

**Turkish Clinical Microbiology and Infectious Diseases Congress (KLIMIK 2019)**

Gloria Golf Resort Hotel Belek, Antalya

# **Pathogenesis of Gram positive Bacterial Infections - Optimizing Treatment**

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Zurich<sup>UZH</sup>**



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# The top 10 causes of death in 2019

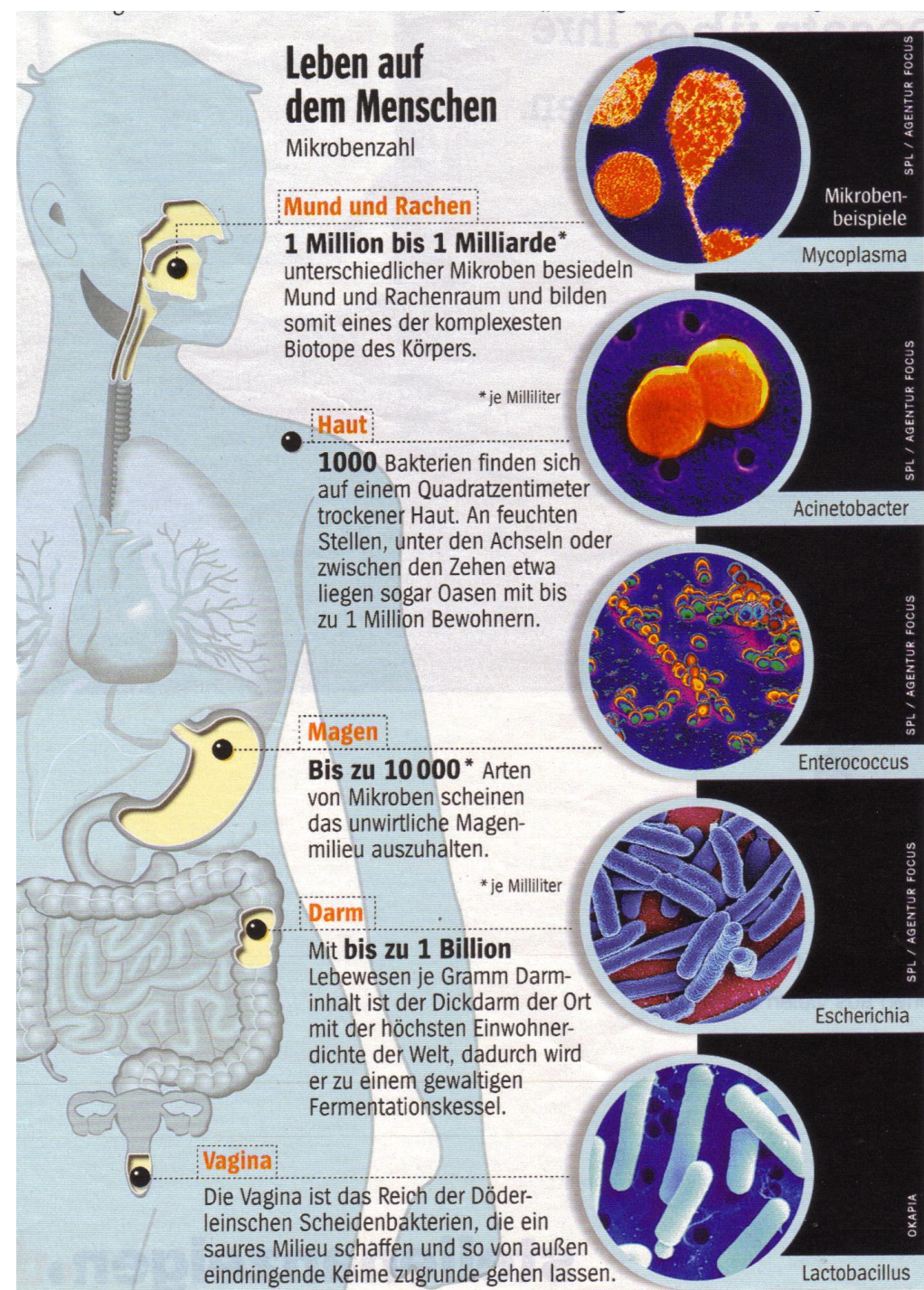
6 out of 10 infectious diseases

- Global influenza pandemic
- Antimicrobial resistance
- Ebola and other high-threat pathogens
- Vaccine hesitancy
- Dengue
- HIV

