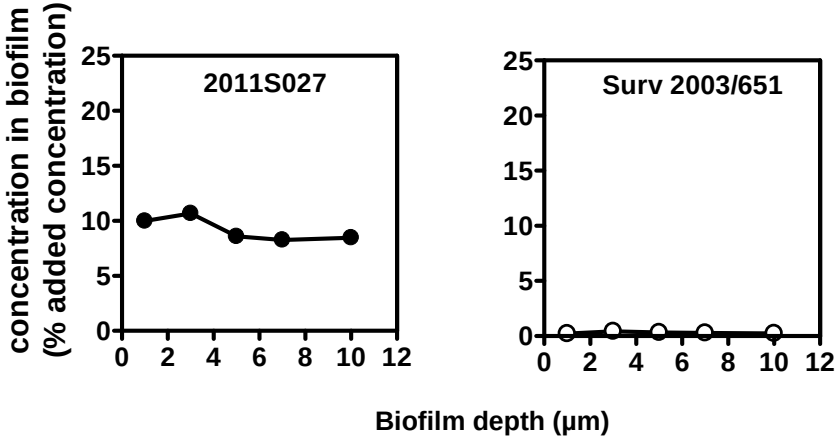
Siala et al, in preparation

PK parameter: antibiotic penetration

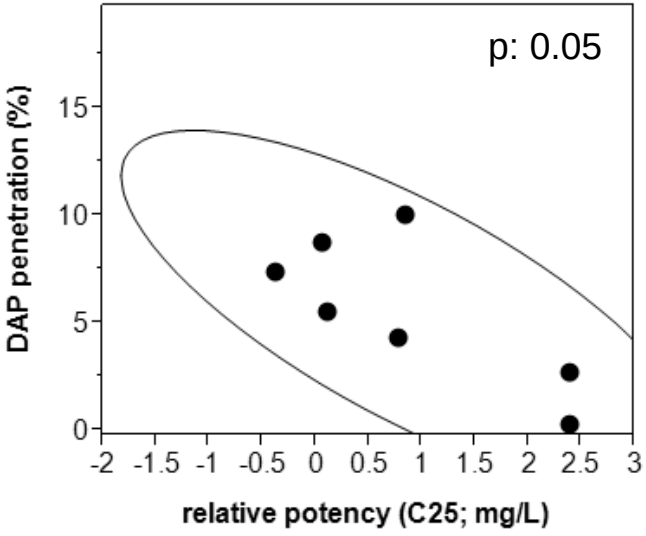




Bodipy-DAP
CTC



more strains

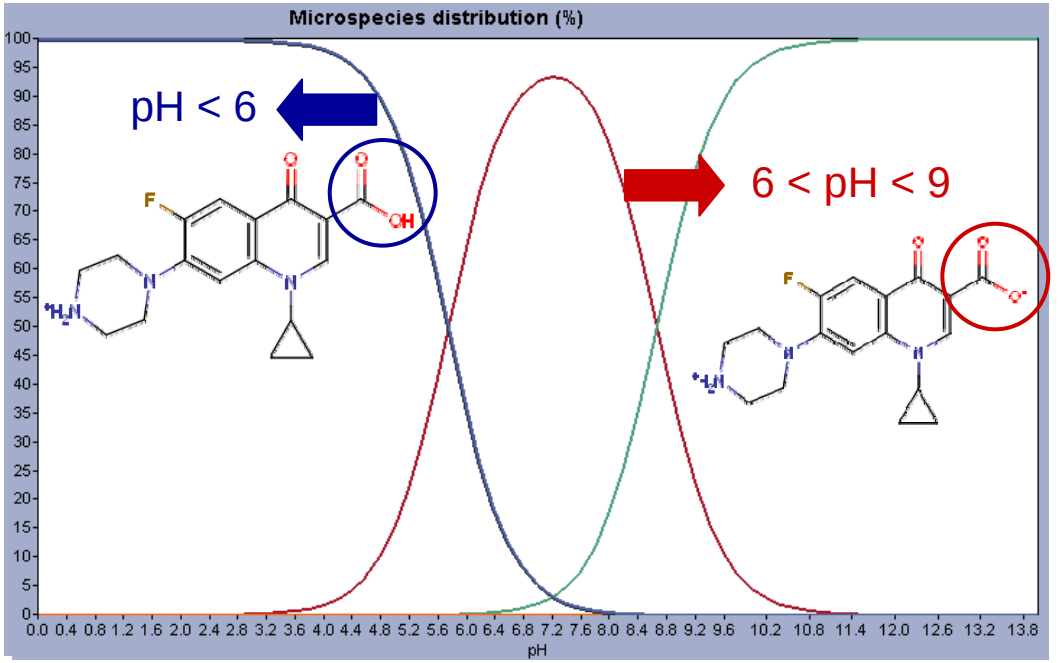


→ more potent if better penetration

Siala et al, in preparation

PD parameter: environmental pH

pH and fluoroquinolones



Proteus mirabilis
planktonic cultures *vs.* biofilms

	Ciprofloxacin (mg l^{-1})		
pH	MIC	MBC	MBEC
5	0.66	250	250
7	0.08	0.31	125
9	0.04	0.04	25
10	0.04	0.08	25

→ less potent in acidic biofilms

Irwin et al, J. Appl. Microbiol. 2013; 15:382-9

PK/PD : on the way to biofilm bkpts ?

