ULB

SEMINAIRES DE MICROBIOLOGIE
ET MALADIES INFECTIEUSES

Antibiotics and biofilm: in vitro evidence and new clinical applications

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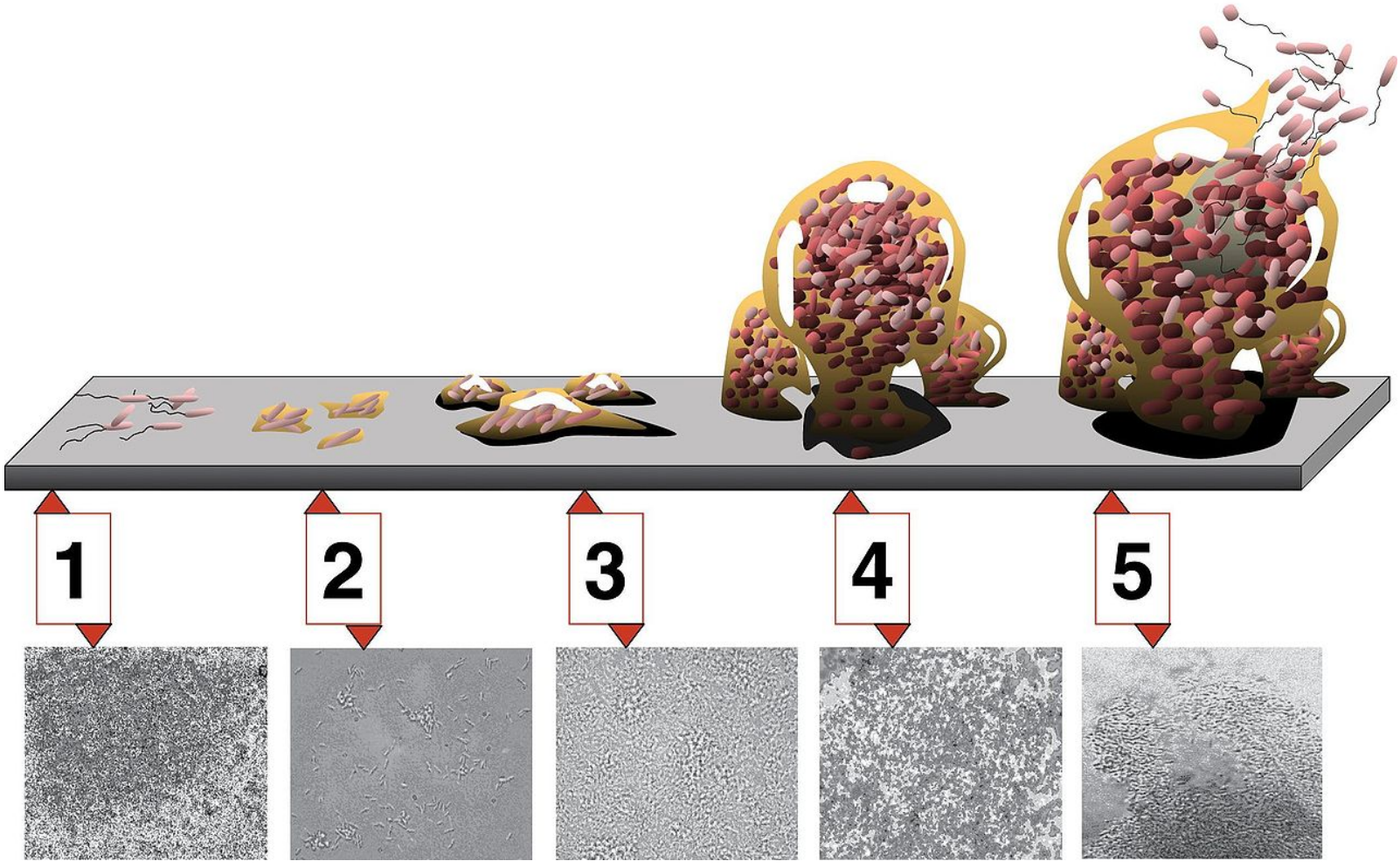
UCL Université catholique de Louvain

LDRI LOUVAIN DRUG RESEARCH INSTITUTE

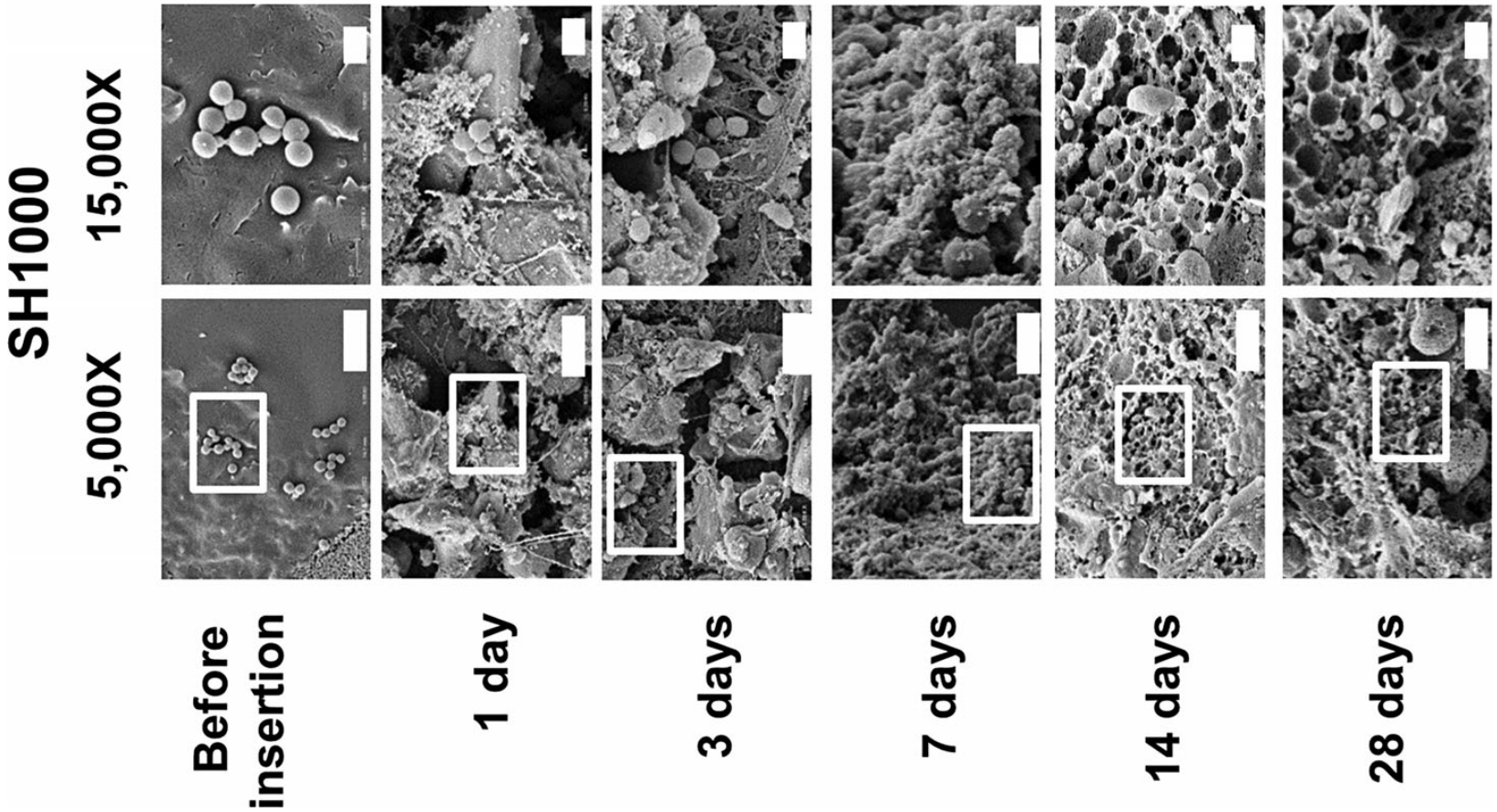
Biofilms: what are we speaking about ?



Attachment ⟶ Maturation ⟶ Dispersion

History of biofilm development *in vivo*

Natural history of biofilm formation *in vivo* during the establishment of chronic implant-associated *S.aureus* osteomyelitis in mice

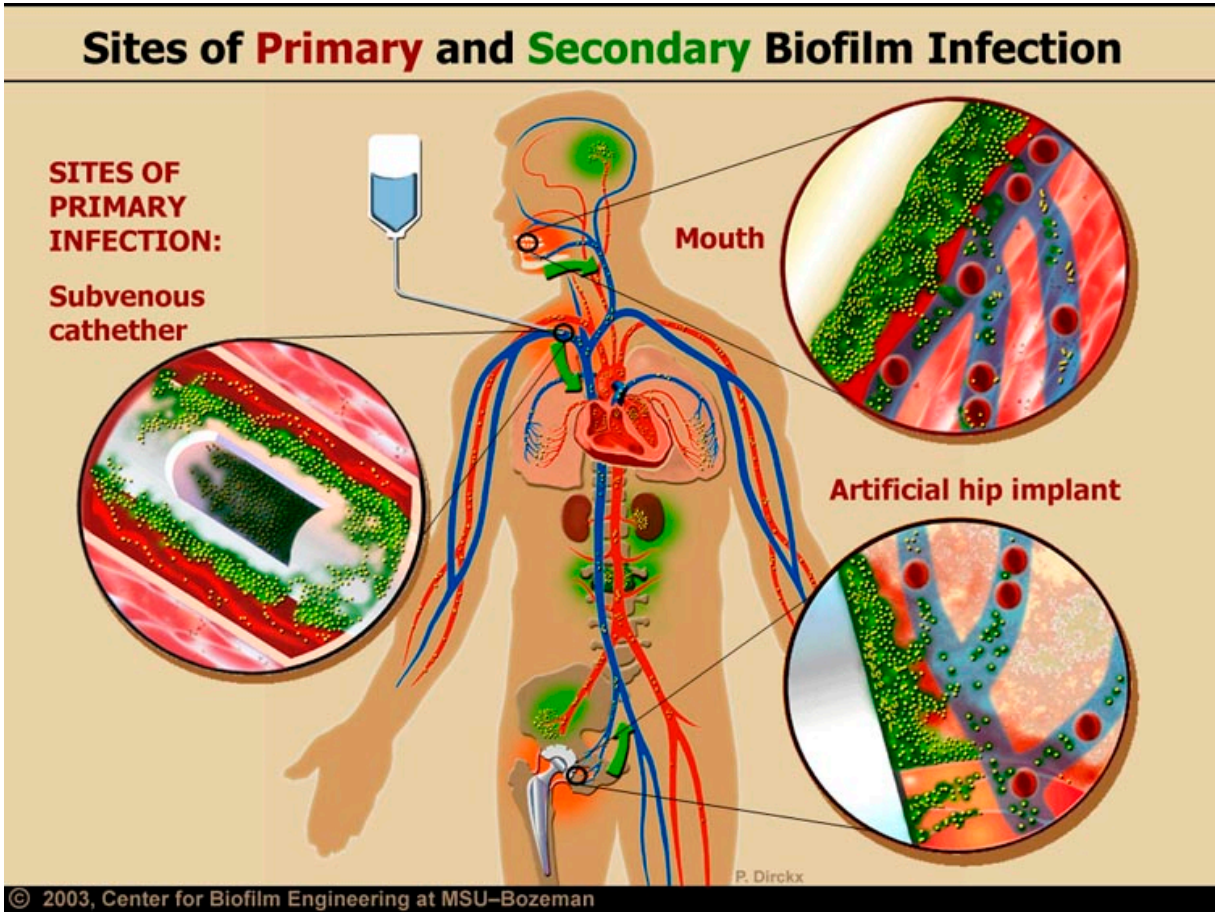


Attachment → Maturation → Dispersion

Nishitani et al; J Orthop Res. 2015;33:1311-9

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

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]*

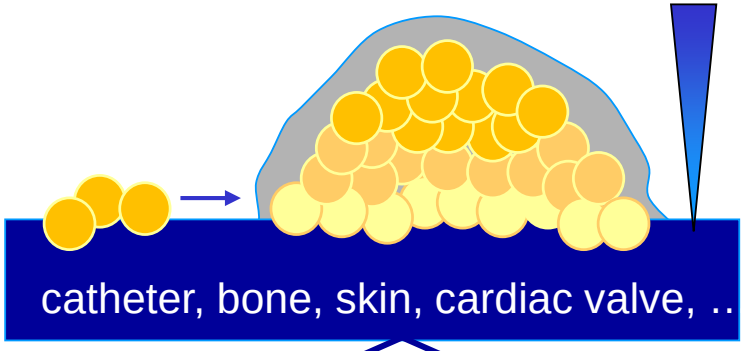
Pathog Dis. 2013 Nov;69(2):142-8. doi: 10.1111/2049-632X.12100. Epub 2013 Oct 7.

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…

PK/PD parameters in biofilms

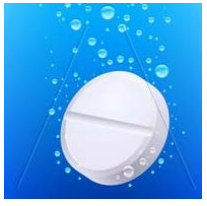


pharmacokinetics

- 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, ..])

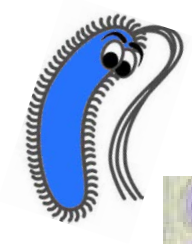


In vitro evidence : models in 96-well polystyrene plates

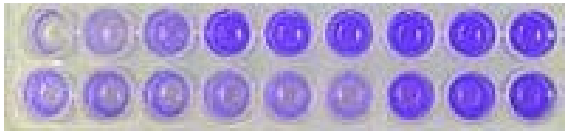
appropriate
dyes
to evaluate biomass or
bacterial load

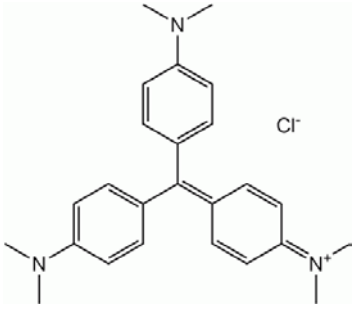
Quantifying biomass and metabolic activity in biofilms

biofilm mass



crystal violet

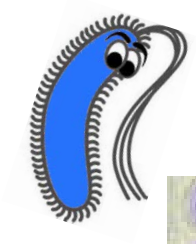




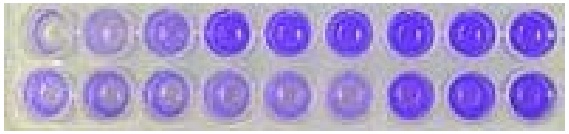
Christensen et al, Infect. Immun. 1982; 37:318–26

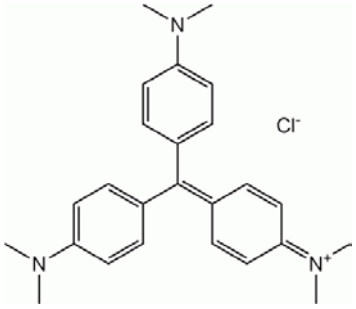
Quantifying biomass and metabolic activity in biofilms

biofilm mass



crystal violet



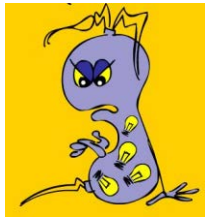


Christensen et al, Infect. Immun. 1982; 37:318–26

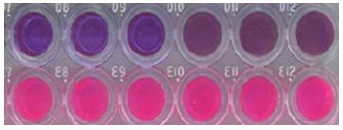
Gram(+) bacteria

metabolic activity

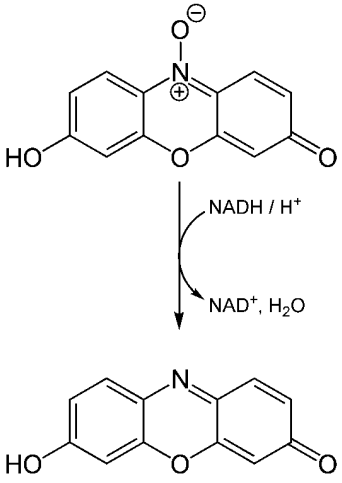
Gram(-) bacteria



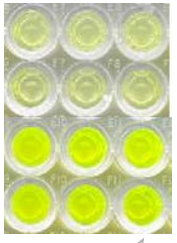
resazurin



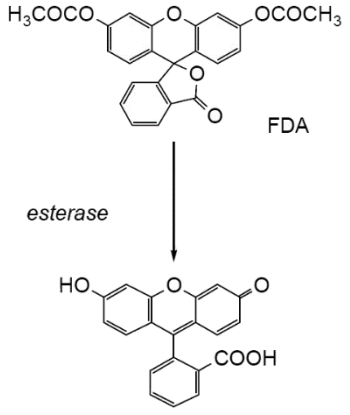
resorufin



fluorescein diacetate



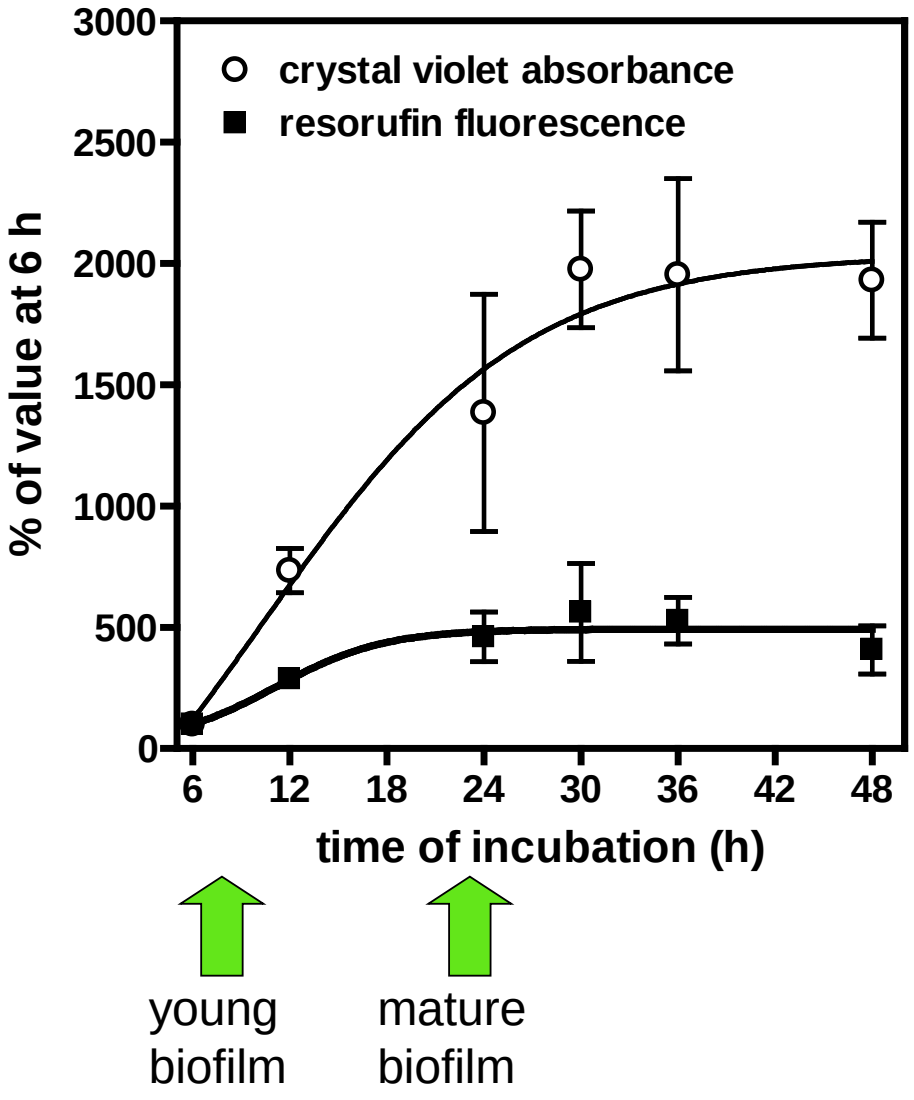
fluorescein



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

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

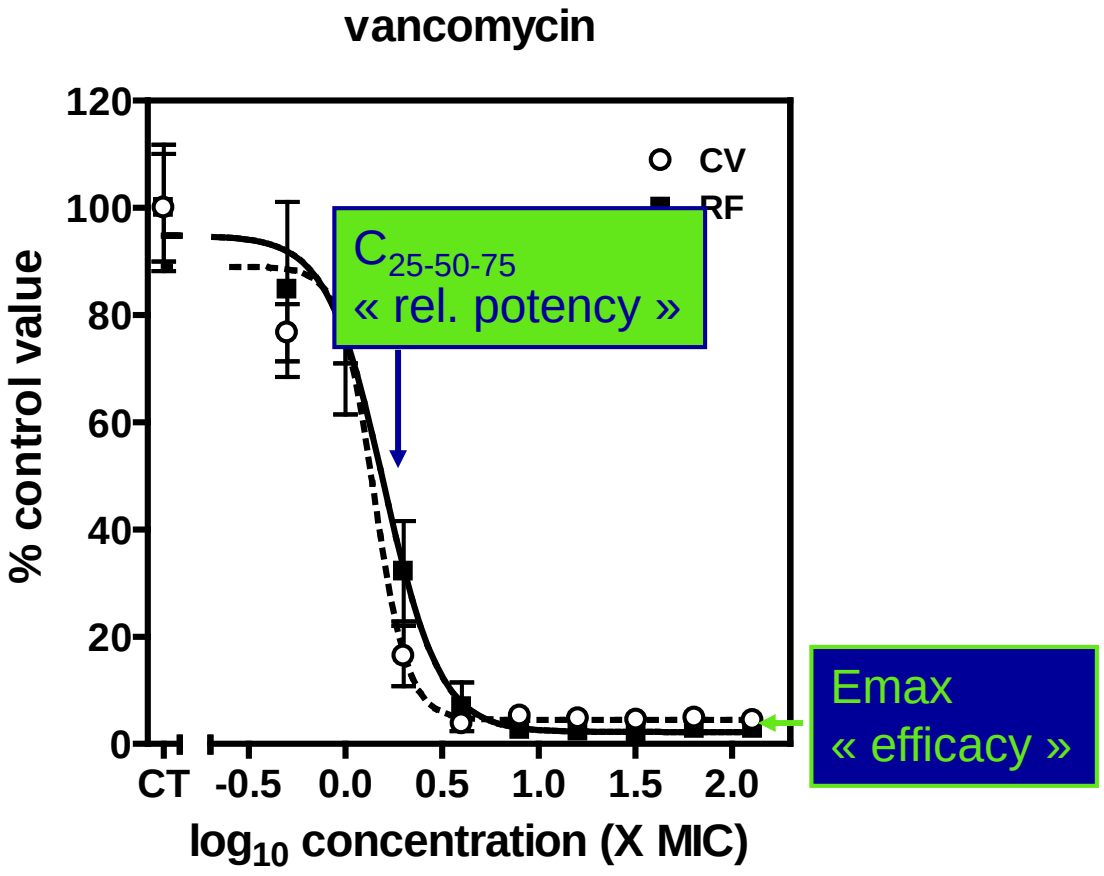
Kinetics of biofilm formation



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

Pharmacodynamic model for antibiotic activity

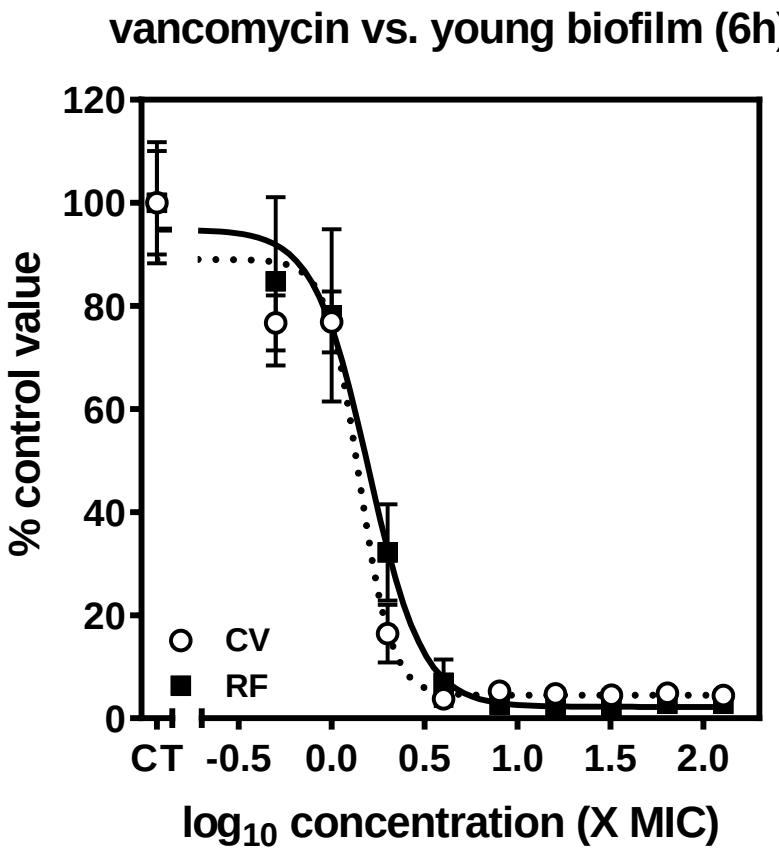
An example with a young biofilm of *S. aureus* - ATCC MSSA

