



Det Sundhedsvidenskabelige Fakultet



Biofilms and antibiotic resistance

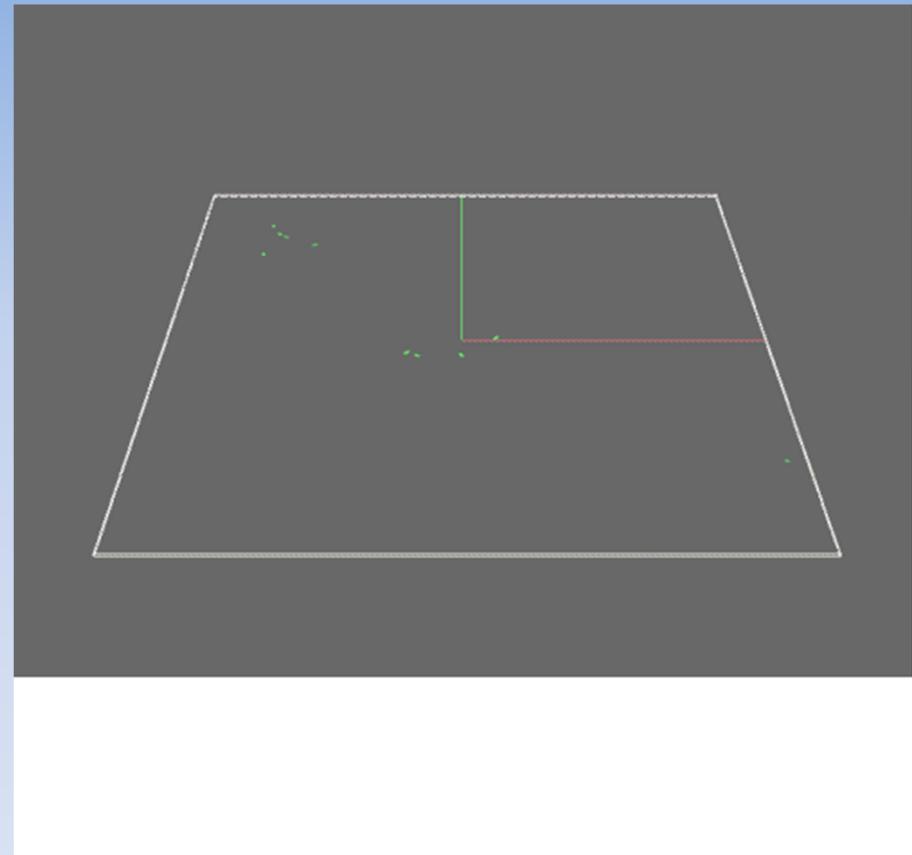
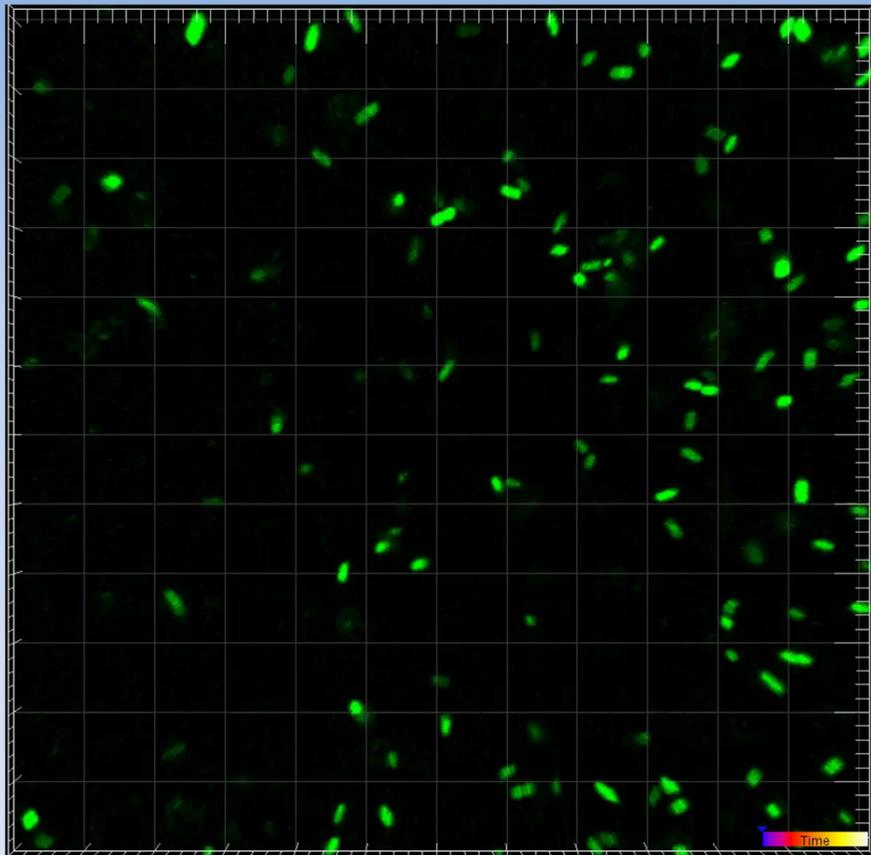
Thomas Bjarnsholt
Professor, DMSc & PhD



Rigshospitalet



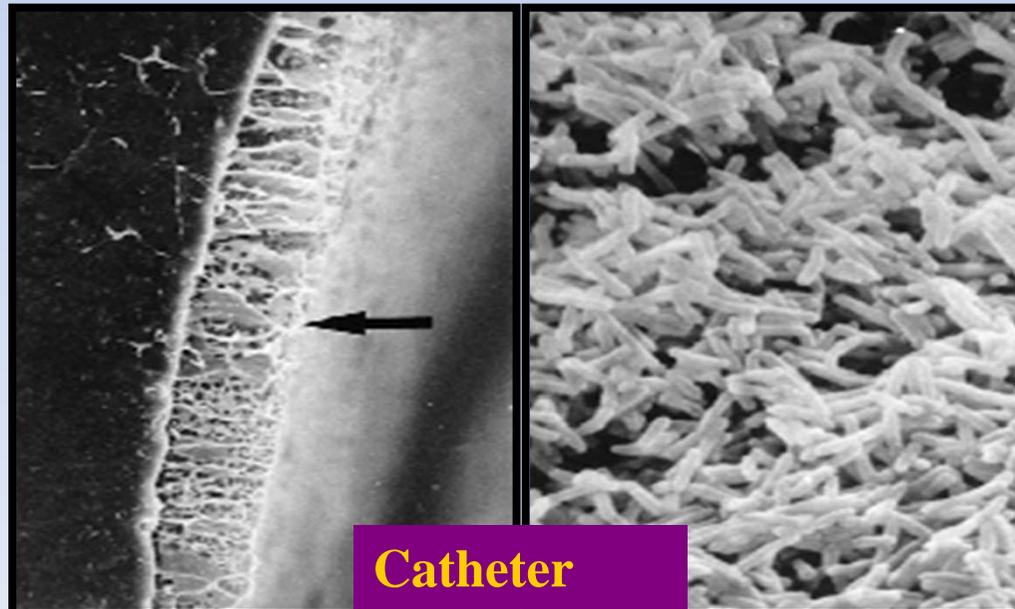
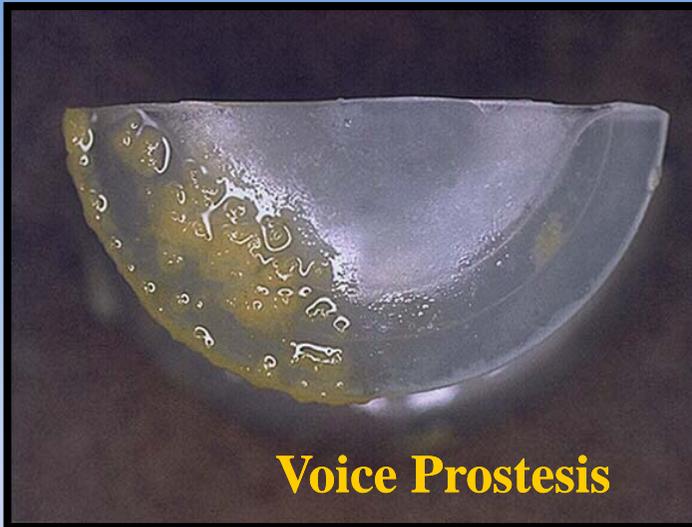
Planktonic vs. Biofilm



Everywhere a liquid flows across a surface



In the human body



Planktonic vs. biofilm

- Study from 1956.
- Injected 7,500,000 CFU *S. aureus* in skin of human volunteers = only 50% infected, all resolved
- < 100 CFU onto an implant in humans = 100% infected, did not resolve
- Implants or dead tissue ↑ virulence over 75,000 fold.

EXPERIMENTAL STAPHYLOCOCCAL INFECTIONS IN THE SKIN OF MAN

By Stephen D. Elek

St. George's Hospital Medical School, University of London, London, England

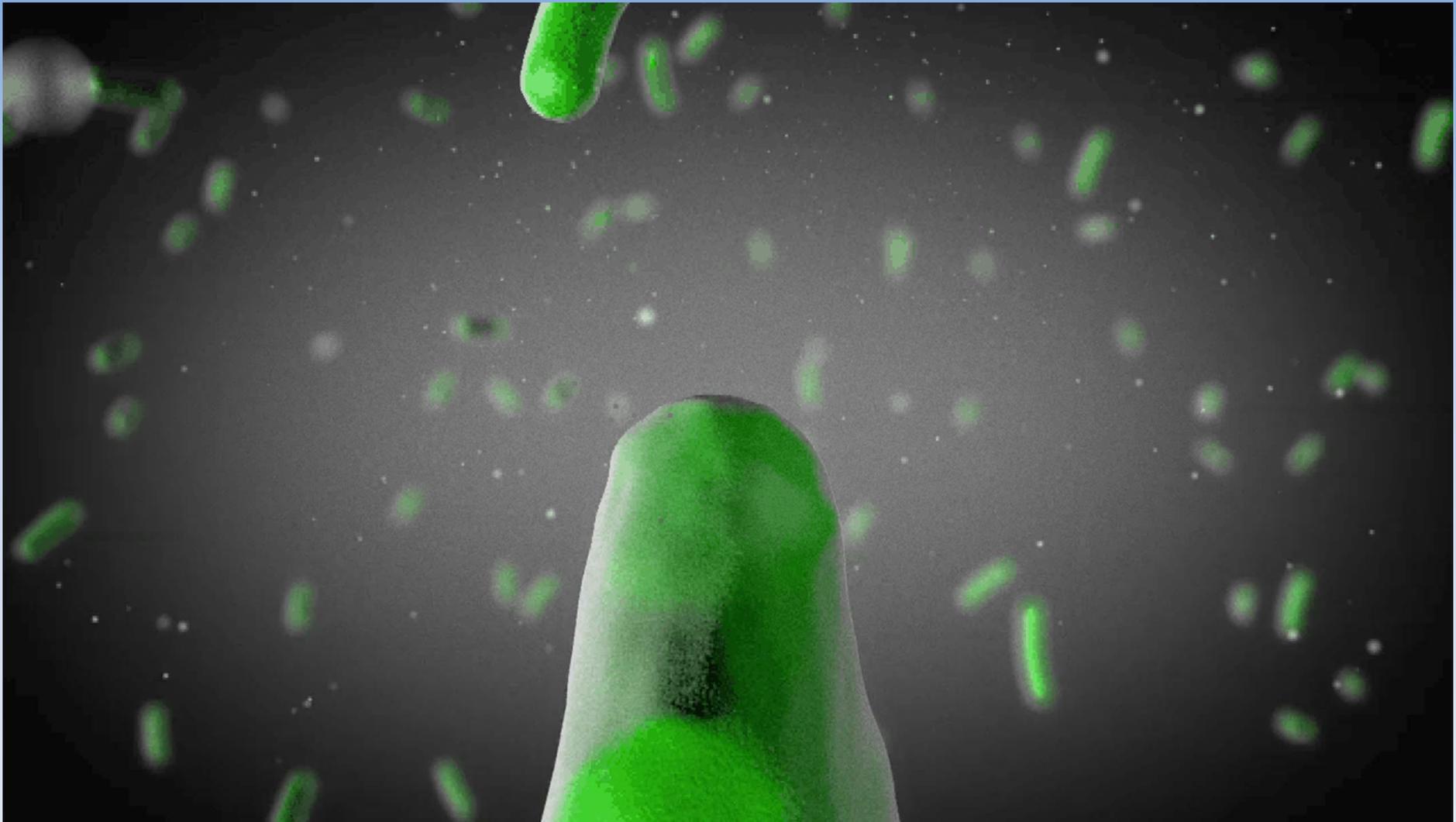
The concept of virulence of a microbe is relative to a given host species. The fact that an organism is capable of setting up lesions in 1 experimental animal cannot be used as a basis for comparing the virulence of microbial strains in relation to another species of host. The traditional approach of using any convenient laboratory animal in virulence studies may yield completely fallacious findings if different metabolic products in other species of animals contribute to varying degrees of virulence in those species. There is evidence that the virulence of coagulase positive staphylococci does vary when tested on rabbits, mice, or other animals, but there is no information concerning the existence of similar differences in man (Frappier *et al.*, 1955).