Ceftobipole and comparators vs. *S. aureus*

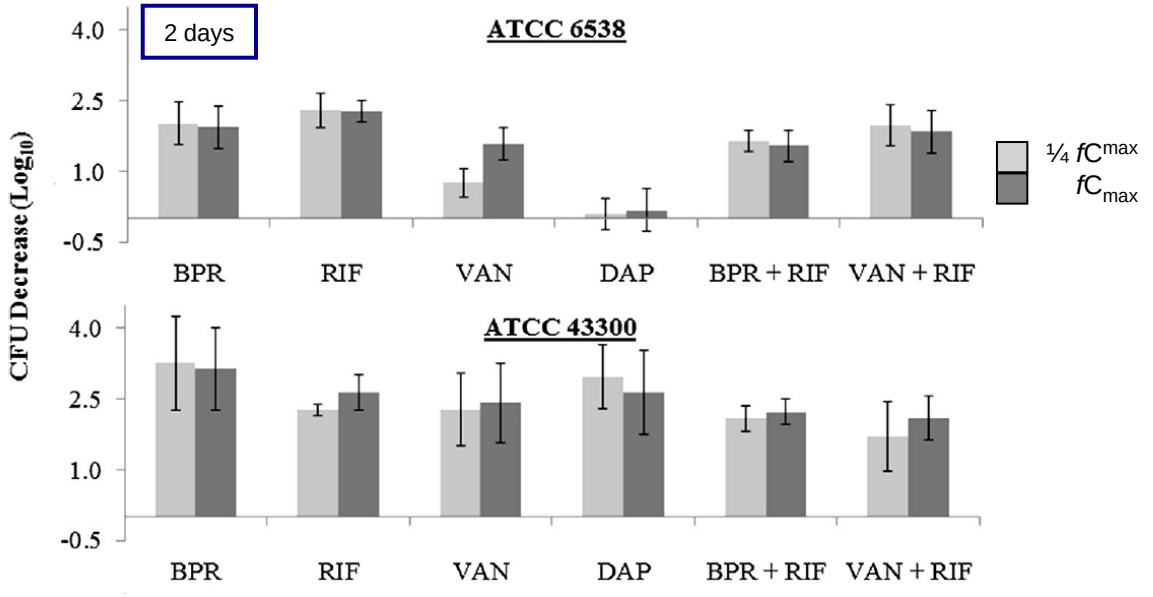


Table 2
Dose–response of ceftobiprole (BPR) and rifampicin (RIF) with 11-day *Staphylococcus aureus* colony biofilms.

Antibiotic concentration	CFU decrease ($\log_{10}$) after 7 days of antibiotic exposure[a]					
	BPR		RIF		BPR + RIF	
	MSSA ATCC 6538	MRSA ATCC 43300	MSSA ATCC 6538	MRSA ATCC 43300	MSSA ATCC 6538	MRSA ATCC 43300
fC_{max}	1.6	2.8	0.07	0.43	3.8	4.9
1/2 fC_{max}	1.6	2.5	0.03	0.33	3.1	4.1
1/4 fC_{max}	1.9	1.7	0.03	0.30	4.7	4.7
1/8 fC_{max}	2.1	0.68	0.09	0.14	3.8	3.6
1/16 fC_{max}	2.7	0.20	0.08	0.19	2.8	1.5
1/32 fC_{max}	3.8	0.14	0.24	0.47	3.4	0.44
1/64 fC_{max}	2.7	N/T	0.10	N/T	3.1	N/T

fC_{max}, maximum free-drug plasma concentration attained during clinical use; MSSA, meticillin-susceptible *S. aureus*; MRSA, meticillin-resistant *S. aureus*; N/T, not tested.
[a] CFU decreases were calculated relative to biofilm CFUs of Day 11 non-drug controls and were averaged from ≥4 individual biofilms.