<https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death>

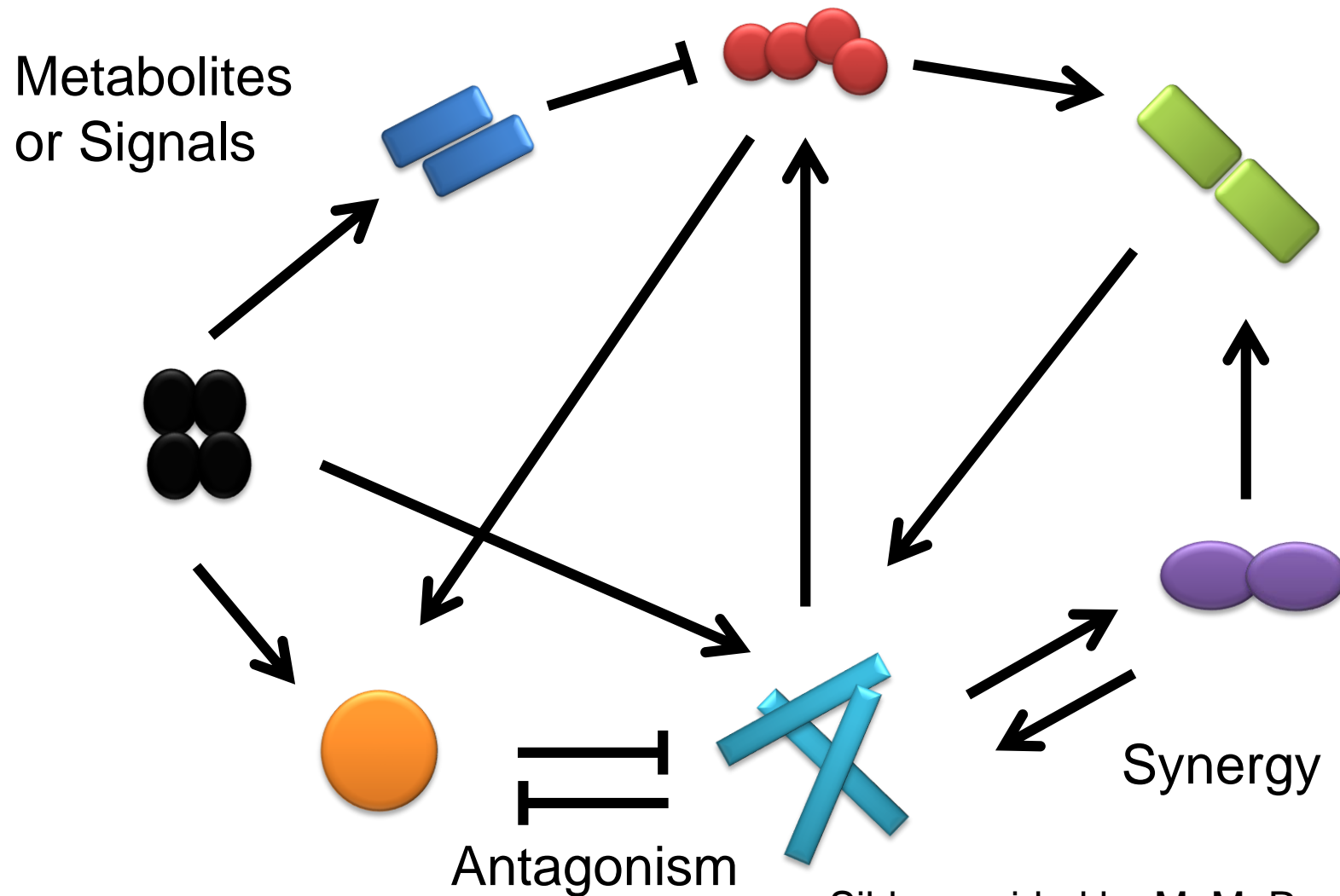


# Bacteria - Host



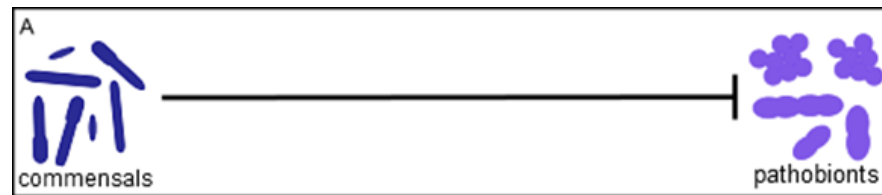
# Commensals can prevent (nasal) colonization of pathobionts