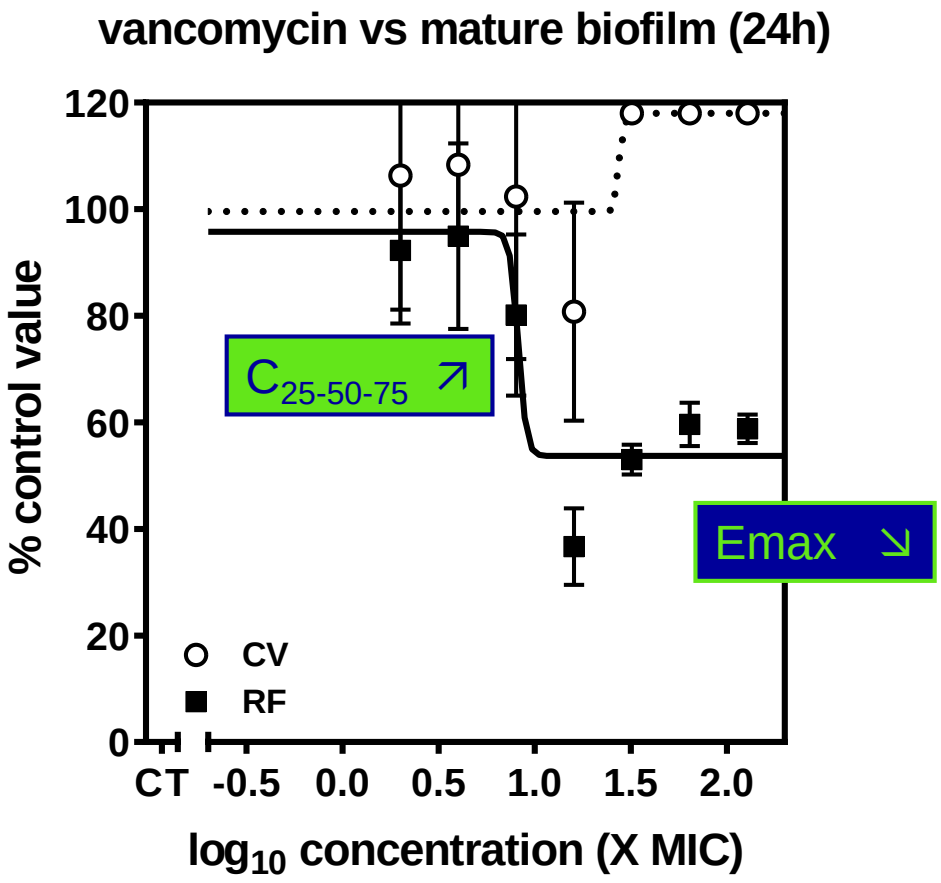


Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37

Pharmacodynamic model for antibiotic activity

Young vs. mature biofilm of *S. aureus* - ATCC MSSA

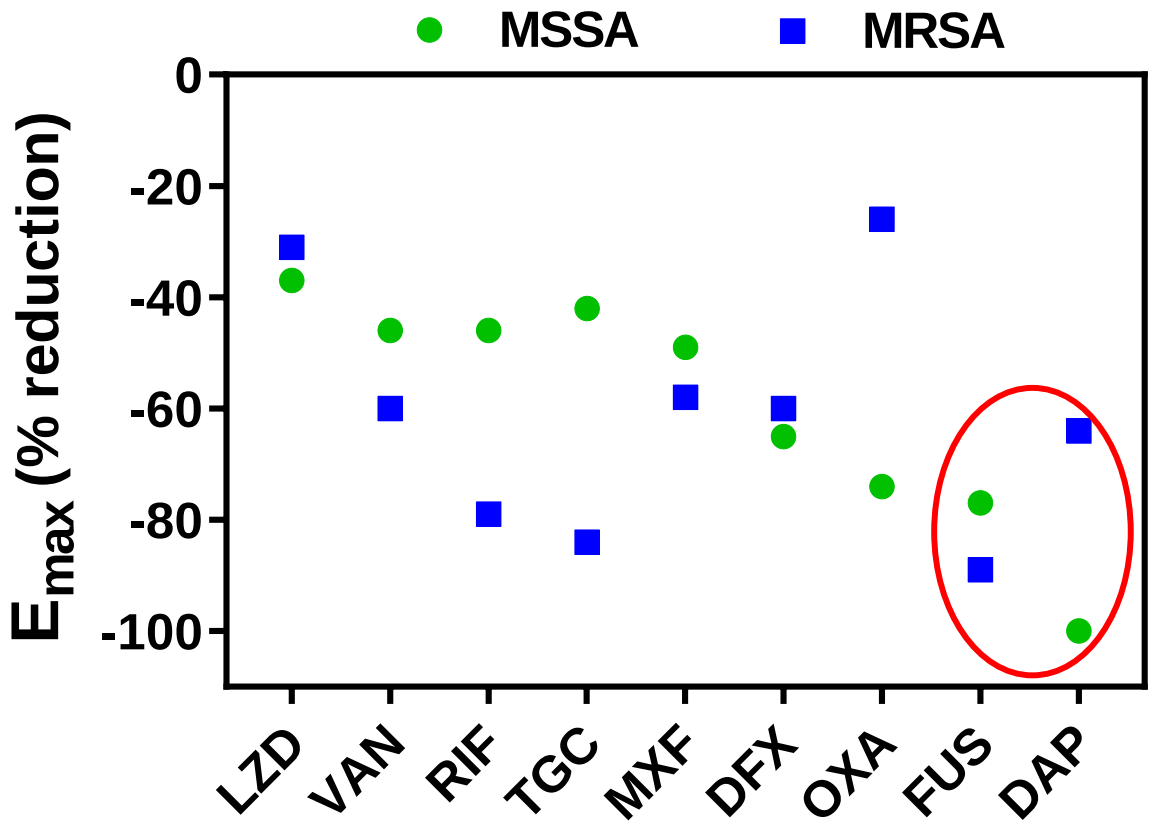




Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37

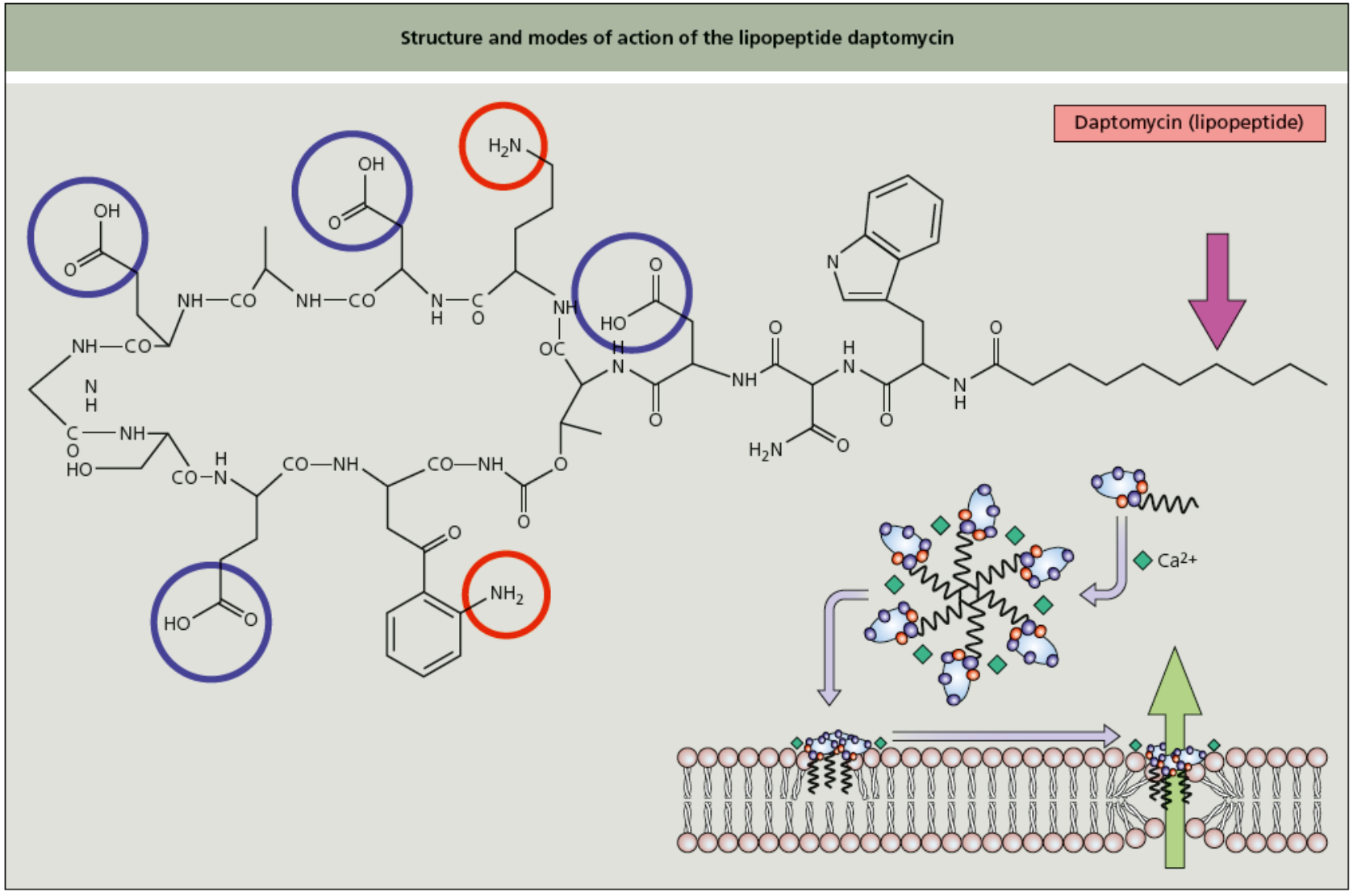
Pharmacodynamic model for antibiotic activity

Comparison of antibiotic efficacy – ATCC reference strains



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

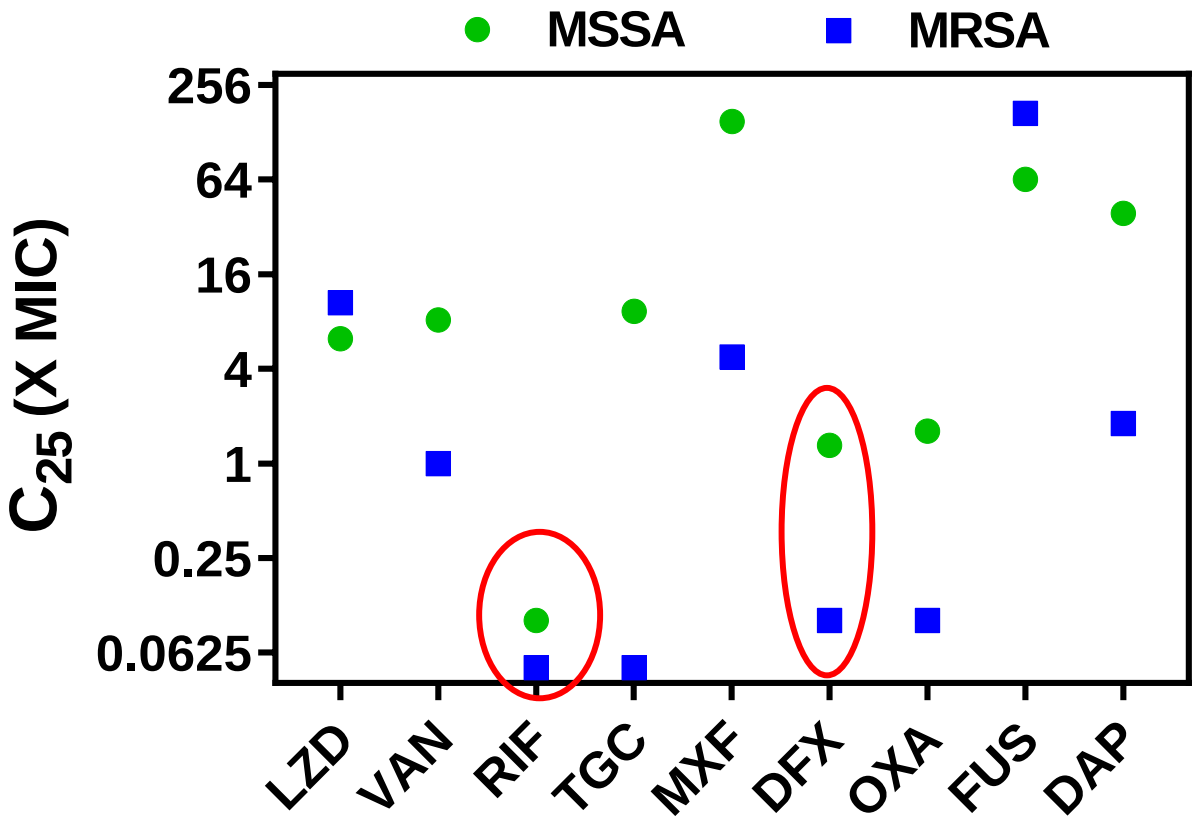
Daptomycin, a lipoglycopeptide



Van Bambeke et al; Amstrong & Cohen – Infectious diseases 2016

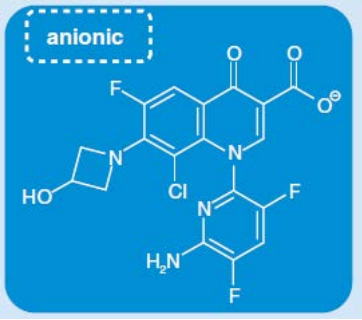
Pharmacodynamic model for antibiotic activity

Comparison of antibiotic relative potency - ATCC MSSA



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

Delafloxacin, a new fluoroquinolone



Future MICROBIOLOGY

DRUG EVALUATION

Delafloxacin, a non-zwitterionic fluoroquinolone in Phase III of clinical development: evaluation of its pharmacology, pharmacokinetics, pharmacodynamics and clinical efficacy

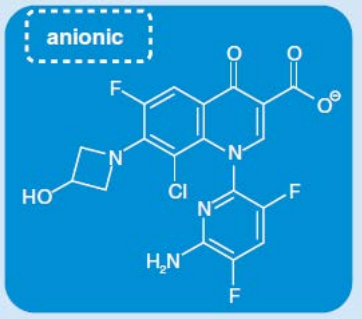
Françoise Van Bambeke*

ABSTRACT Delafloxacin is a fluoroquinolone lacking a basic substituent in position 7. It shows MICs remarkably low against Gram-positive organisms and anaerobes and similar to those of ciprofloxacin against Gram-negative bacteria. It remains active against most fluoroquinolone-resistant strains, except enterococci. Its potency is further increased in acidic environments (found in many infection sites). Delafloxacin is active on staphylococci growing intracellularly or in biofilms. It is currently evaluated as an intravenous and intravenous/oral stepdown therapy in Phase III trials for the treatment of complicated skin/skin structure infections. It was also granted as Qualified Infectious Disease Product for the treatment of acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia, due to its high activity on pneumococci and atypical pathogens.

Delafloxacin, a new fluoroquinolone

Future MICROBIOLOGY

DRUG EVALUATION



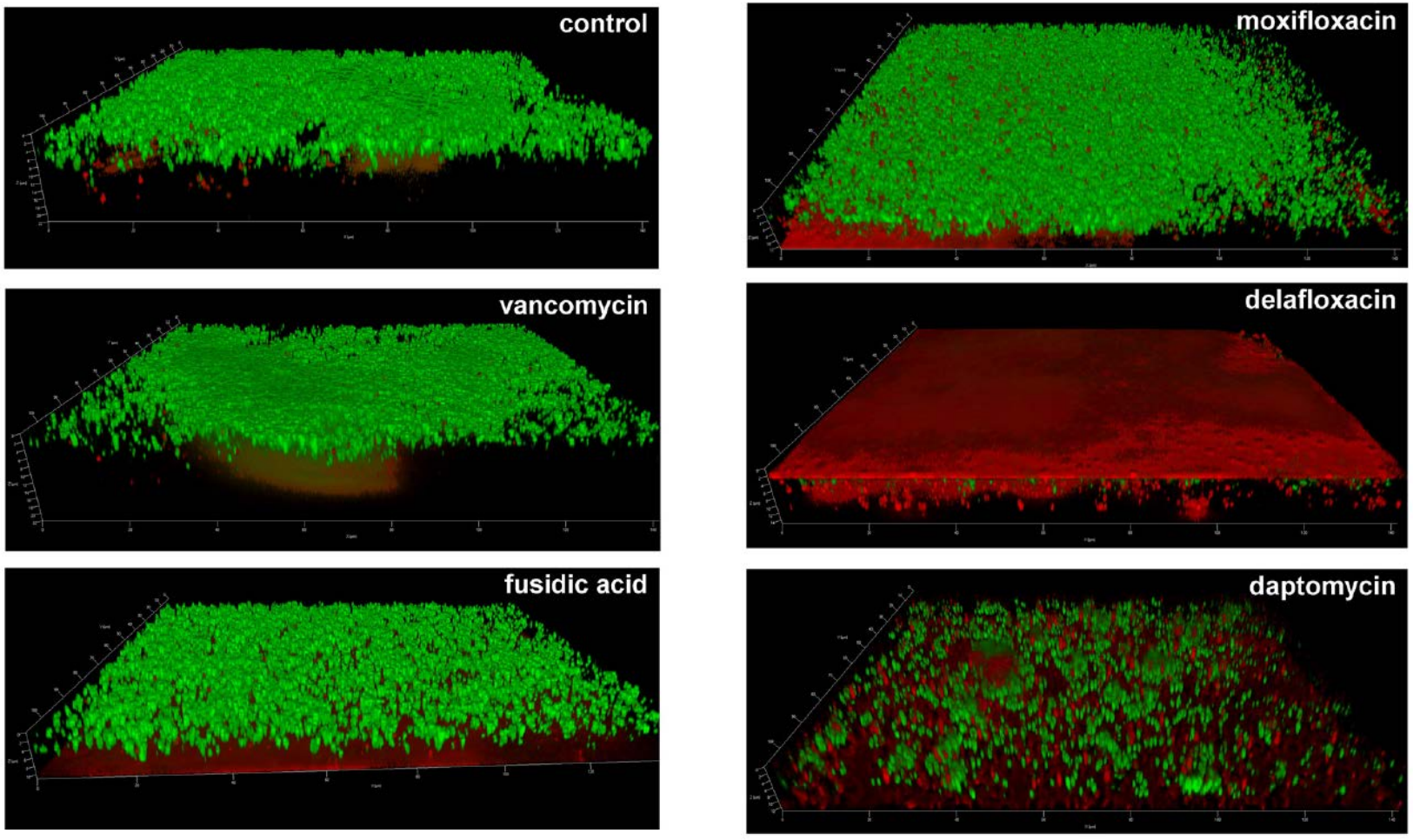
Delafloxacin, a non-zwitterionic fluoroquinolone in Phase III of clinical development: evaluation of its pharmacology, pharmacokinetics, pharmacodynamics and clinical efficacy

Table 1. Susceptibility of relevant Gram-positive pathogens to delafloxacin and other commercially available fluoroquinolones.

Species	Phenotype	Number of strains	Antibiotic	MIC_{50} (mg/l)	MIC_{90} (mg/l)	MIC range (mg/l)	Ref.†
S. aureus	All	681	Levofloxacin	0.12	>32	0.03–>32	[41]
		681	Delafloxacin	0.12	0.5	≤0.004–16	[41]
	FQ-S	70	Levofloxacin	0.25	0.5	0.06–0.5	[23]
		88		0.12	0.25	0.06–1	[42]
		70	Moxifloxacin	0.06	0.1	0.015–0.5	[23]
		70	Delafloxacin	0.004	0.008	0.002–0.008	[23]
		88		0.002	0.008	≤0.001–0.06	[42]
	FQ-R	71	Levofloxacin	16	32	4–64	[23]
		100		4	8	2–32	[42]
		71	Moxifloxacin	4	8	0.25–16	[23]
		71	Delafloxacin	0.25	1	0.015–1	[23]
		100		0.006	0.12	0.015–2	[42]

Comparison of antibiotic activity in confocal microscopy

Live/dead staining (antibiotics at 32 X MIC) – ATCC MRSA



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

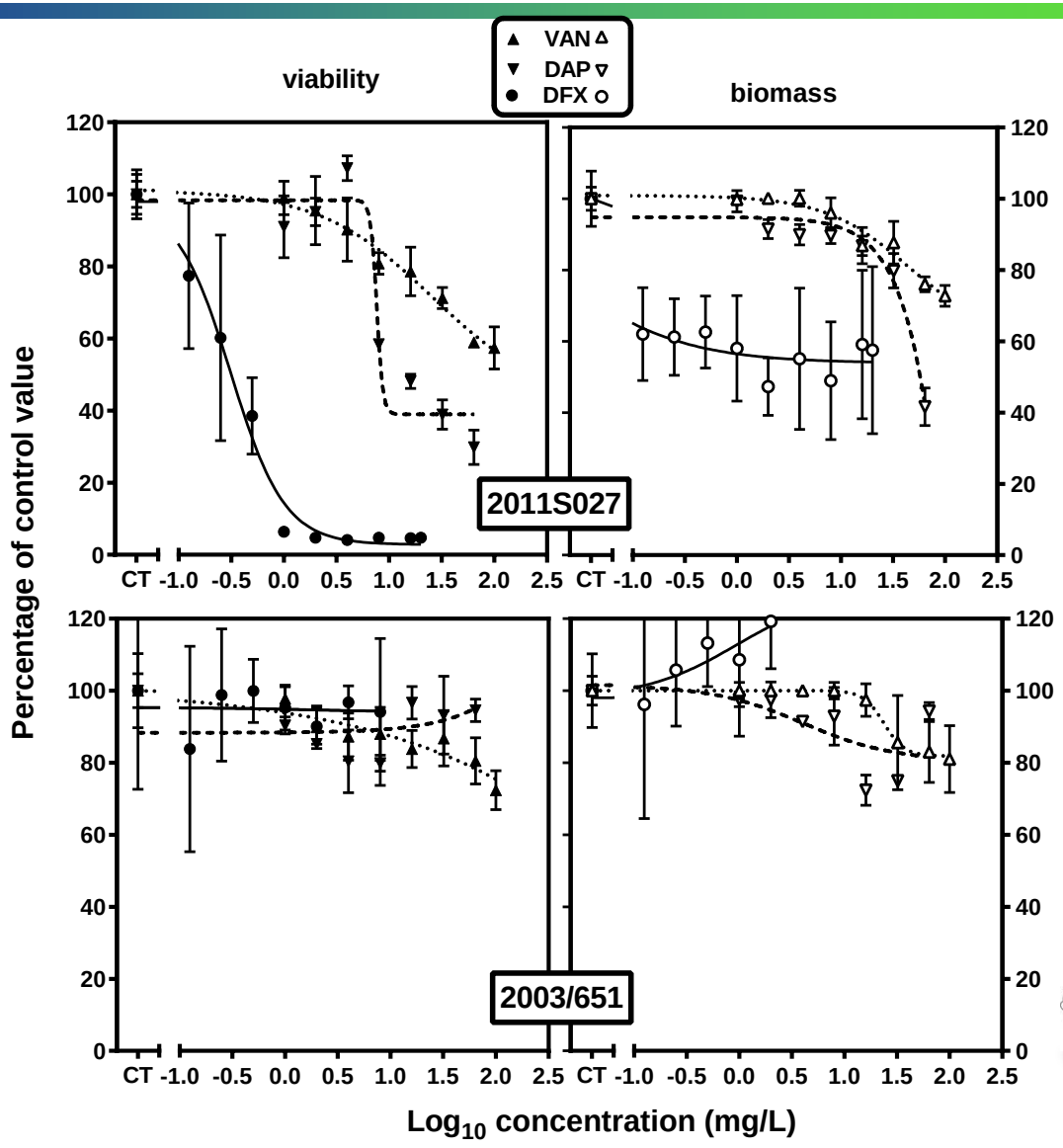
Moving to clinical isolates from pandemic lineages

Description of clinical strains included in the study

	Molecular characterization[f]							
Strain	16S[a]	*nuc*[a]	*mecA*[b]	*spa* type[c]	MLST[d]	TSST-1[e]	PVL[e]	Clinical origin
2011S027	+	+	−	t002	CC5	+	−	Cellulitis and bacteremia
Surv 2003/1083	+	+	−	t002	CC5	+	−	Chirurgical wound
Surv 2005/104	+	+	+	t002	CC5	−	−	Skin
2009S028	+	+	+	t002	CC5	+	−	Nasal carriage
2009S025	+	+	+	t002	CC5	+	−	Ear
Surv 2005/179	+	+	+	t008	CC8	−	−	Skin
Surv 2003/651	+	+	+	051	CC8	−	−	Respiratory infection