Abbanat et al, Int J Antimicrob Agents. 2014;43:32-9

In vitro dynamic models

permanent fluid stirring

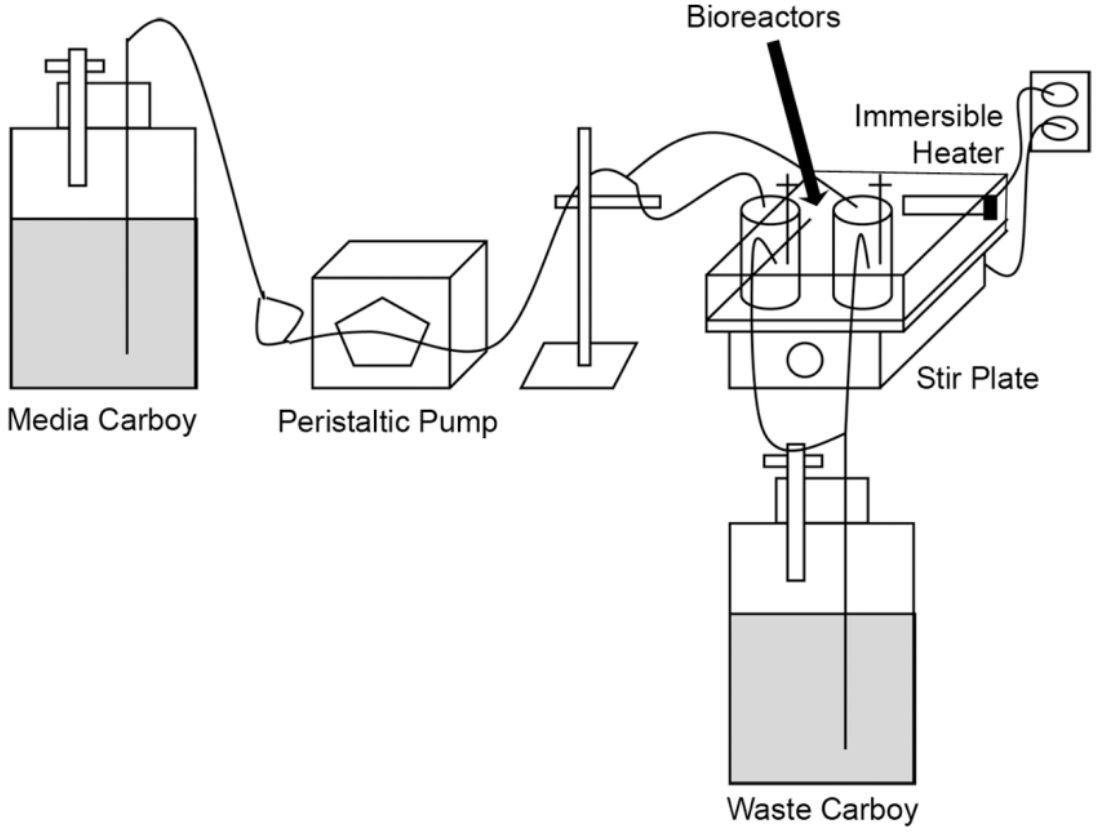
unidirectional flow replacement

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

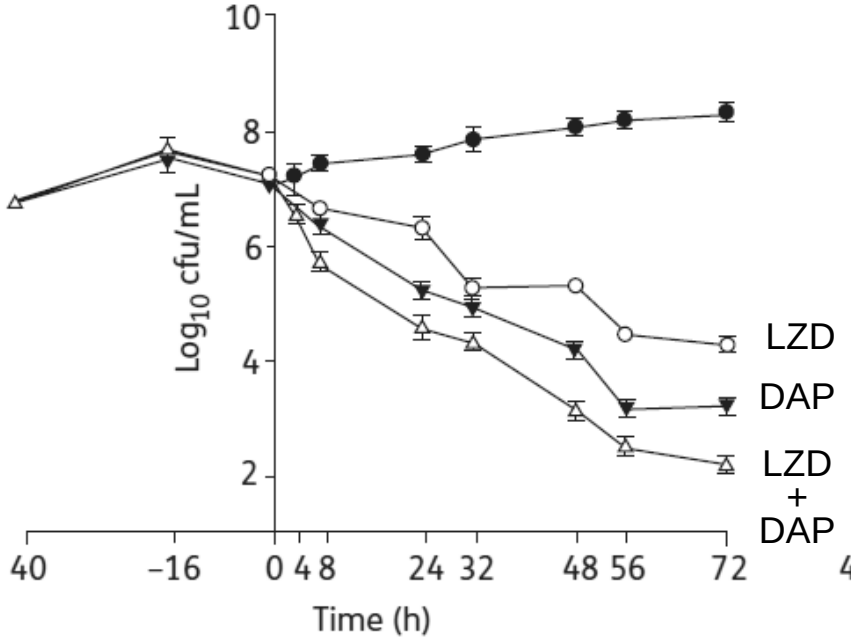
Antibiotic activity - mimicking human exposure

