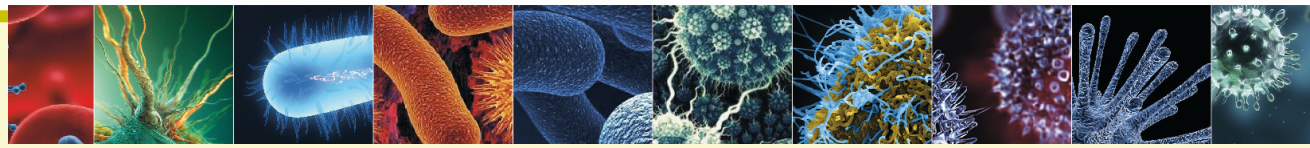
Hospital Vila Franca de Xira

arsalentejo
Administração Regional de Saúde do Alentejo, I.P.

1º Encontro do GCR-PPCIRA da ARSAlentejo,I.P.



Biofilmes

Da investigação à clínica

Resistências aos Antibióticos

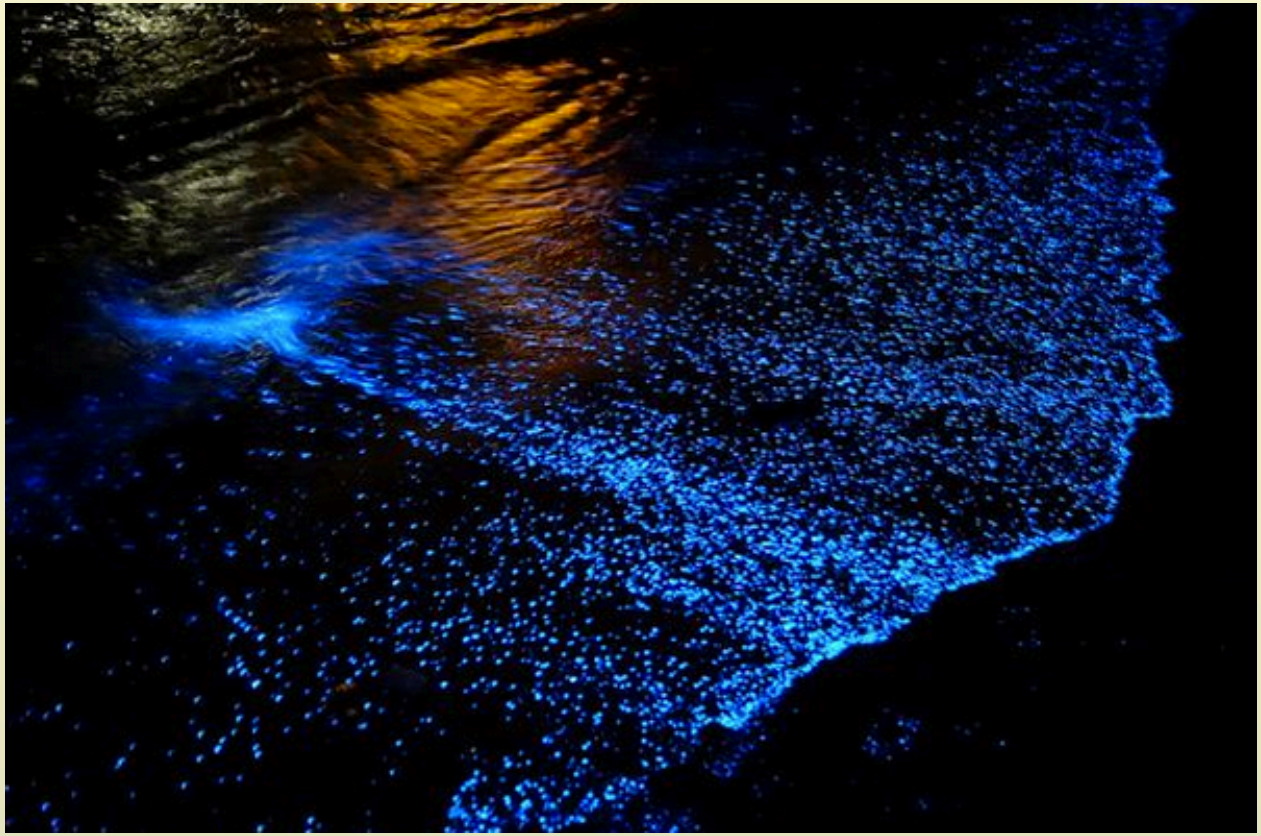
João Gonçalves Pereira

MD, Ph D

Director UCI, Hospital Vila Franca Xira

quorum sensing

Tipo de comunicação célula a célula, através da qual as bactérias reconhecem a sua densidade populacional, pela produção de pequenas moléculas; Assim coordenam a expressão genética de acordo com a densidade bacteriana.

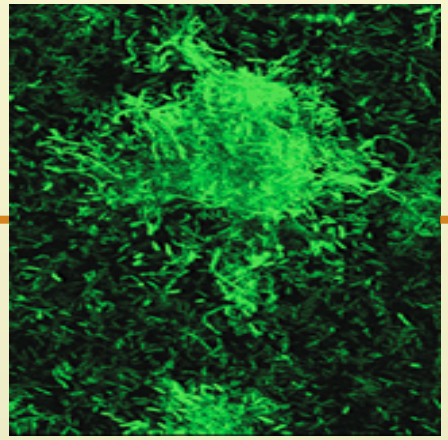


Aliivibrio fischeri

Vibrio harveyi

V. harveyi Are Bilingual

V. harveyi communication systems



AI-1 molecule

Speaks language unique to *V. harveyi*

AI-2 molecule

Speaks universal language used by many quorum-sensing bacteria

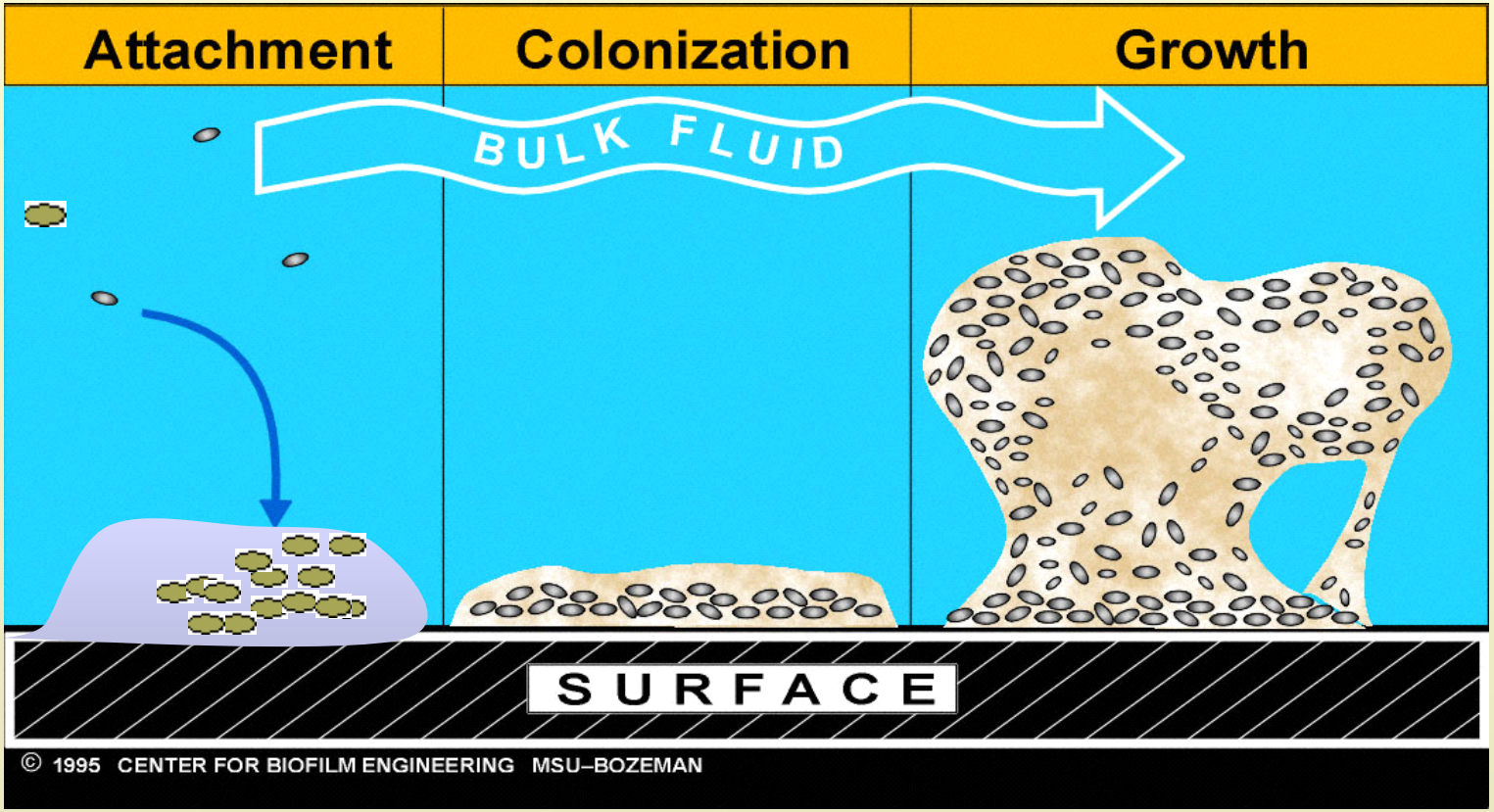
What is a Biofilm?