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

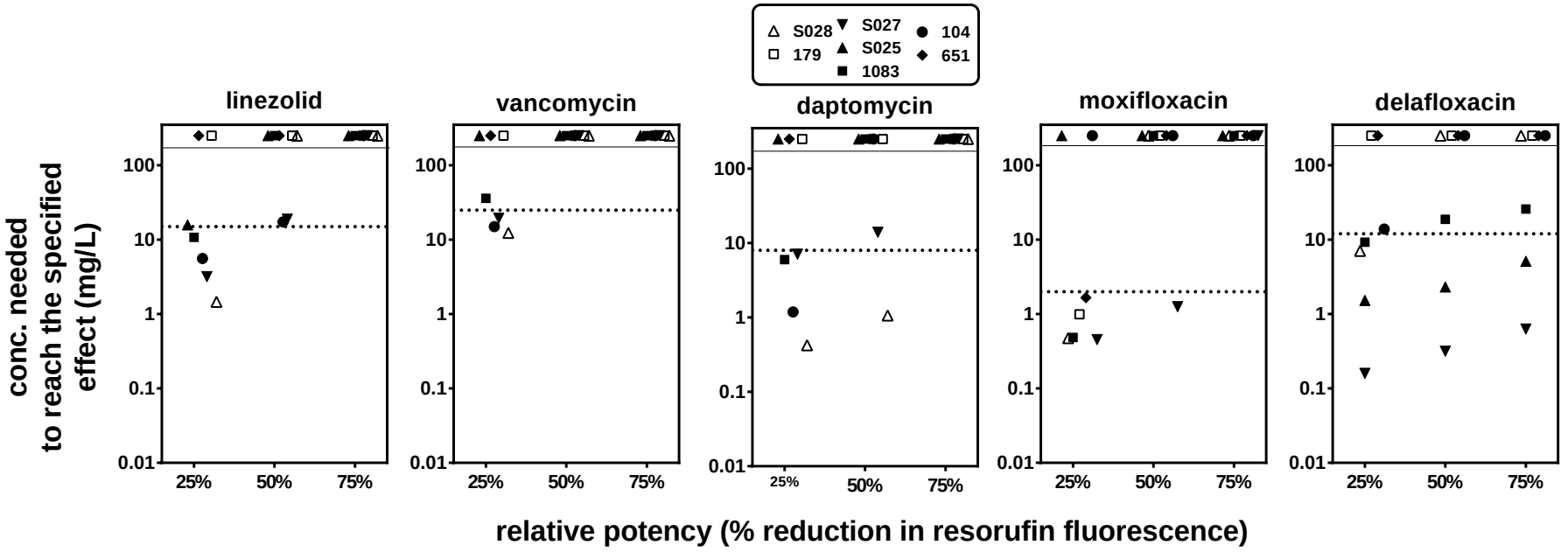
Comparison of 2 strains & 3 antibiotics



Huge variability in activity among strains …

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

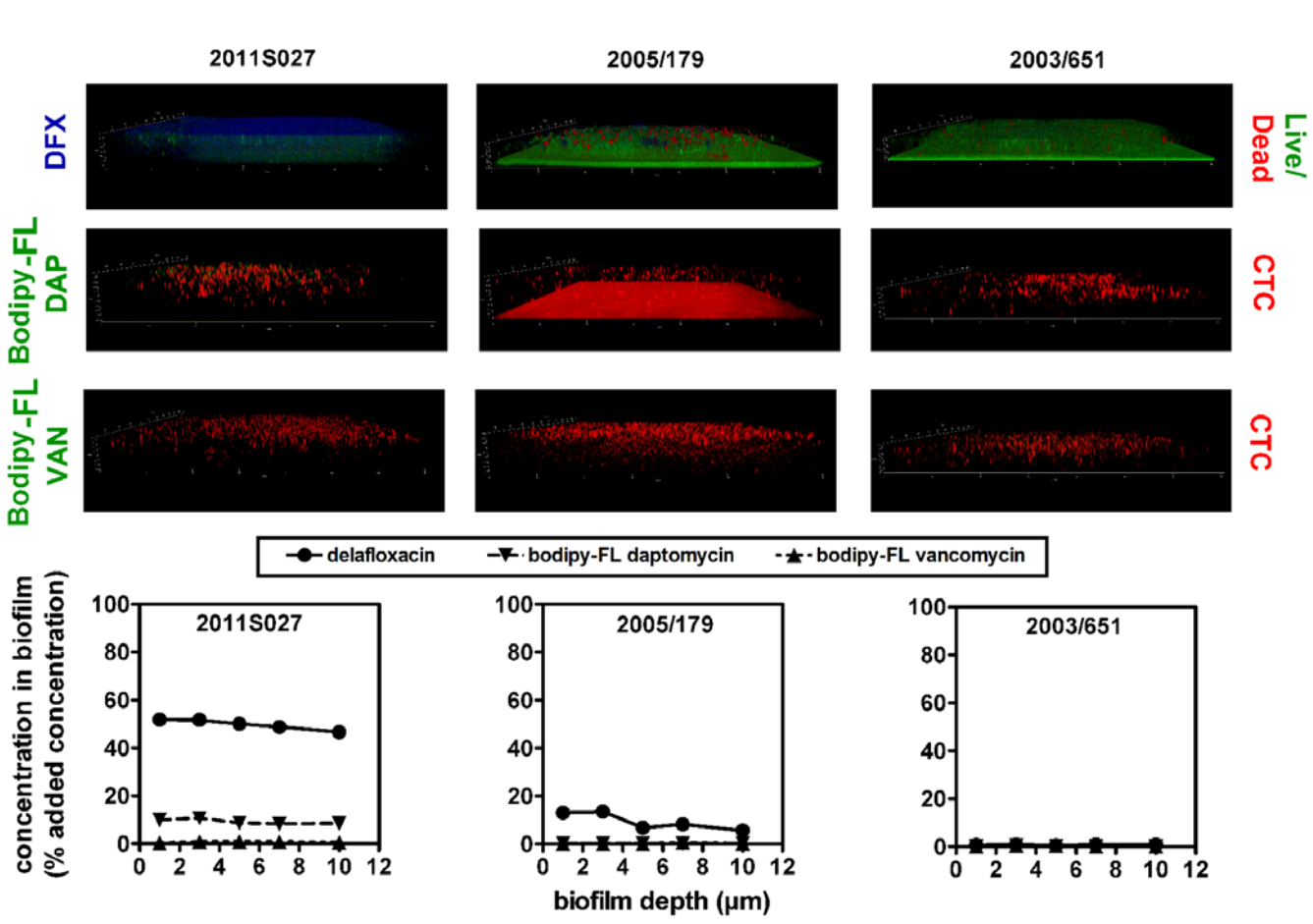
Comparison of 7 strains & 5 antibiotics

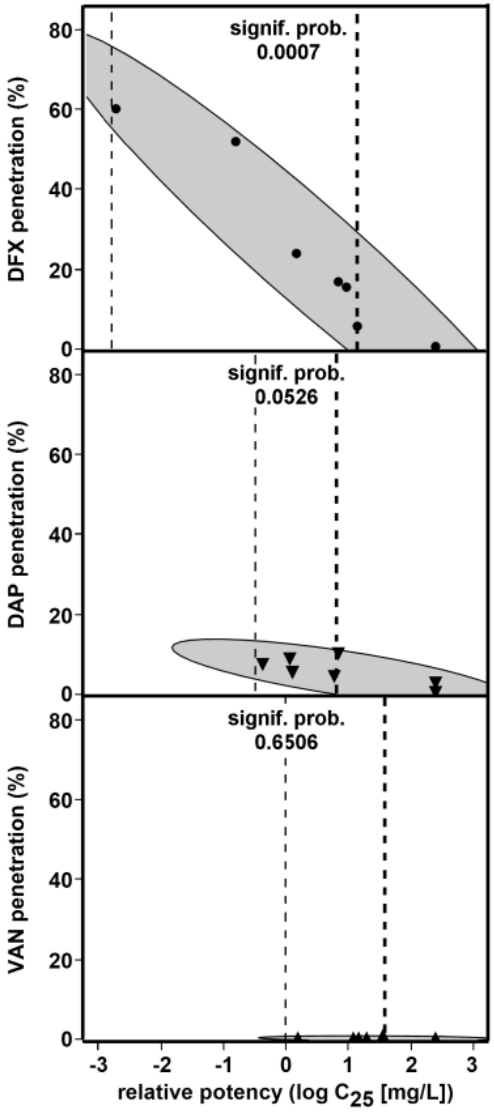


Daptomycin and fluoroquinolones more potent…
but again, high variability among strains

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

Importance of antibiotic concentration inside biofilms for activity

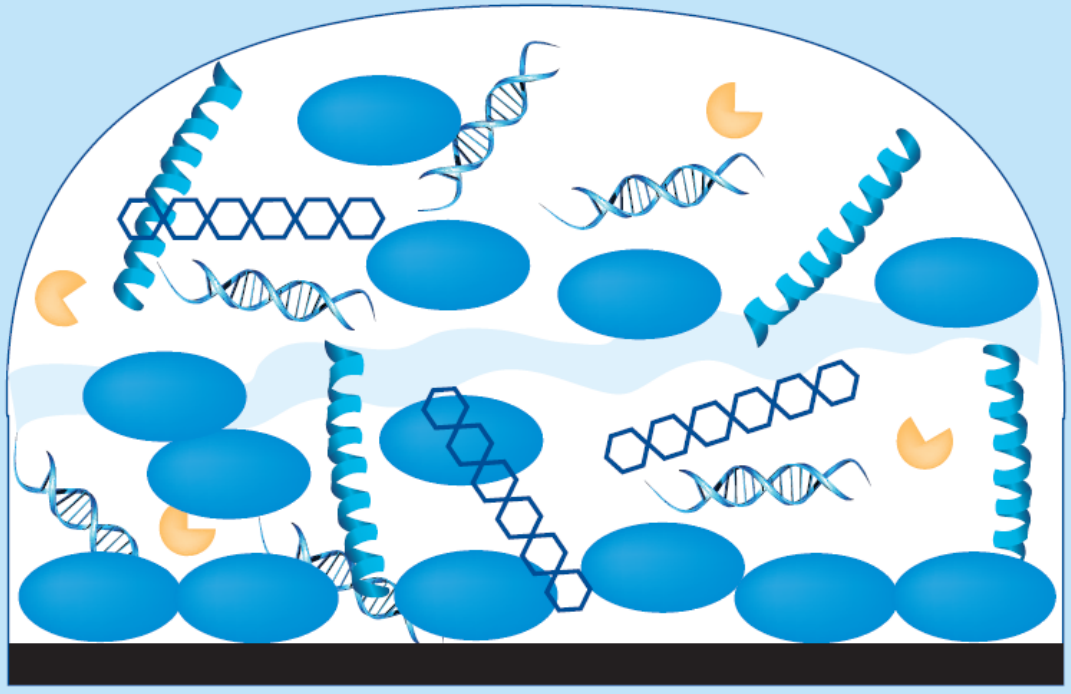


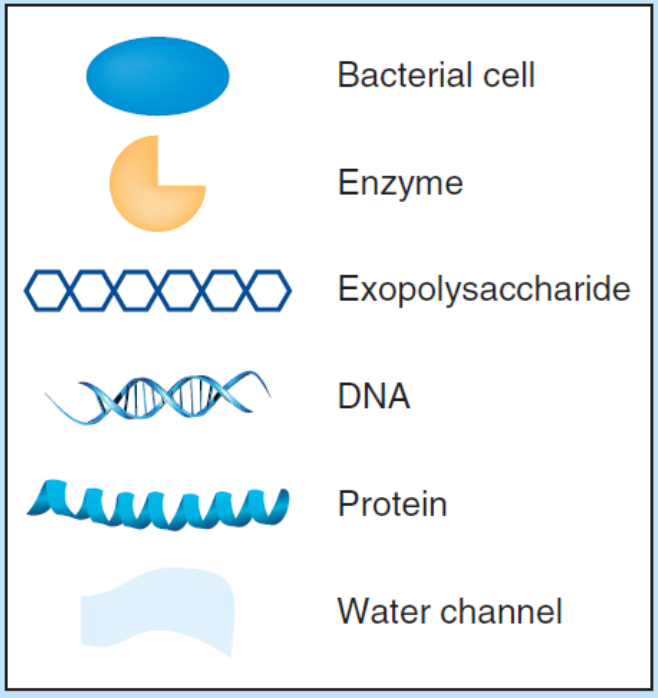


Activity in biofilm is correlated with antibiotic penetration

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

Biofilm matrix: what is it made of ?

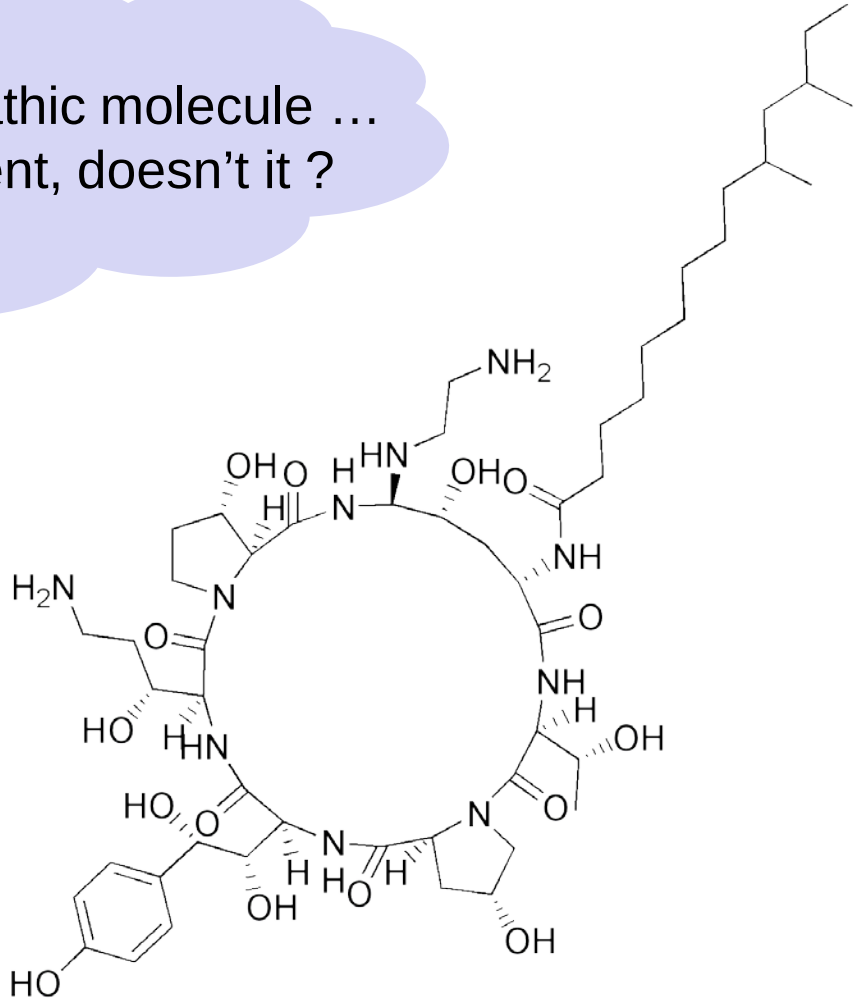




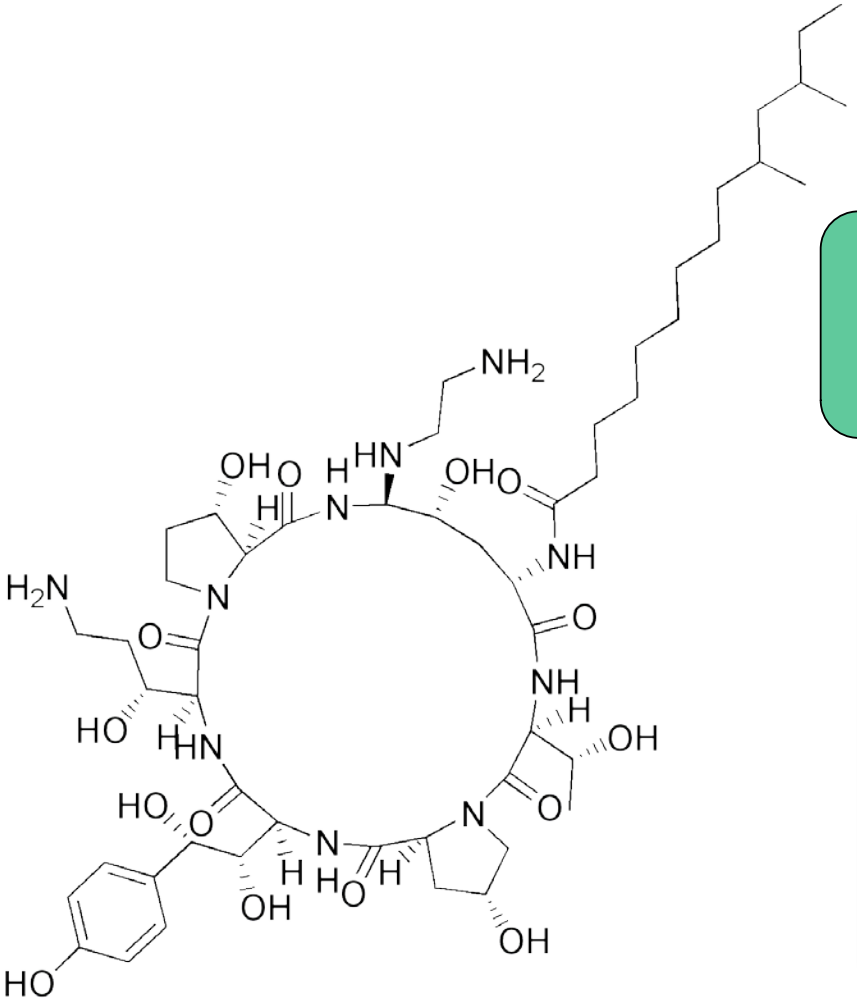
Rabin et al., Future Med. Chem. 2015; 7:493–512

Adjuvants acting on the matrix

Let's try this amphipathic molecule …
It looks like a detergent, doesn't it ?



Adjuvants acting on the matrix

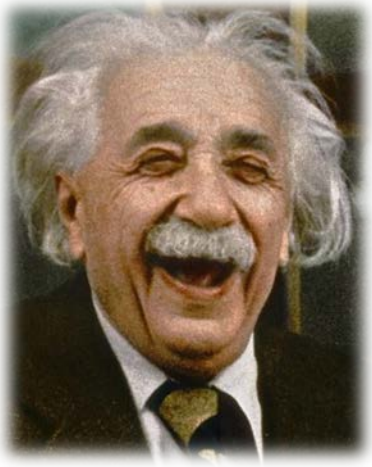


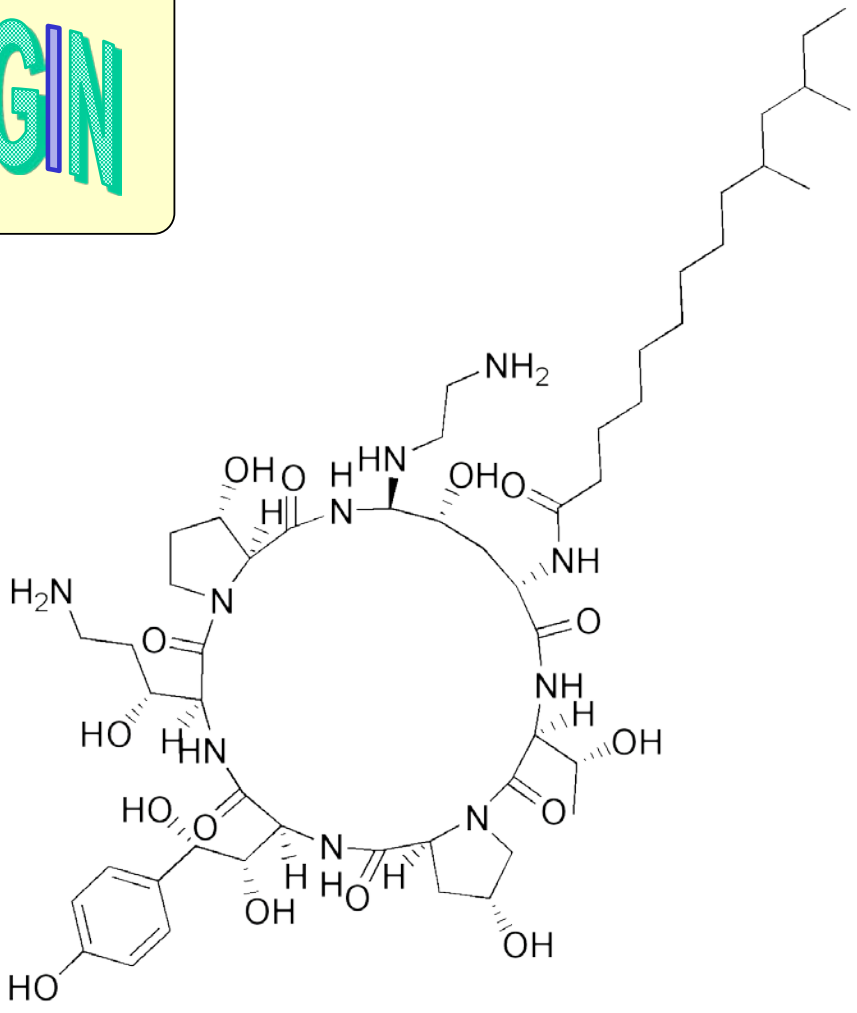
But do you recognize this molecule ?

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

Adjuvants acting on the matrix

CASPOFUNGIN





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

Adjuvants acting on the matrix

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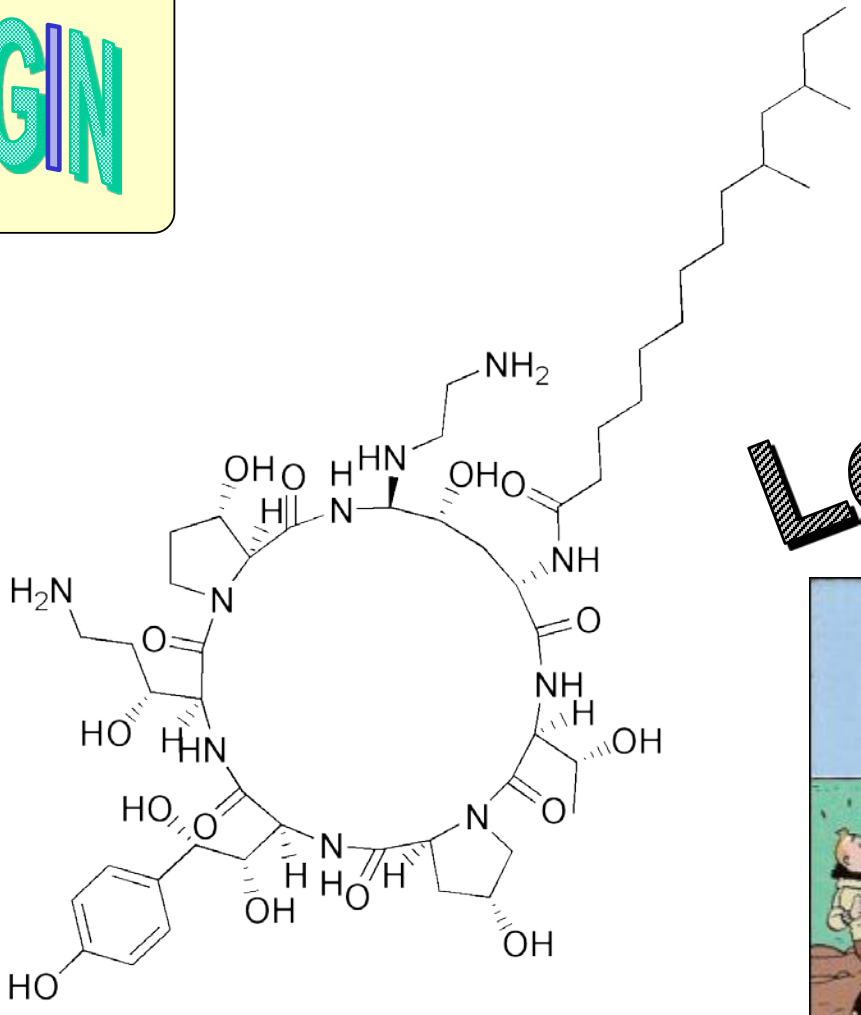
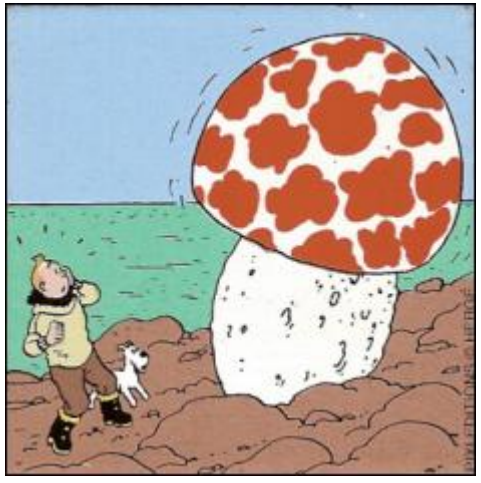
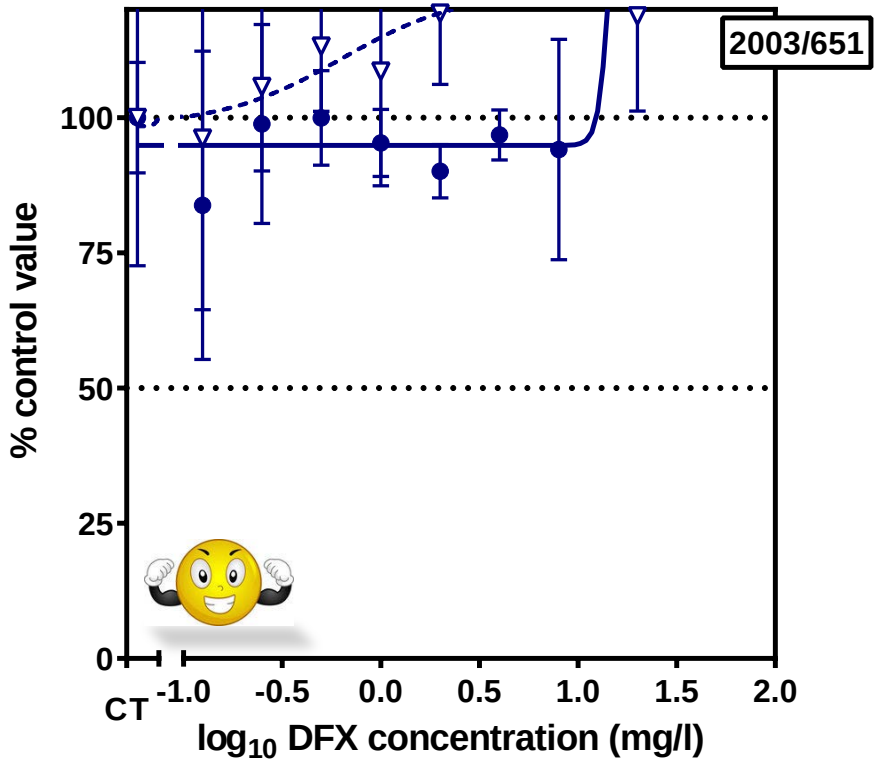


Let's try



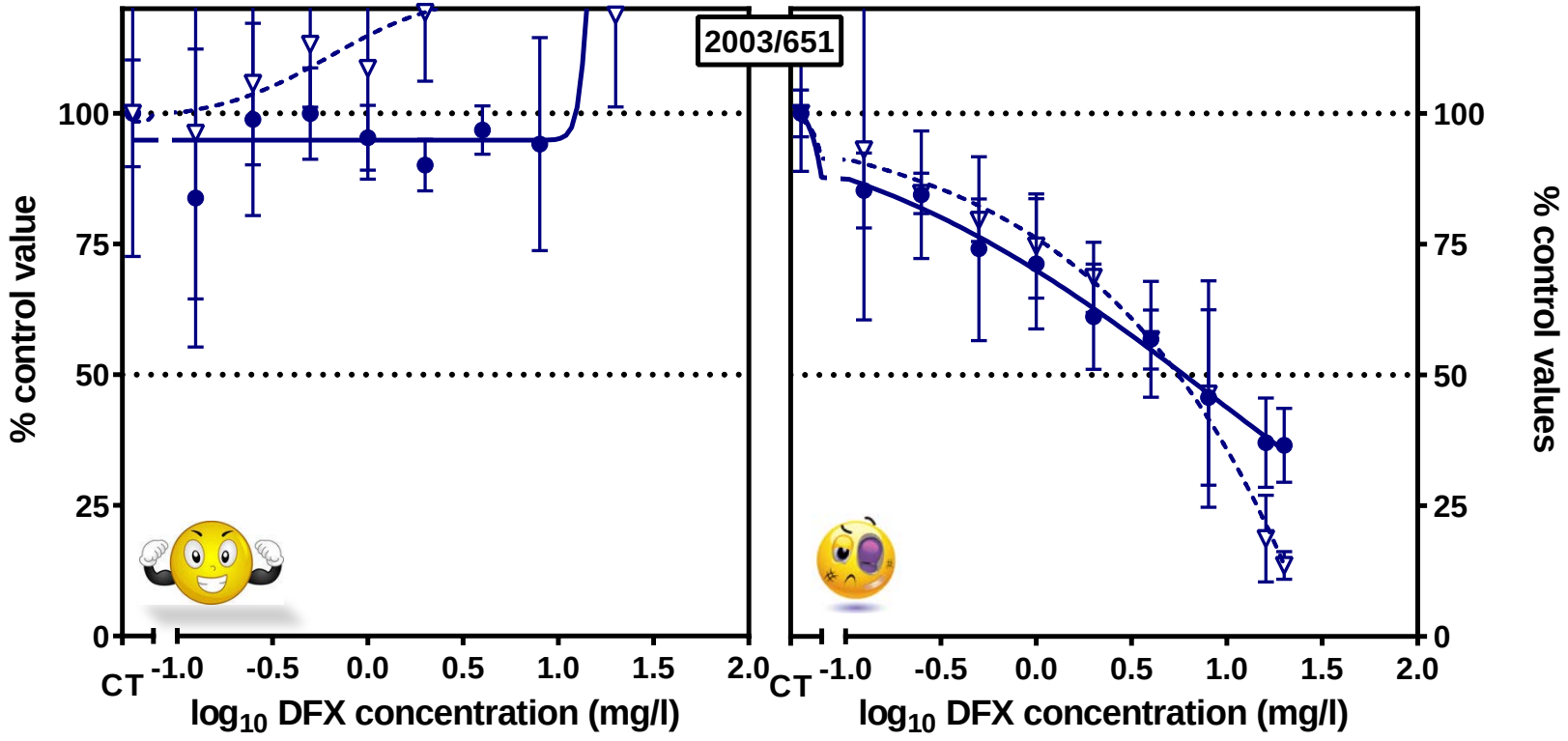
*Siala et al, Nature Communications 2016; 7:*13286

delafloxacin -/+ caspofungin on strain 2003/651



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

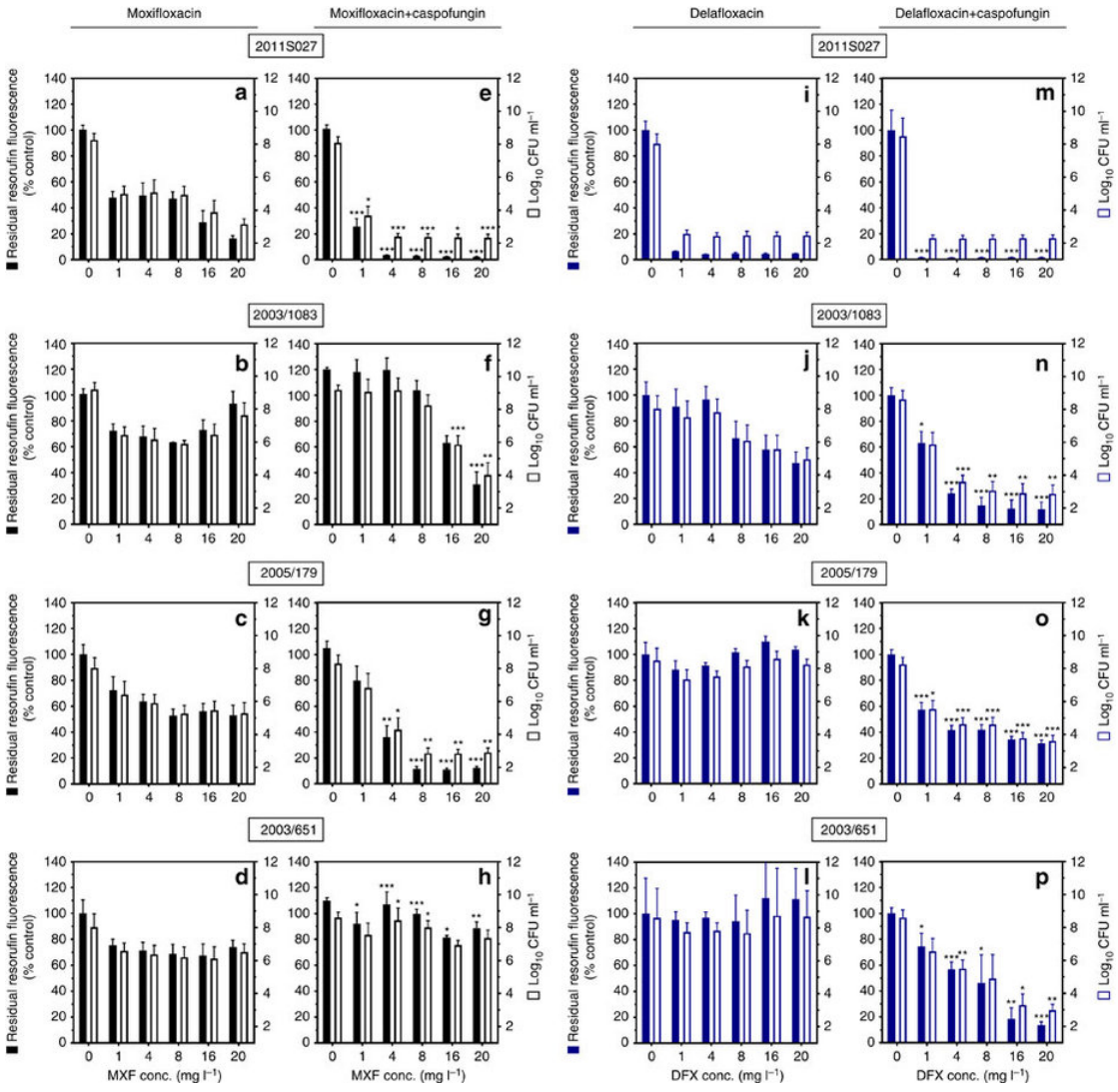
delafloxacin -/+ caspofungin on strain 2003/651