CDC reactor

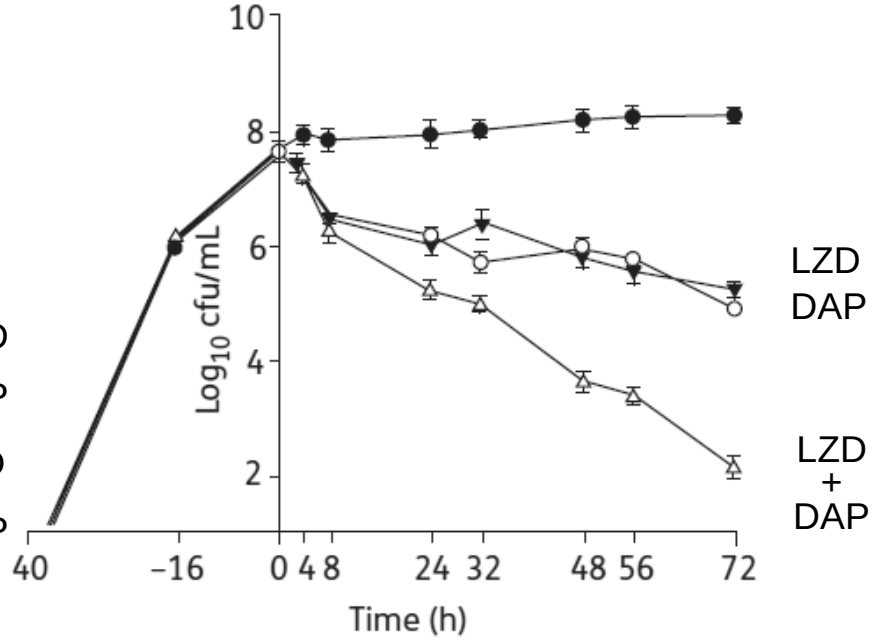
S. aureus

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

Planktonic cultures



Biofilm (CDC reactor)



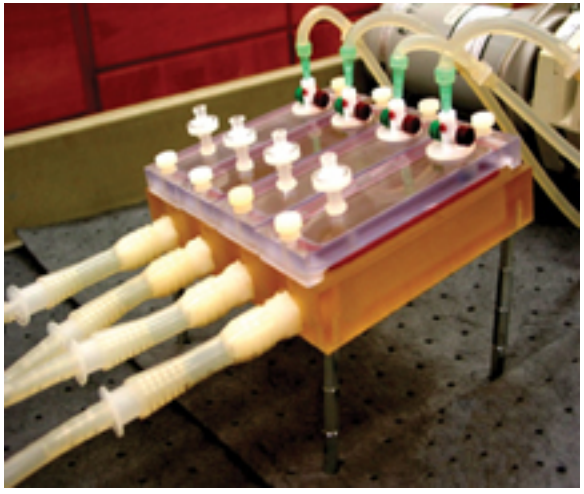
Combination more useful against biofilm than planktonic bacteria

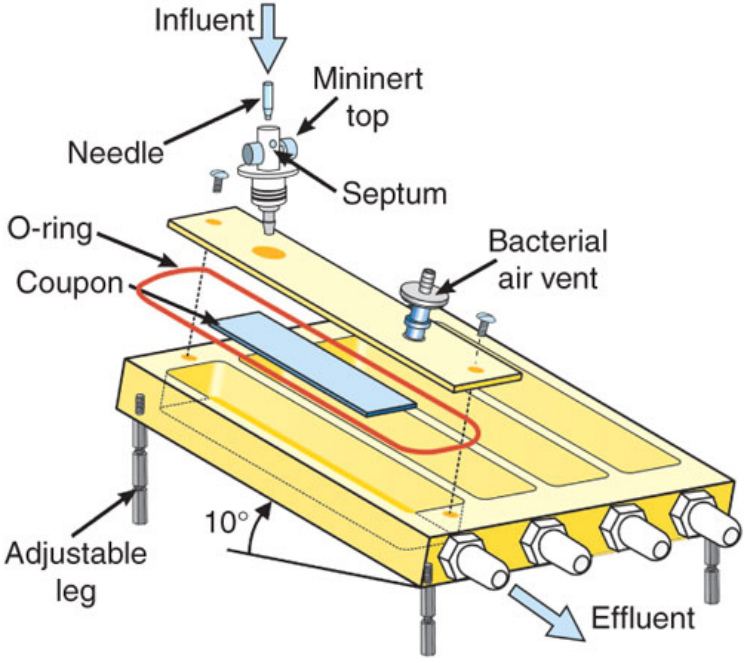
Parra-Ruiz et al, J Antimicrob Chemother 2012; 67:2682–5

Dynamic models: bioreactors

Drip flow reactor :