- Structured, co-operative microbial community embedded in an extracellular matrix, usually attached to a surface

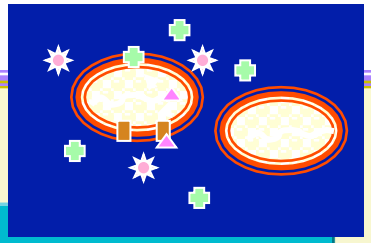
- Different species may be competing or co-operating

•Free-floating (planktonic) cells attach to become sessile
•Biofilm organisms usually express a different phenotype

What is a Biofilm?

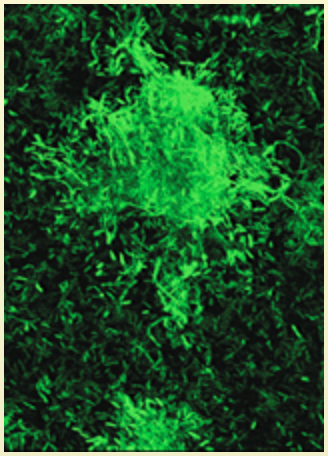


Biofilms



Colonization: *Quorum sensing*

Bacteria cells seems harmless when multiplying



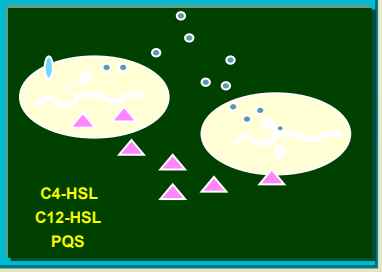
These changes in bacteria are responsible for most infections

Signaling moleculles

- Self / non Self
- Number

When their number reaches a significant threshold (the "*quorum*") phenotypical changes changes occur in its

- Behaviour
- Metabolism



☞ **Virulence Factors**

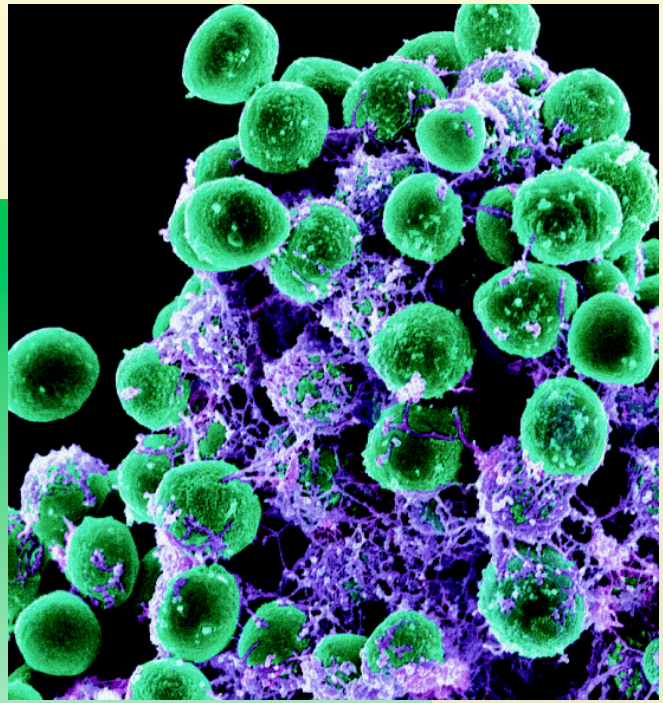
☞ **Biofilms**

Structured, cooperative microbial community embedded in an extracellular matrix, usually attached to a surface

Biofilmes e próteses

Fixação

Implicações clínicas dos Biofilmes



- A maioria das infecções nosocomiais estão associadas a dispositivos médicos invasivos

- 97% ITU - algália
- 87% bacteriémia – Catéter venoso central
- 83% pneumonia – Tubo traqueal

Implicações clínicas dos Biofilmes

- **Os biofilmes podem formar micro-colónias e fragmentar**
- **A embolização infecciosa pode ser responsável por**
  - Invasão de líquidos orgânicos → sépsis
  - Entrada na circulação capilar→ colonização dos tecidos, infecção ou enfarte