Caspofungin increases efficacy and relative potency of delafloxacin against a recalcitrant stain

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

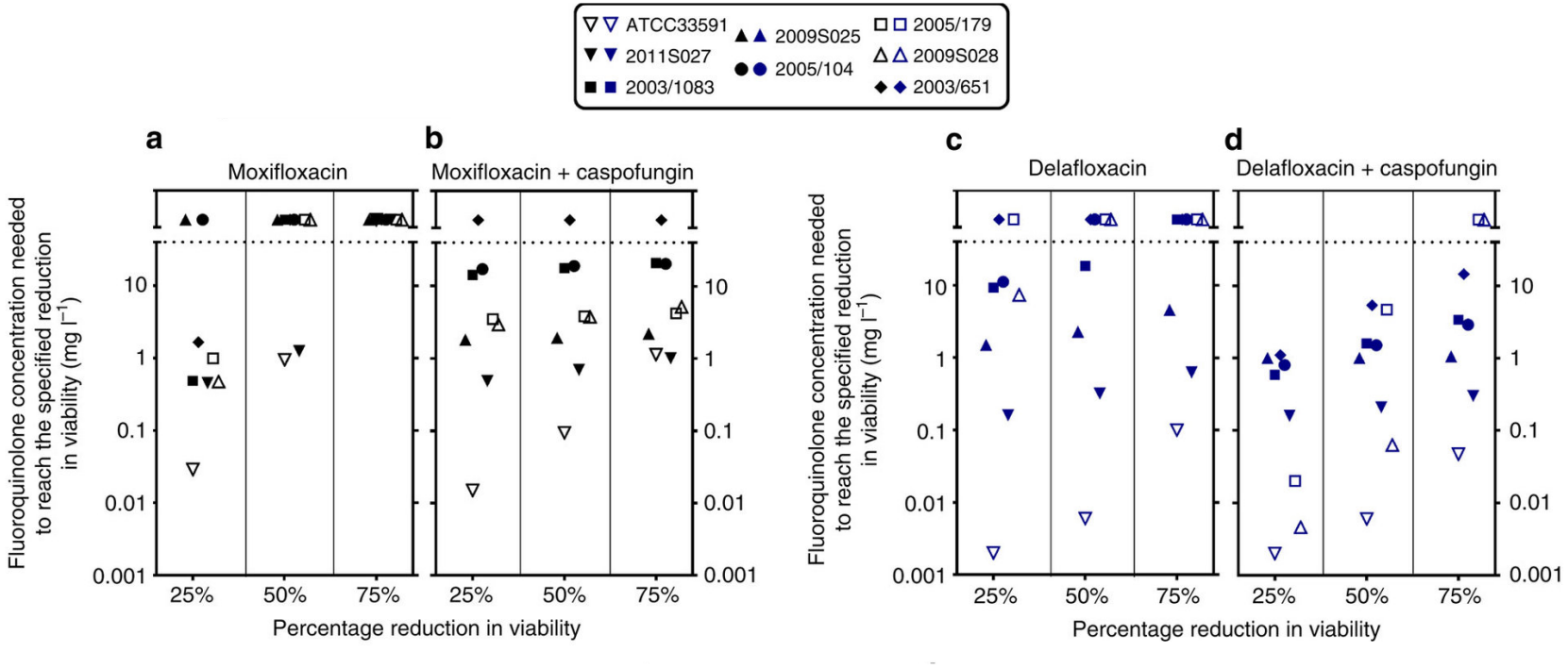
Caspofungin-fluoroquinolone combinations



The combination works for two FQs and against several strains, but to different extents …

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

Caspofungin increases FQ potency within biofilms

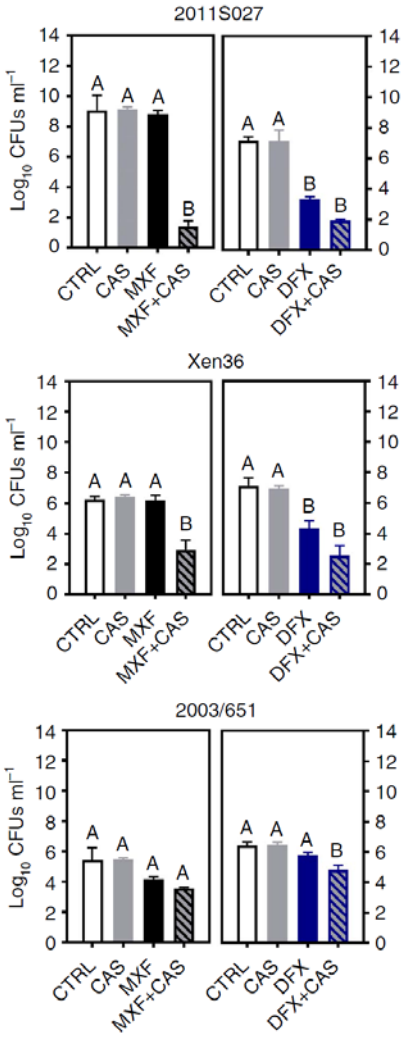


The combination works for two FQs and against several strains,
but to different extents …

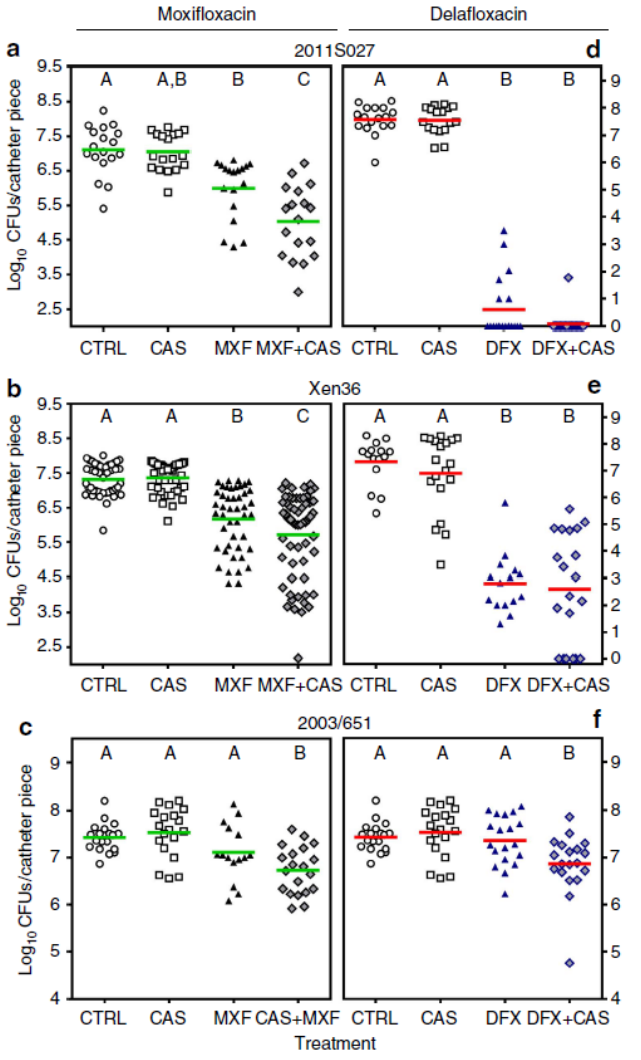
*Siala et al, Nature Communications 2016; 7:*13286

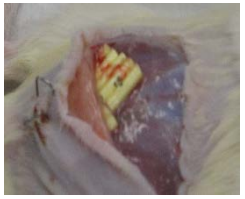
Caspofungin increases fluoroquinolone activity *in vitro* and *in vivo*

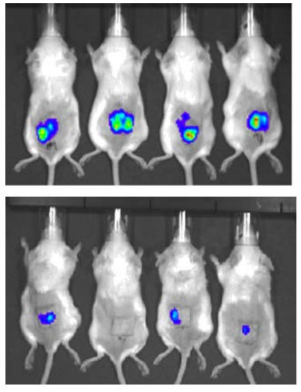
Catheters in vitro



Catheters in vivo

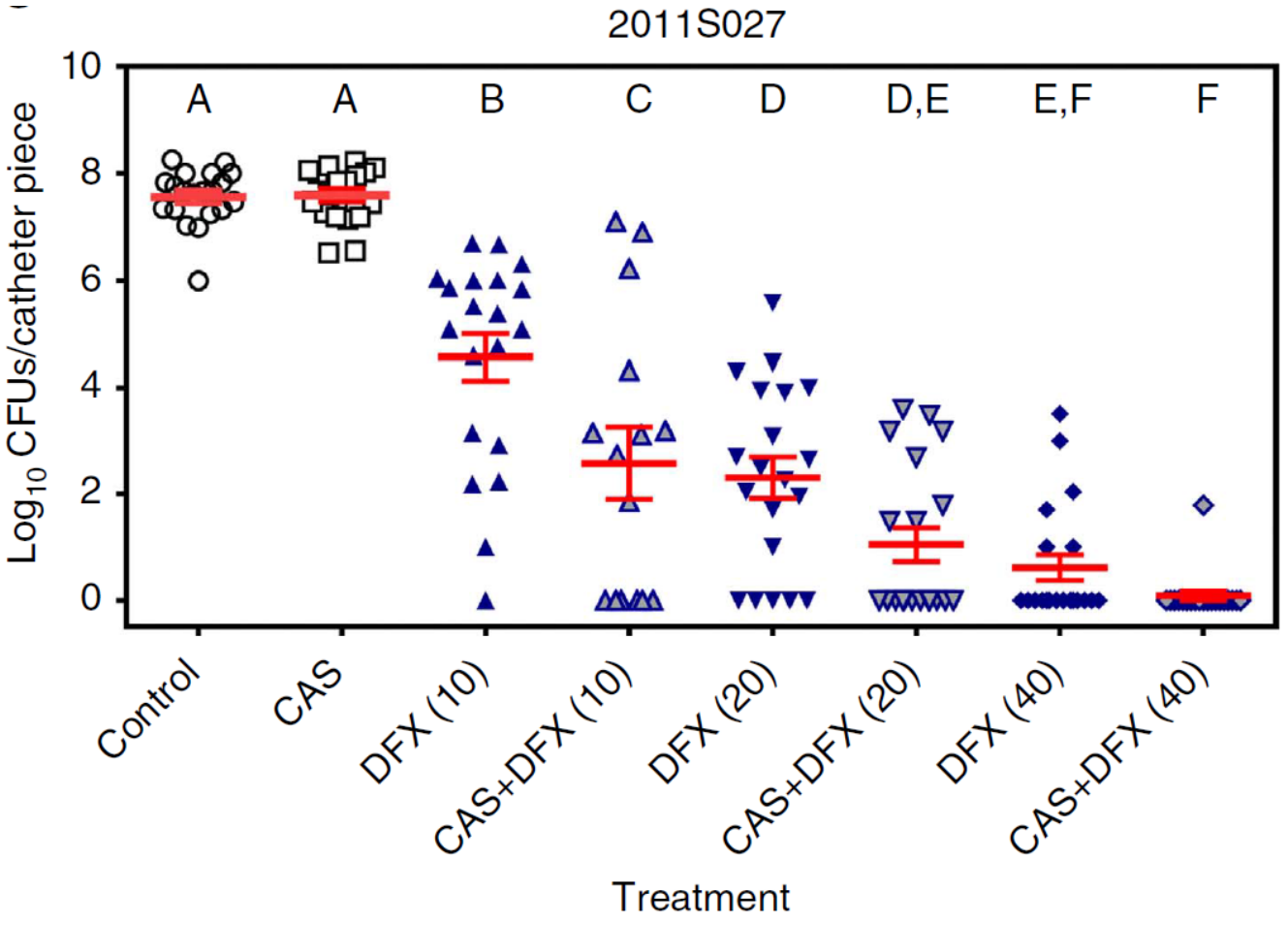






*Siala et al, Nature Communications 2016; 7:*13286

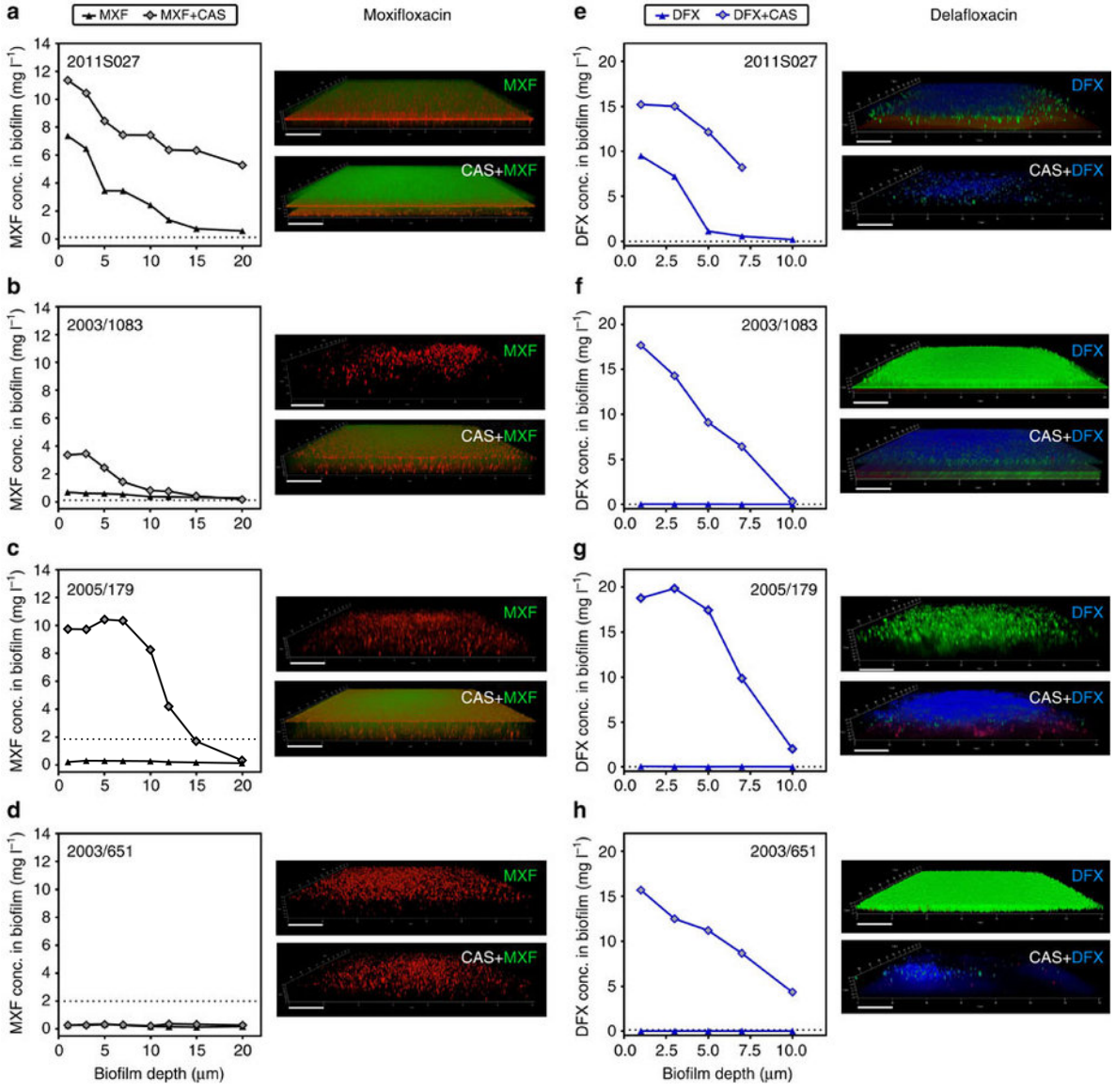
Caspofungin increases fluoroquinolone activity *in vitro* and *in vivo*



Caspofungin makes fluoroquinolones active at lower concentrations

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

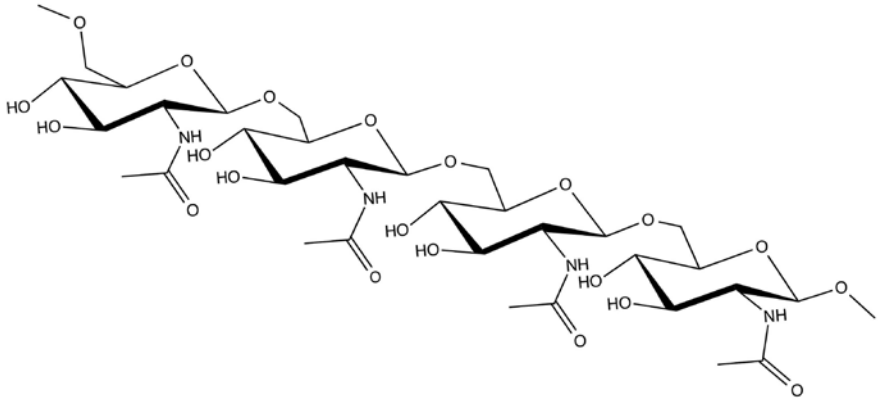
Caspofungin increases fluoroquinolone penetration



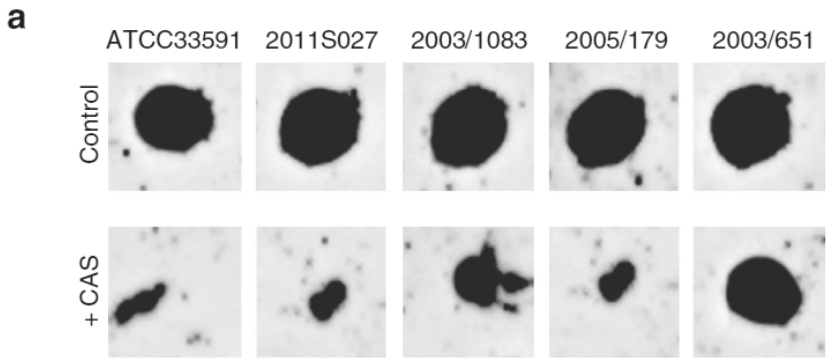
CAS ↗ fluoroquinolone penetration in biofilms (strain dependent)

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

Effect of caspofungin on PNAG in biofilm matrix



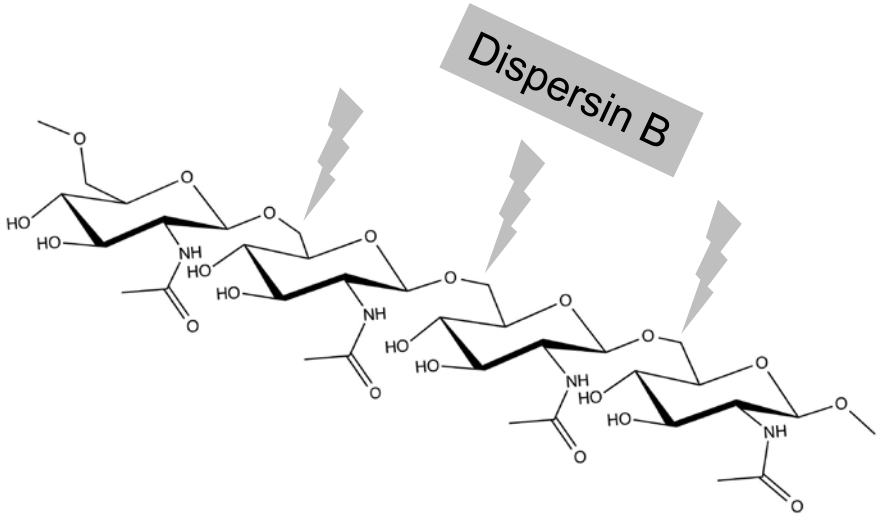
Poly-N-acetylglucosamine



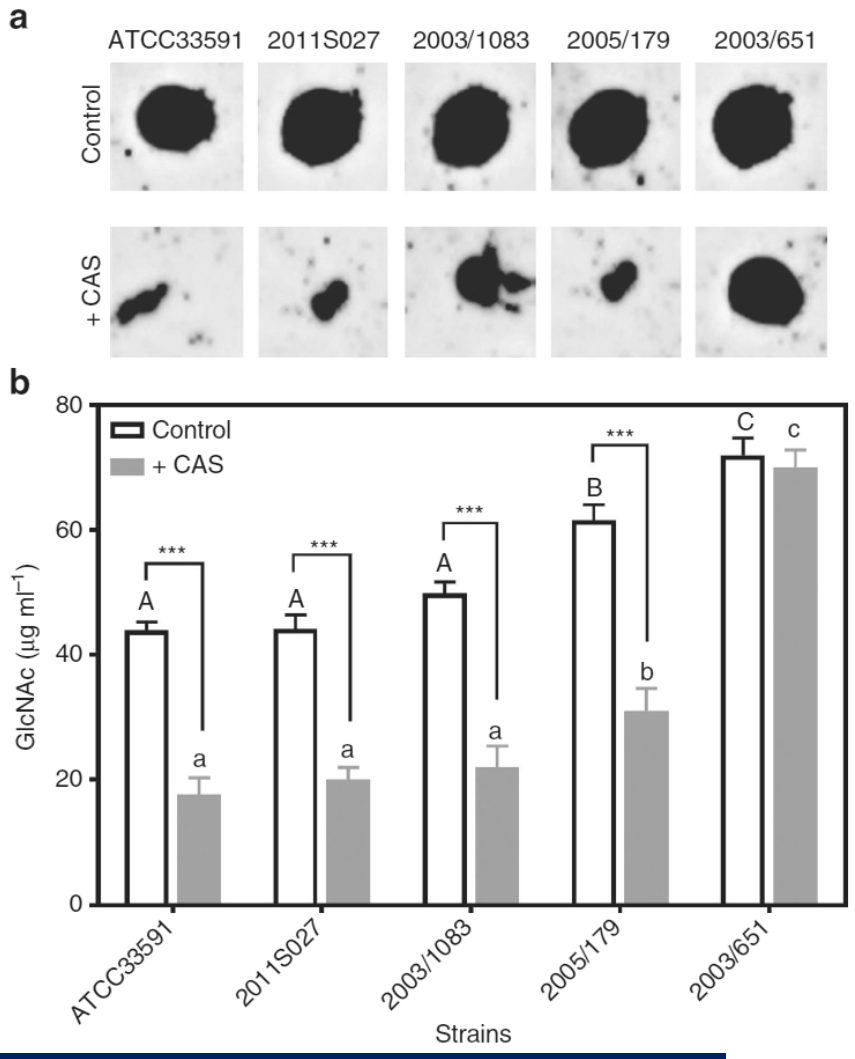
CAS ↘ poly-N-acetylglucosamine content in biofilms