- progressive, unidirectional change in medium
- low shear stress

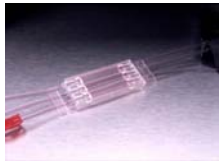




Goeres et al, Nature Protocols 2009; 4: 783-8

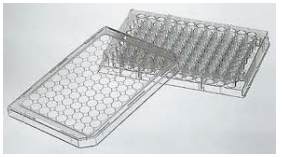
Expression of antibiotic resistance

Flow cell reactor



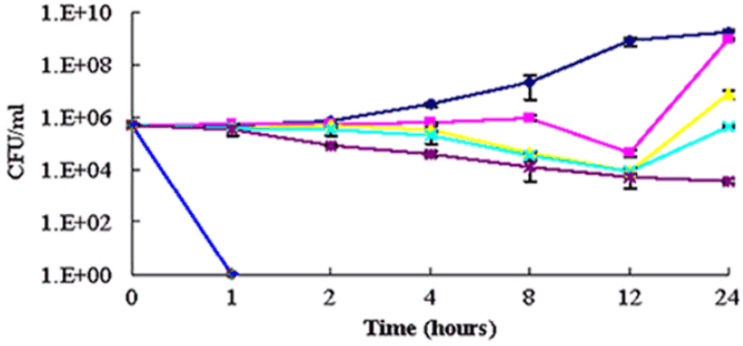
P. aeruginosa

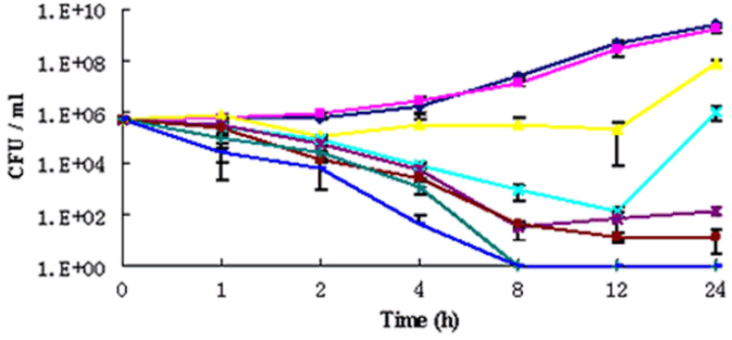
96-well plates, static



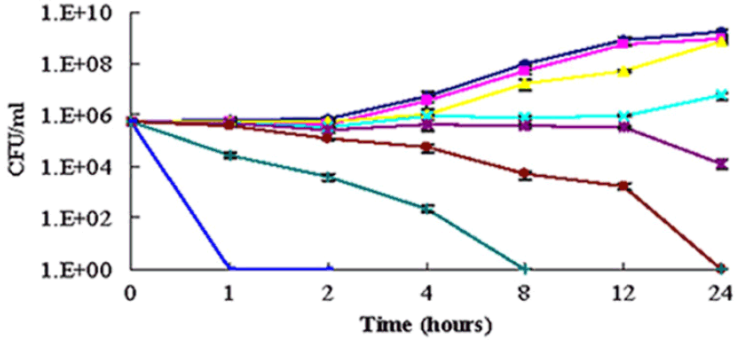
Fixed conc. of ceftazidime over time

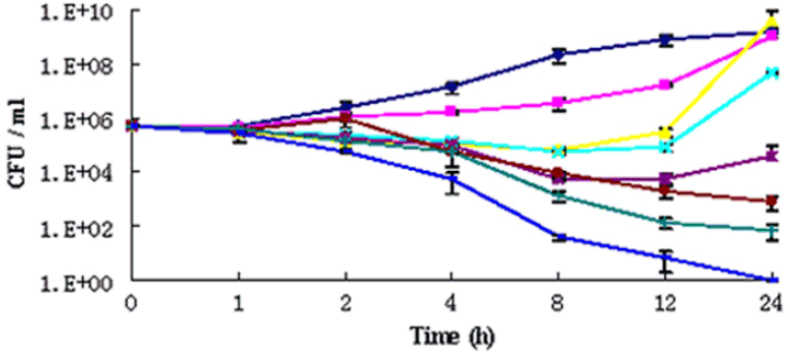
PAO1





β-lactamase overproducer

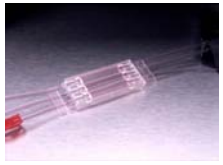




Hengzhuang et al, Antimicrob. Ag. Chemother. 2013; 57:196-204