The carrier rate of coagulase-positive staphylococci and their distribution in dust, clothing, and elsewhere is very high, but clinical staphylococcal infection in man is comparatively rare. This suggests that either a minority of nasal strains are endowed with special virulence or that the circumstances of the infection determine the occurrence of disease. Since virulence testing on animals could not be expected to provide the correct answer, it was decided to compare a number of nasal strains with staphylococci isolated from human lesions by injecting volunteers intradermally. All the strains tested were coagulase-positive but, as the other biological properties of such material have frequently been described, no further details were regarded as relevant. Virulence testing in man being necessarily restricted in extent, our primary aim was to establish, within the limitations of the approach, whether or not differences in the resulting lesions could be demonstrated between randomly selected nasal strains of *Staphylococcus pyogenes* and other strains obtained from human lesions, and therefore presumed to be virulent. The indicator effect chosen was pus formation. Since staphylococci typically produce purulent lesions, the minimum inoculum surviving and progressing to pus formation can be used as the basis of comparison between the virulence to man of different strains.

Experiments on man with *Staph. pyogenes* have been carried out before (Garé, 1885; Bumm, 1885; and Bockhart, 1887), but only to show that they cause disease. No information was available concerning the minimal pus-forming dose or concerning differences between strains. Since the number of volunteers available was insufficient to test an adequate number of strains with different sizes of inocula it was decided to determine the minimal pus-forming dose for a strain freshly isolated from a severe lesion and then to compare the effects of the same dose of nasal and of some further known pyogenic strains. If the hypothesis that only a minority of nasal strains are virulent is correct, these strains would be expected not to cause pus formation at that dose.

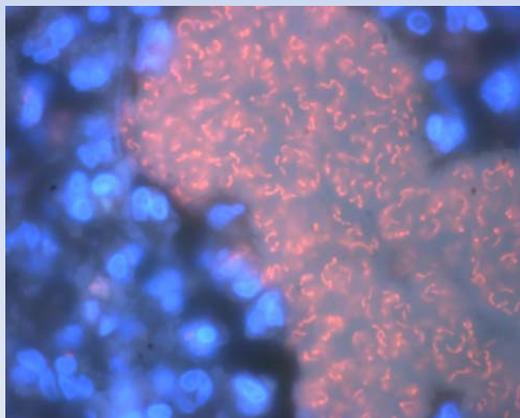
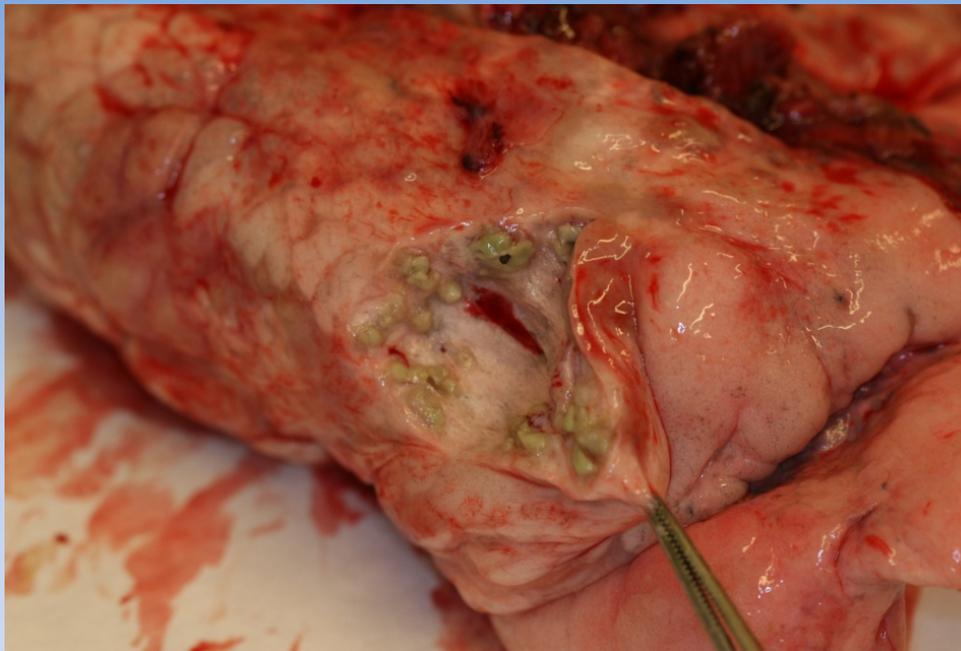
A strain obtained from an abscess of the neck was injected in varying dilu-

The problem



Cystic fibrosis – the classical example

CF male, 28 years of chronic PA infection



2 week anti PA treatments
20 years daily colistin/tobramycin inhalations
1 kg tobramycin,
10 kg beta-lactam anti-pseudomonas antibiotics
and 1 kg inhaled colistin

A present problem:

Adverse reactions to polyacrylamide gel are seen as swellings or nodules, and controversy exists whether these are due to bacterial infection or an autoimmune reaction to the filler.



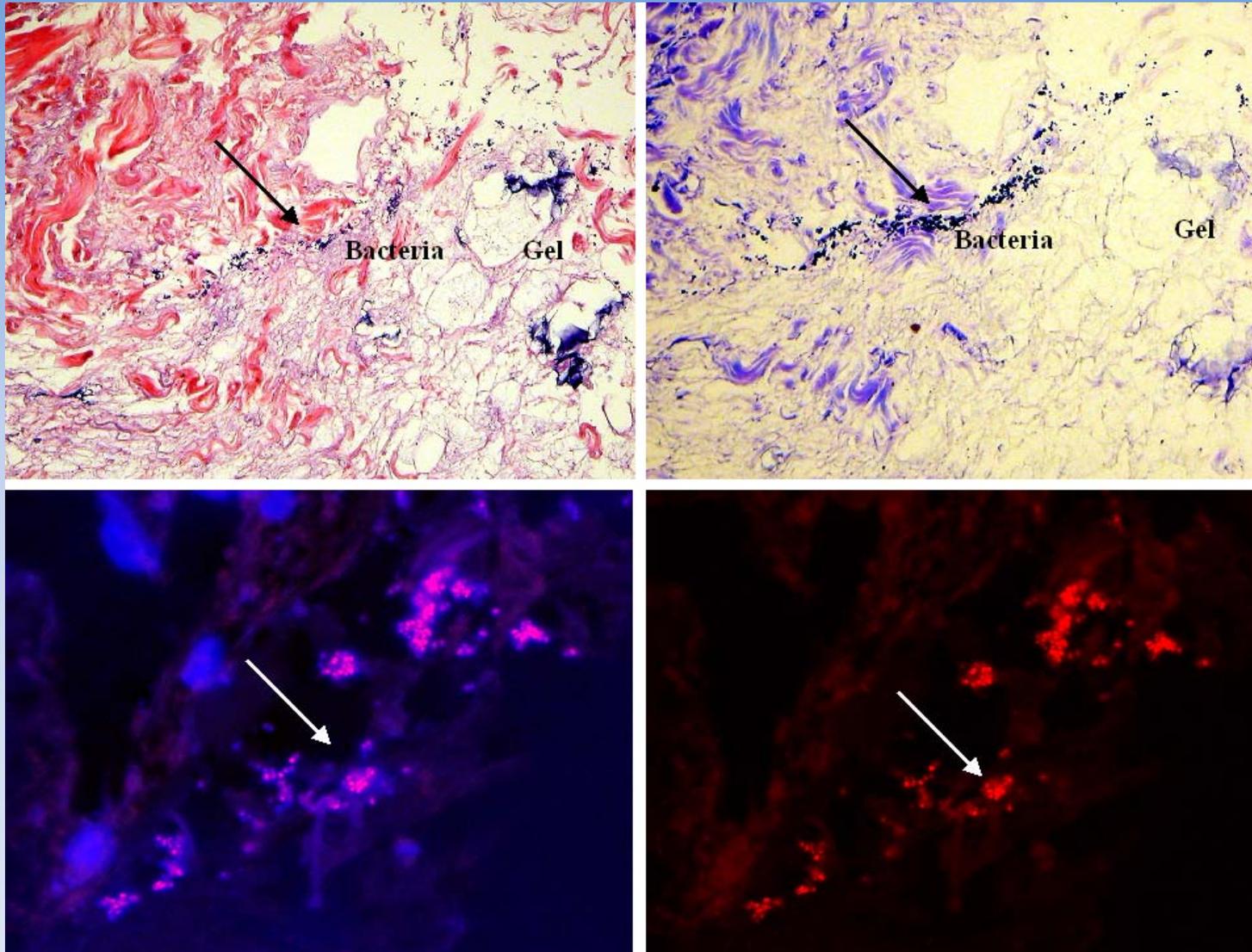
4 days after steroid

Courtesy of Lise Christensen

site	Type of PAAG	Time since inj	Initial treatment	Time with AE
Cheek	Aquamid	2 years	Steroid inj Later AB inj	7 months
Lip	Aquamid	½ year	Steroid inj Later ABs	½ year
Lip	Aquamid	1 month	Steroid + ABs Later ABs	2 years
Breast	Amazing gel	2 years	ABs liposuction	5 months
Tear-trough	Aquamid	2 years	Steroid inj Later ABs	½ year
Naso-labial fold	Aquamid	14 days	Steroid ABs+surgery	1½ year
Lip	Interfall gel	½ year	Steroid ABs+surgery	2½ years
Penis	Aquamid	2 years	Steroid AB inj+surgery	1½ years