Os mecanismos adquiridos de resistência podem permanecer

Clinical Implication of biofilms

Ability to colonize any foreign material

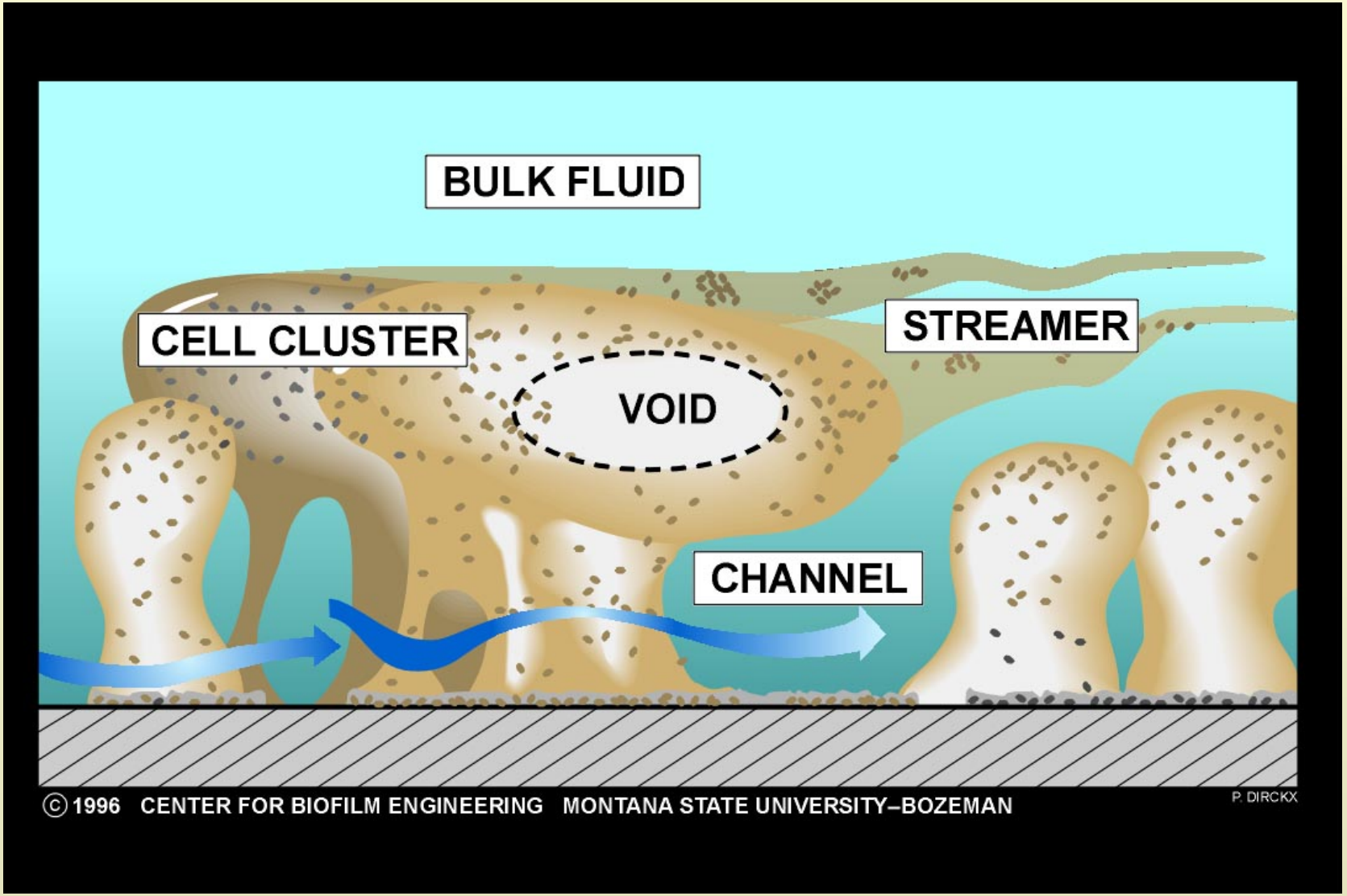
- Cardiac valves
- Central venous lines
- Bone prothesis
- Contact lenses
- Intrautherin devices
- Endotracheal tube
- Peritoneal dialiysis catheter
- Pacemaker

Several bacteria can become virulent

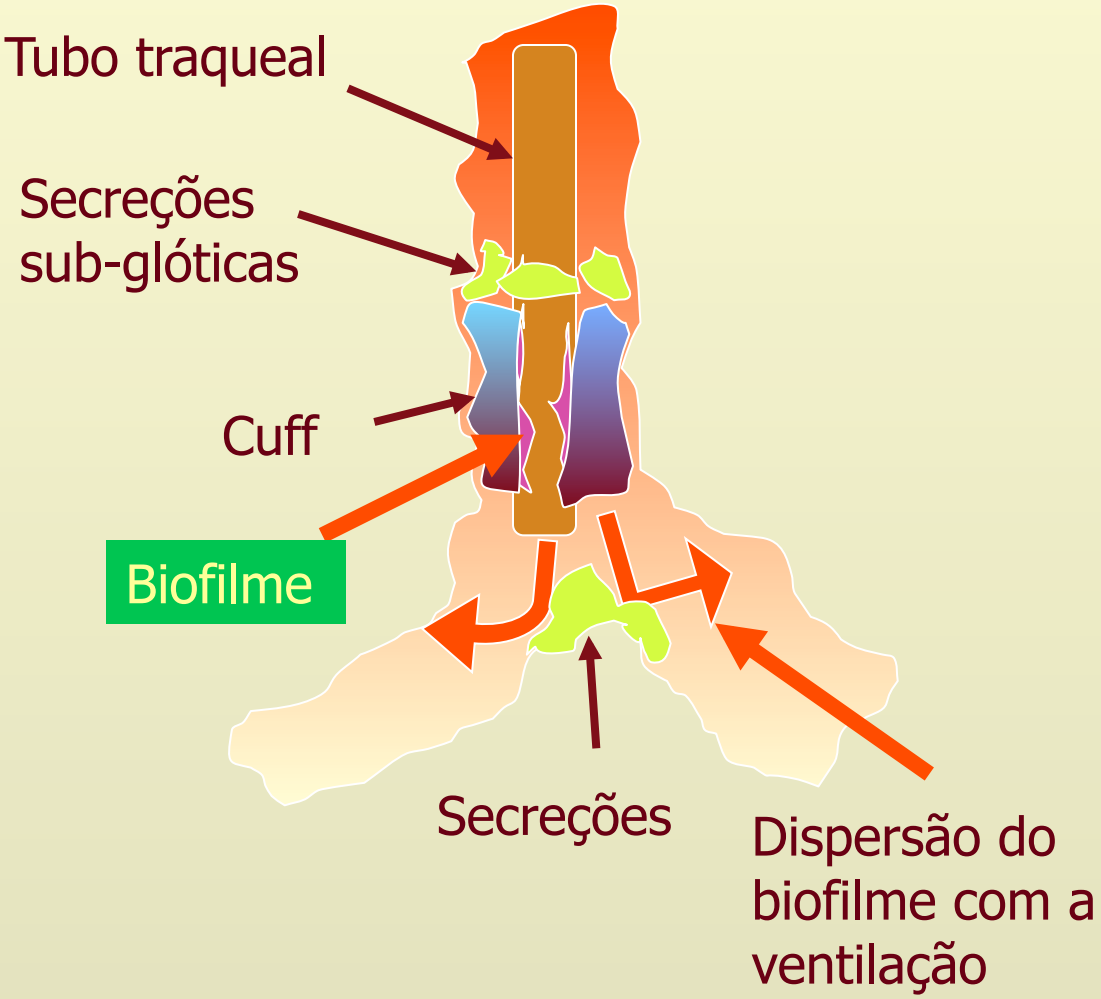
- *Staphylococcus coagulase-negativo*
- *Staphylococcus aureus*
- *Streptococcus* spp
- *Enterococcus* spp
- *Klebsiella pneumoniae*
- *Escherichia coli*
- *Proteus mirabilis*
- *Pseudomonas aeruginosa*
- *Candida albicans*

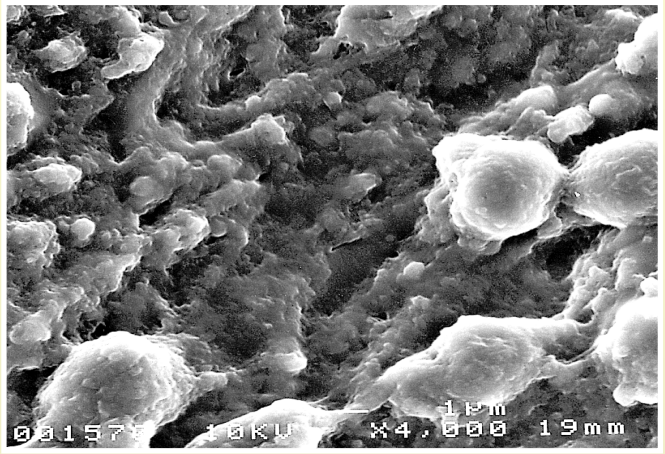
☞ **Once installed a high rate of resistance to antibiotics is noted**