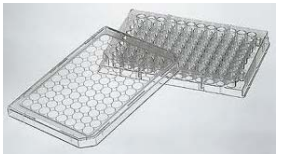
Expression of antibiotic resistance

Flow cell reactor



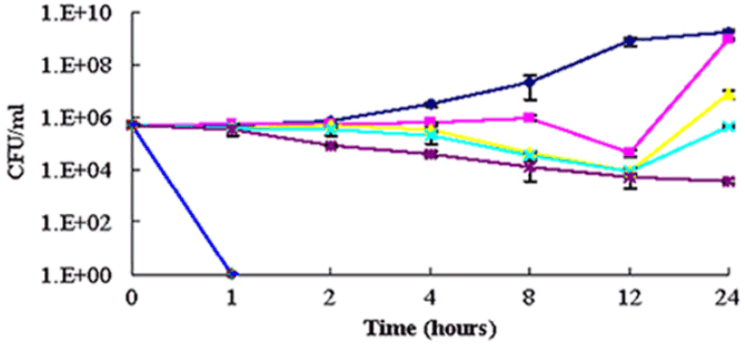
P. aeruginosa

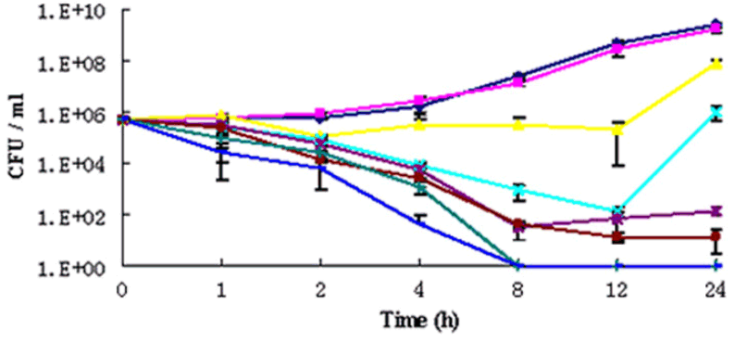
96-well plates, static



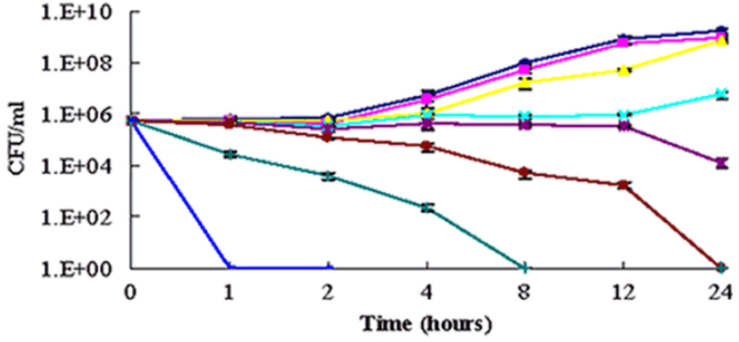
Fixed conc. of ceftazidime over time

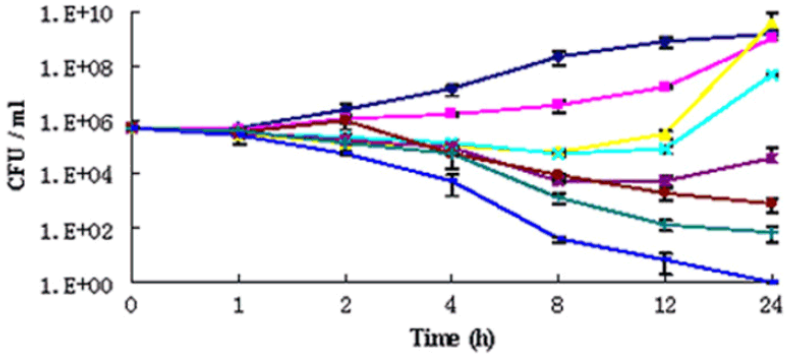
PAO1 → time-dependent





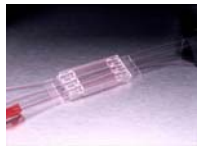
β-lactamase overproducer → concentration-dependent ~ antibiotic access ??





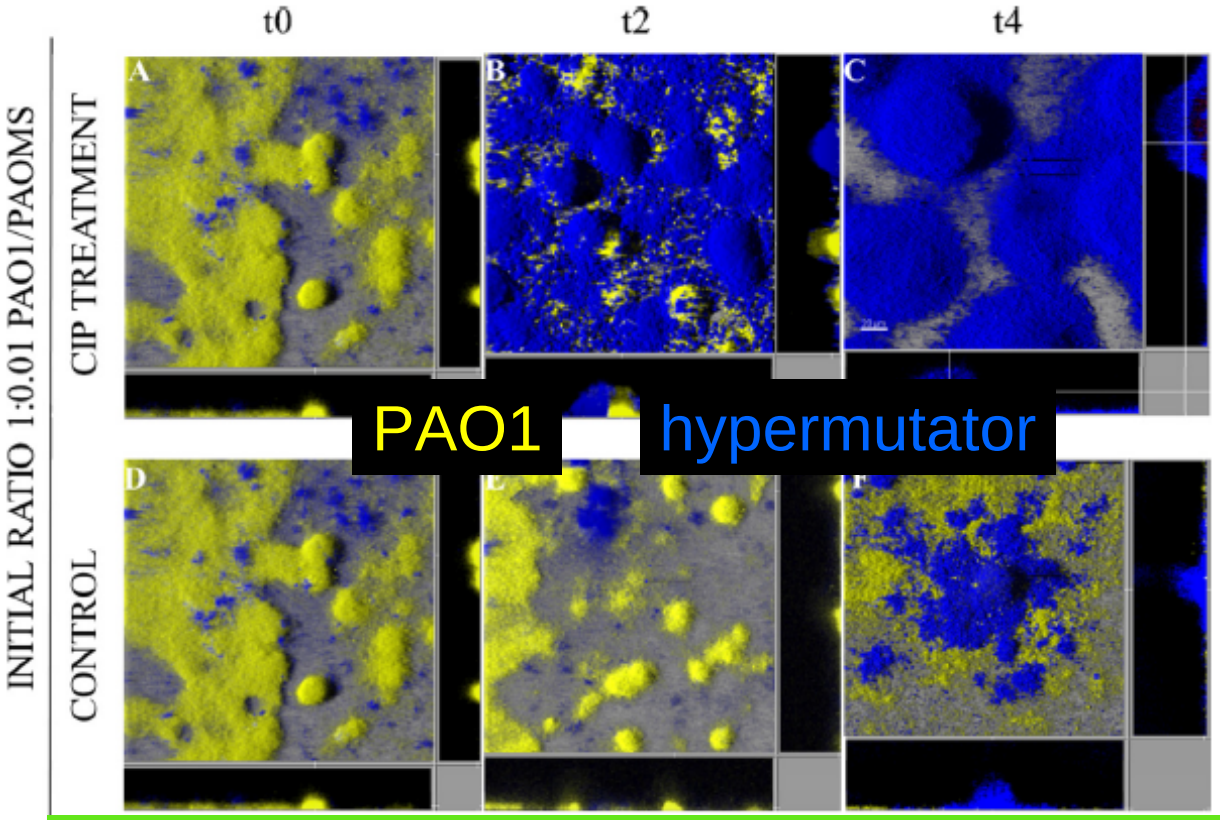
Hengzhuang et al, Antimicrob. Ag. Chemother. 2013; 57:196-204