Courtesy of Lise Christensen

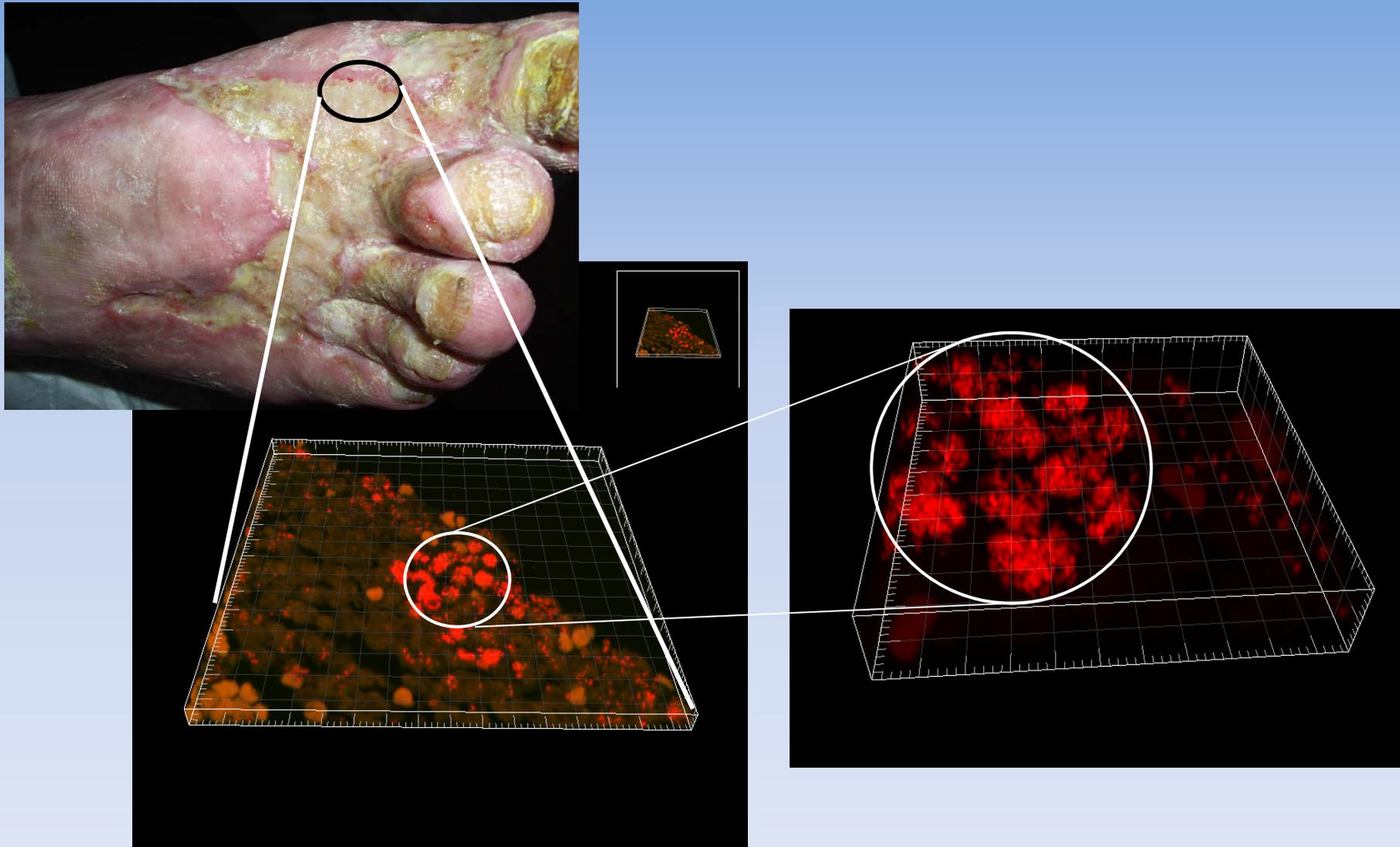
Soft tissue fillers



Bjarnsholt et al;; Dermatol.surg. 2009 (Aug) Followup: Clin Infect Dis. 2013

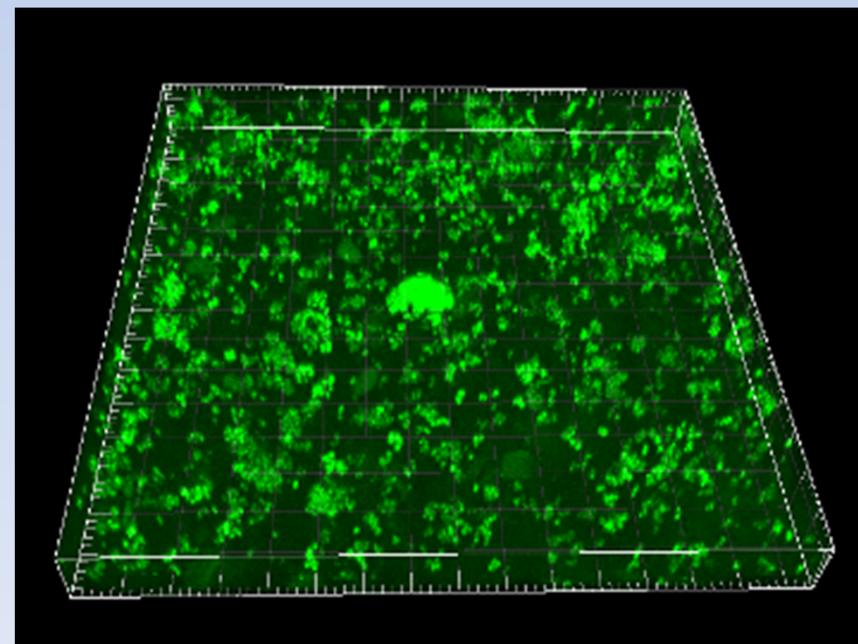
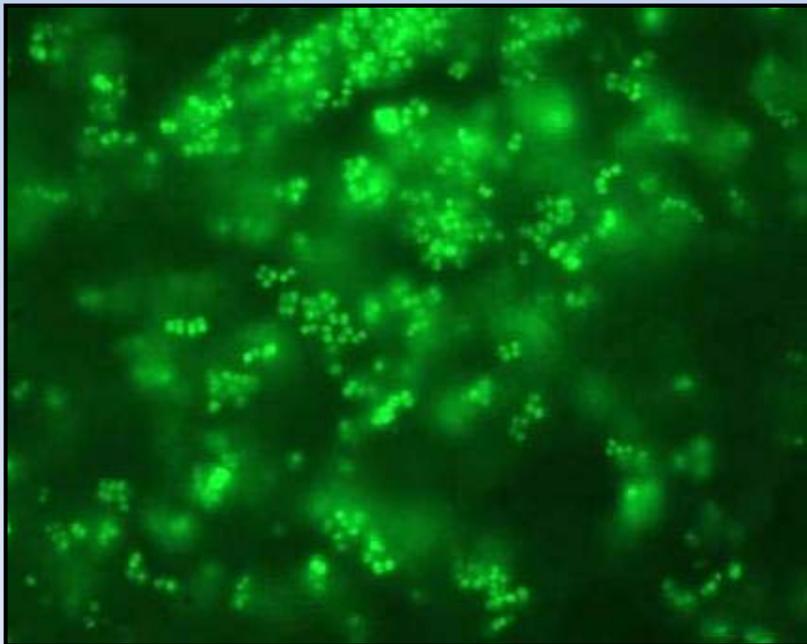
Alhede, M.... Bjarnsholt T; Bacterial biofilm formation and treatment in soft tissue fillers, [Pathog Dis.](#) 2014

Biofilms in chronic wounds



Bjarnsholt et al; Wound Repair and Regeneration, 2008 Jan-Feb;16(1):2-10.

Chronic otitis media



DEVICE-RELATED INFECTIONS

Ventricular derivations

Contact lenses

Endotracheal tubes

Vascular central catheters

Prosthetic cardiac valves,
pacemakers and
vascular grafts

Peripheral vascular catheters

Tissue fillers,
breast implants

Urinary catheters

Orthopedic implants
and prosthetic joints

TISSUE INFECTIONS

Chronic otitis media,
chronic sinusitis

Chronic tonsillitis,
dental plaque,
chronic laryngitis

Endocarditis

Lung infection in
cystic fibrosis

Kidney stones

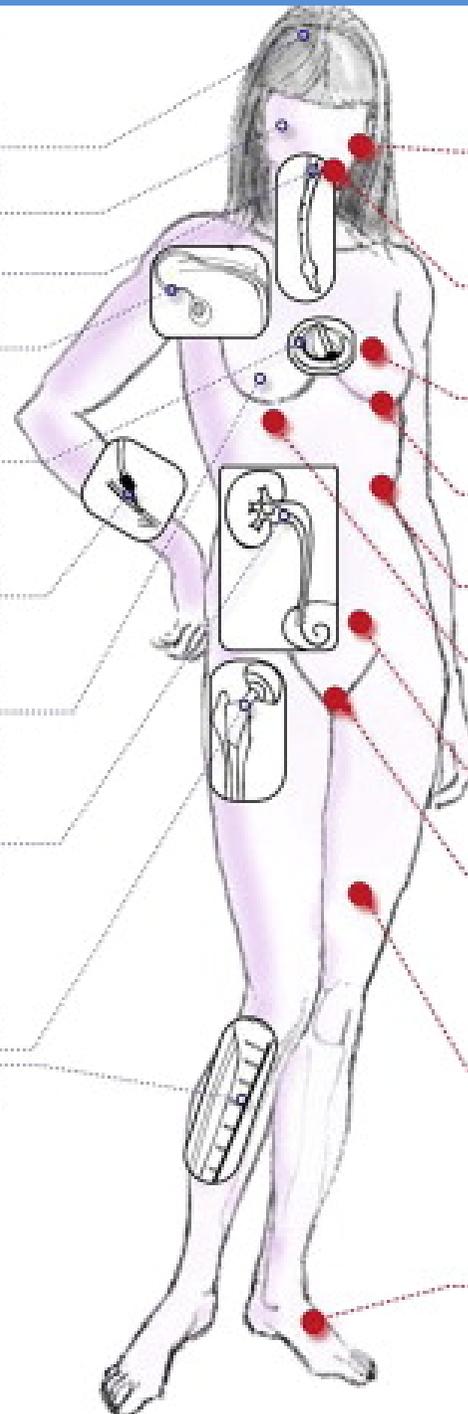
Biliary tract infections

Urinary tract infections

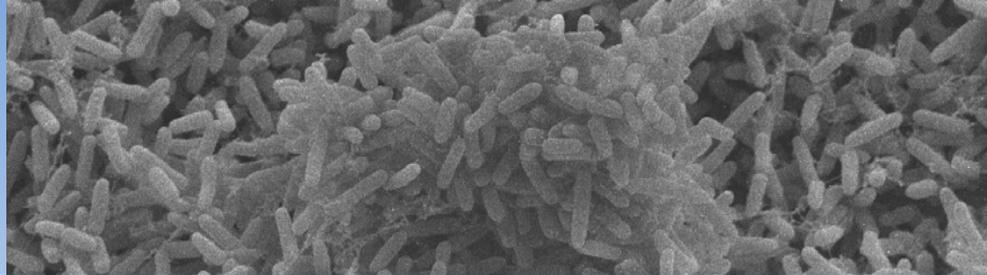
Vaginosis

Osteomyelitis

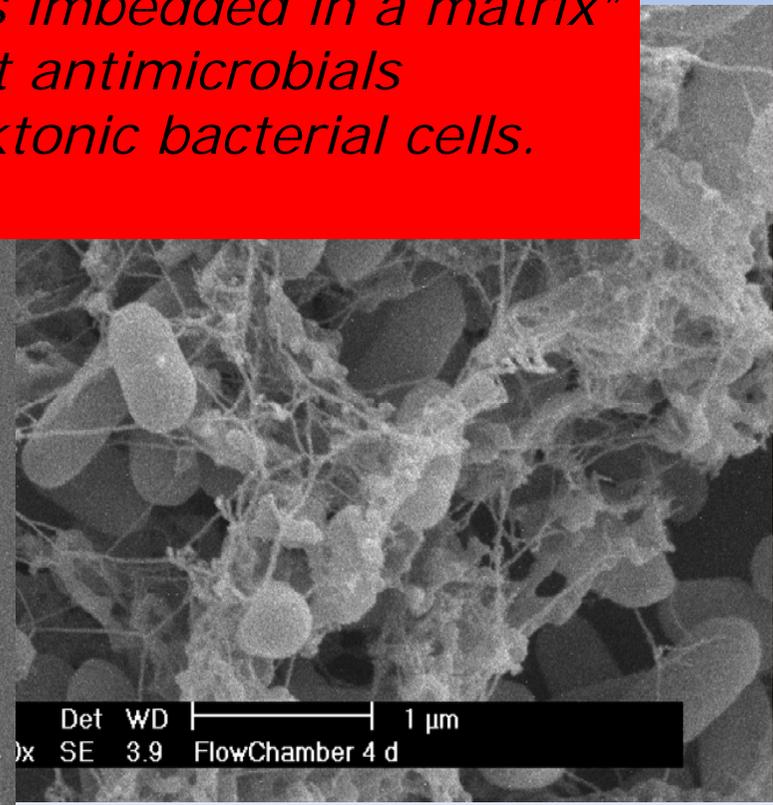
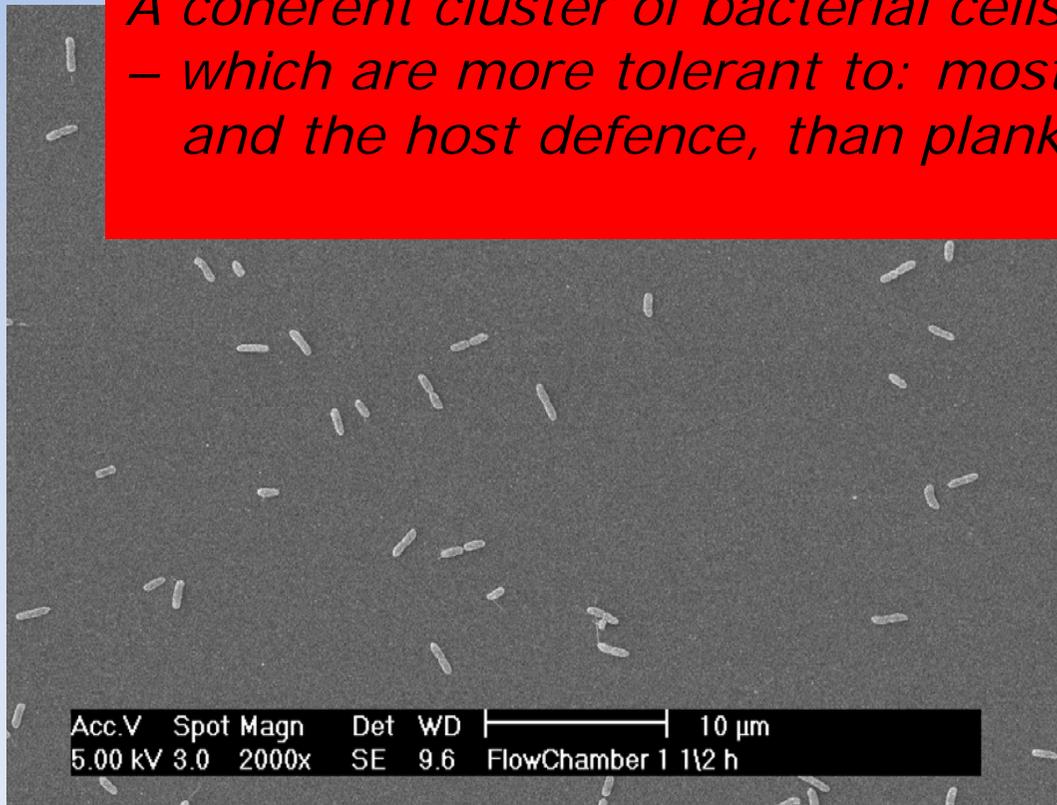
Chronic wounds



What is a biofilm



*A coherent cluster of bacterial cells imbedded in a matrix”
– which are more tolerant to: most antimicrobials
and the host defence, than planktonic bacterial cells.*



Planktonic vs Biofilm

0 h

24 h

72 h

In vitro

Single cells
Rapid growth
No matrix
High susceptibility to AB

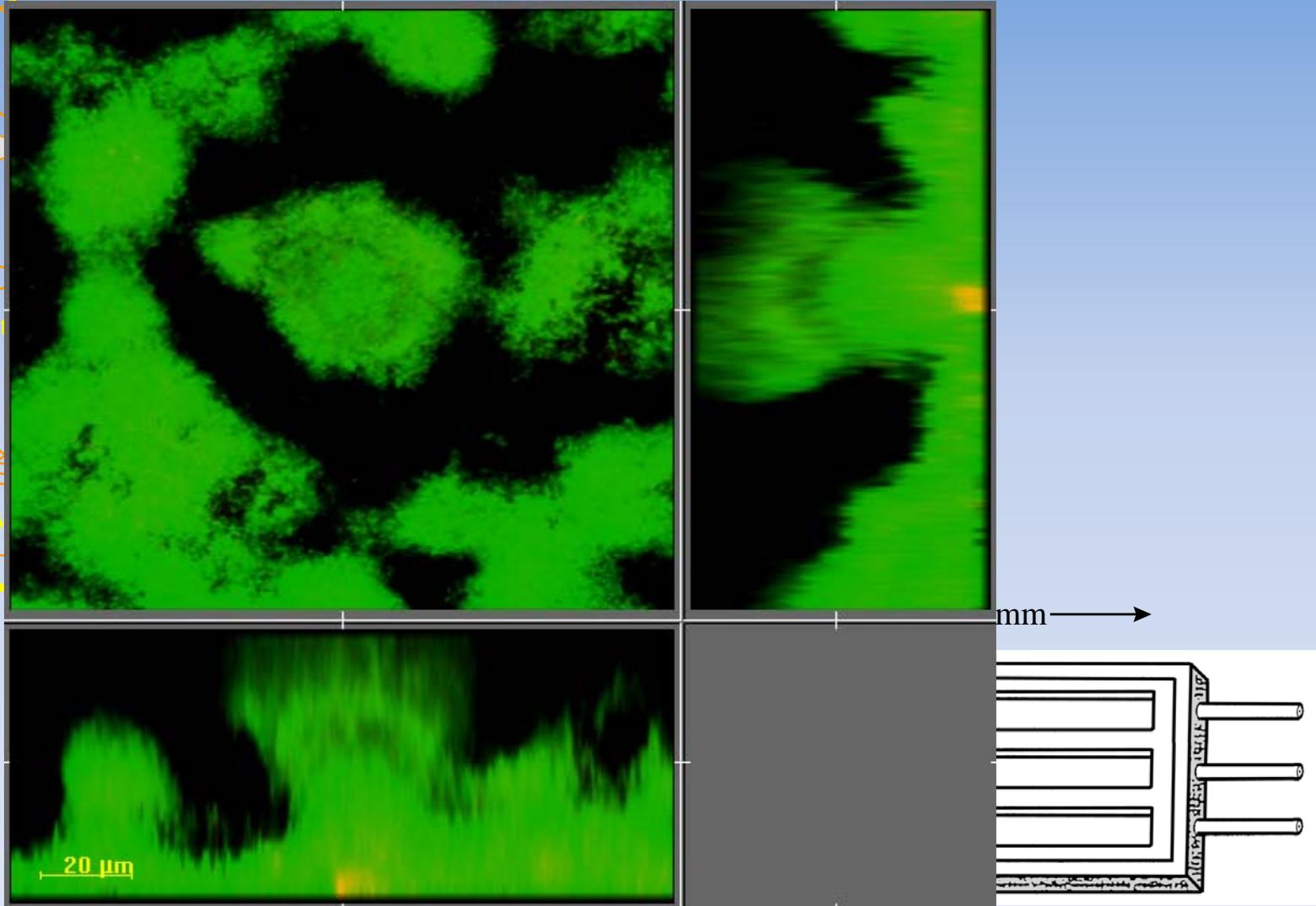
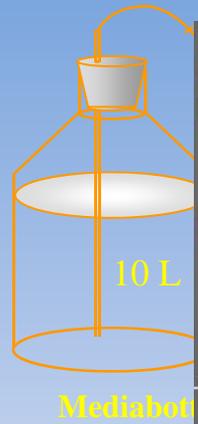
Aggregates
Slow growth
Matrix
Low susceptibility to AB