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

Effect of caspofungin on PNAG in biofilm matrix



Poly-N-acetylglucosamine

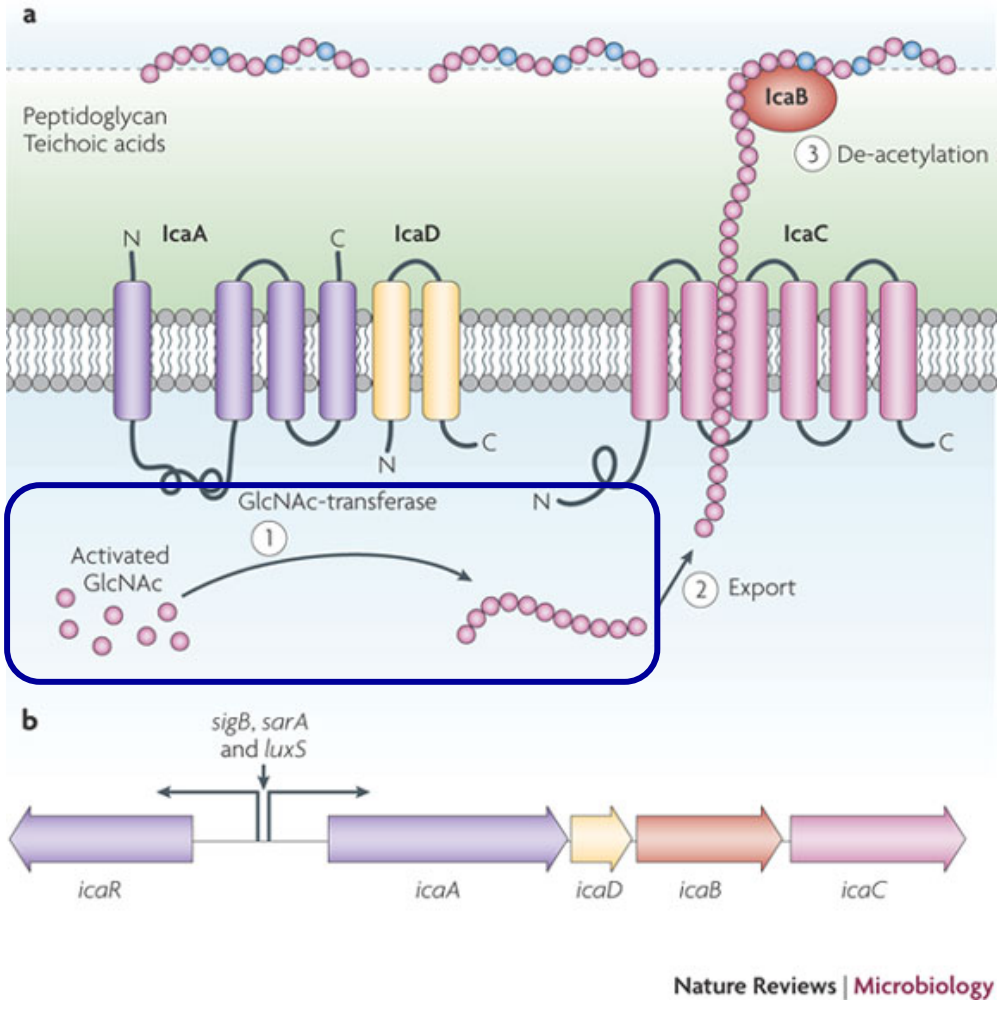


CAS ↘ poly-N-acetylglucosamine content and polymerization in biofilms

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

IacA and polysaccharide synthesis in *S. aureus*

Ica A is involved in N-acetylglucosamine homopolymer synthesis

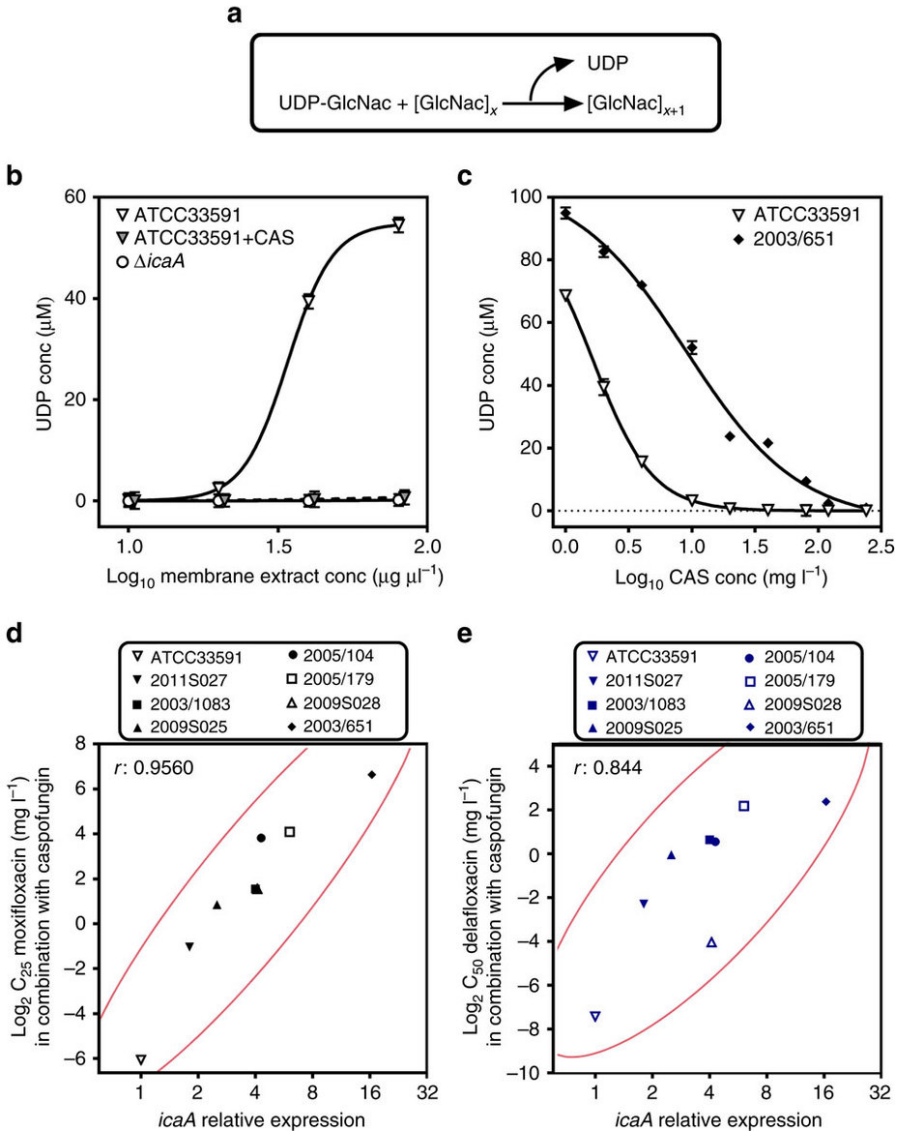


Otto et al., Nat. Rev. Microbiol. 2009; 7:555-67

Caspofungin, an unexpected IcaA inhibitor !

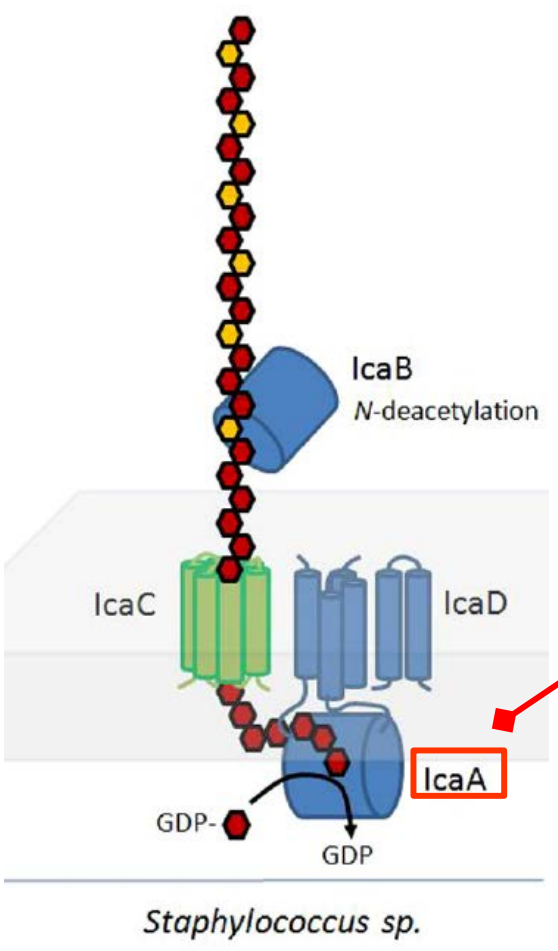
CAS inhibits IcaA and increases FQ potency in inverse proportion to *icaA* expression

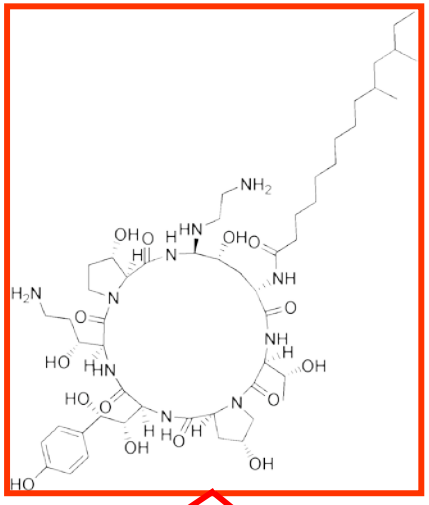
strain	icaA expression
ATCC33591	1
2011S027	1.8 ± 0.5*
2003/1083	4.0 ± 0.6 *
2009S025	2.5 ± 0.5*
2005/104	4.2 ± 0.4*
2005/179	6.0 ± 0.9*
2009S028	4.1 ± 0.2*
2003/651	16.3 ± 0.7*



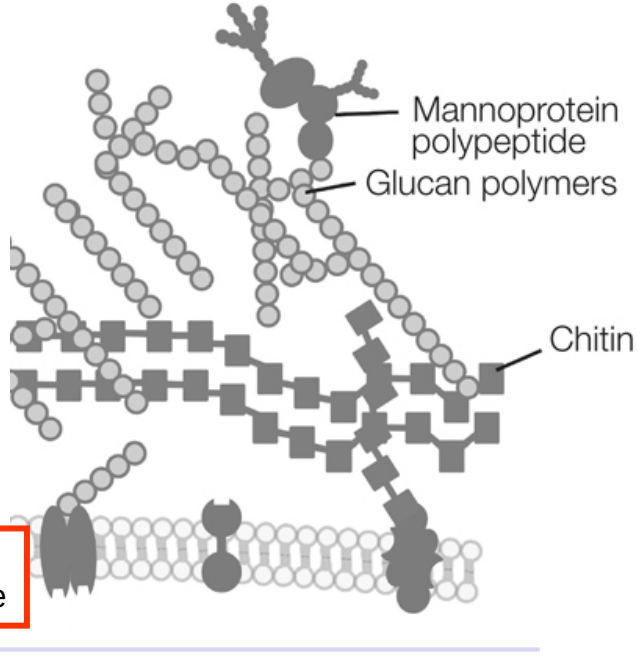
*Siala et al, Nature Communications 2016; 7:*13286

The antifungal caspofungin as an inhibitor of polysaccharide synthesis





Glucan synthase

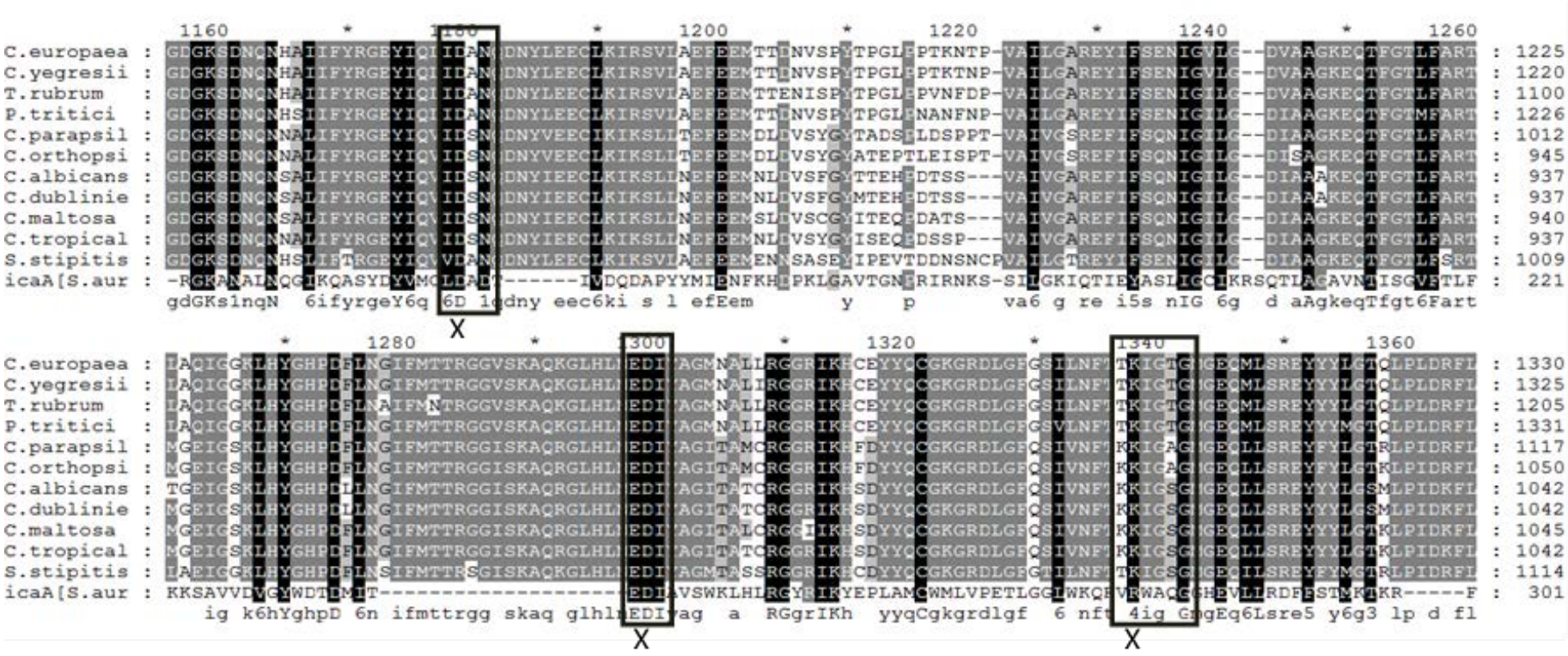


Atkin et al, FEBS Lett. 2014;588:1869-72

Adapted from Arnold, Kucer's 6the edition

Caspofungin, an unexpected IcaA inhibitor ?

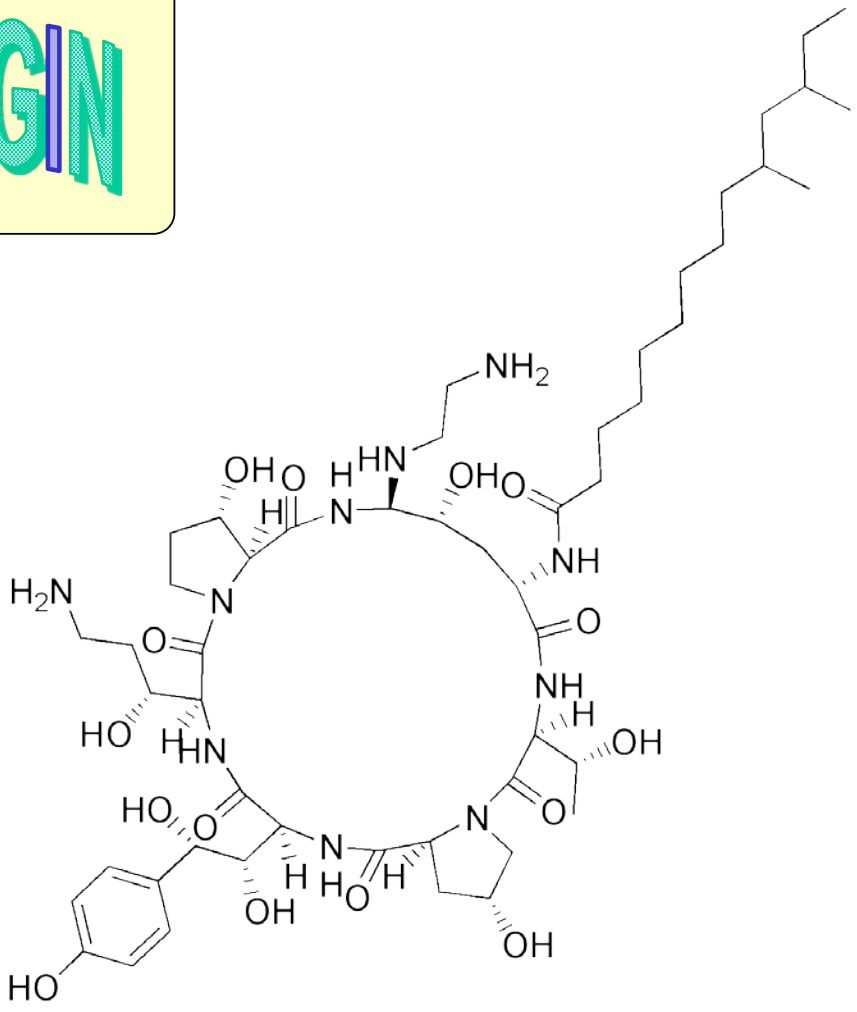
IcaA is homologous to glucan synthases (caspofungin target in fungi)

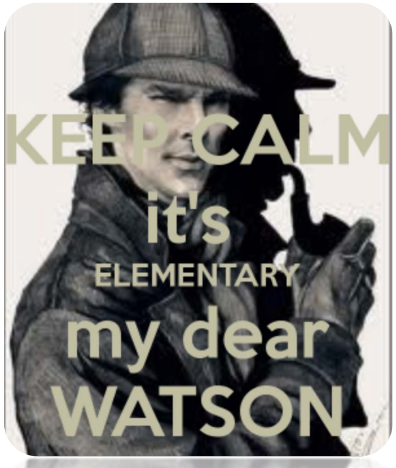


*Siala et al, Nature Communications 2016; 7:*13286

Caspofungin as a prototype for icaA inhibitors

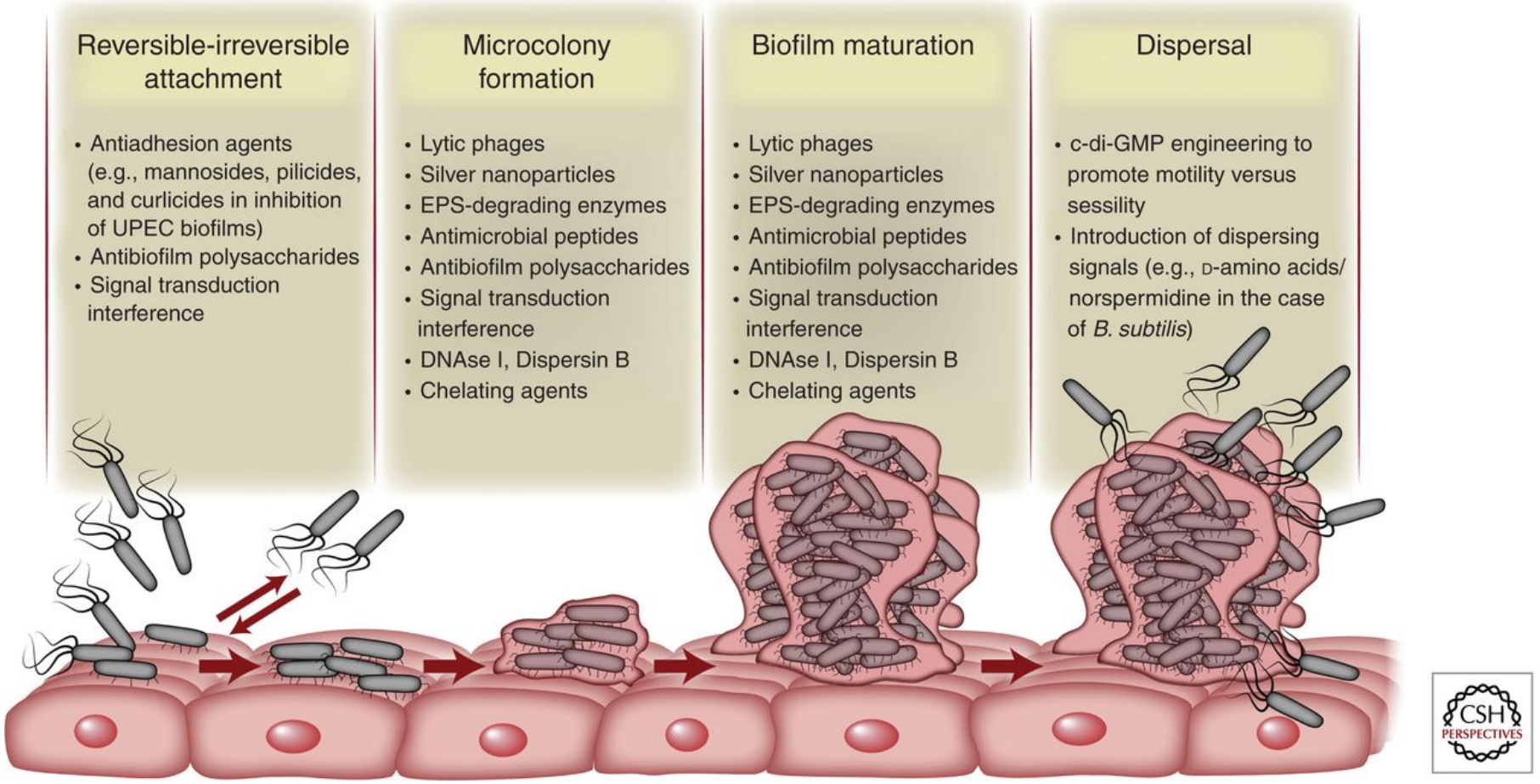
CASPOFUNGIN





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

Antibiofilm strategies under study in the lab …



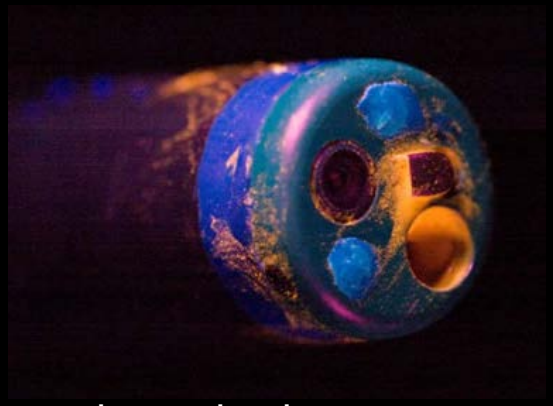
Kostakioti et al. Cold Spring Harb Perspect Med 2013;3:a010306

New clinical applications

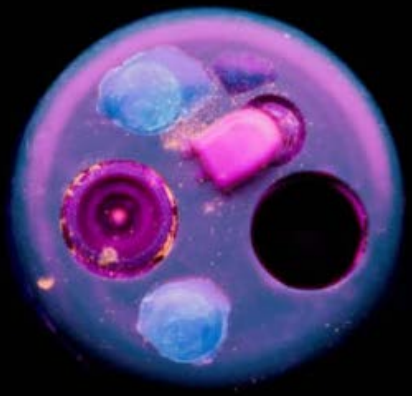
1. Infections on medical devices

This is not the main reason for contamination …

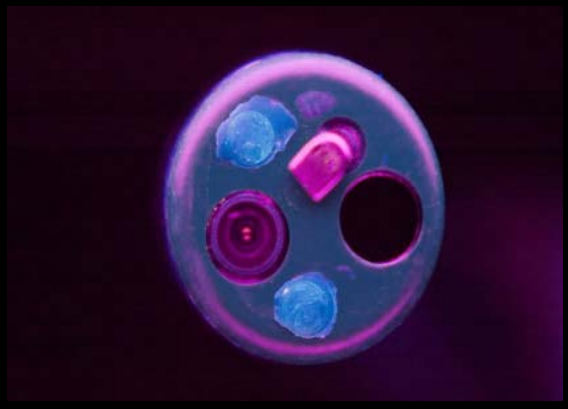
Biofilms on endoscopes and cleaning procedures



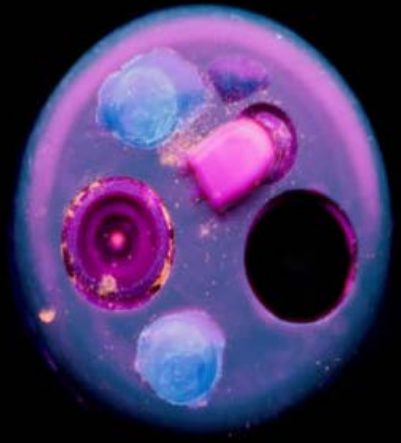
uncleaned colonoscope



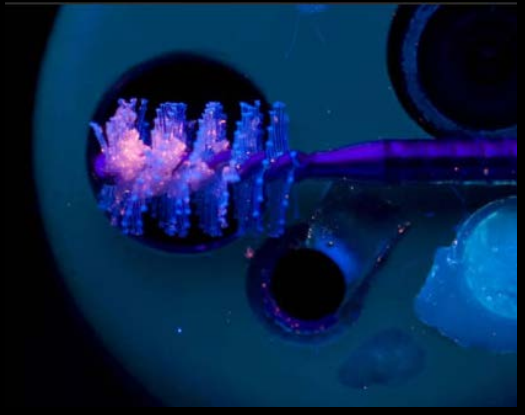
manual cleaning



paracetic acid



glutaraldehyde/alcool



removal of air/water noozle

https://www.educationaldimensions.com/eLearn/biofilm/photograph.php

Importance of cleaning procedure

Summary of answers to the follow-up questionnaire for endoscope reprocessing procedures in 66 hospitals

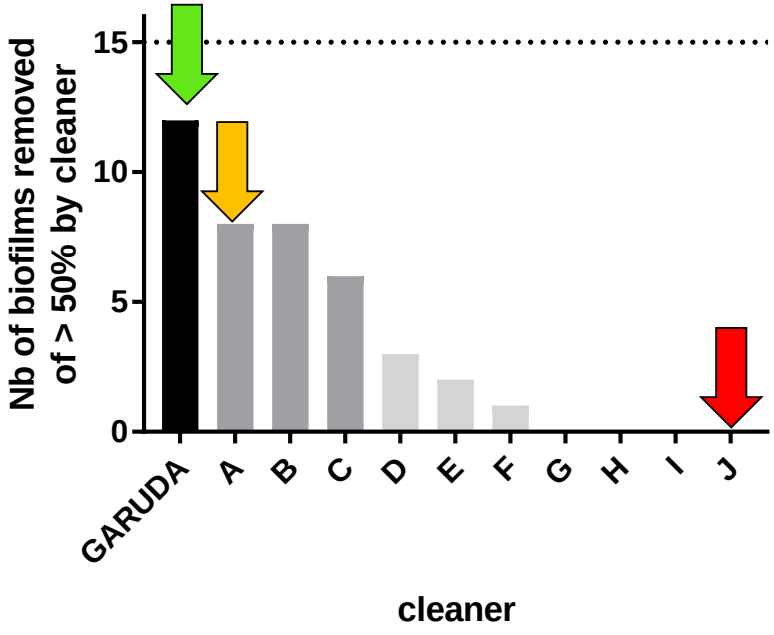
Characteristic and Recommendation	biofilm (n = 30)	No biofilm (n = 36)	Total (N = 66)	*P* value
Daily surgical volume				.239
<5	70.0 (21/30)	83.3 (30/36)	78.8 (51/66)	
50-100	16.7 (5/30)	13.9 (5/36)	15.2 (10/66)	
>100	13.3 (4/30)	2.7 (1/36)	7.6 (5/66)	
Proportion of manual cleaning	50.0 (15/30)	91.7 (33/36)	72.7 (48/66)	.001
Suctioning all channel	90.0 (27/30)	83.3 (30/36)	86.4 (57/66)	.670
Use of biofilm removal detergent	26.7 (8/30)	0 (0/36)	12.1 (8/66)	.003
Repeated use of detergent	63.3 (19/30)	91.7 (33/36)	78.8 (52/66)	.005
Sterile water used to rinse	60.0 (18/30)	61.1 (22/36)	60.6 (40/66)	.927
Alcohol dry	76.7 (23/30)	38.9 (14/36)	56.0 (37/66)	.002

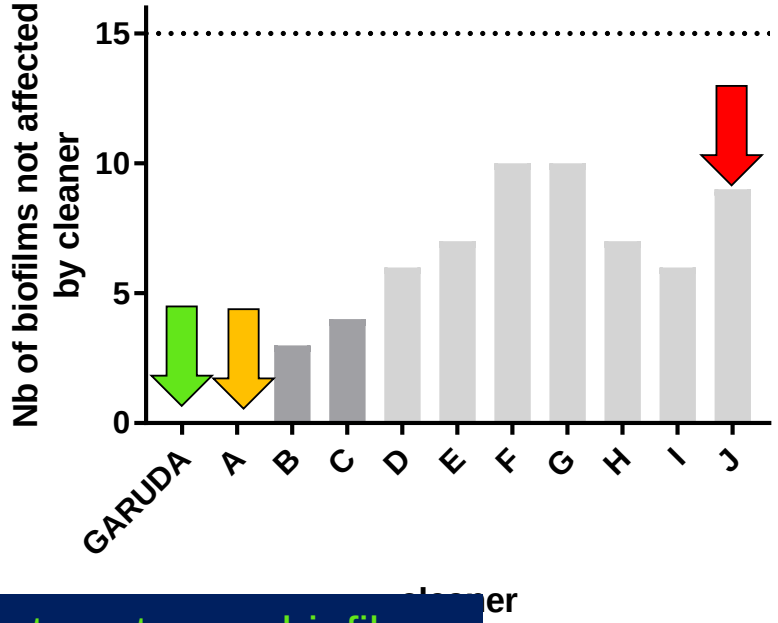
NOTE. Values are percentages (compliance with recommendations for reprocessing or characteristic).