Selection of resistant populations



P. aeruginosa

Fixed conc. of ciprofloxacin over time (MPC)



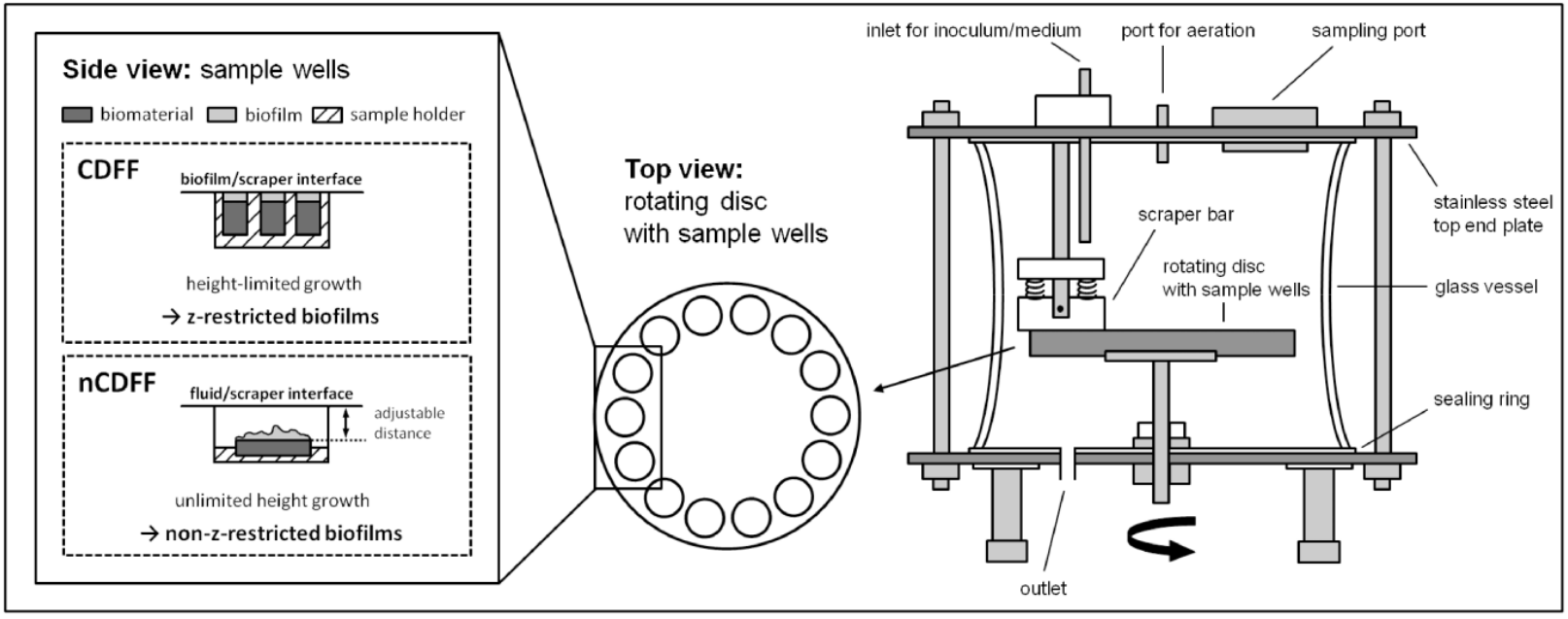
Amplification of mutator population during antibiotic treatment and accumulation of resistance mechanisms

Macià et al, Antimicrob. Ag. Chemother. 2013; 55:5230–7

Dynamic models: bioreactors

(non)-constant depth biofilm fermenter:

- constant conditions
- low shear stress
- ~ biofilms on implants

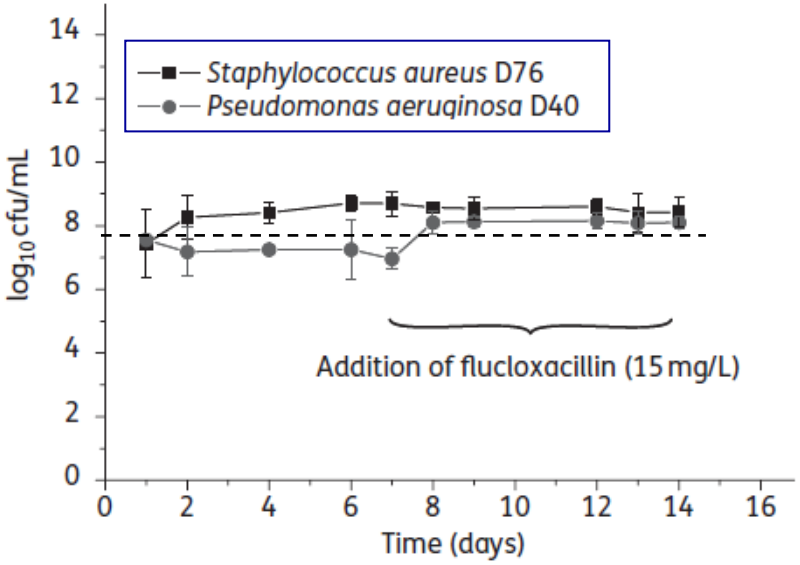


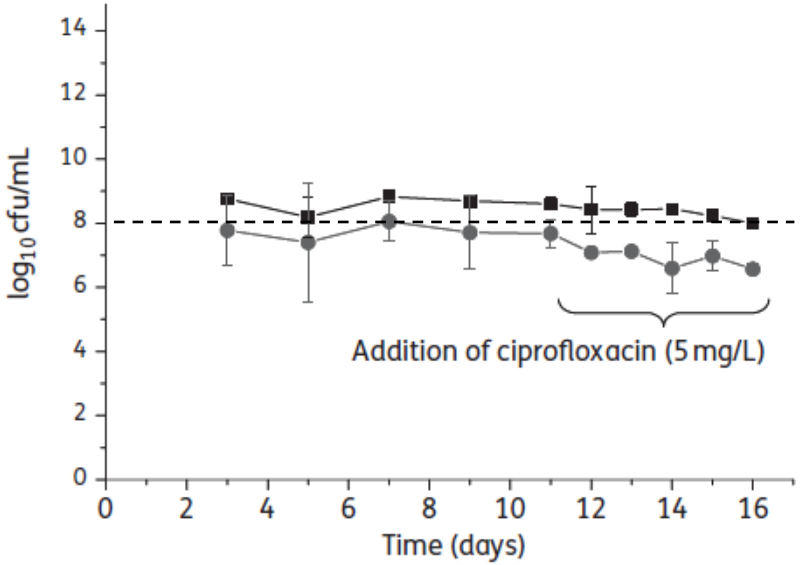
Lüdecke et al, PLoS One 2014; 9(1):e84837

Selection of resistant populations

Constant depth fermenter

Fixed antibiotic conc. over time;
multispecies biofilm (strains isolated from human wounds)





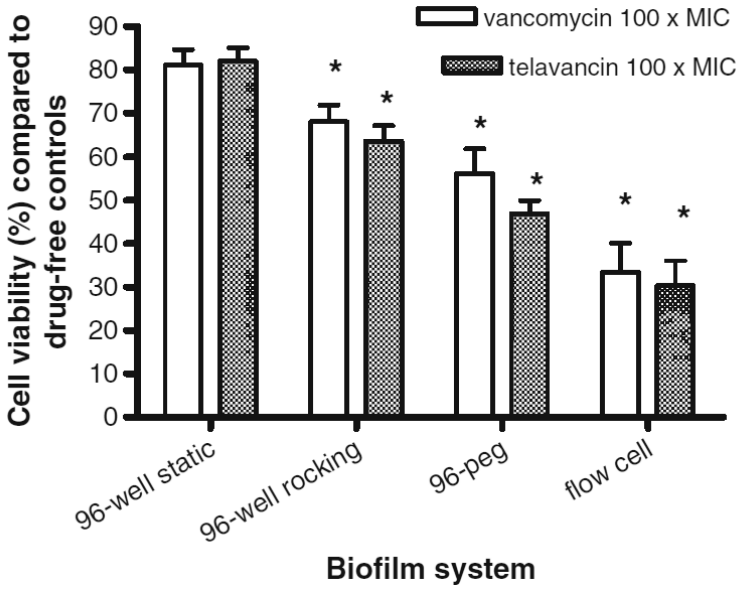
Low activity at clinically-relevant concentration

Hill et al, J. Antimicrob. Chemother. 2010; 65:1195-206

Take home messages ….

Conclusions

- 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 ?



Smith et al, Eur J Clin Microbiol Infect Dis 2013 ; 32:1327–32

Conclusions

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- 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

Conclusions

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  - 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

combination with agents modifying matrix properties or bacterial metabolic state ?

Still a lot of work ahead …

Acknowledgments


Julia Bauer


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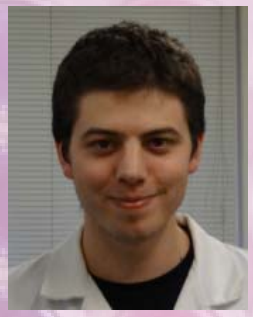
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