In vivo

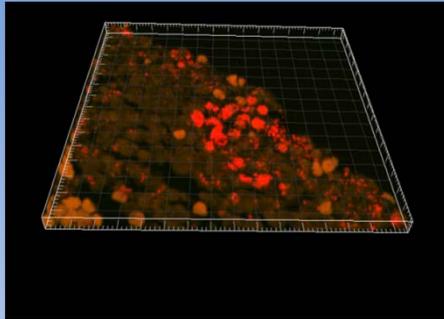
Acute infections
Single cells
Rapid growth
No matrix
High susceptibility to AB
Easily phagocytized

Chronic infections
Slow growth
Dense matrix
Low susceptibility to AB
No phagocytosis

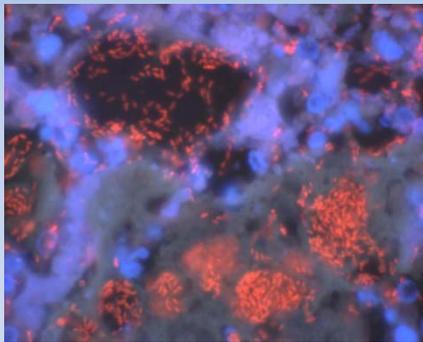
The *in vitro* biofilm



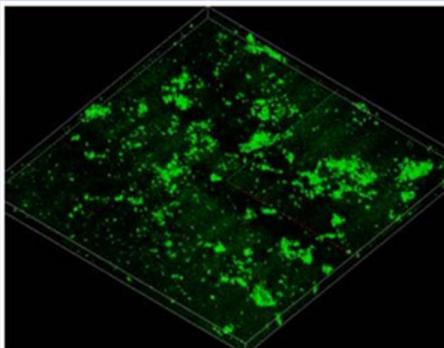
The *in vivo* Biofilm



Wound

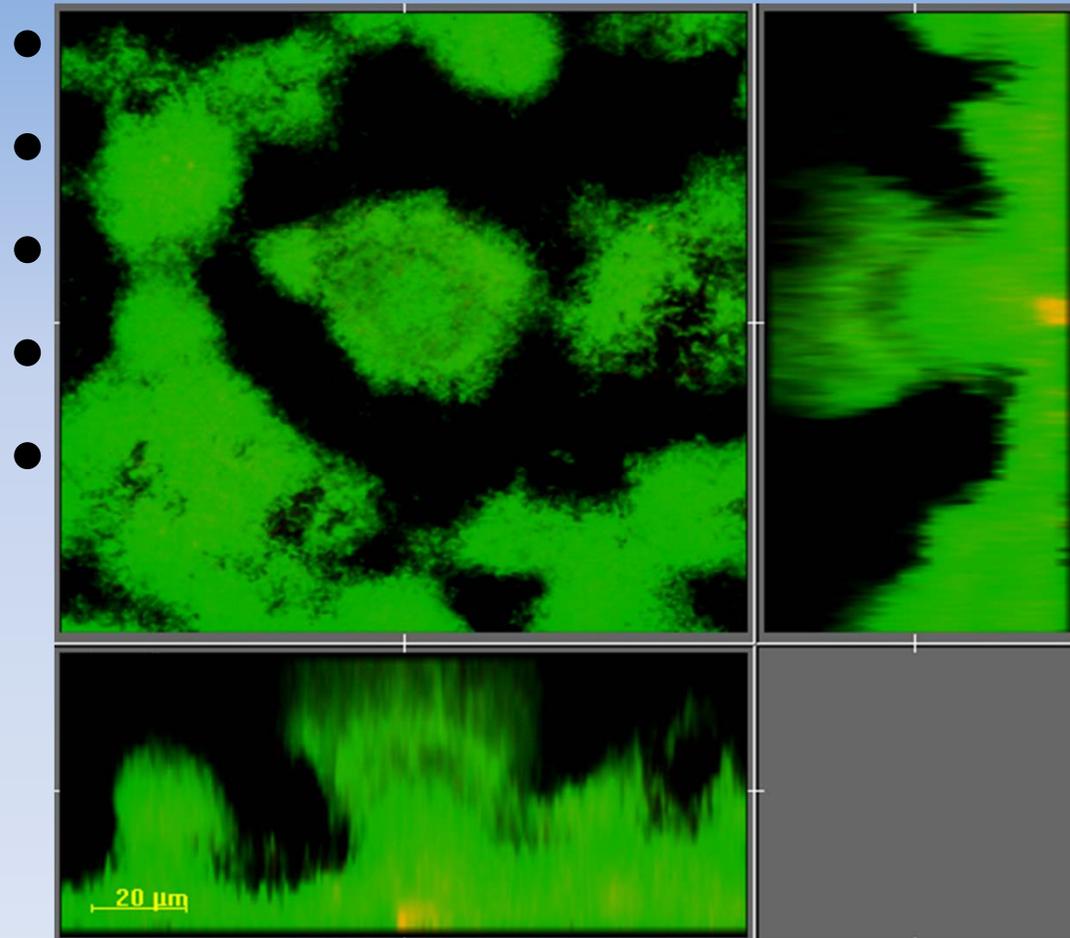


CF lung



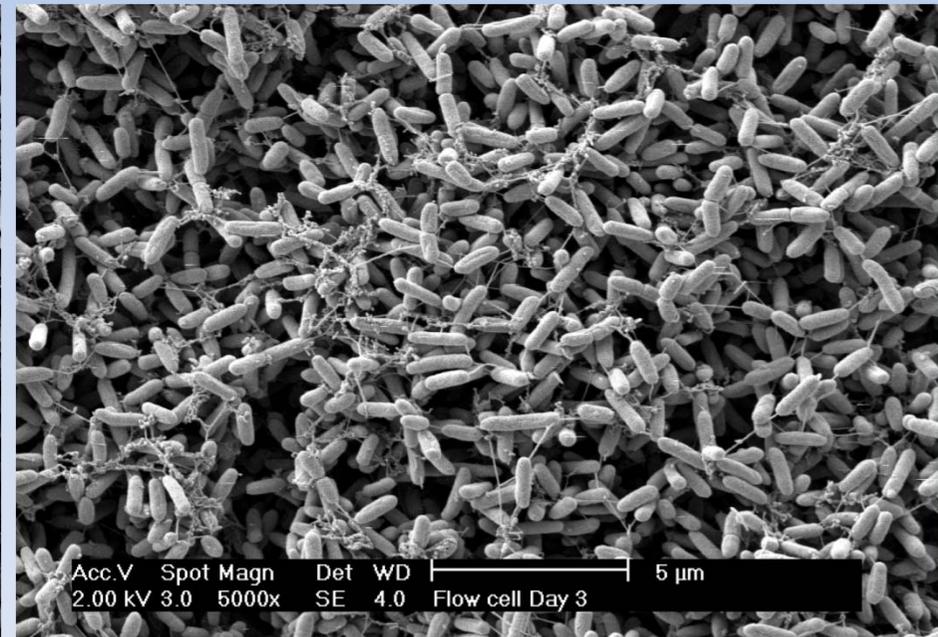
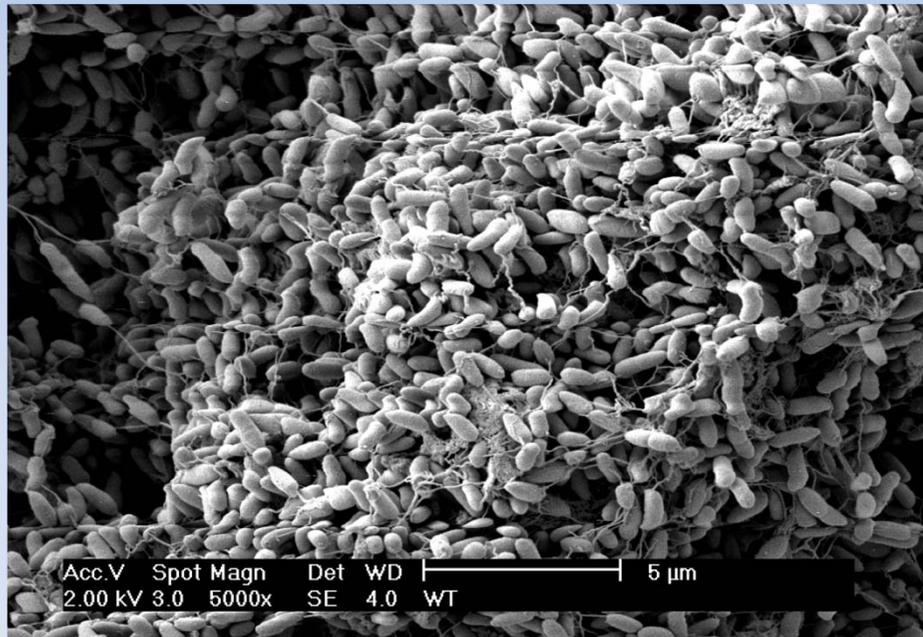
Implant mouse model

- No mushrooms



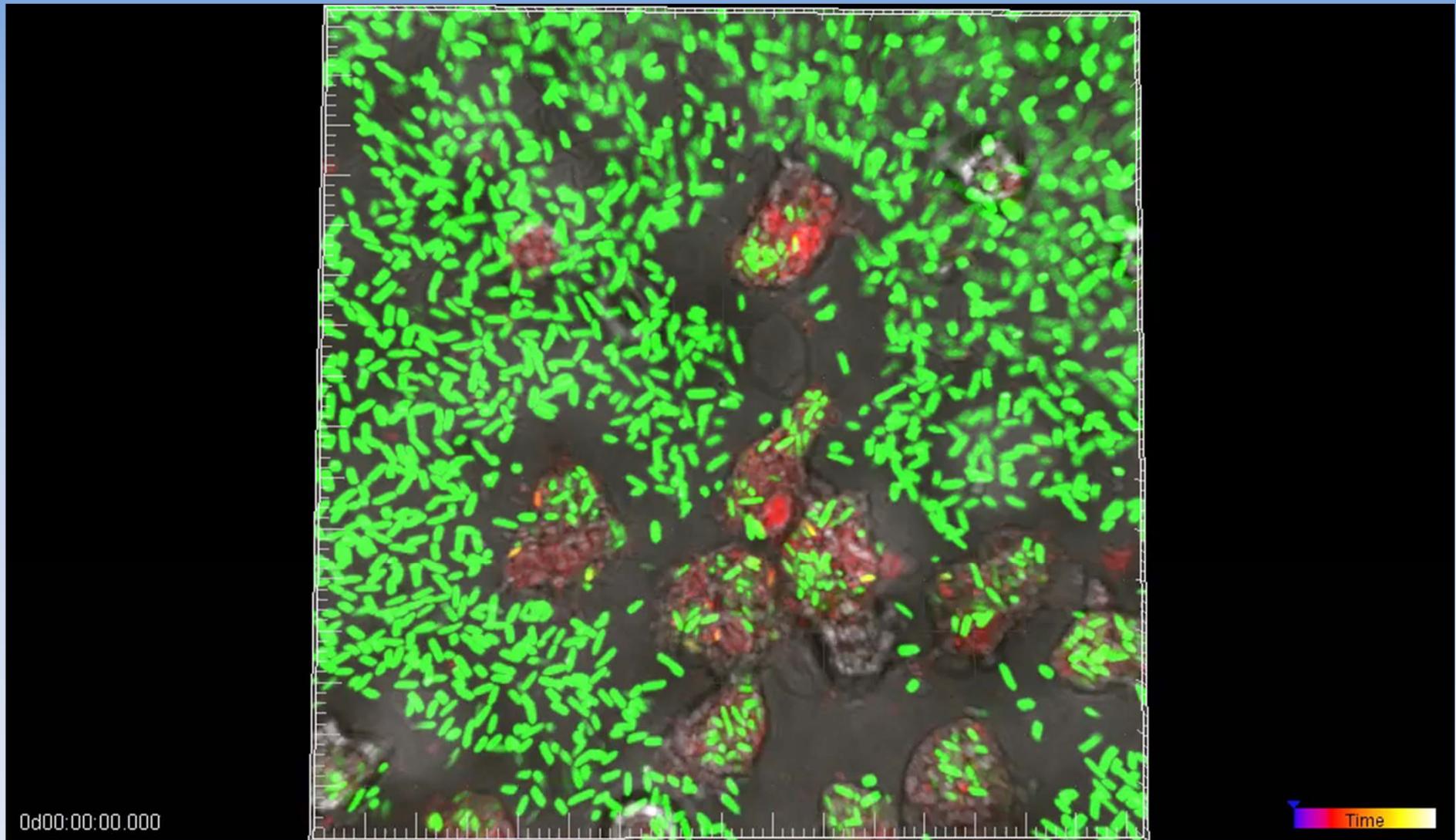
rial

Surface or not to surface that is not the question



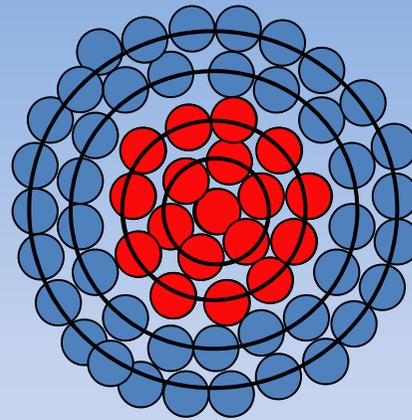
Alhede M et al.; PLoS One. 2011;6(11):e27943