Ren-Pei et al, Am J Infect Control 2014; 42:1203-6

Efficacy of biofilm-removing detergents

In vitro evaluation of 12 detergent solutions against 15 biofilms from different species

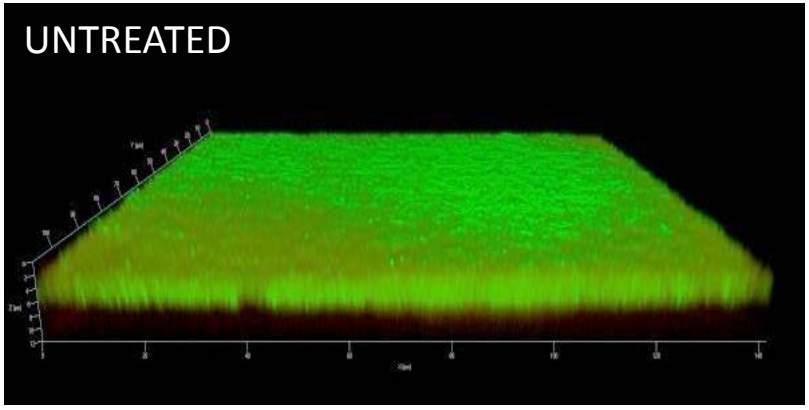


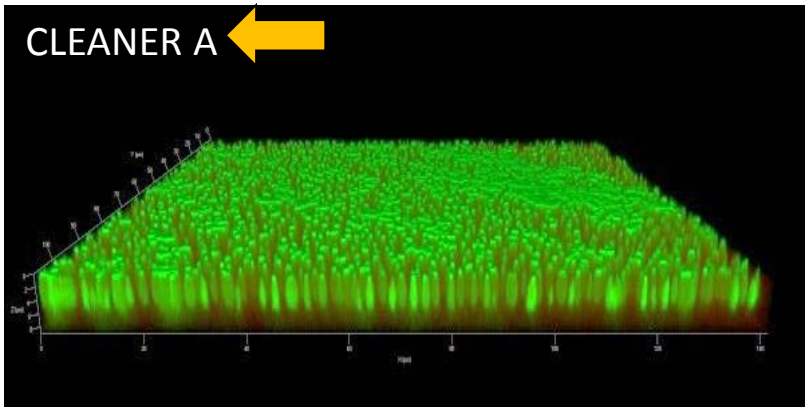


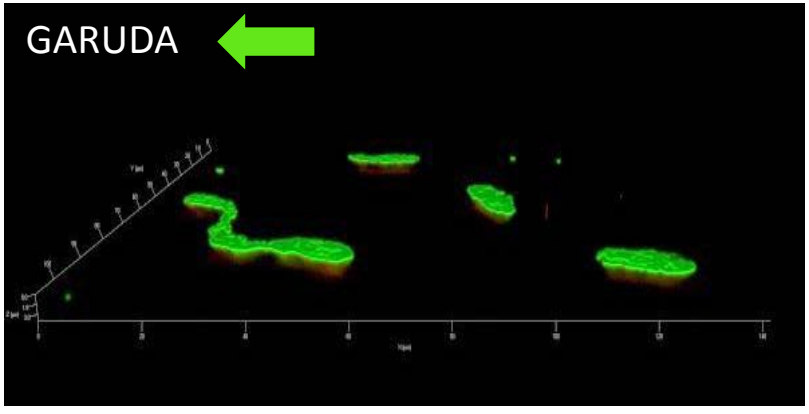
High variability in capacity to act upon biofilms among detergents

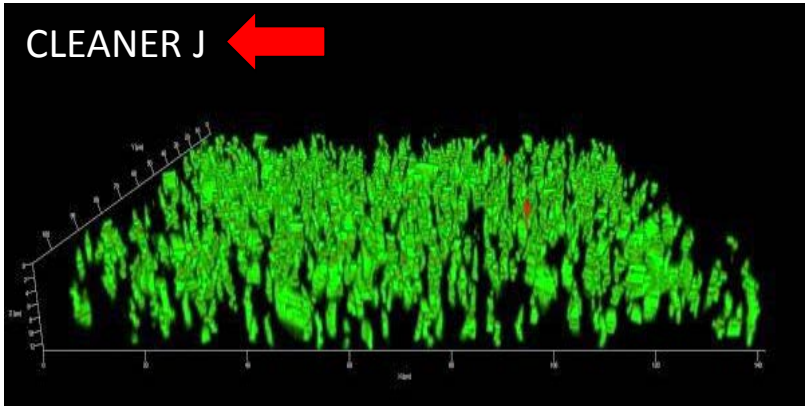
Siala et al, unpublished

Efficacy of biofilm-removing detergents









Siala et al, unpublished

Efficacy of biofilm-removing detergents

OneLIFE
Specialized medical cleaning

Ex vivo efficacy of GARUDA® for endoscope cleaning

Hospital	Endoscope type	First microbiological control (in 100 ml)	Intensive cleaning and disinfection procedure	Second microbiological control (in 100 ml)	Garuda MD corrective cleaning and disinfection	Microbiological control after Garuda MD procedure (in 100 ml)
French University Hospital	Echo-endoscope	**>150 CFU** + *Stenotrophomonas maltophilia*	Yes (double 5 min manual cleaning + AER)	**> 150 CFU** + *Stenotrophomonas maltophilia*	Garuda MD + AER	**< 1 CFU** Absence of *Stenotrophomonas maltophilia*
French University Hospital	Echo-endoscope	**75 CFU** + *Pseudomonas aeruginosa* + *Streptococcus* spp.	Yes (double 5 min manual cleaning + AER)	**16 CFU** + *Stenotrophomonas maltophilia*	Garuda MD + AER	**0 CFU** Absence of *Stenotrophomonas maltophilia*
French University Hospital	Echo-endoscope	Return from maintenance / Not tested	Yes (double 5 min manual cleaning + AER)	**> 150 CFU** + *Stenotrophomonas maltophilia* + *Pseudomonas aeruginosa*	Garuda MD + AER	**< 5 CFU** Absence of *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*
Belgian University Hospital	Gastroscope	**>300 CFU**	Yes (No manual cleaning but longer AER cycle)	**> 300 CFU**	Garuda MD + AER	**0 CFU**
Belgian Hospital # 1	Gastroscope	**> 100 CFU** + *Pseudomonas aeruginosa*	Yes (5 min manual cleaning + AER)	**> 100 CFU** + *Pseudomonas aeruginosa*	Garuda MD + AER	**0 CFU** Absence of *Pseudomonas aeruginosa*
Belgian Hospital # 2	Duodenoscope	**1.000 CFU** + *Pseudomonas aeruginosa*	Yes (5 min manual cleaning + AER)	**10.000 CFU** + *Pseudomonas aeruginosa*	Garuda MD + AER	**< 20 CFU** Absence of *Pseudomonas aeruginosa*
Belgian Hospital # 2	Duodenoscope	**5.000 CFU** + *Pseudomonas aeruginosa*	Yes (5 min manual cleaning + AER)	**5.000 CFU** + *Pseudomonas aeruginosa*	Garuda MD + AER	**< 20 CFU** Absence of *Pseudomonas aeruginosa*

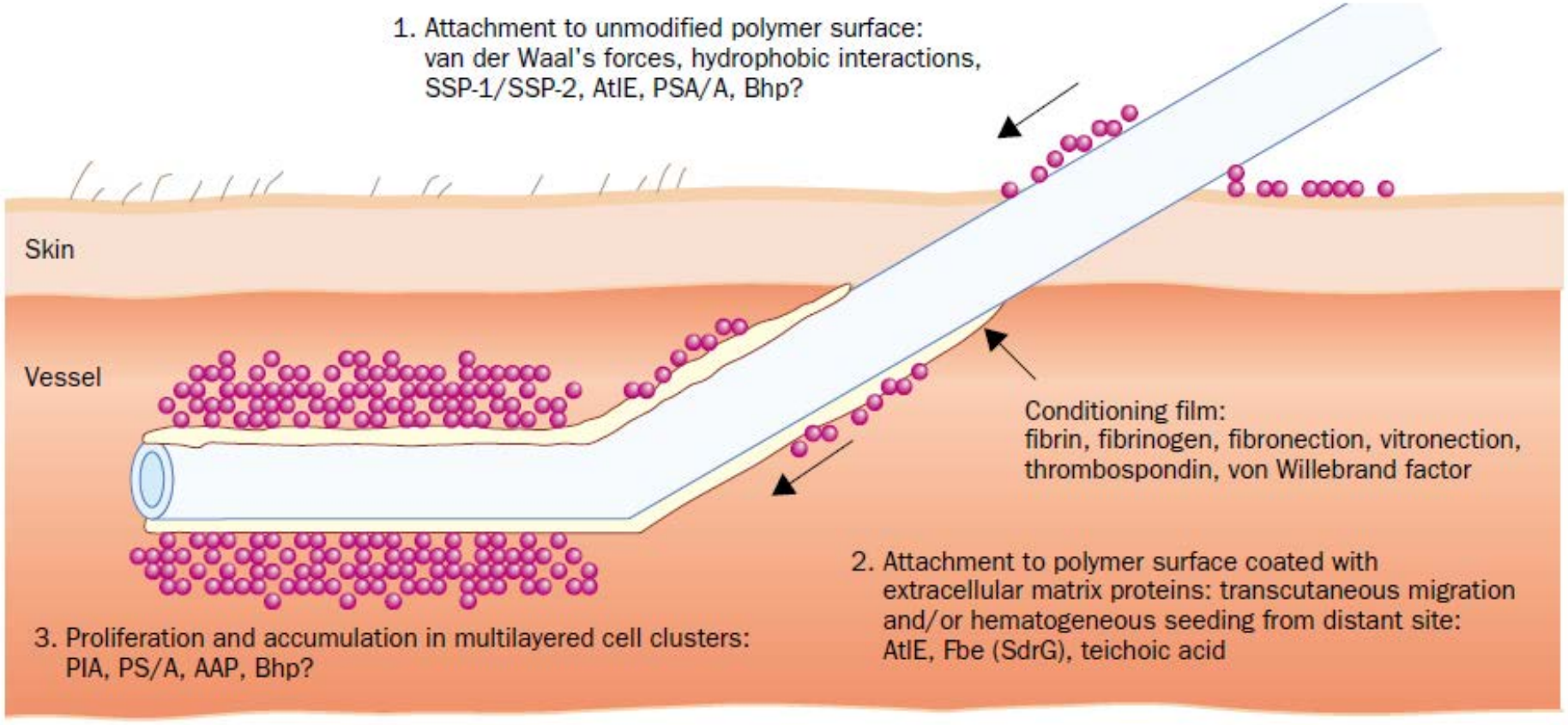
AER: Automated Endoscope Reprocessors

GARUDA® removes most of remaining contamination

Siala et al, unpublished

New clinical applications

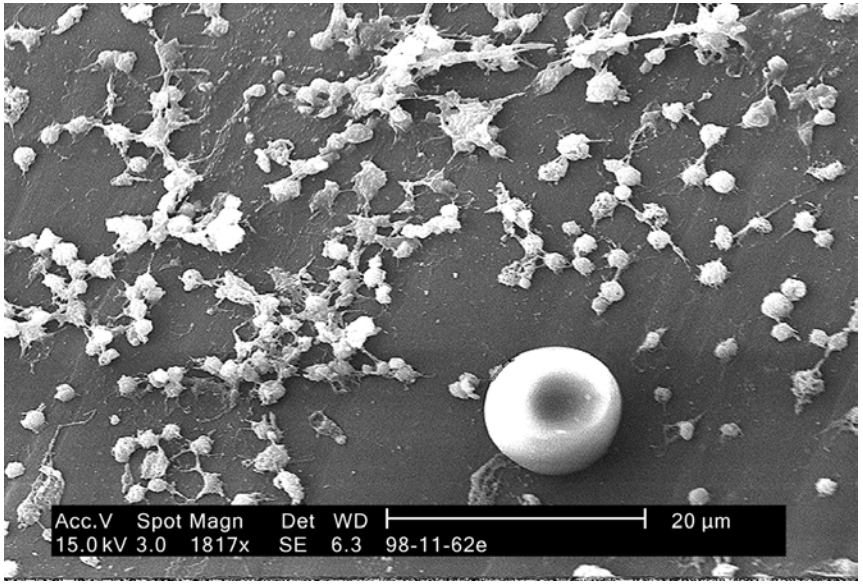
2. Infections on catheters

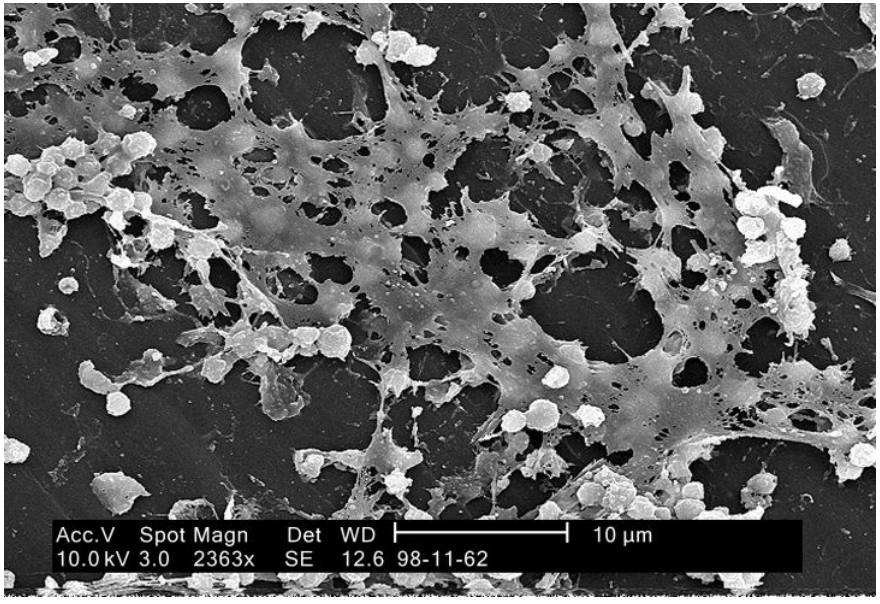


Von Eiff, Lancet ID 2002; 2:677-685

New clinical applications

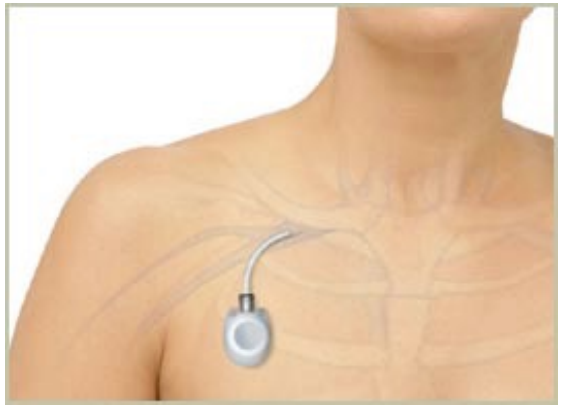
Infections on catheters

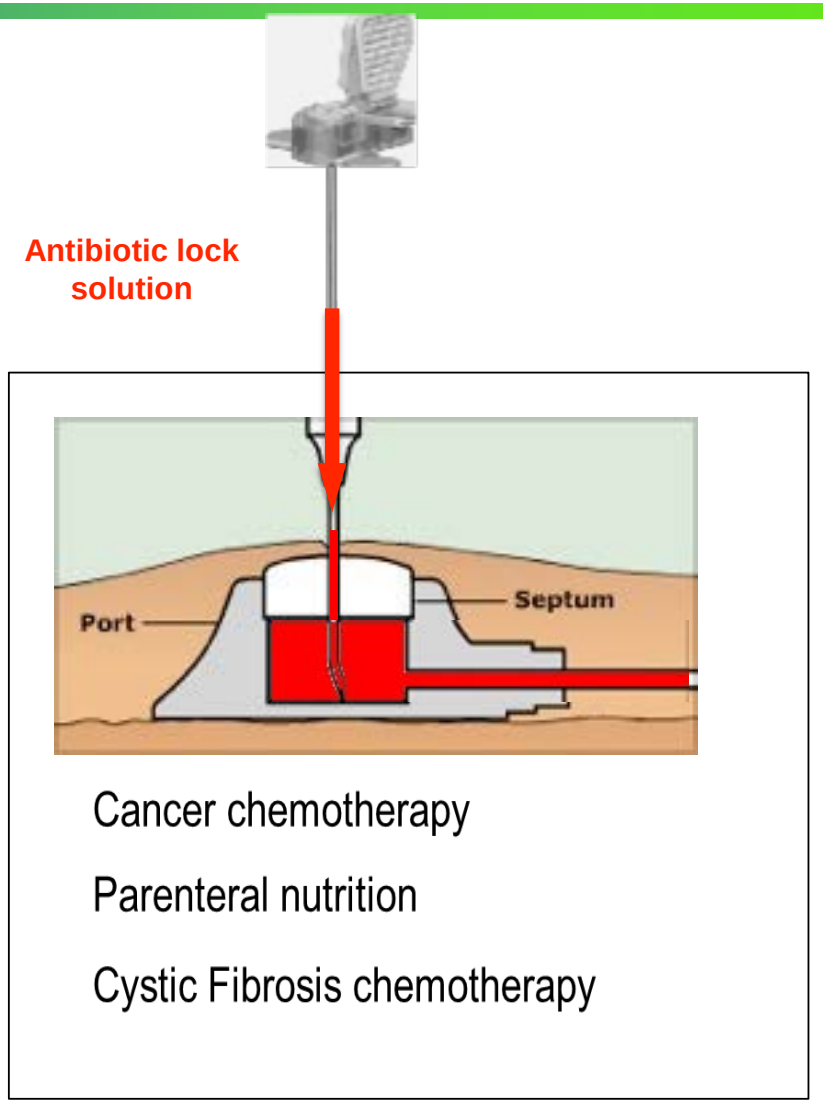




Lock therapy and catheter-related infections

Totally implanted venous access catheters

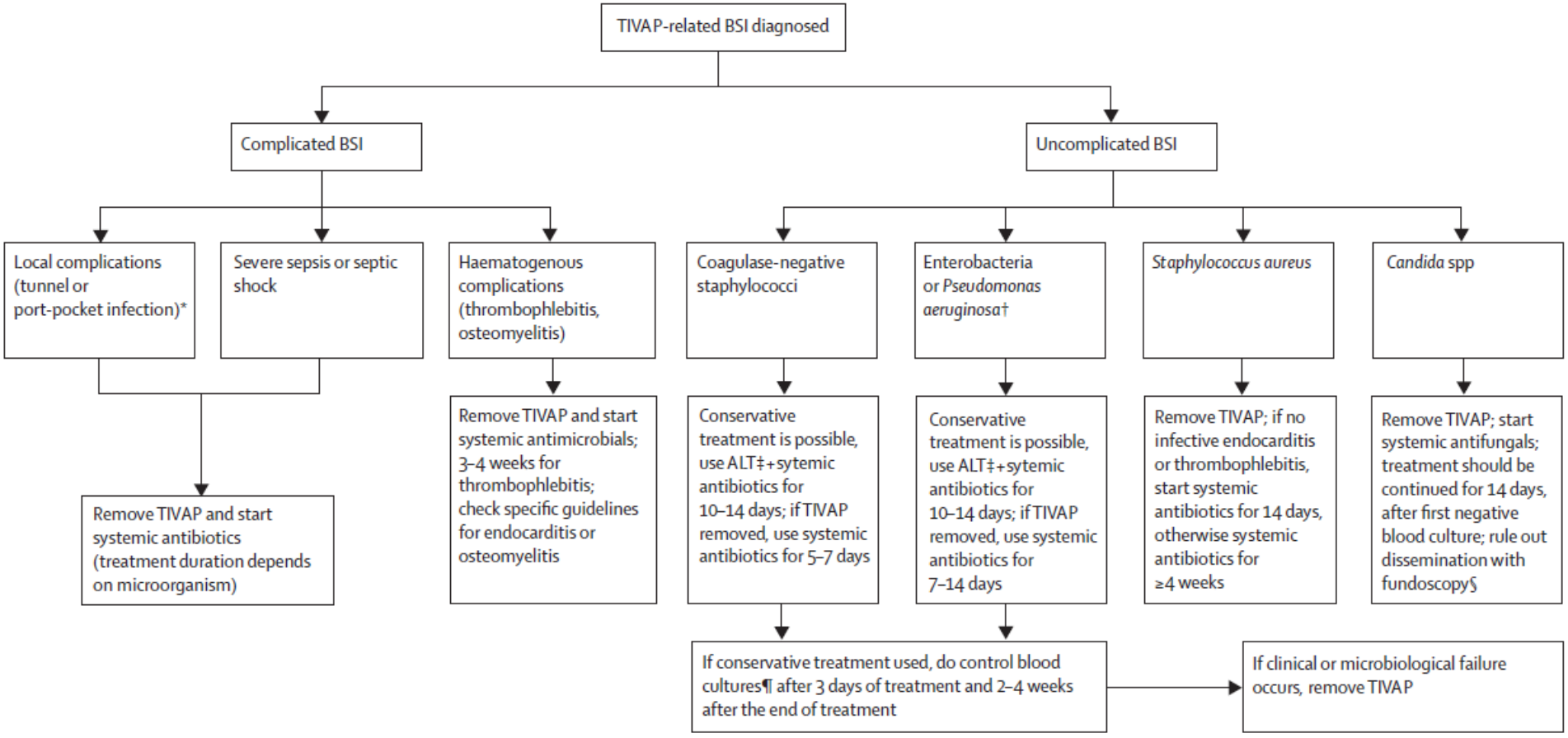




- Closed system but accessible to colonisation
- Possibility to follow colonisation
  - ➢ in the chamber,
  - ➢ in the catheter,
  - ➢ the related infection

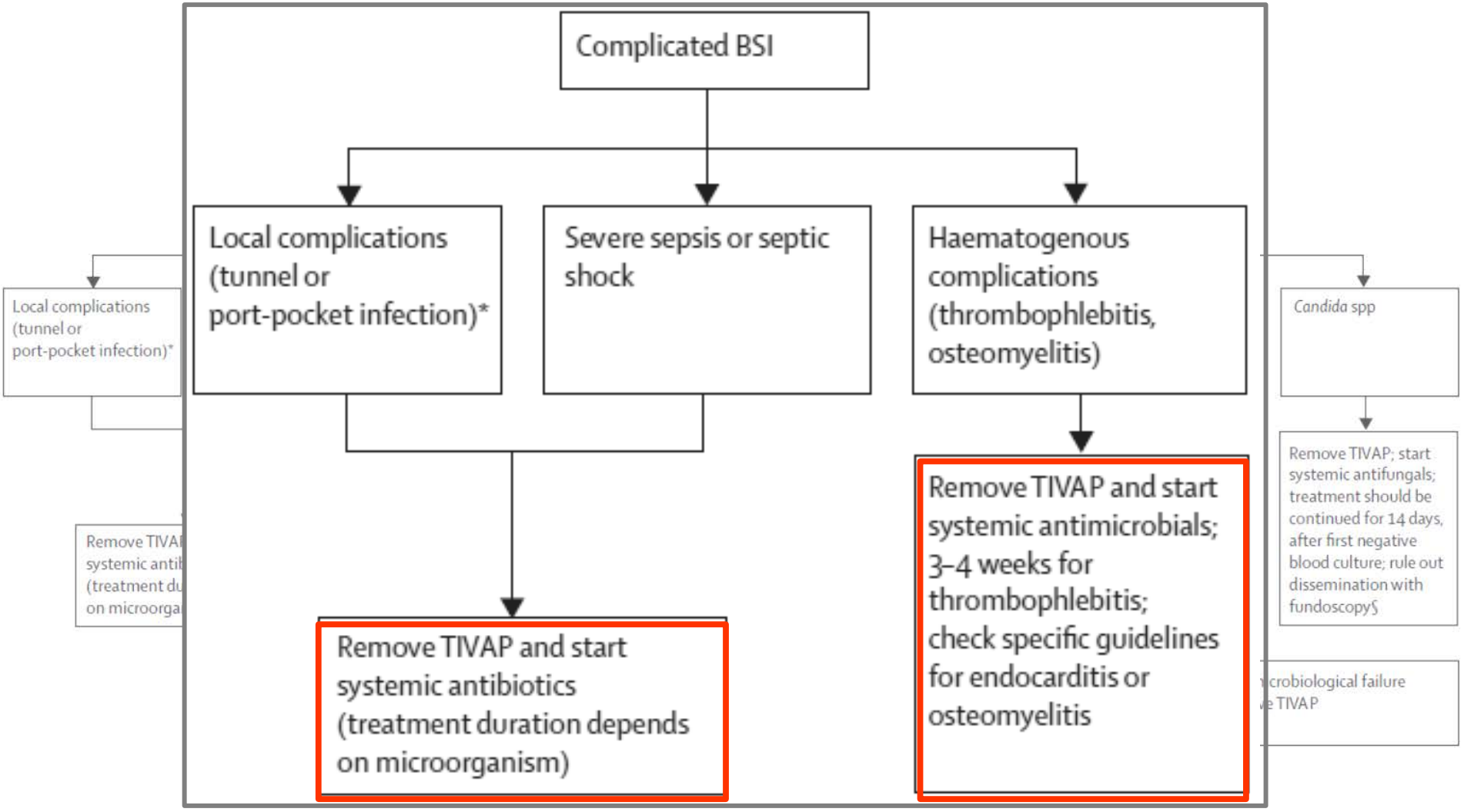
Lebeaux et al. Lancet ID 2014;14:146-59.