Clinical Implication of biofilms



Mecanismos de colonização





Superfície dum tubo endotraqueal removido dum doente de UCI

"In vivo" incluem muco, restos celulares do hospedeiro e bacterianos

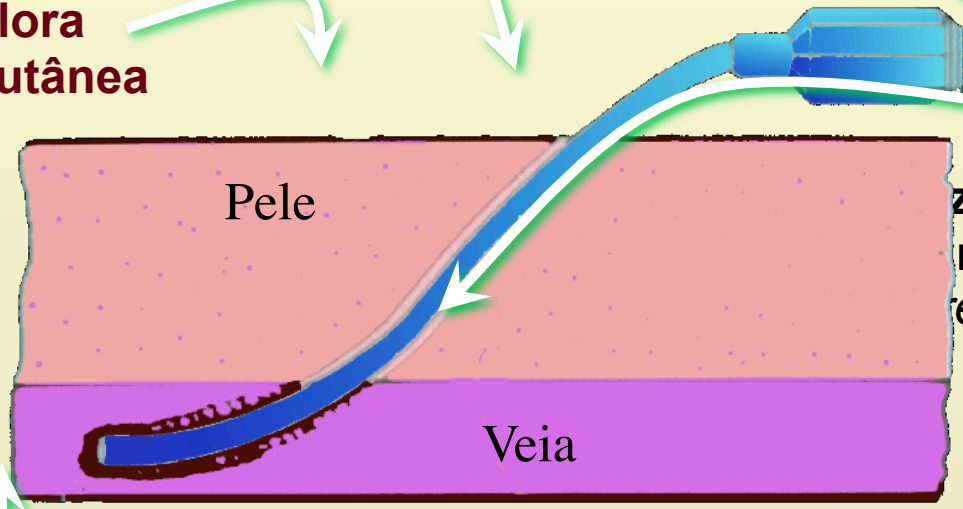
Mecanismos de colonização

Contaminação cruzada

Colonização endoluminal

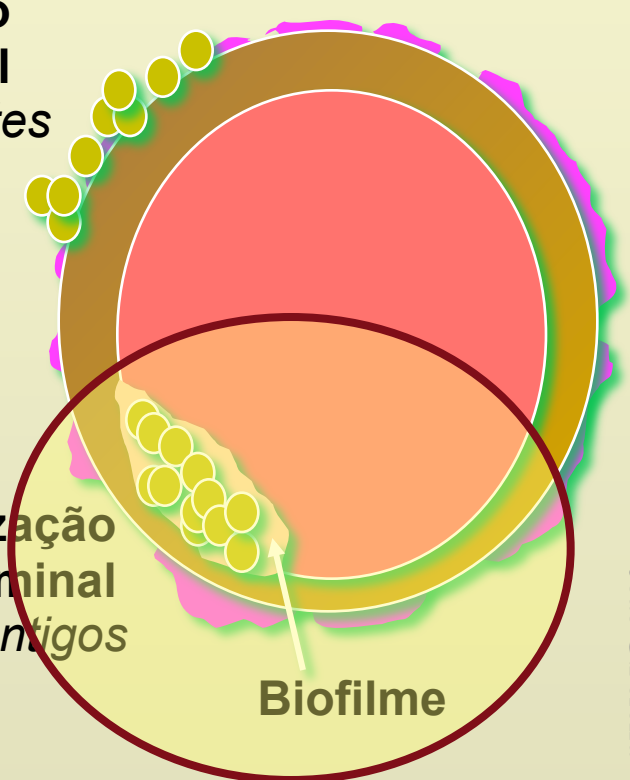
Flora cutânea

Colonização extraluminal



zação
minal
ecentes

Colonização hematogénea



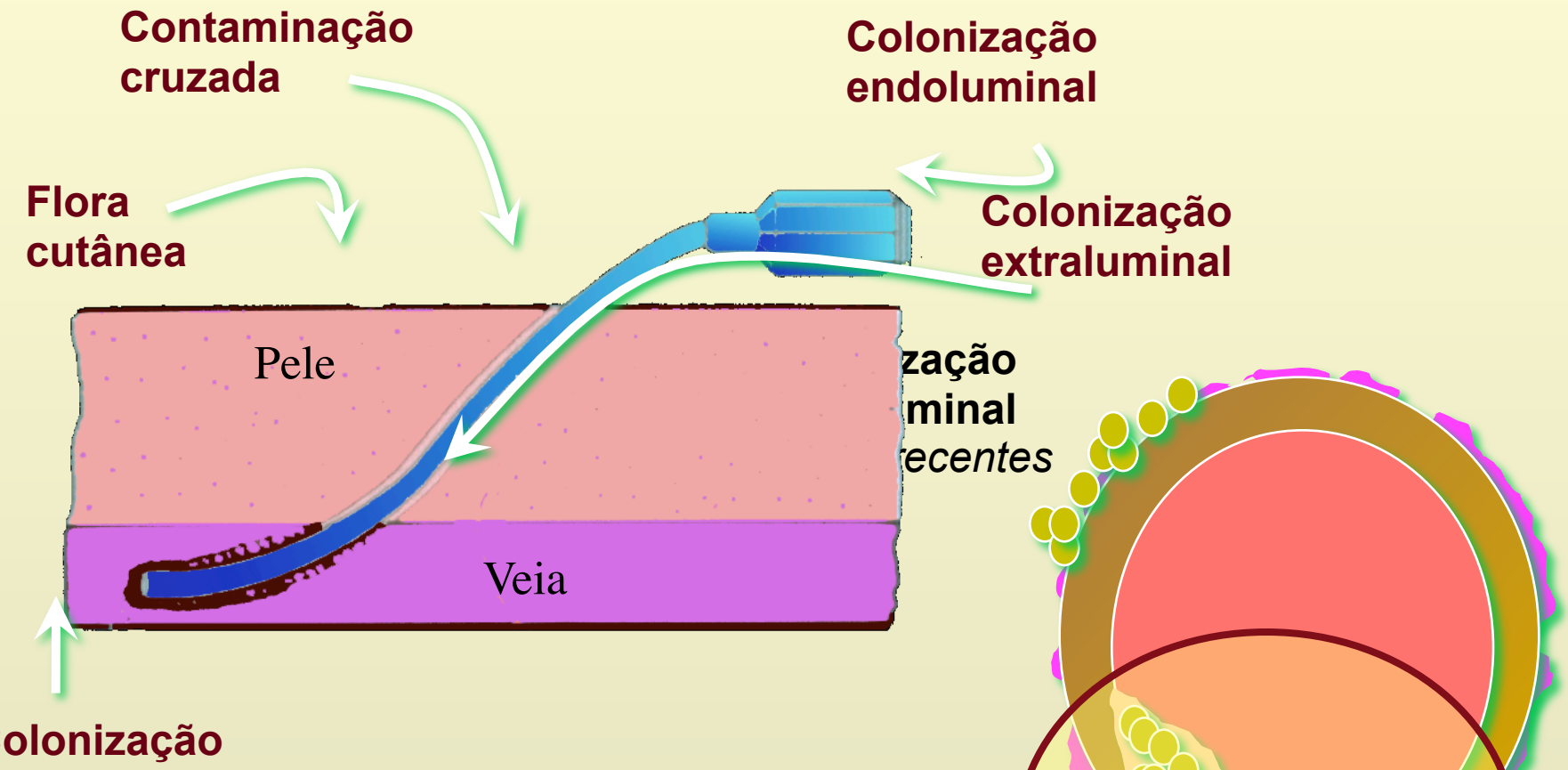
Colonização endoluminal
CVCs antigos

Biofilme

Maki DG Hospital Infections 1992, 849-98.

João G.Pereira

Mecanismos de colonização



Removal of the device

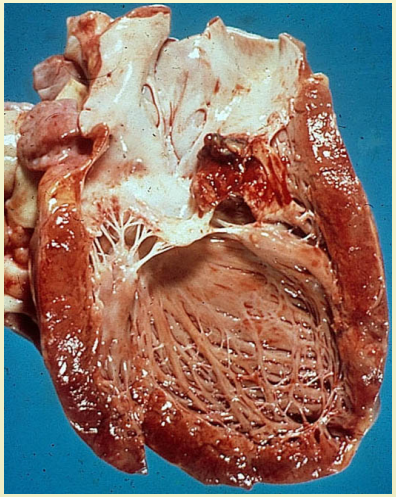
Is the only sure way to remove a biofilm

Maki DG Hospital Infections 1992, 849-98.