Single layer of attached bacteria

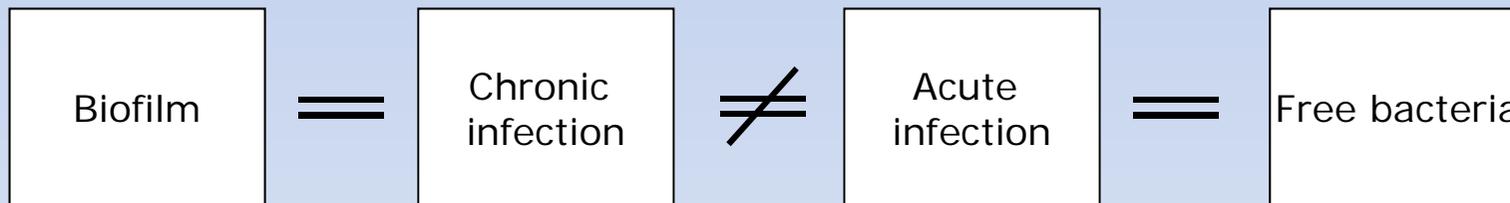


By Maria van Gennip

It is the aggregation



- Growing cells
- Stationary cells (tolerant)

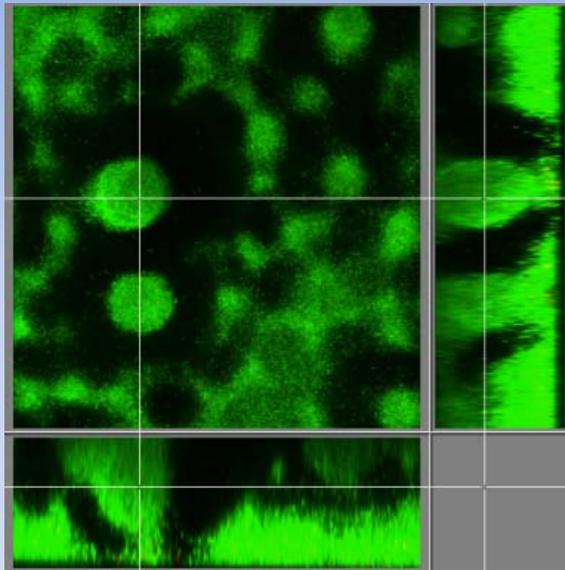


Host defense and antibiotics incapable of clearing

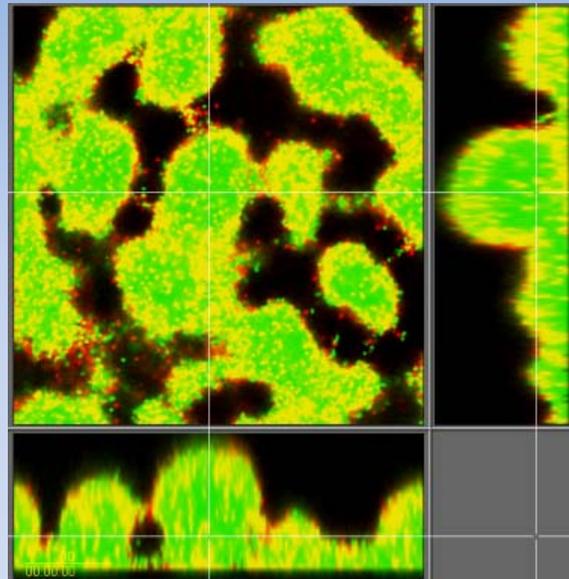
Vs.

Host defense and antibiotics capable of clearing (most often)

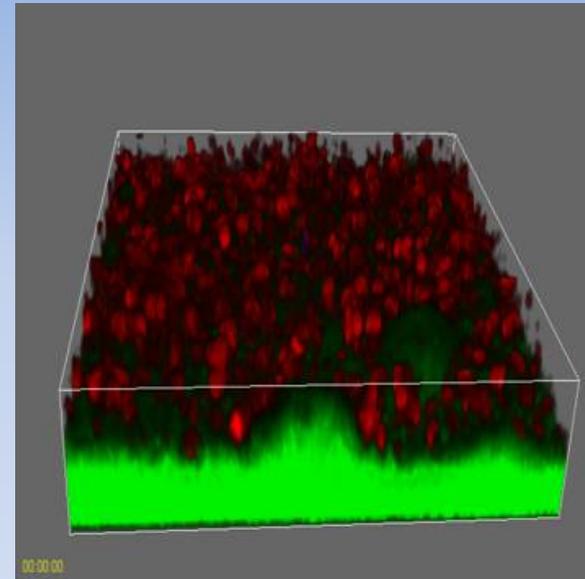
The problem



Wild type biofilm

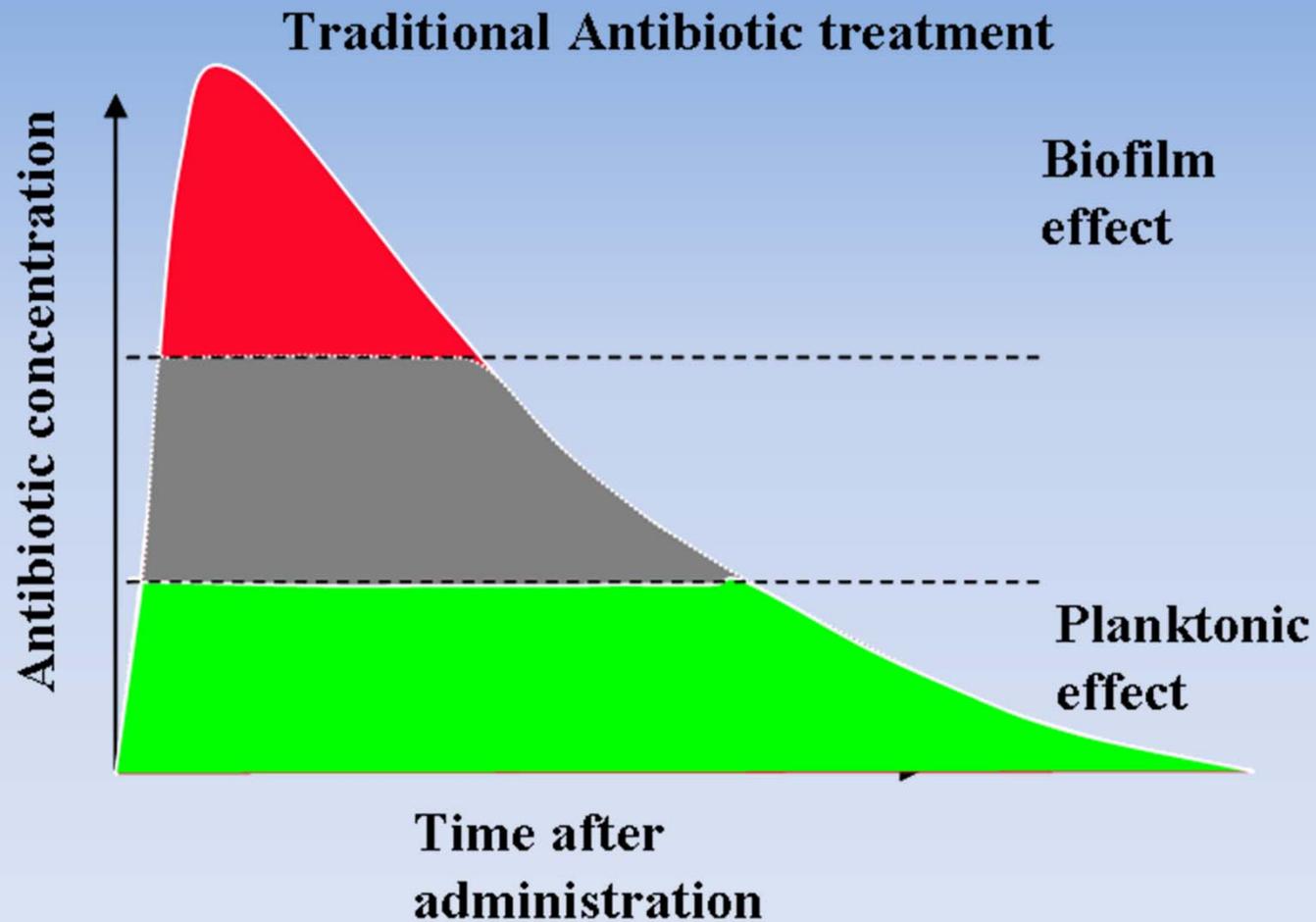


Live/dead stain of an antibiotic treated biofilm



Paralysis of neutrophils
(white blood cells)

Biofilms and antibiotics



Biofilm cells tolerate high doses of antibiotics

Review

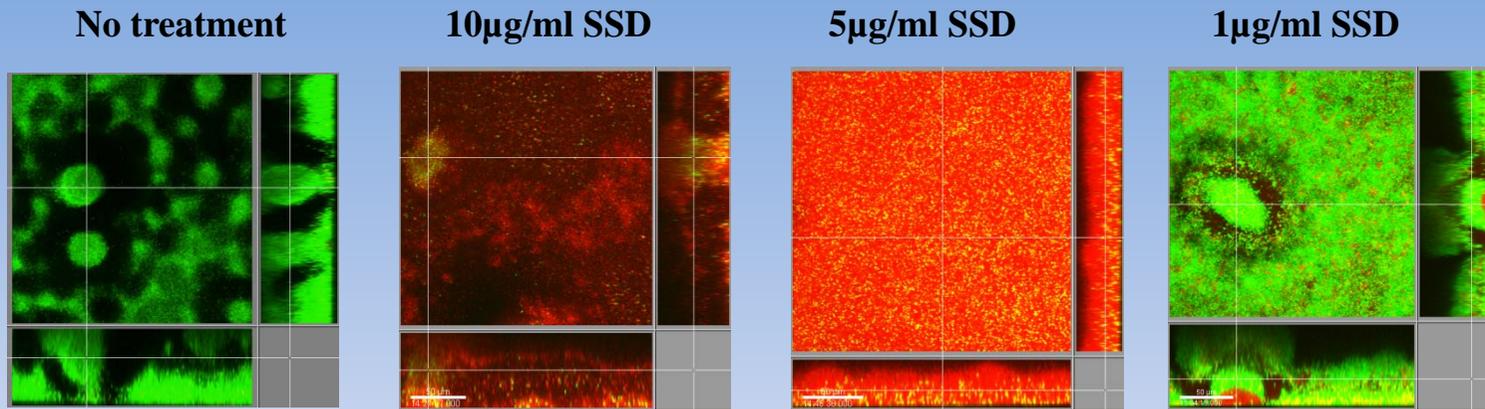
Høiby et al.

Table I. Susceptibility in vitro of planktonic and biofilm cells of non-mucoid and mucoid *P. aeruginosa* to piperacillin and tobramycin¹.