Antibiotic lock therapy: current practice



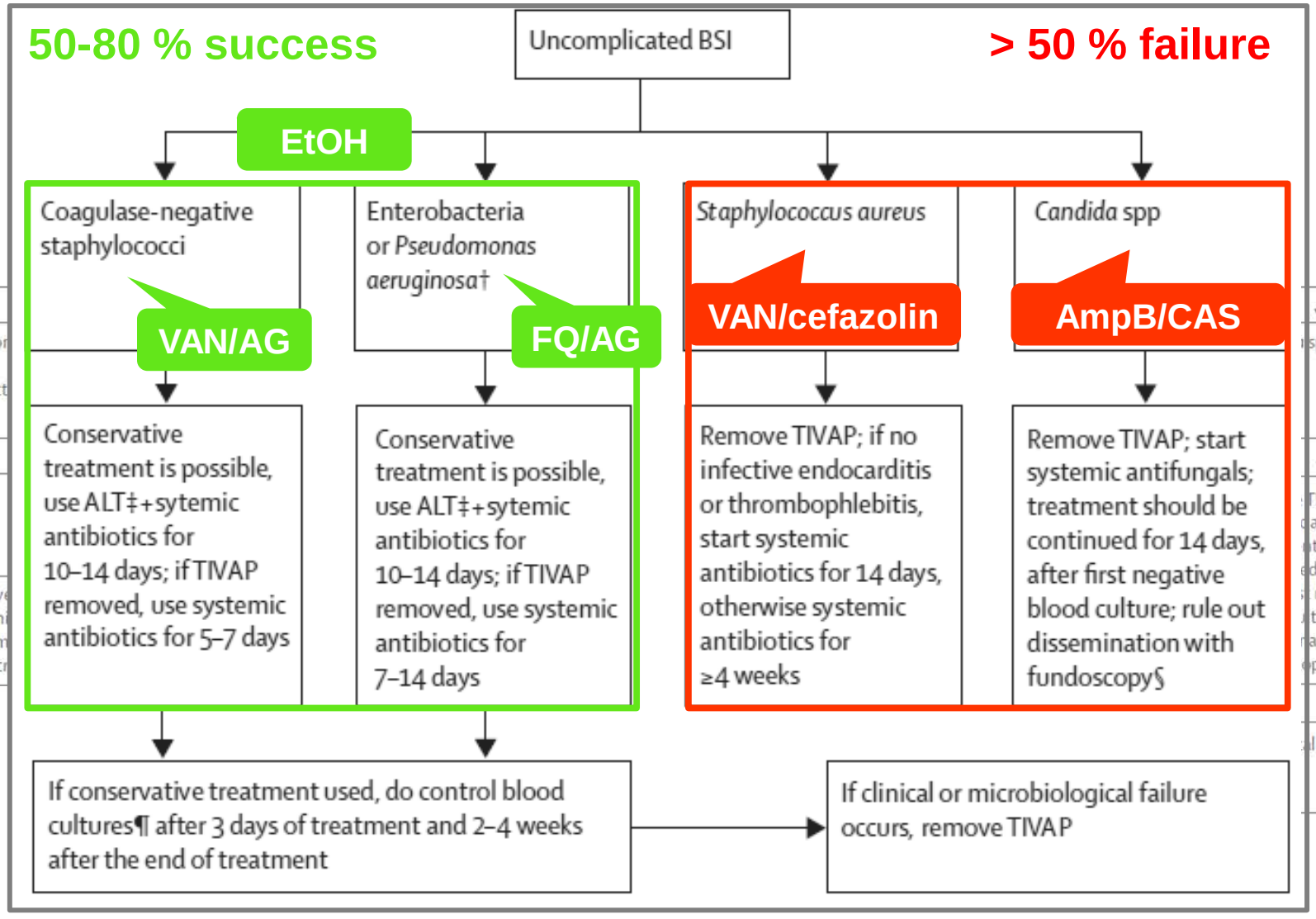
Lebeaux et al, Lancet ID 2014; 14:146–159

Antibiotic lock therapy: current practice



Lebeaux et al, Lancet ID 2014; 14:146–159

Antibiotic lock therapy: current practice



Lebeaux et al, Lancet ID 2014; 14:146–159

Antibiotic locks: clinical efficacy

Antibiotics compared with no antibiotics prior to long-term CVC insertion to prevent catheter-related infections

Patient or population: adults with a newly inserted long-term CVC who were at risk of neutropenia due to chemotherapy or disease
Settings: inpatient and outpatient
Intervention: intravenous antibiotics (vancomycin, teicoplanin or ceftazidime)
Comparison: placebo or no antibiotics

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Antibiotics				
Catheter-related sepsis	200 per 1000	144 per 1000 (66 to 316)	RR 0.72 (0.33 to 1.58)	360 (5)	⊕⊕⊕◯ moderate	The difference between the comparison groups was not significant (P = 0.41). We downgraded this evidence to moderate due to the substantial heterogeneity (I^2 = 52%) between studies

*The basis for the **assumed risk** is the mean control group risk across studies. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio

Van de Wetering et al, Cochrane Database of Systematic Reviews 2013; 11

Anti-coagulant + antibiotic locks: clinical efficacy

Antibiotic and heparin solution compared with a heparin only solution for flushing or locking long-term CVCs to prevent Gram positive catheter-related sepsis

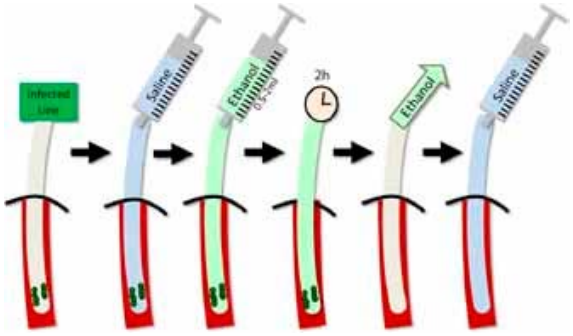
Patient or population: adults and children with a newly inserted long-term CVC who were at risk of neutropenia due to chemotherapy or disease
Settings: inpatient and outpatient
Intervention: antibiotic (vancomycin, vancomycin and amikacon, or taurolidine) plus heparin solution
Comparison: heparin only solution

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Heparin-only	Antibiotic/heparin				
Catheter-related sepsis	200 per 1000	94 per 1000 (56 to 160)	RR 0.47 (0.28 to 0.80)	468 (6)	⊕⊕⊕◯ moderate	Data consistent across included studies; I^2 = 0%; P = 0.005. For an assumed risk of 15%, the NNT = 12 (9 to 33). We downgraded this evidence to moderate as the sample was clinically heterogeneous

*The basis for the **assumed risk** is the mean control group risk across included studies. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **RR:** Risk ratio; **NNT:** number needed to treat

Van de Wetering et al, Cochrane Database of Systematic Reviews 2013; 11

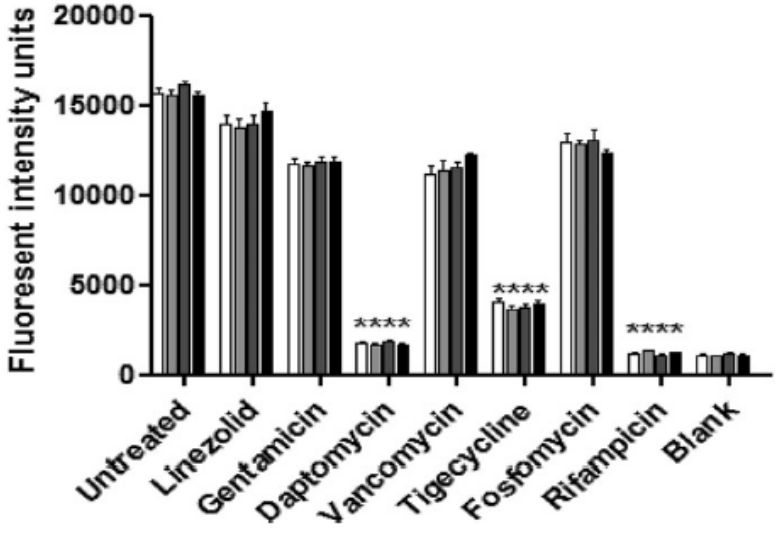
Lock therapy in the lab: screening of antibiotics



Results of treatment of experimental MRSA catheter-related sepsis

Treatment	MRSA 7 strain		MRSA 16 strain	
	negative cultures/total	log_{10} total cfu (SD)	negative cultures/total	log_{10} total cfu (SD)
Control	0/15	5.90 (1.13)	0/9	5.94 (1.36)
Linezolid	0/10	5.13 (0.94)[a]	0/12	4.64 (1.62)
Vancomycin	0/13	5.11 (1.05)[a]	0/13	5.00 (1.41)
Gentamicin	4/10[a]	2.19 (1.78)[a,b]	2/8[a]	3.00 (2.67)[a]

[a] $P < 0.05$ versus control.
[b] $P < 0.01$ versus linezolid and vancomycin.



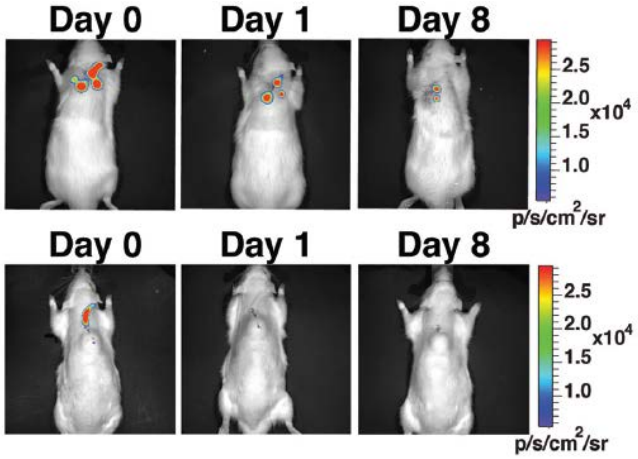
Fernandez-Hidalgo et al,
J Antimicrob Chemother 2010; 65: 525–530

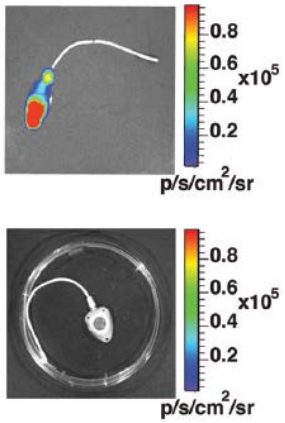
Hogan et al,
Antimicrob Agents Chemother. 2016;60:5968-75.

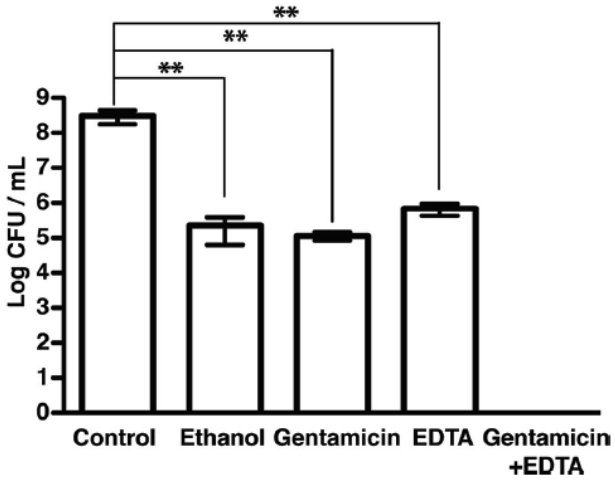
Aminoglycosides + EDTA

control

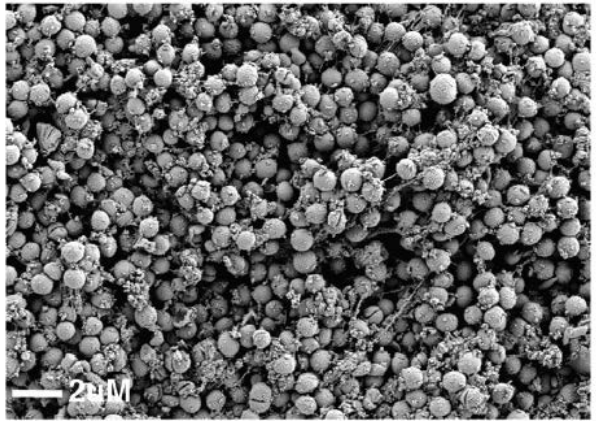
Genta + EDTA



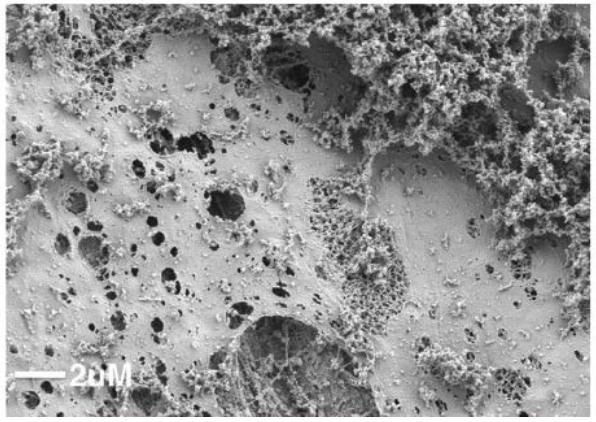




control



Genta + EDTA

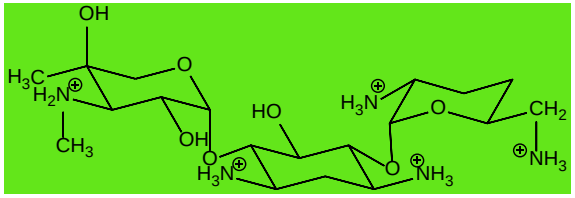


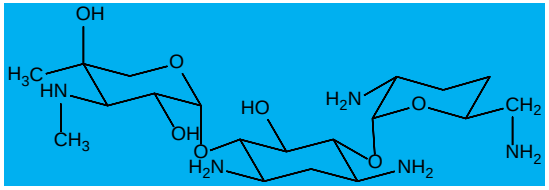
Genta + EDTA : highly synergistic combination

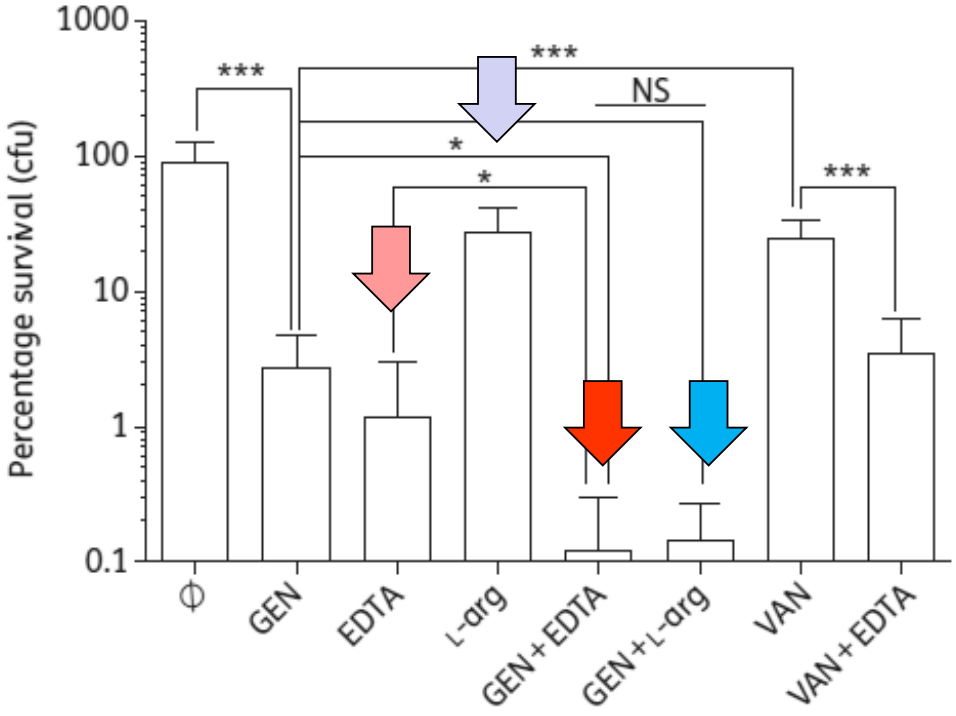
Hogan et al, Antimicrob Agents Chemother. 2012;56:6310-18.

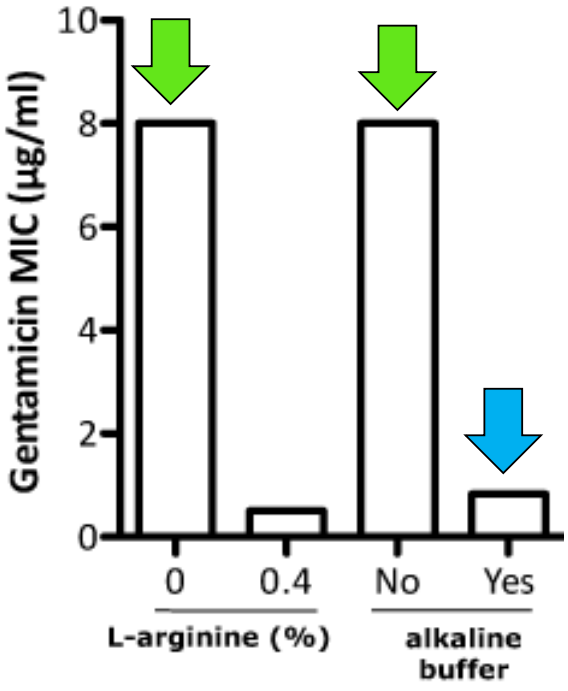
Modulation of gentamicin activity: L-Arginine

In vitro synergism







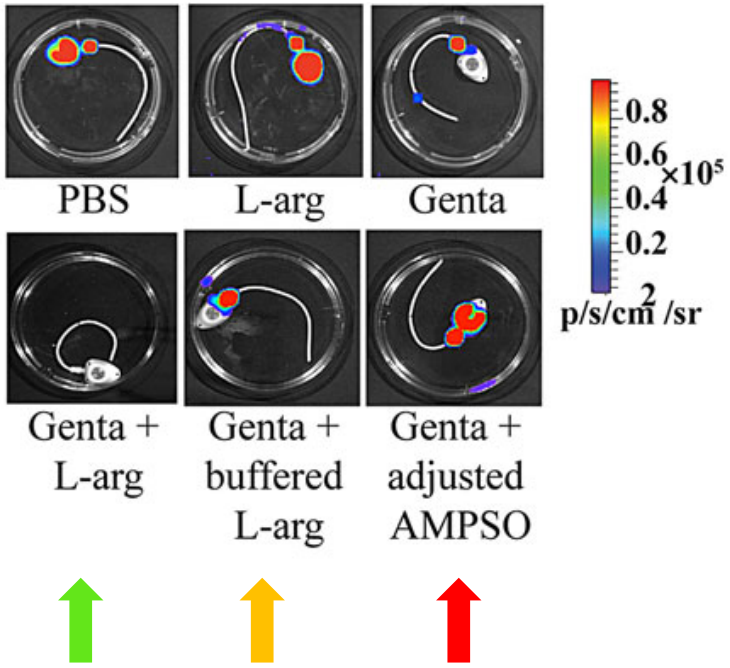


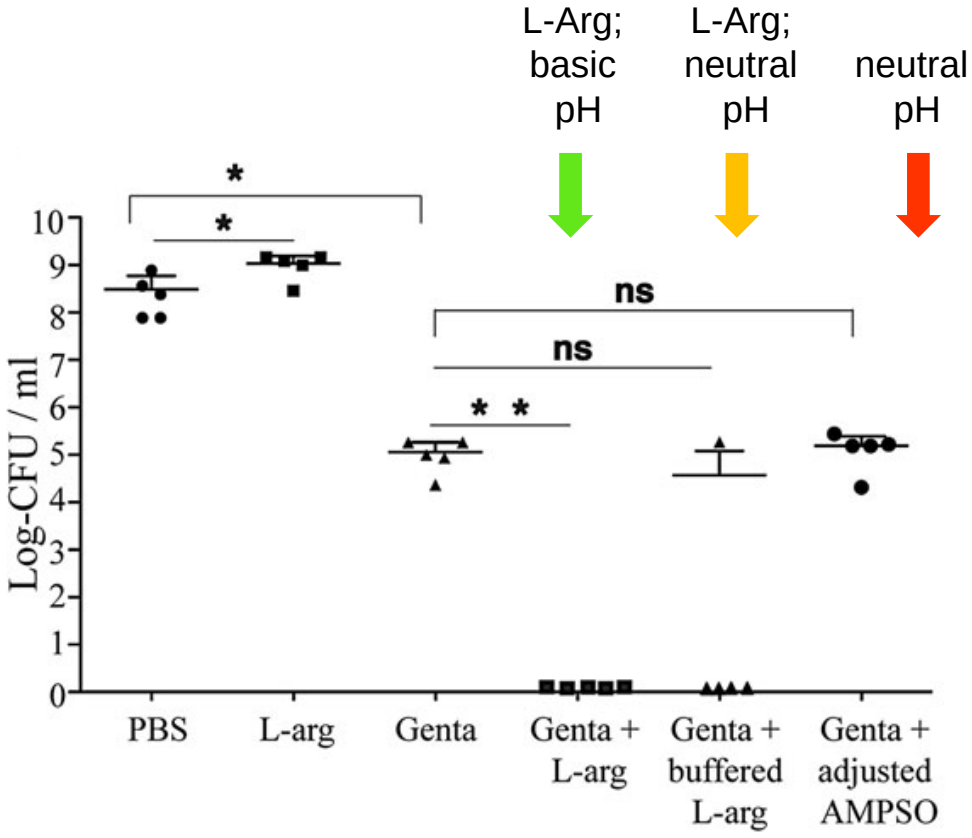
L-Arginine improves Genta activity by increasing pH

Lebeaux et al, J. Infect. Dis. 2014; 210; 1357–66.

Modulation of gentamicin activity: L-Arg

In vivo synergism

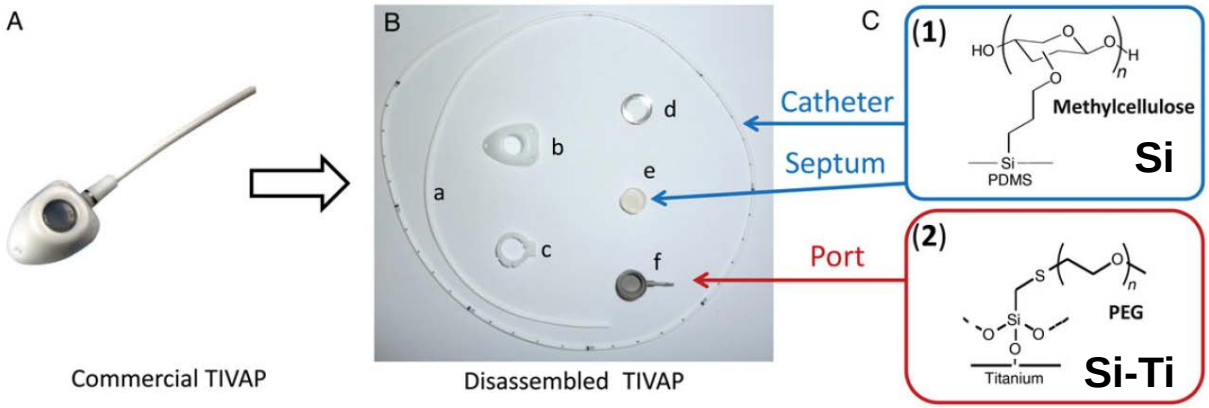


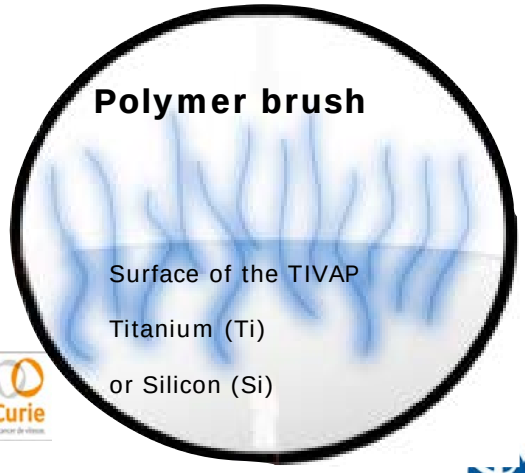


In vivo, some of the L-Arg effects are pH independent

Lebeaux et al, J. Antimicrob. Chemother. 2015; 70:1704-1712

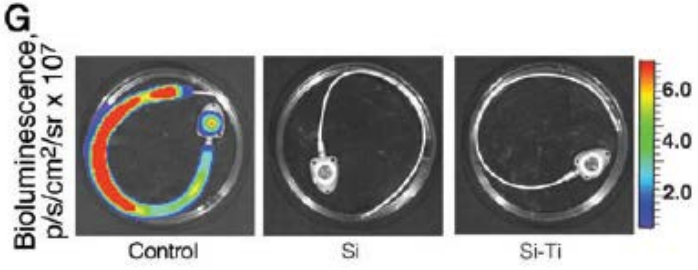
Grafting non-biocidal anti-adhesion molec. on catheters

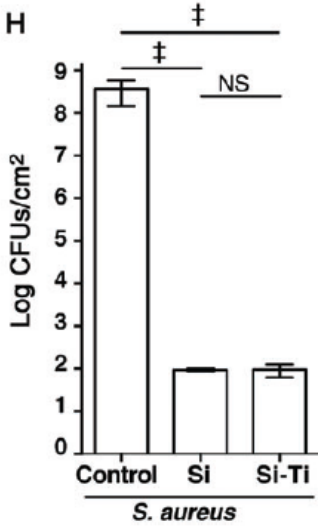




institutCurie

Institut Pasteur



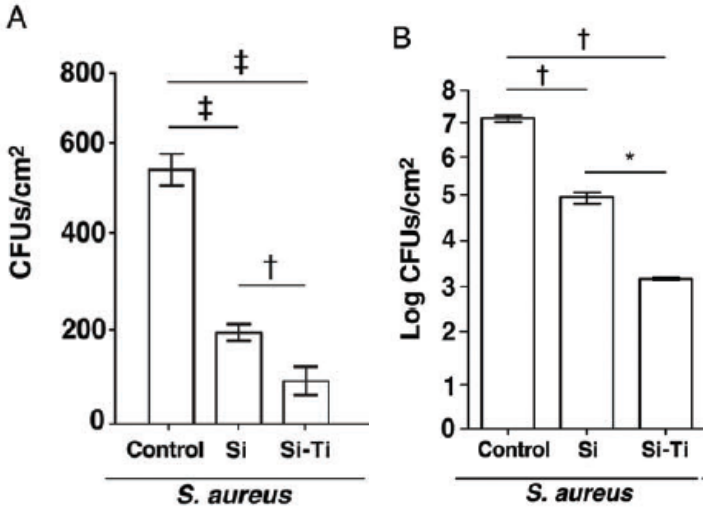


Coating prevents bacterial adhesion

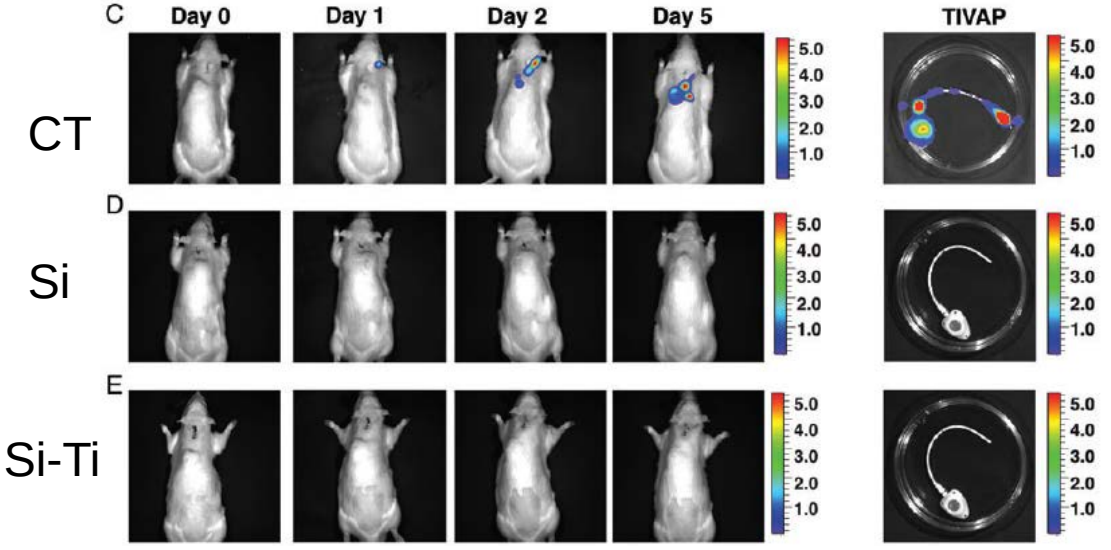
Chauhan et al. J. Infect. Dis. 2014; 210:1347–1356.