Infections with *S. aureus* Biofilms

- Chronic Infections/Diseases:

Osteomyelitis



Endocarditis

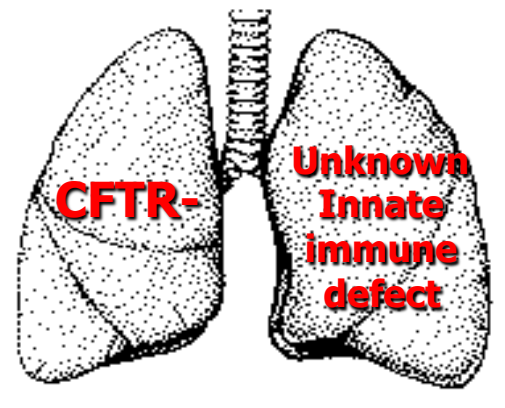
- Biofilms are resistant to antibiotic levels 10-1,000 times higher than planktonic bacteria
  - Concentrations of antibiotics required to kill biofilms may not be therapeutically achievable

http://www.who.int/buruli/photos/Osteomyelitis_Nigeria_large.jpg
http://www.pathology.vcu.edu/education/cardio/images/2g-a.jpg

Chronic *Pseudomonas aeruginosa* Infection in Cystic Fibrosis

Hospital Vila Franca de Xira

Environmental *Pseudomonas*



Bacterial Adaptation

Unique surface modifications

Increased airway inflammation

Biofilms/ Resistance to antimicrobials

Chronic Lung Disease

Innate Immune Selective Pressure

PA colonization - ASYMPTOMATIC | **Increased bacterial burden - SYMPTOMATIC**

Biofilms
Growth and maturation

Antibiotics and Biofilms

srepression “quorum”

sm

han the one identified

owitz SM. JAC 2005; 43: 5085

J Clin Microbiol 2005; 43: 5085

Biofilms
Growth and maturation

Antibiotics and Biofilms

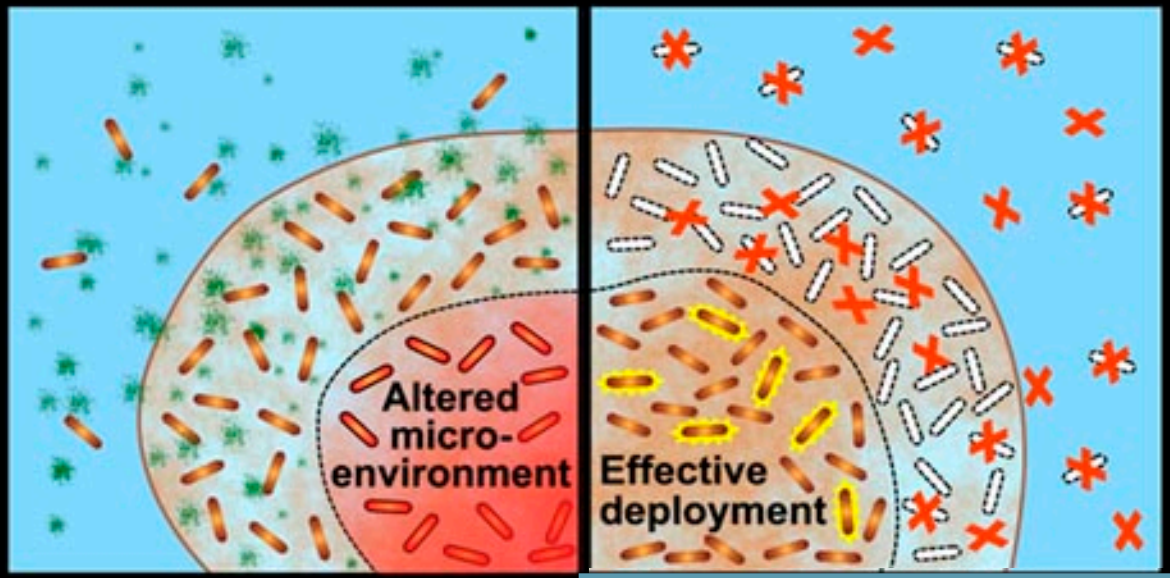
- ✓ Neutralisation of the antibiotics by the polissacaride matrix
- ✓ Extracellular Beta lactamases
- ✓ Inactivation of antibiotic compounds by simultaneous desrepression “quorum” of resistance related genes
- ✓ Plasmidium mediated transference of resistance genes
- ✓ Latent bacterial subpopulations, with low grade metabolism

- ✓ **Bacteria may possess a different sensitivity profile than the one identified “in vitro”**

Moskowitz SM. JAC 2005; 43: 5085
Hill D. J Clin Microbiol 2005; 43: 5085

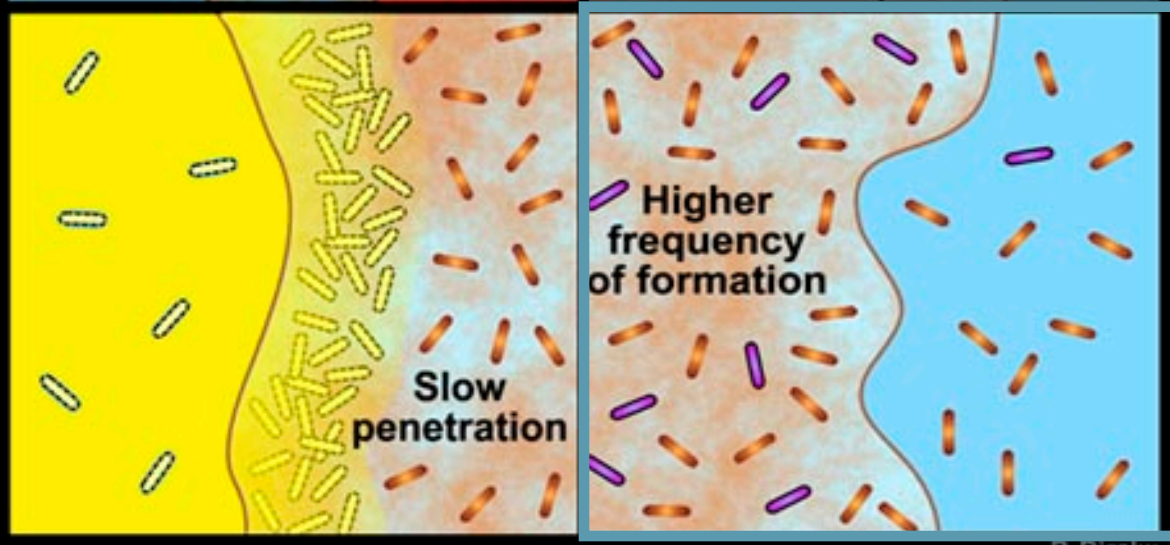
MIC: Biofilms

Nutrient depletion creates zones of altered activity.



Inner layers of biofilm cells have more time to initiate stress response.

Outer layers of biofilm cells absorb damage.



"Persister" cells may be present in higher numbers.