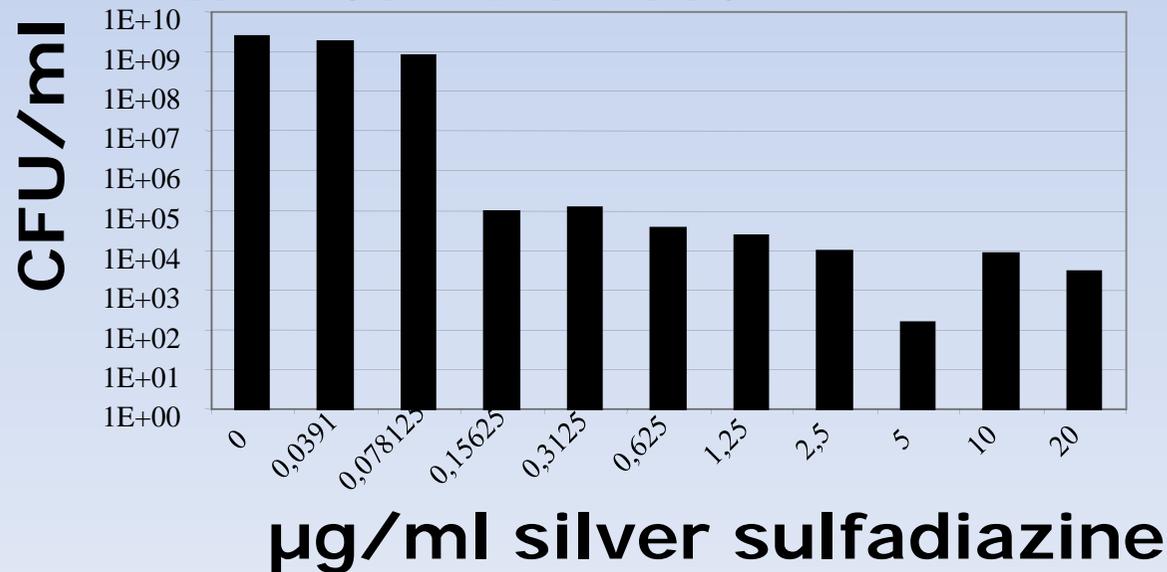
	% survival after 4–5 h				
	Planktonic cells		Young biofilm (2 days)		Old biofilm (7 days)
	NM*	M**	NM	M	NM and M
Piperacillin 200 µg/mL	4%	4%	5–10%	5–10%	31%
Tobramycin 10 µg/mL	0.001%	0.001%	0.001%	2%	50% (50 µg/mL tobramycin)
Piperacillin 200 µg/mL + Tobramycin 10 µg/mL	< 0.001%	< 0.001%	< 0.001%	0.1–0.01%	10% (25 µg/mL tobramycin)

¹ Inoculum, 10⁸ CFU/mL. MIC tobramycin: 1 µg/mL, MIC piperacillin: 16 µg/mL. Modified from [126]. * NM: non-mucoid. ** M: mucoid.

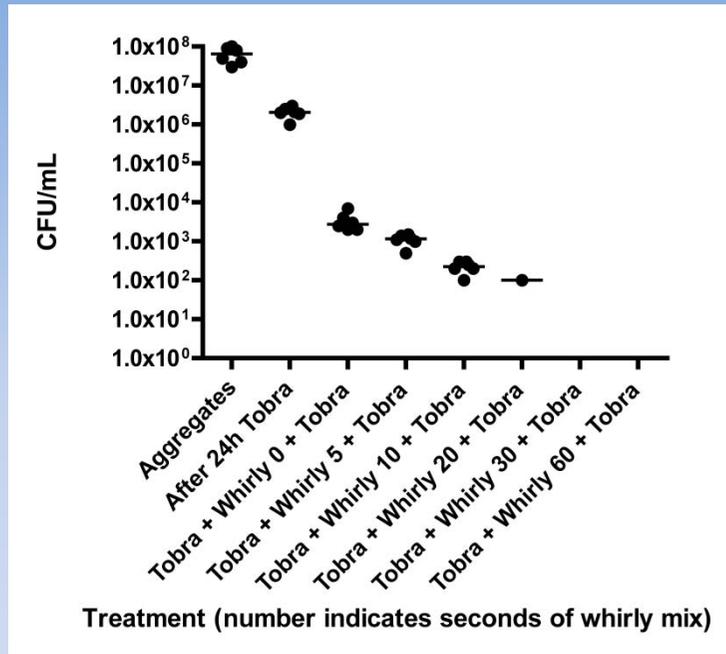
Silver sulfadiazine (SSD)



Planktonic effect



Physical disruption revert the tolerance

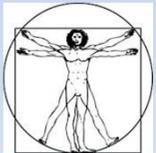


Resistance vs. Tolerance



Resistance: Resistance is the inherited ability of a bacteria to survive and reproduce following exposure to a lethal dose of antimicrobial

- Heritable active processes (e.g. production of beta-lactamases and efflux pumps)



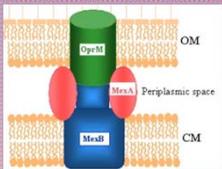
Tolerance: Tolerance is caused by a reversible physiological state in a small subpopulation of genetically identical cells.

- Non-heritable processes (e.g. biofilm formation and slow growth)

Resistance vs. Tolerance

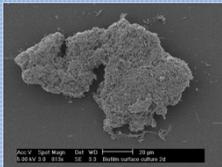


- Acquired resistance (mutation or horizontal gene transfer)

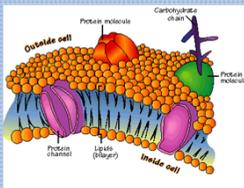


- Efflux pumps (e.g. *mexAB*, *mexXY*)
- Degrading enzymes (e.g. *beta-lactamases*)

Resistance



- Grows as aggregates (so called biofilms)



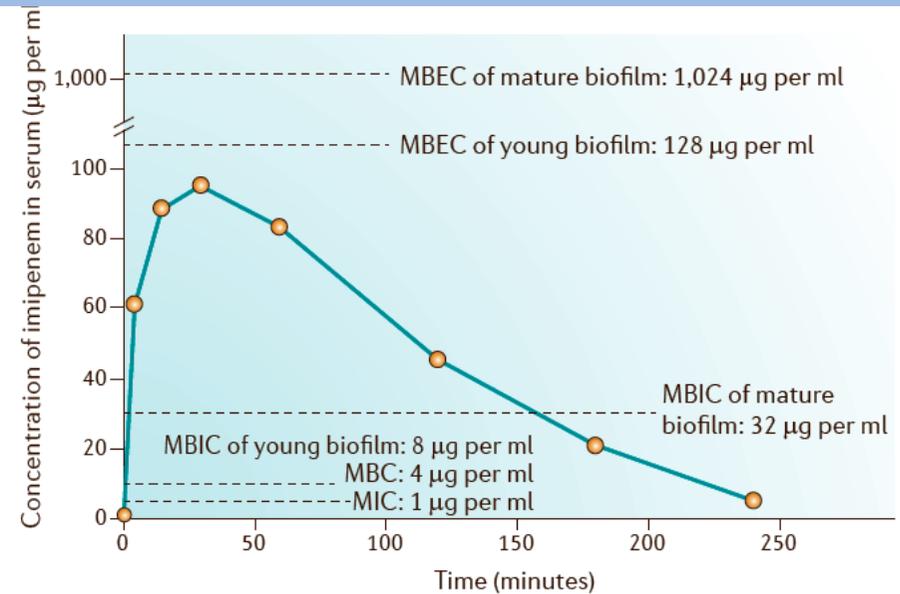
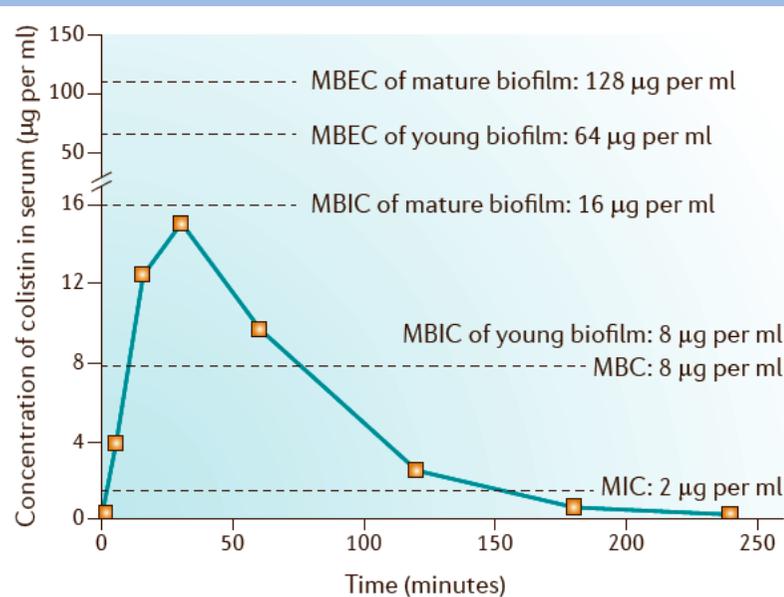
- Slow growth
- Low permeability of matrix

Tolerance

MBEC and MBIC

MBIC – Minimal Biofilm Inhibitory Concentration

MBEC – Minimal Biofilm Eradication Concentration



Mice were treated with a single intraperitoneal dose of either colistin (16 mg per kg) or imipenem (64 mg per kg).

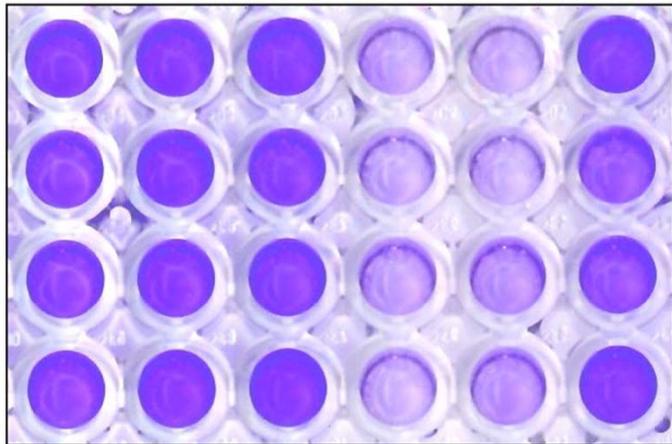
Bjarnsholt et al Nature Review Drug Discovery.

2013 Oct 1;12(10):791-808

In vitro biofilms

Crystal Violet

High throughput

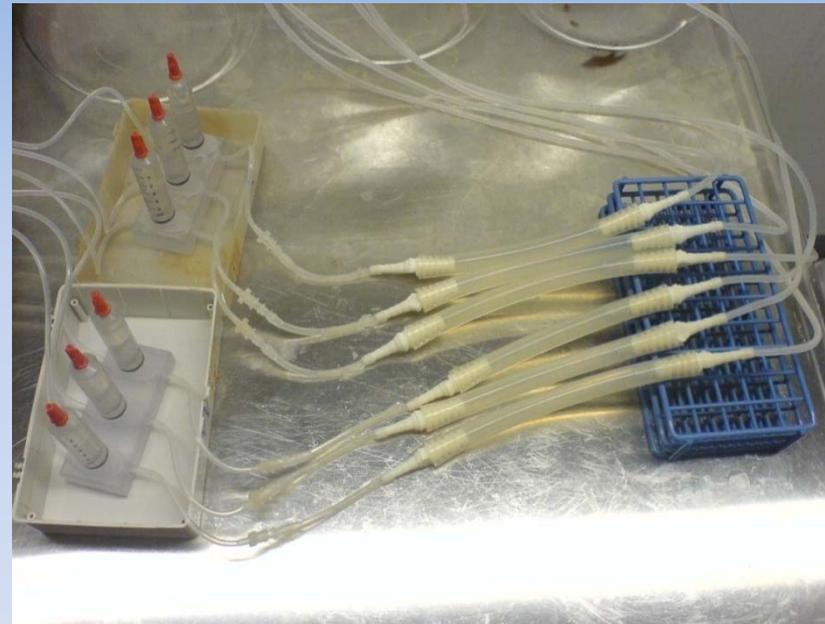


LB Glycerol Glucose LB Glycerol Glucose
2% 1% 2% 1%

Attached biomass NOT viability

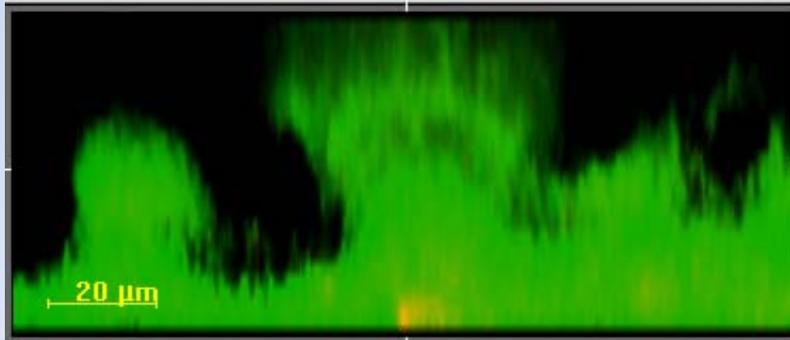
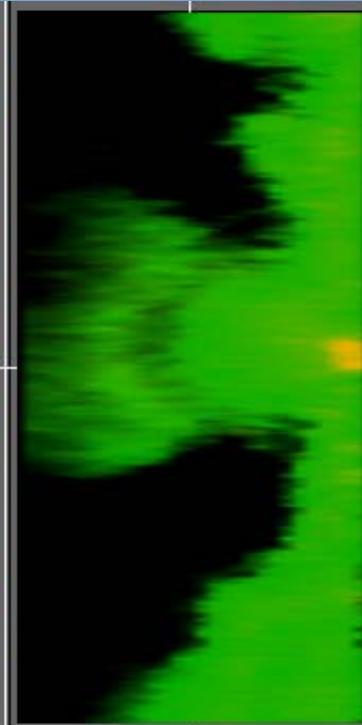
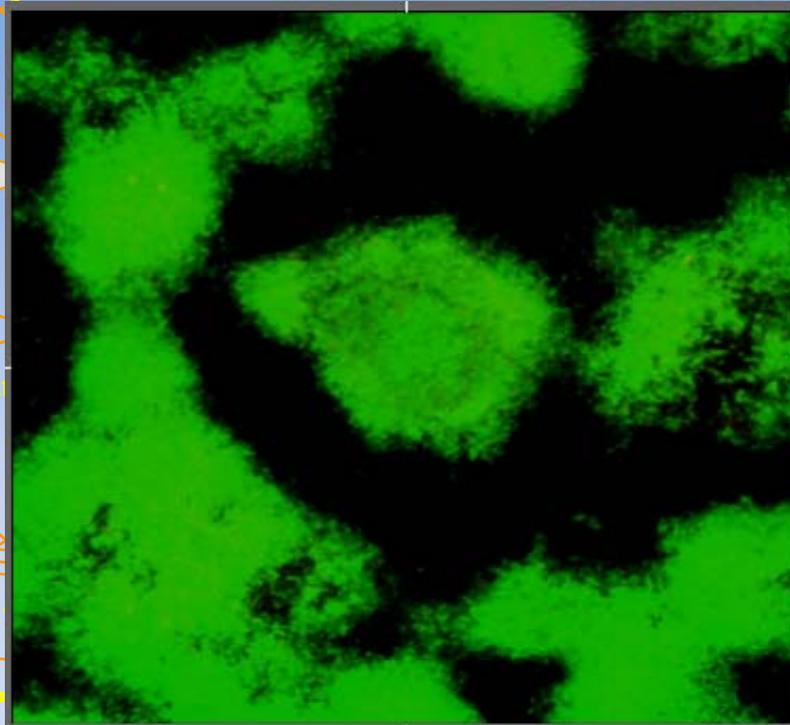
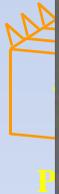
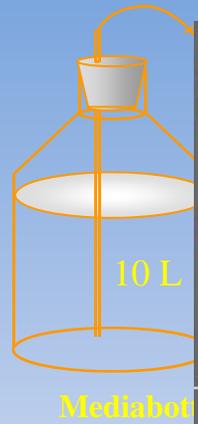
Flow cell biofilm