Grafting non-biocidal anti-adhesion molec. on catheters

adherence colonization at day 5



Coating prevents bacterial adhesion and further colonization

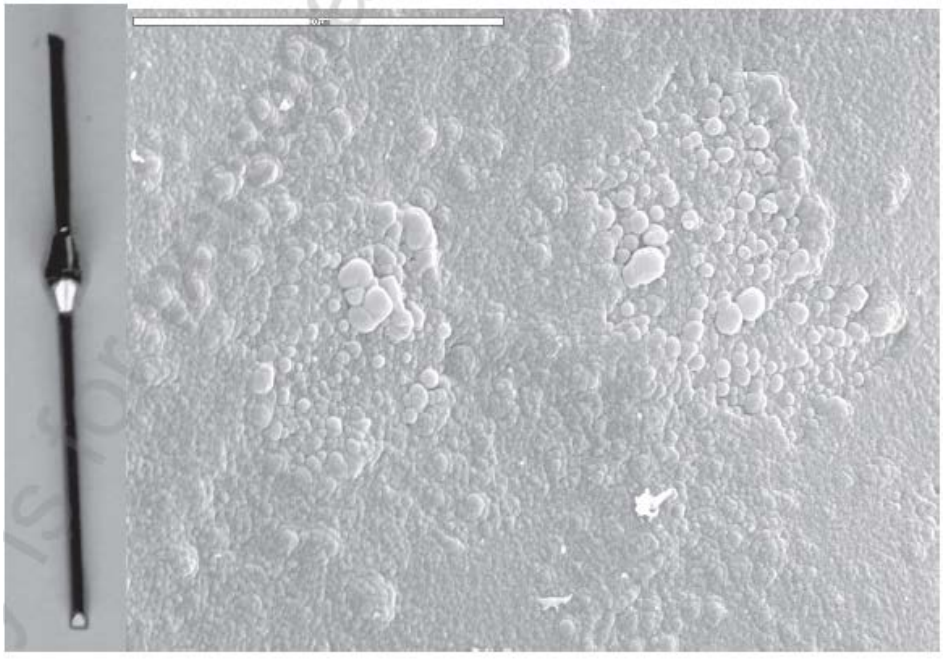


Chauhan et al. Journal of Infectious Diseases 2014; 210:1347–1356.

New clinical applications

3. Orthopedic infections

biofilm observed in electron microscopy
on a steel component of an Ilizarov device
obtained from a patient with clinical infection (*S. aureus*)



Bartoszewicz et al; Orttopediia Traumatologia Rehabilitacja 2007; 9:310-8

Evidence for biofilm in orthopedic infections

Increased risk of treatment failure for biofilmogenic *S. epidermidis* in Device-Related Osteomyelitis of the Lower Extremity in Human Patients

Descriptive and Univariable Analysis of Prognostic Factors for "cure" in the Lower Extremity Cohort

	Cured		Univariable Regression Model for "cure"		
	No	Yes			
Prognostic Factor	n (%)	n (%)	Odds Ratio for "cure"	95%-Confidence Interval	*p*-Value
Total	19 (25.7)	55 (74.3)			
Biofilm formation[a]			0.53	(0.26; 1.07)	0.076
Non	4 (16.0)	21 (84.0)			
Weak	7 (24.1)	22 (75.9)			
Marked[b]	8 (40.0)	12 (60.0)			

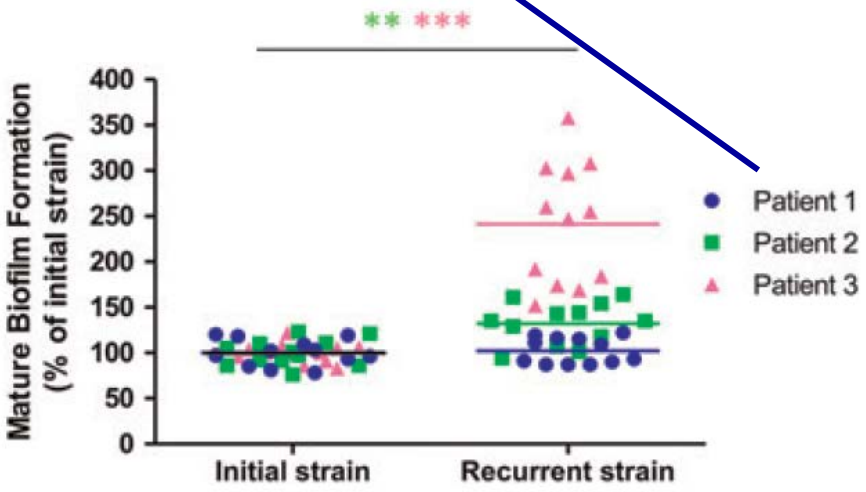
Morgensten et al, J Orthop Res. 2016 doi: 10.1002/jor.23218.

Evidence for biofilm in orthopedic infections

Persistent isolates of *S. aureus* are higher biofilms producers ….

Table 1. Characteristics of the patients and isolates.

Patient no.	Sex, age (year)	Site of infection	Duration of symptoms (days)[a]	Surgical treatment	Duration of antibiotherapy (days)	Time to failure or relapse (days)[b]
1	M, 26	Tibia osteosynthesis material	12	Material removed	82	0
2	M, 80	Total knee arthroplasty	3	Irrigation and debridement	191	10
3	F, 82	Total hip arthroplasty	3	Irrigation and debridement	98	36



Trouillet-Assant et al, Cellular Microbiology 2016; 18:1405–14

Biomaterials for antibiotic delivery

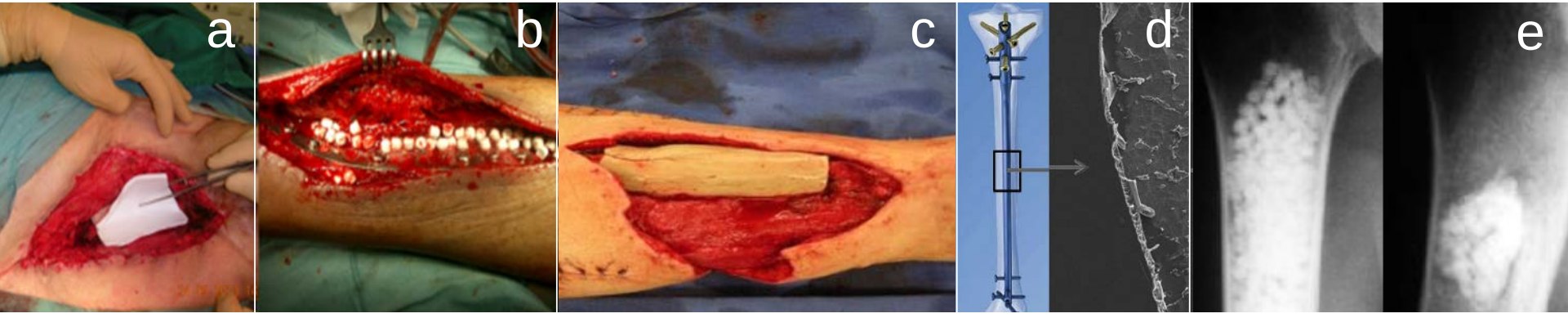
Available prophylactic biomaterials vehicles:

a. Collagen (hypersensitivity, poor handling)

b-c: PMMA [methylmetacrylate] beads or spacers (non degradable)

d. PDLLA [poly-D,L-lactide] (acidic degradation products)

e. Calcium sulfates (osteoconductive)

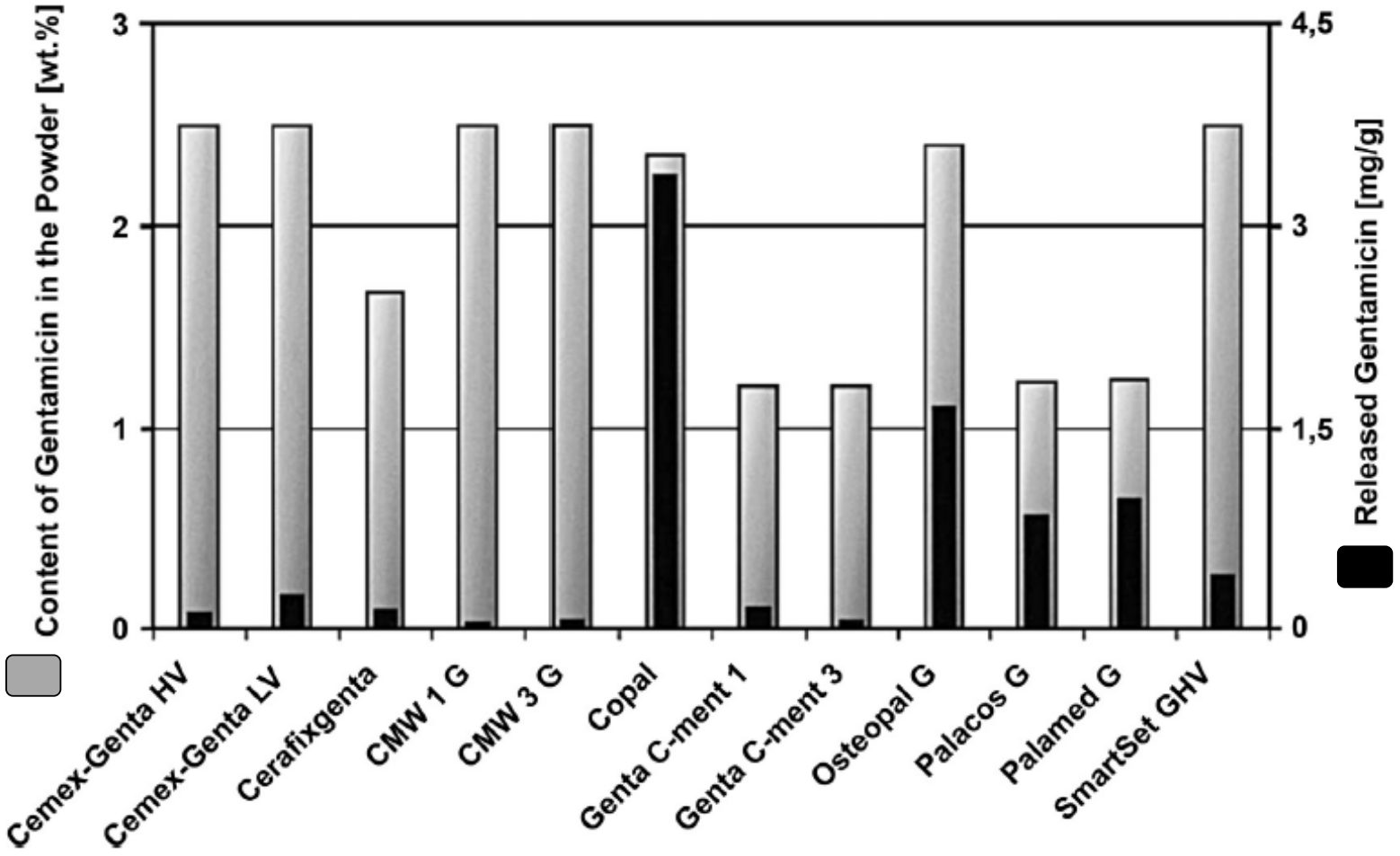


Images: Dr. Mario Morgenstern BGU Murnau, Germany; Dr Menendez, Jobe Orthopaedic Clinic, Los Angeles, CA

ter Boo et al, Biomaterials 2015, 52:113-25.

Biomaterials for antibiotic delivery

Variable antibiotic release from commercial cements



ter Boo et al, Biomaterials 2015, 52:113-25.

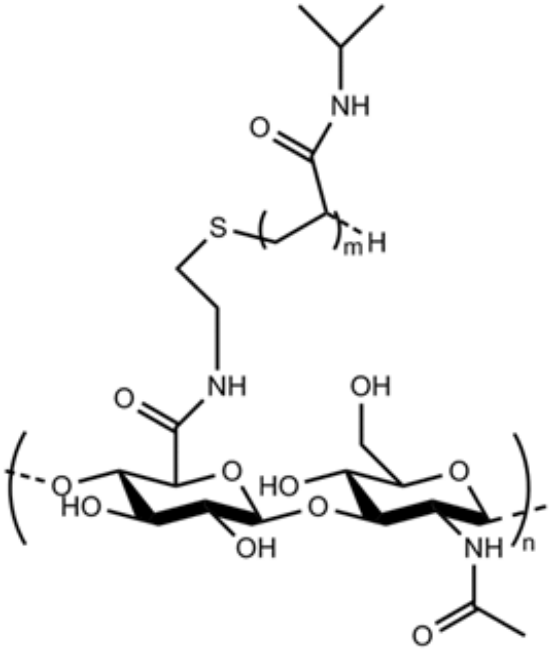
Antibiotic-loaded bone cements: clinical experience

Study by Arthroplasty Site	Study Period	Patients, No./ Joints, No.	Spacer Antibiotic Content (Dose, g/40 g Cement)	Infection Eradication Rate[a]		Deaths[b]
				By Review	As Reported by Authors	
Knee						
[43]	Not reported	12/12	Tobramycin (4.8) + vancomycin (4)	12/12 (100)	12/12 (100)	0
[17][c]	1995–2002	29/31	Tobramycin (4.6) + vancomycin (4)	25/31 (81)	29/31 (93)	0
[15][d]	1998–2005	102/102	Tobramycin (3.6) + vancomycin (4)	47/102 (46)	70/96 (73)	0
[20]	1986–1994	48/48	Tobramycin (3.6) + vancomycin (2)	43/48 (90)	44/48 (92)	0
[13][d]	1997–1999	58/58	Tobramycin (3.6) + vancomycin (1.5)	48/58 (83)	45/47 (96)	NA[e]
[44]	1998–2001	24/24	Tobramycin (2.4) + vancomycin (1)	22/24 (92)	22/24 (92)	0
[45]	1996–2001	28/28	Tobramycin (1.2) or gentamicin (1) + vancomycin (1)	25/28 (89)	25/28 (89)	0
[46]	2000–2005	36/36	Piperacillin-tazobactam (4.5) + vancomycin (2) + erythromycin (1)	32/36 (89)	32/36 (89)	0
[18]	1989–2001	50/50	Tobramycin (4.8)	44/50 (88)	44/50 (88)	NA
[22]	1994–2002	44/44	Tobramycin (4.8)	43/44 (98)	43/44 (98)	0
[14][d]	1986–1999	40/40	Tobramycin (1.2)	36/40 (90)	36/40 (90)	0
[19]	1989–1993	69/69	Tobramycin (1)	60/69 (87)	61/69 (88)	0
[47][f]	1998–2003	48/48	Vancomycin (1)	30/48 (63)	42/48 (88)	0
Hip						
[17][c]	1995–2002	16/23	Tobramycin (4.6) + vancomycin (4)	18/23 (78)	22/23 (96)	0
[48]	Not reported	12/12	Tobramycin (3.6) + vancomycin (1)	12/12 (100)	12/12 (100)	0
[12][d]	1998–2001	22/22	Tobramycin (2.4) + vancomycin (1)	20/22 (90)	20/20 (100)	2 (9)
[10][d]	1993–1997	24/24	Gentamicin (1) + vancomycin (1) + cefotaxime (1)	21/24 (88)	21/22 (95)	2 (8)
[16][d,f]	1998–2003	43/44	Gentamicin (0.25) + vancomycin (2)	35/44 (80)	38/41 (93)	3 (7)
[11][d]	1991–2001	42/42	Tobramycin (4.8)	26/42 (62)	26/27 (96)	8 (19)
[9][g]	1996–2003	38/38	Vancomycin (1)	32/38 (84)	34/38 (89)	2 (5)
[23][h]	2001–2006	40/40	Gentamicin (0.76)	38/40 (95)	39/40 (97.5)	0

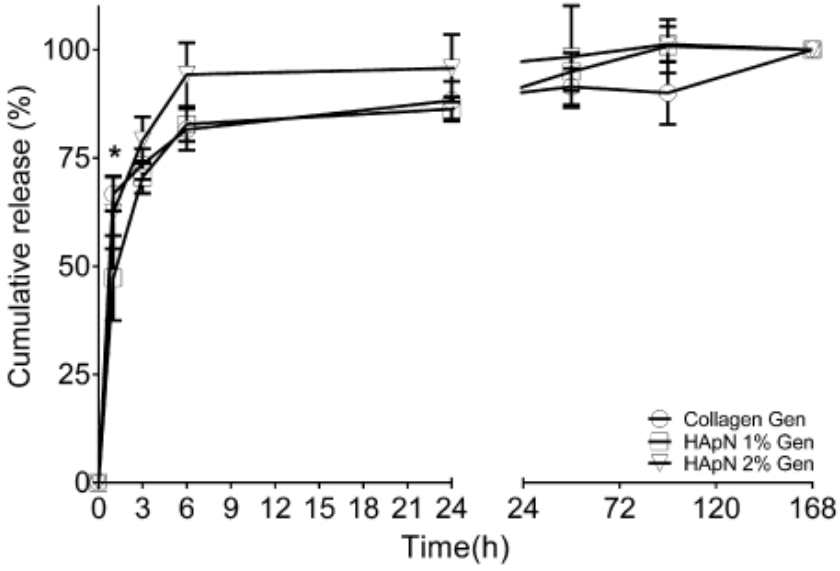
Iarikov et al., Clin Infect Dis 2012; 55:1474-80

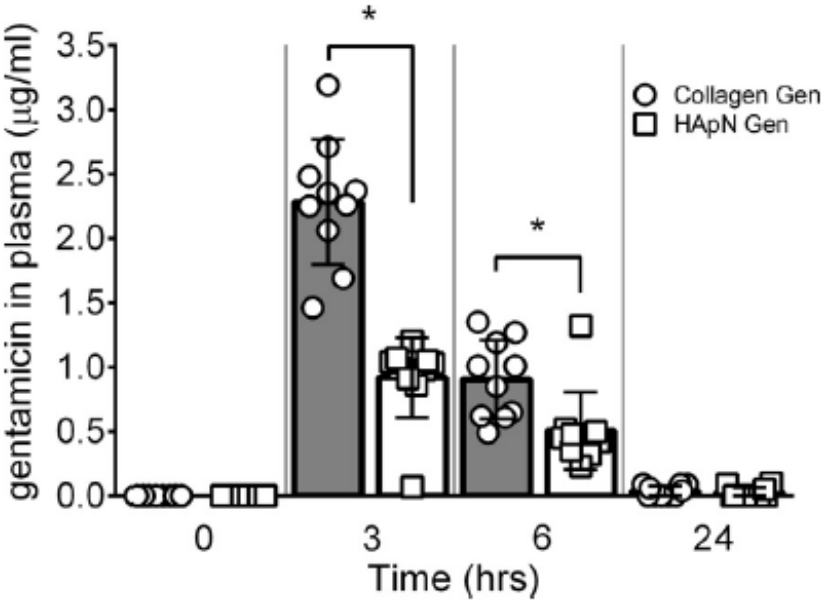
New developments: an example

Thermoresponsive Hyaluronan hydrogel



Rapid release of gentamicin from the gel; low serum levels





Ter Boo et al., Acta Biomaterialia 2016; 43:185–194

New developments: an example

Thermoresponsive Hyaluronan hydrogel

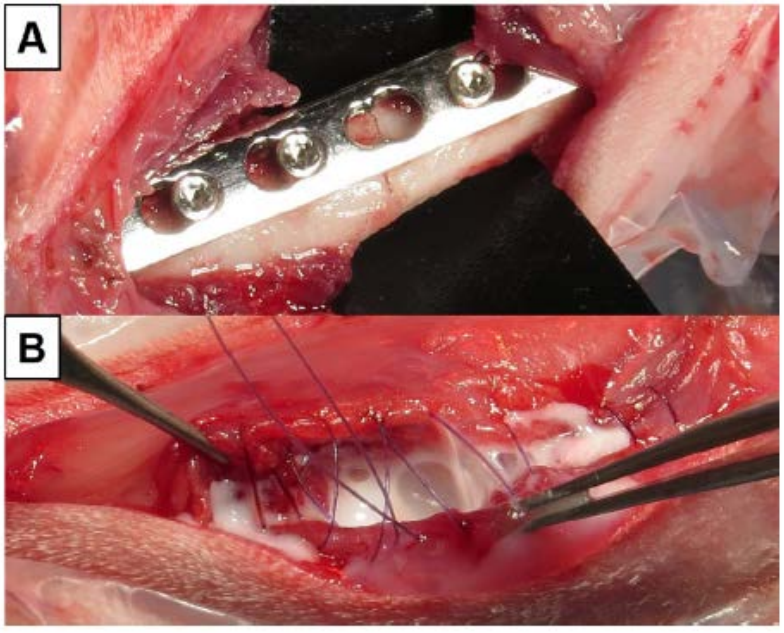
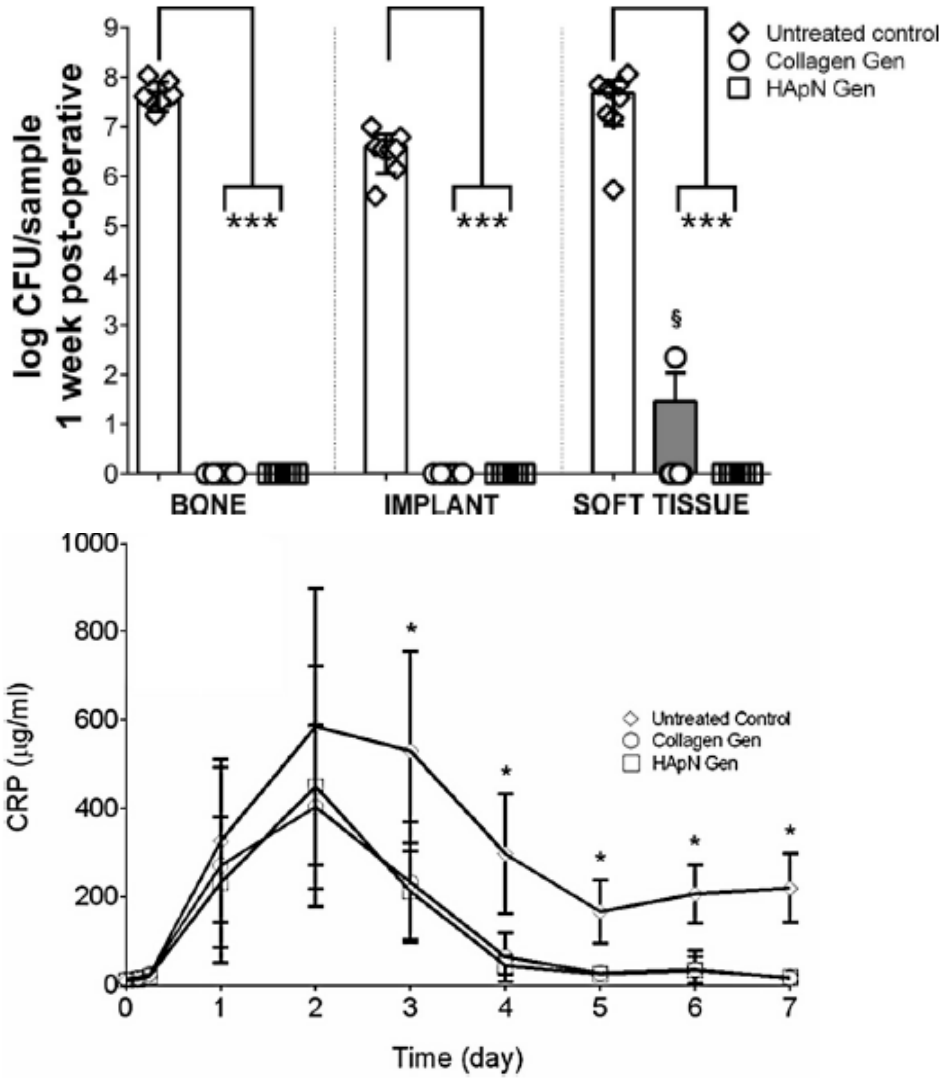


Fig. 1. Intra-operative image before (A) and after (B) application of the gentamicin-loaded HApN hydrogel within the surgical field. The HApN hydrogel (white color) fills the surgical field and turns from a sol to a gel state upon contact with the tissue.

Genta-loaded hydrogel reduces infection



Ter Boo et al., Acta Biomaterialia 2016; 43:185–194

Take home messages

- Antibiotic activity poor against biofilms due to PK/PD issues
- Combinations with adjuvants effective in animal models
- Prevention easier than cure …

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Sona Kucharíková


Patrick Van Dijck

Disclosures

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