P. Dirckx

Tolerance and Persistence

Small groups of persisters cells in the colony

- Roughly 300 genes differentially overexpressed
- Inhibition of translation, replication, modulation of proteins

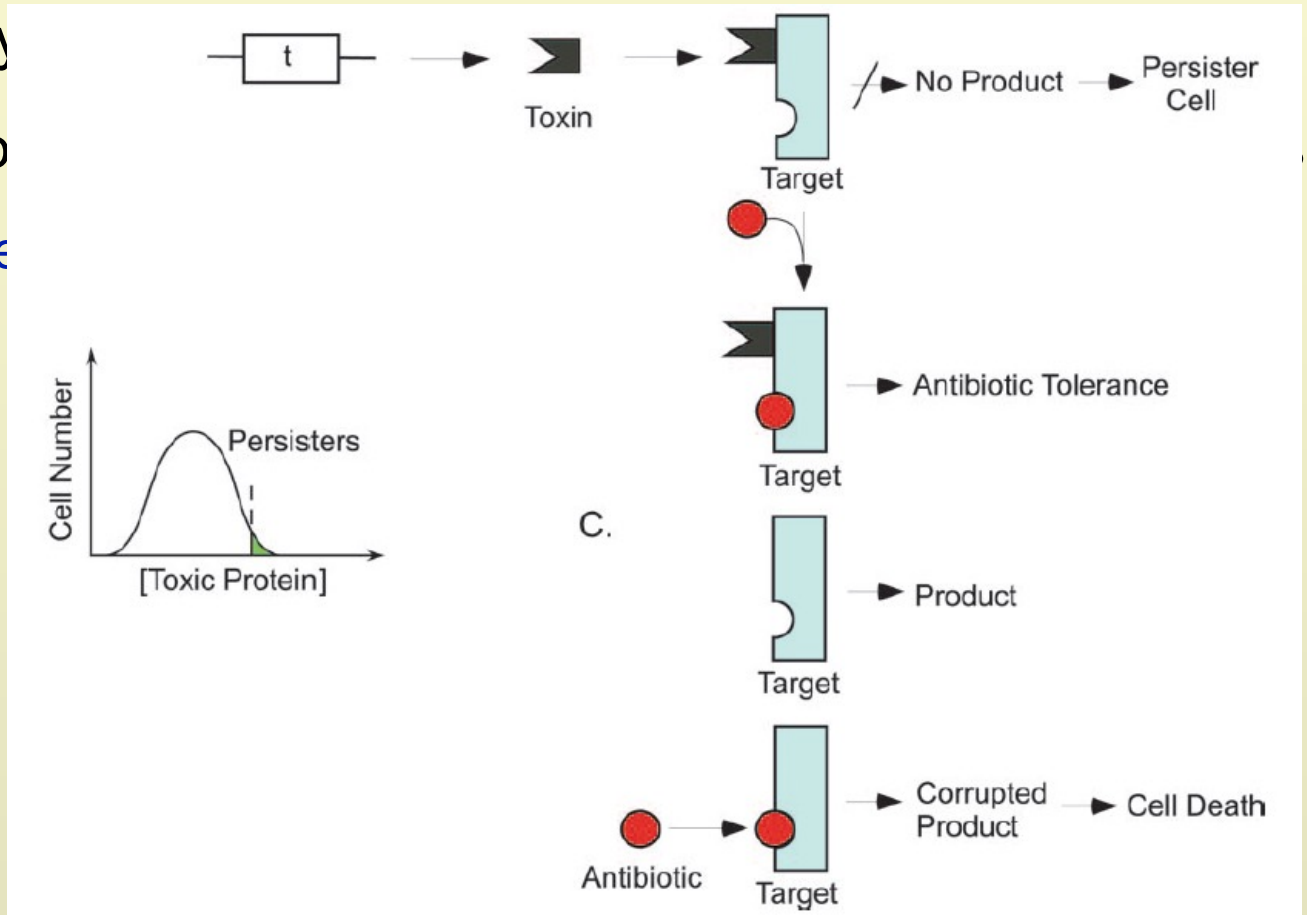
  Tolerance to most antibiotics
- Able to reactivate its functions. Same MIC
- Ensure survival of all population

Keren J Bacteriology 2004;186:8172

Tolerance and Persistence

Small groups of persisters cells in the colony

- Roughly
- Inhibitio
  - Tole
- Able to
- Ensure



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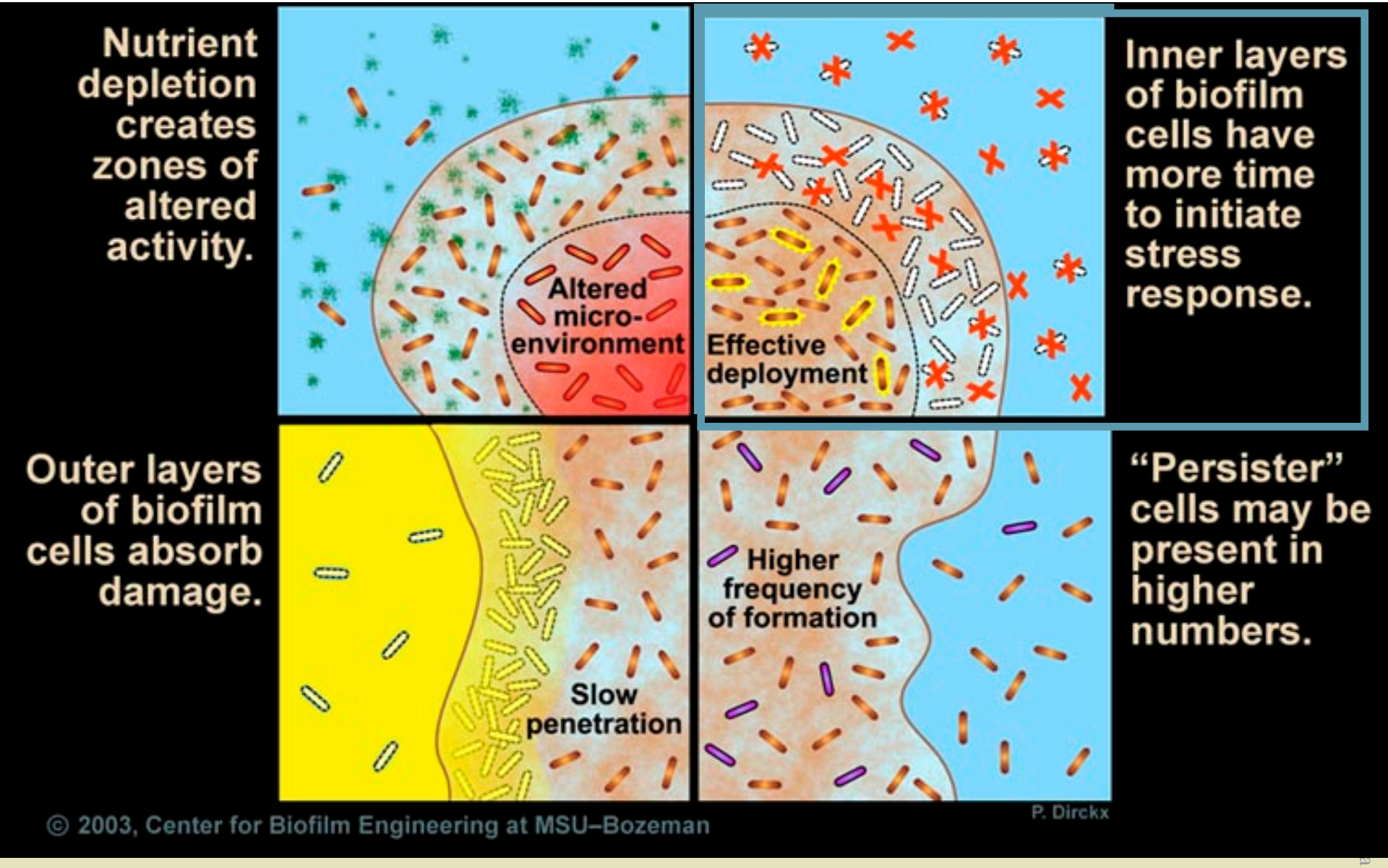