Low throughput

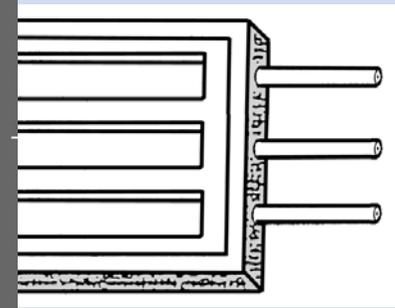


Viability/geneexpression

The *in vitro* biofilm



mm

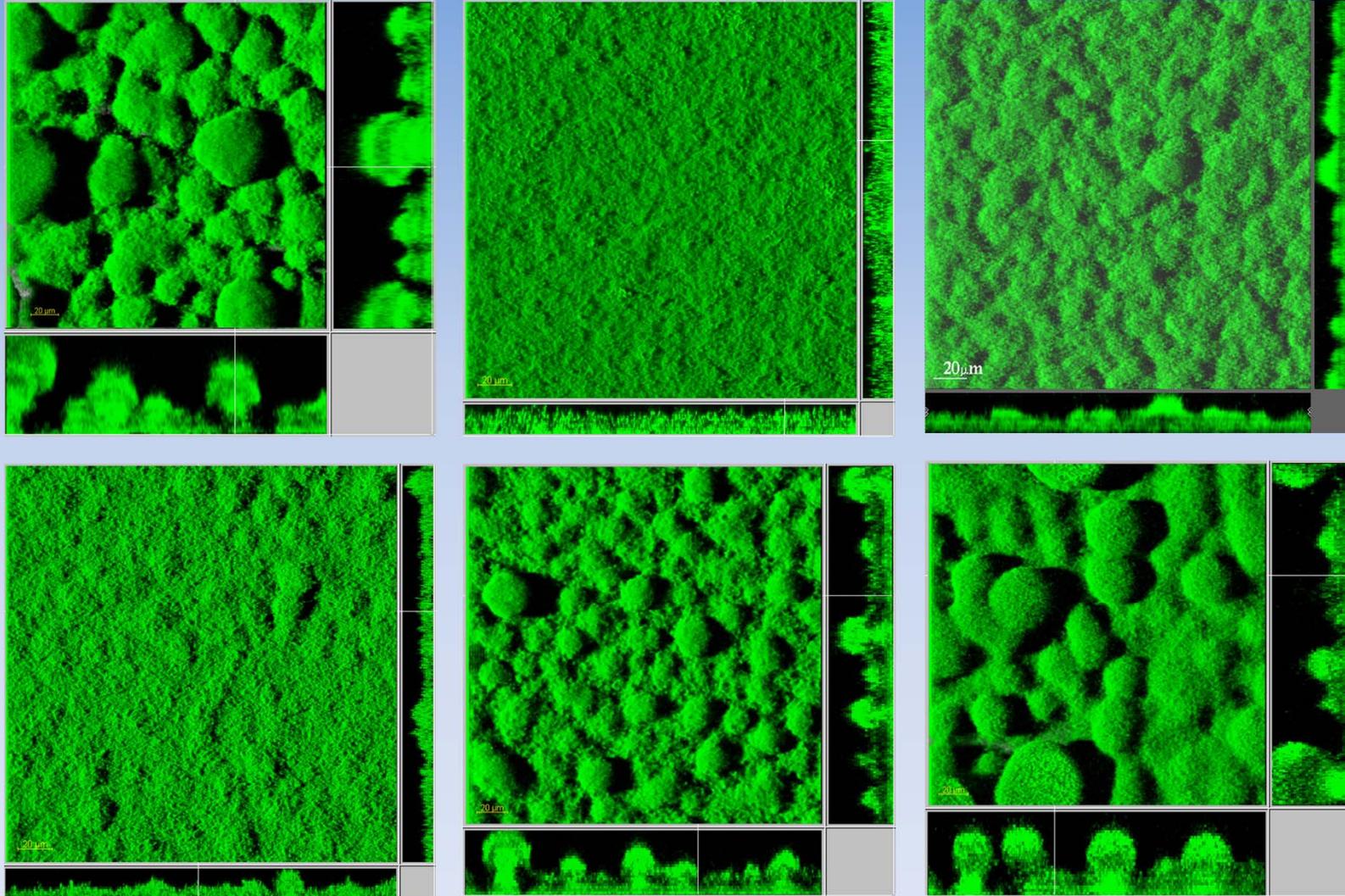


4 mm

Coverglass

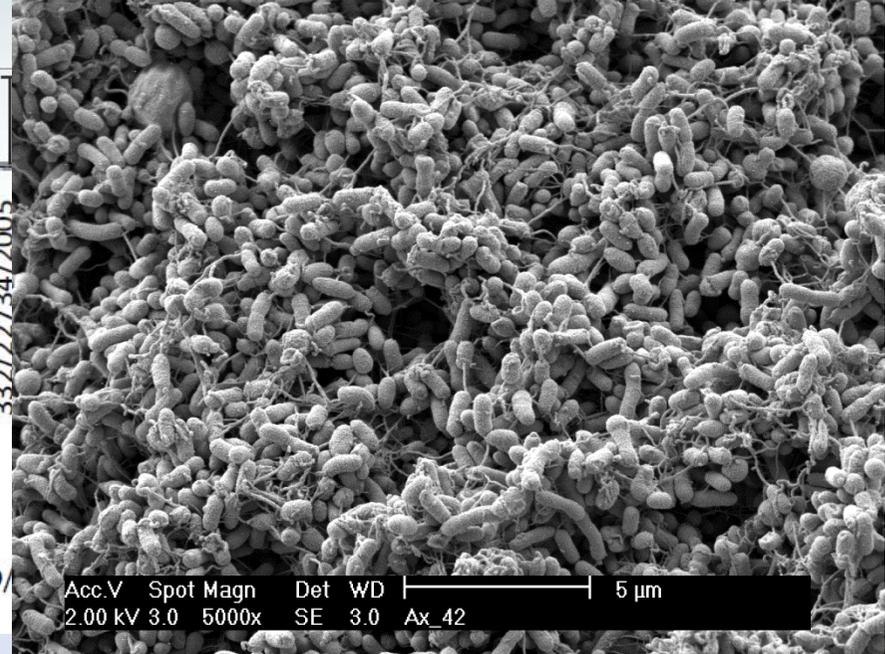
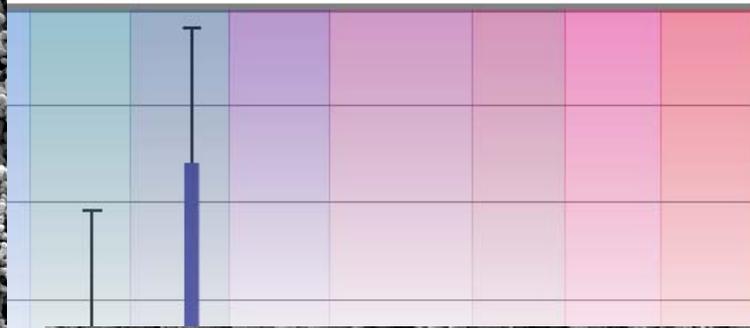
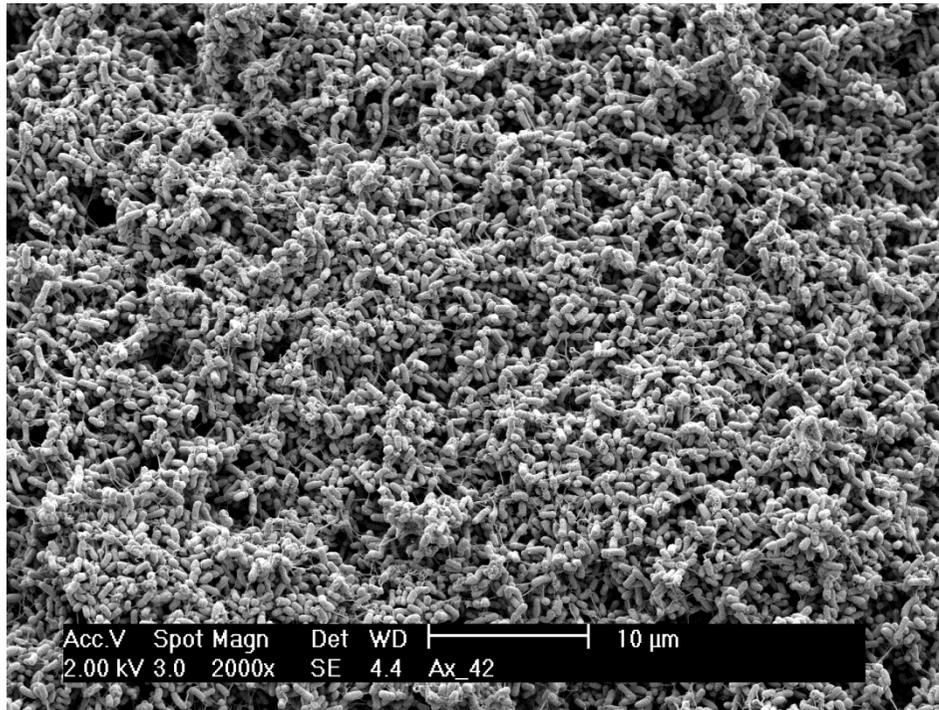
Janus Haagensen

P. aeruginosa day 4,



Anders A. Jørgensen 2003

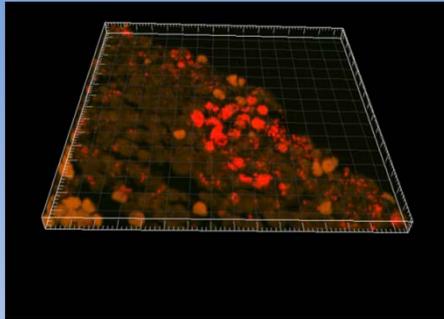
Clinical isolates



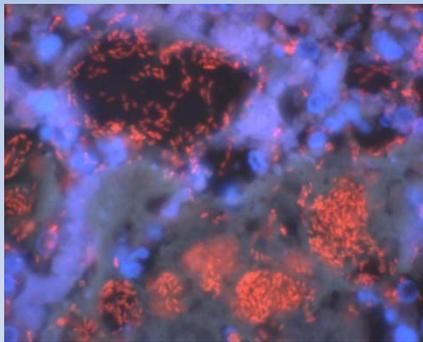
256/
256/NH44784
256/6
256/3
256/4
256/
256/
334/6
334/
334/
1136/
335/4

CF nr./Strain ID/

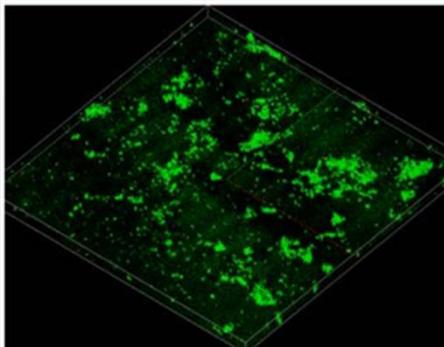
The *in vivo* Biofilm



Wound

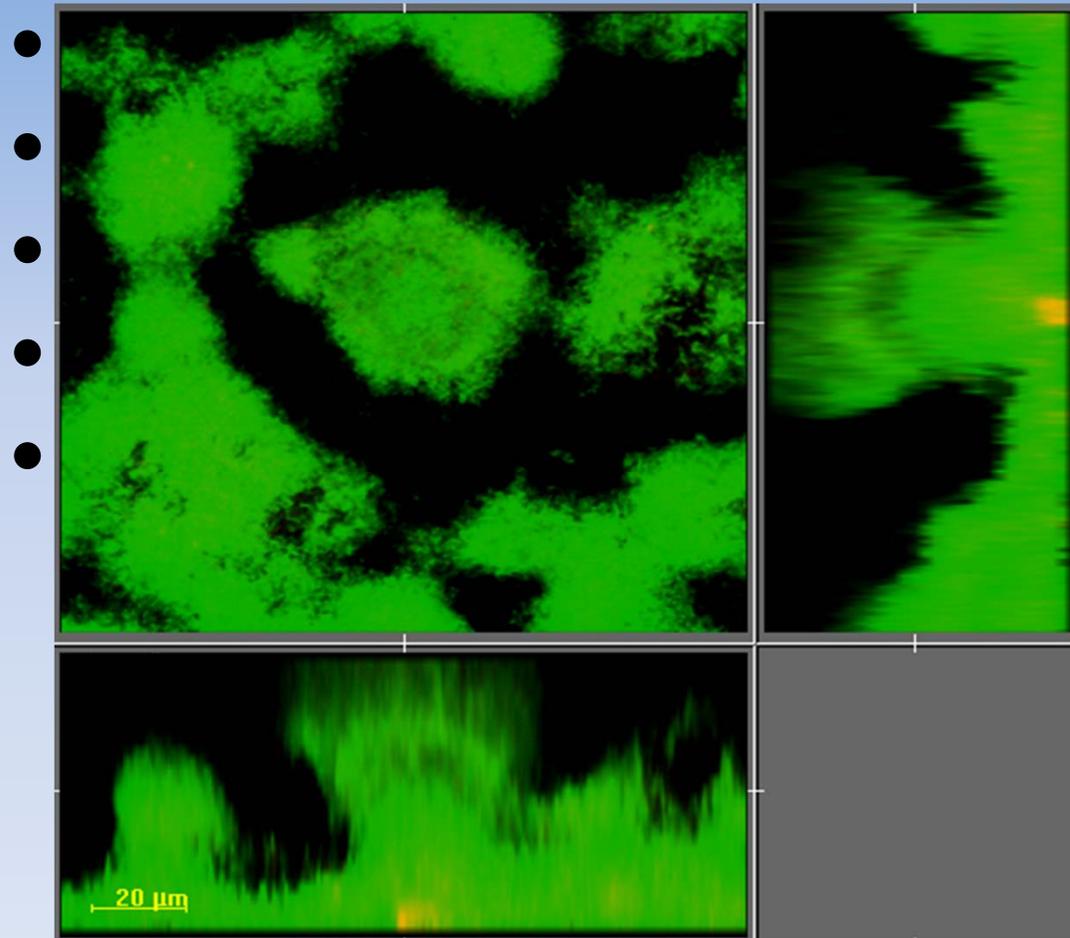


CF lung

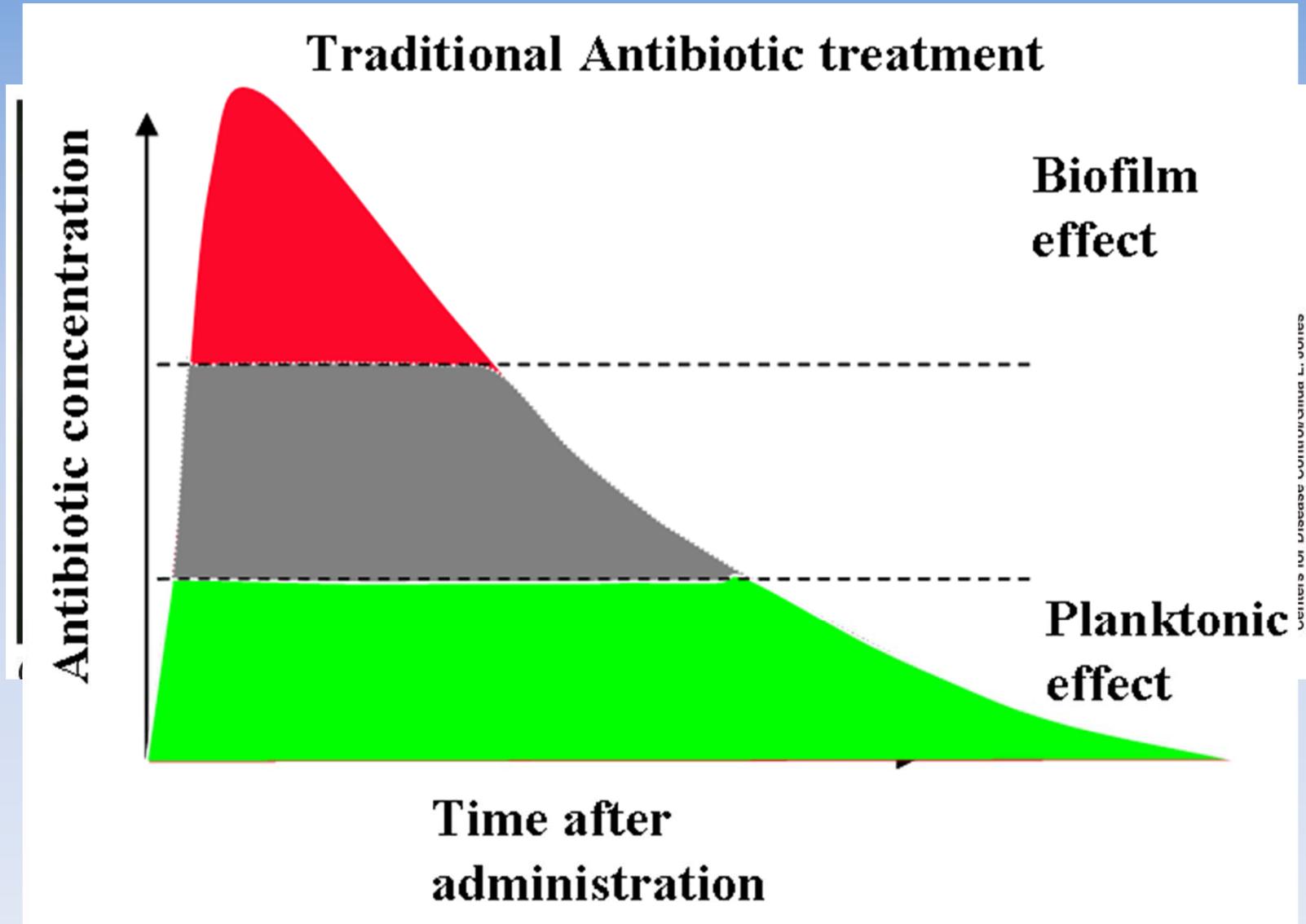


Implant mouse model

- No mushrooms

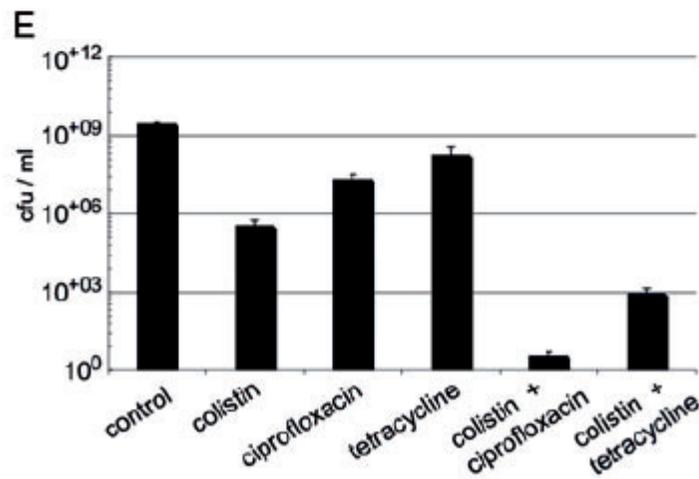
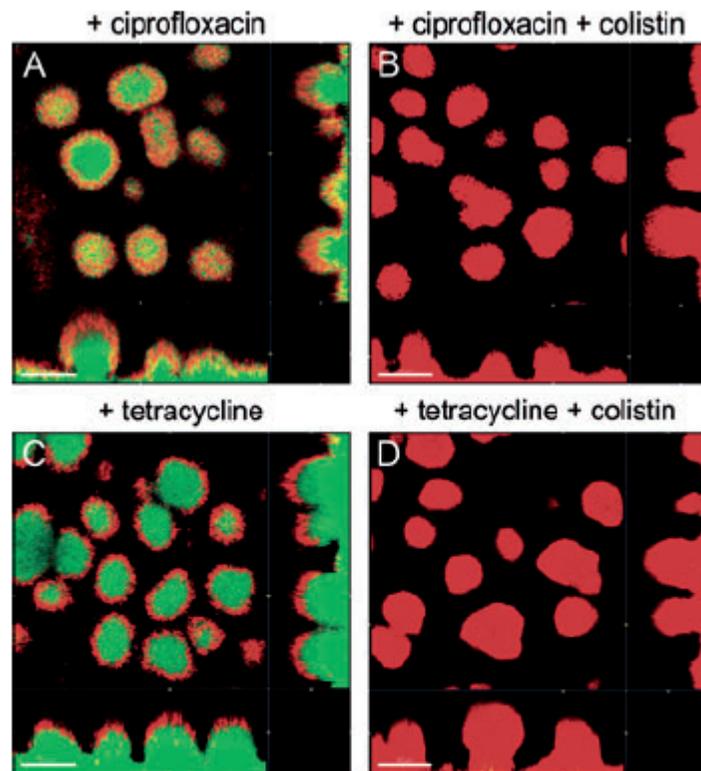


Antibiotic susceptibility

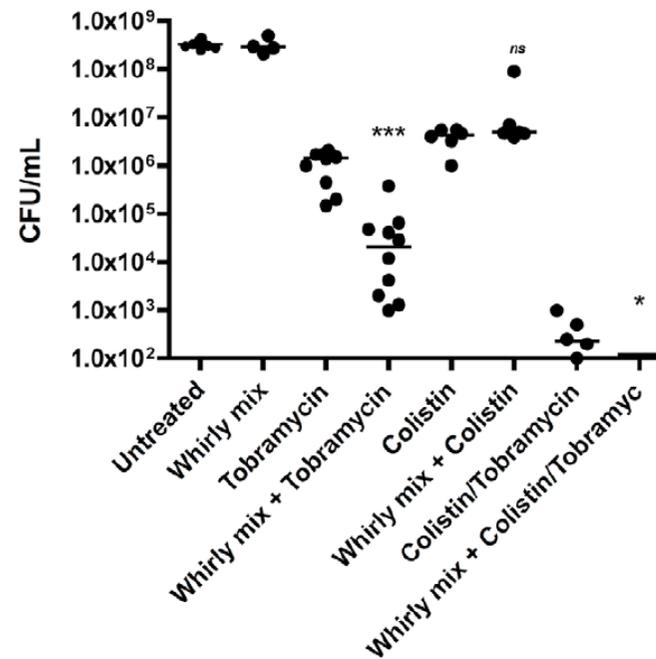


What to do?

- Combinatory treatment



Pamp et al Mol Micro 2008



Alhede et al PlosOne 2011

What to do?

- Combinatory treatment
 - Minimizes resistance
- Highest doses possible
- Long term
- **Removal of foreign body**
- **Diagnostics are just as important**

Acknowledgments

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Stephanie G Crone

Majken Sønderholm

Blaine Fritz

Camilla Stavnsbjerg

Marie Thaysen

Lasse Kvich

Lene Bay

Klaus Qvortrup

Oana Ciofu

Søren Sørensen

Michael Kühl

Mette Burmølle

Hans Petter Hougen

Michael Givskov

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Niels Høiby

Peter Østrup Jensen

Claus Moser

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Lars Christophersen

Michael Tvede

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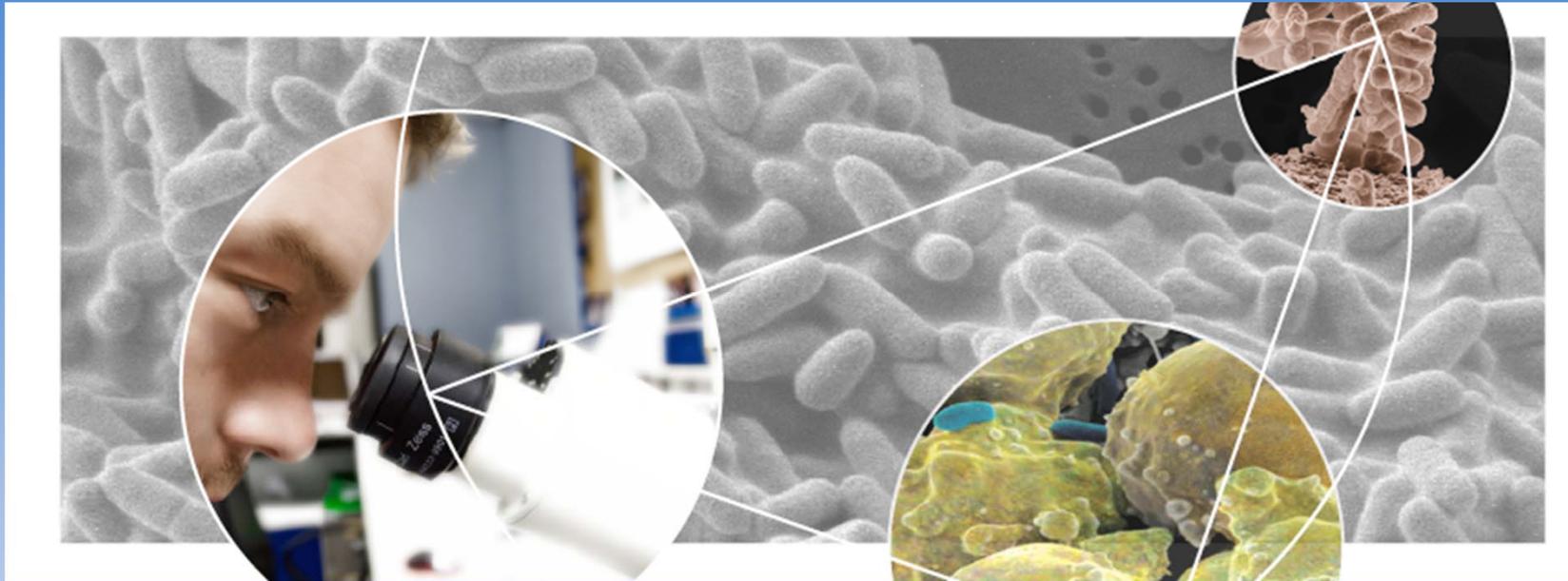
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