MIC: Biofilms

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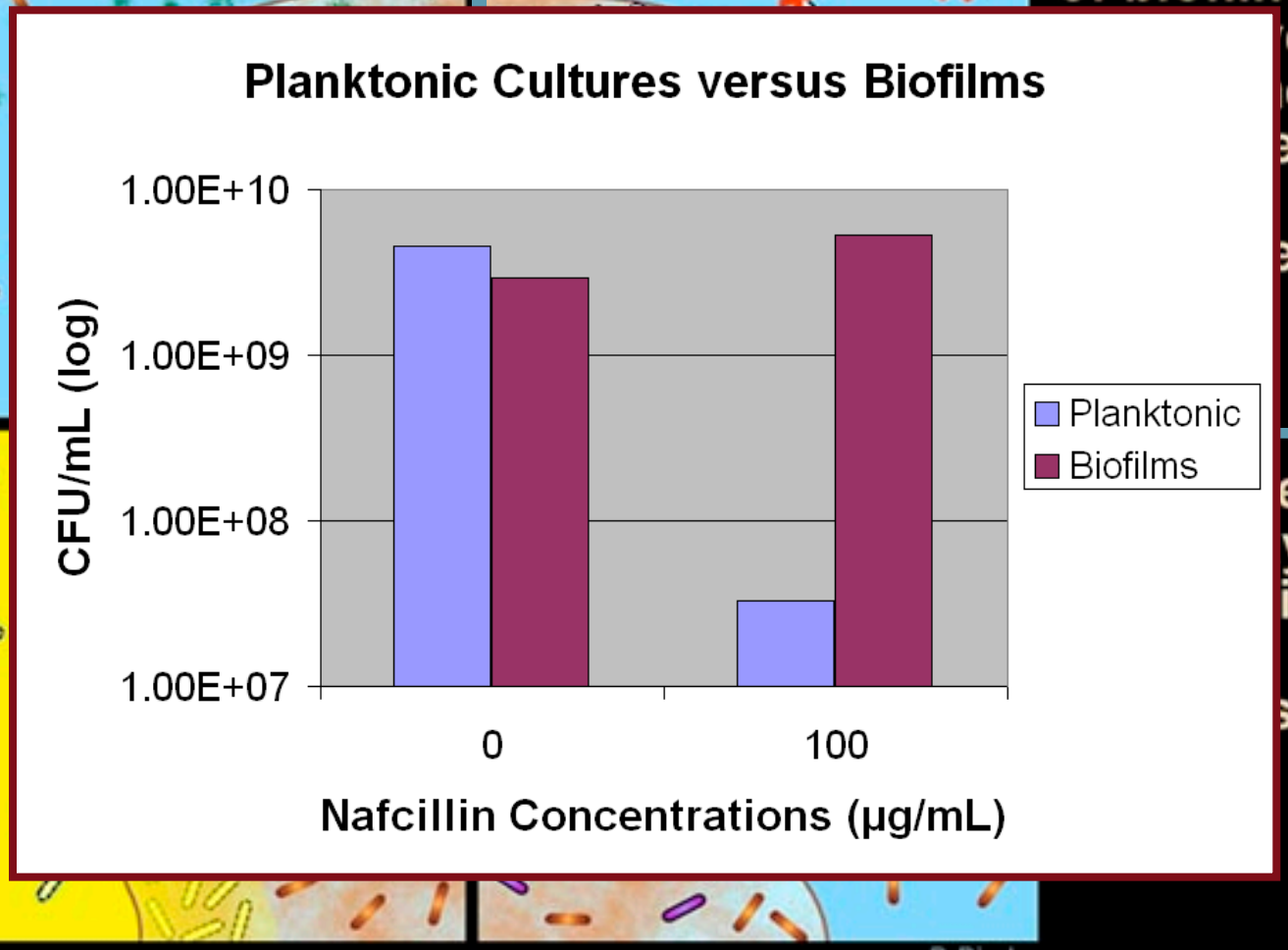
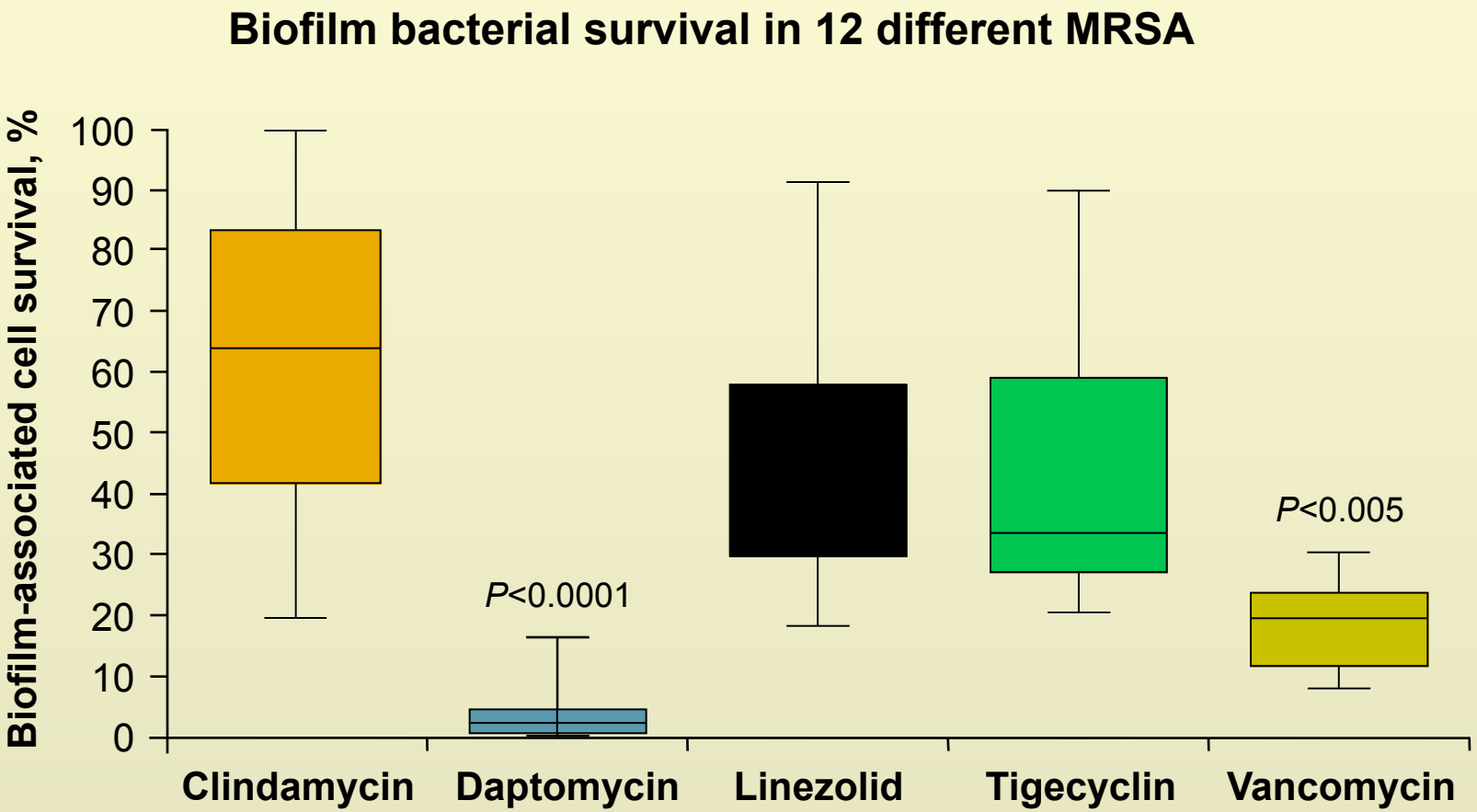
Outer layers of biofilm cells absorb damage.

Inner layers of biofilm



P. Dirckx

MRSA Biofilms

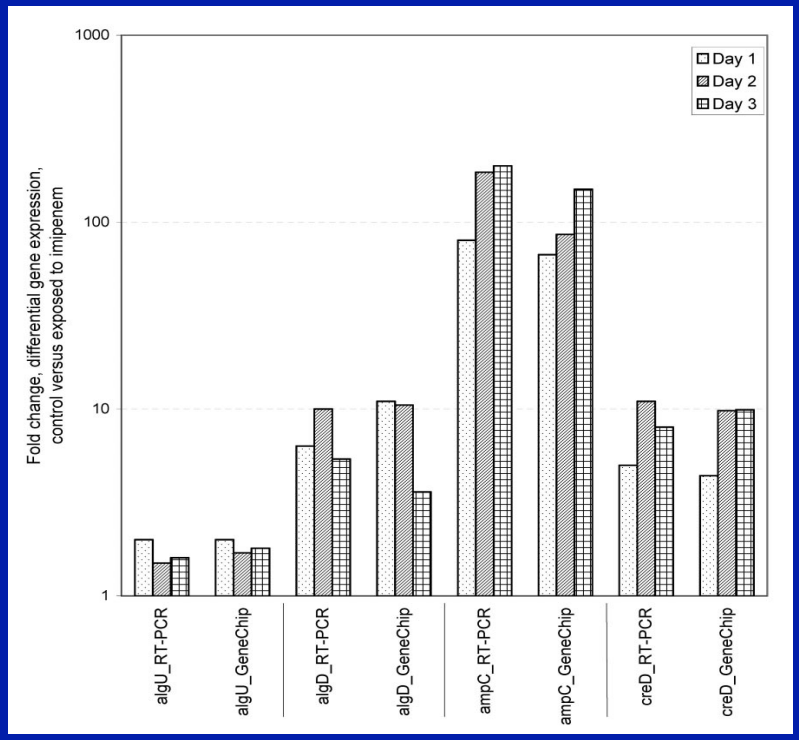


MRSA exposed to antibiotics at concentrations of 64 µg/ml. Each box plot represents the spread of cell survival across the different clinical isolates; error bars are the standard deviation

Smith K Int J Antimicrob Agents 2009;33:374–378

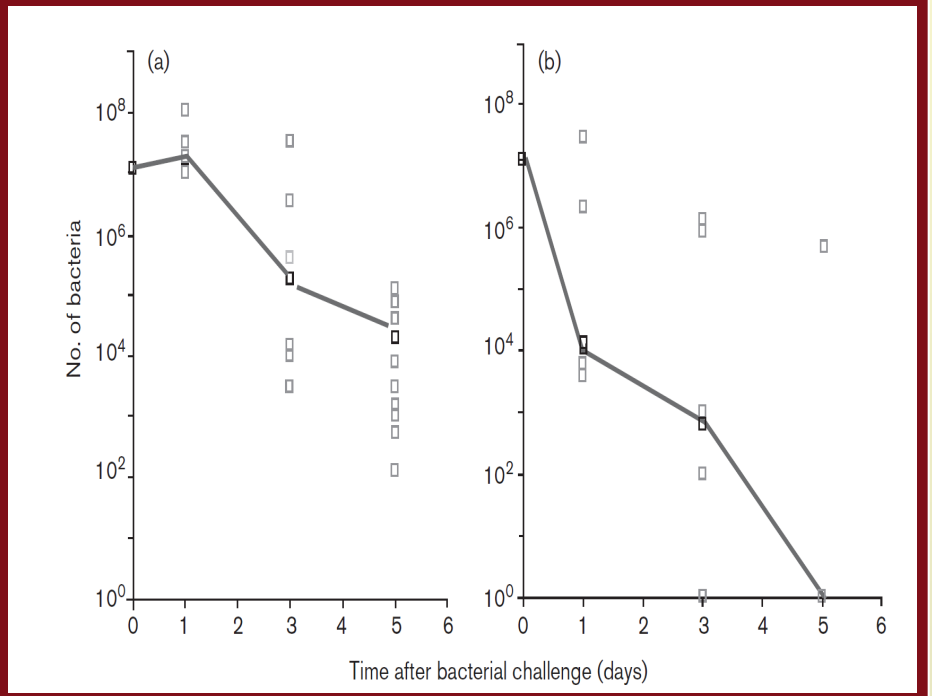
Biofilms

Pseudomonas aeruginosa gene induction in biofilms by subinhibitory concentrations of **imipenem**

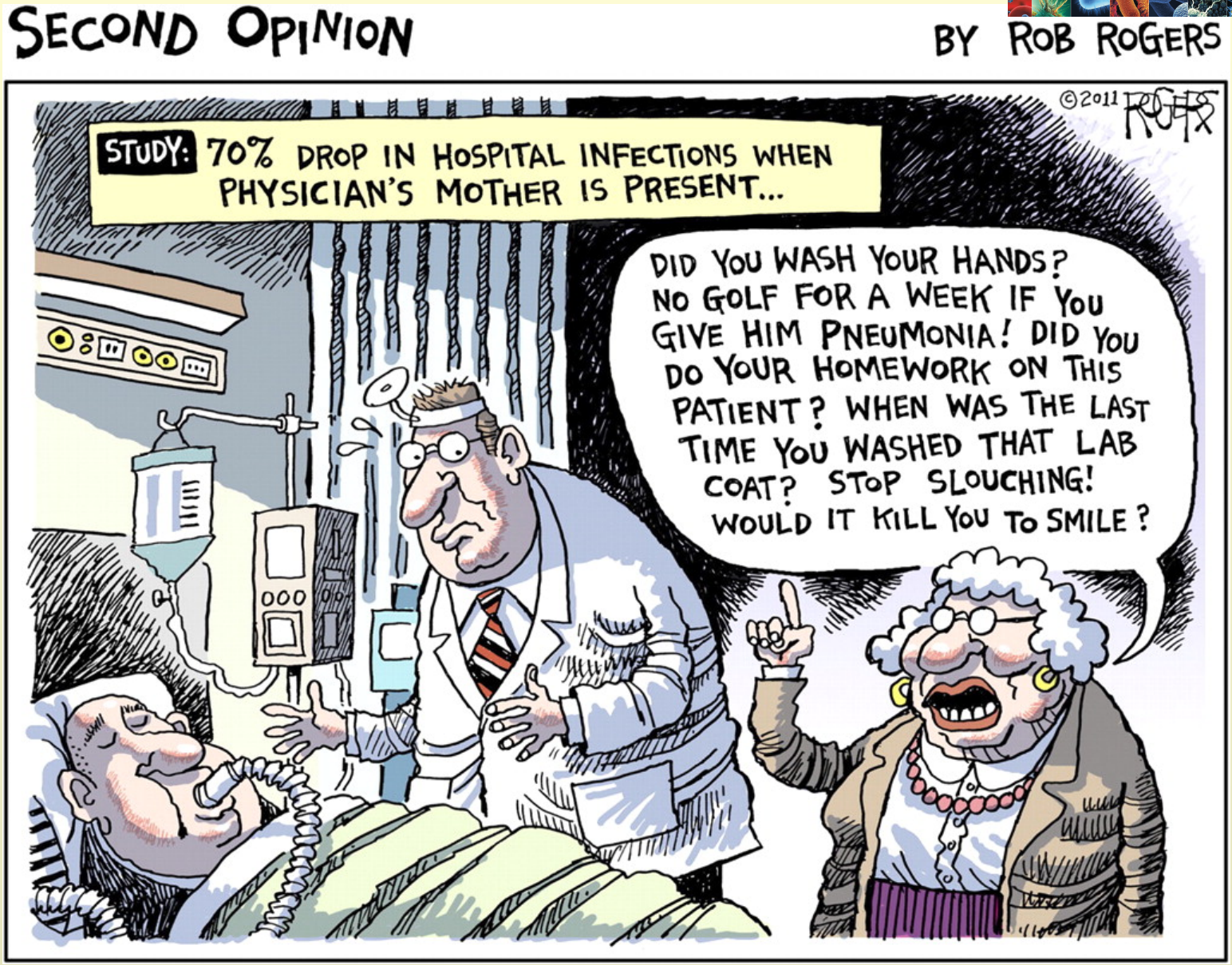


Bagge Antimicrob Agents Chemother 2004;48:1175

Garlic inhibits *Pseudomonas aeruginosa* biofilms in a pneumonic mice model



Bjarnsholt Microbiology 2005;151:3873



Rogers R Chest 2011;139:980-980