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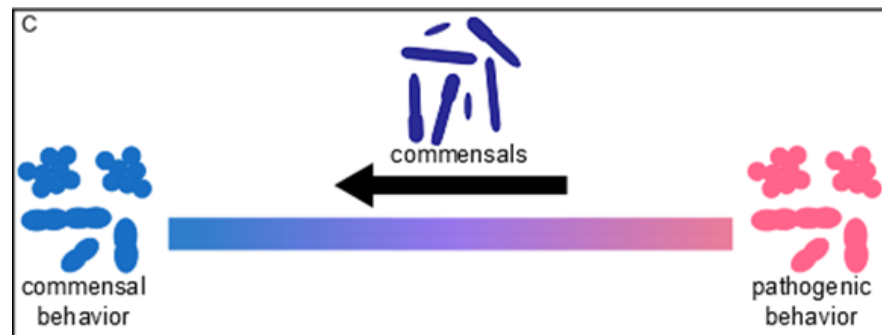


# Commensal – pathobiont - interactions

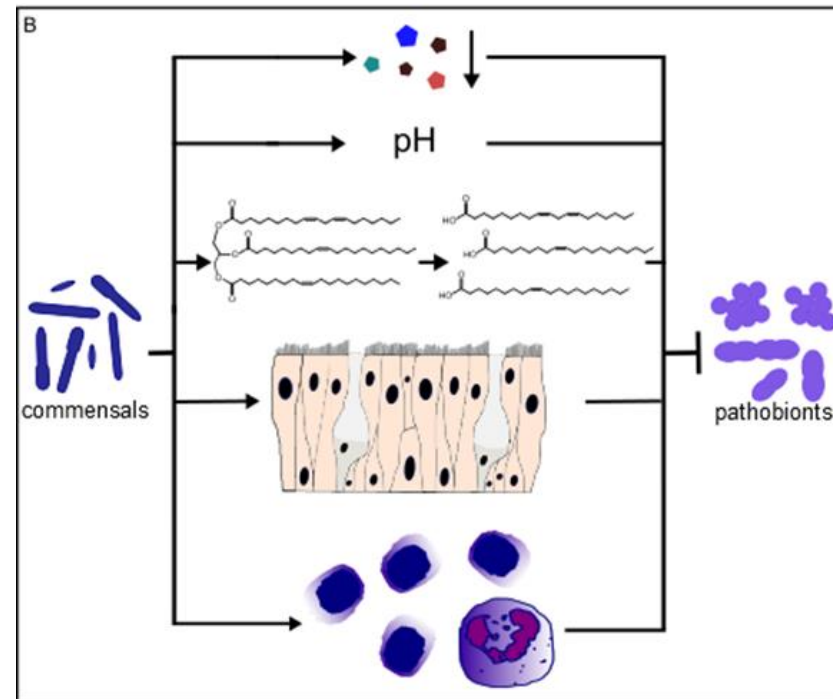
direct inhibition



behavior shift



indirect inhibition



Zipperer, Nature, 2016, Bomar, mBio, 2016, Ramsey, FrontMicro, 2016

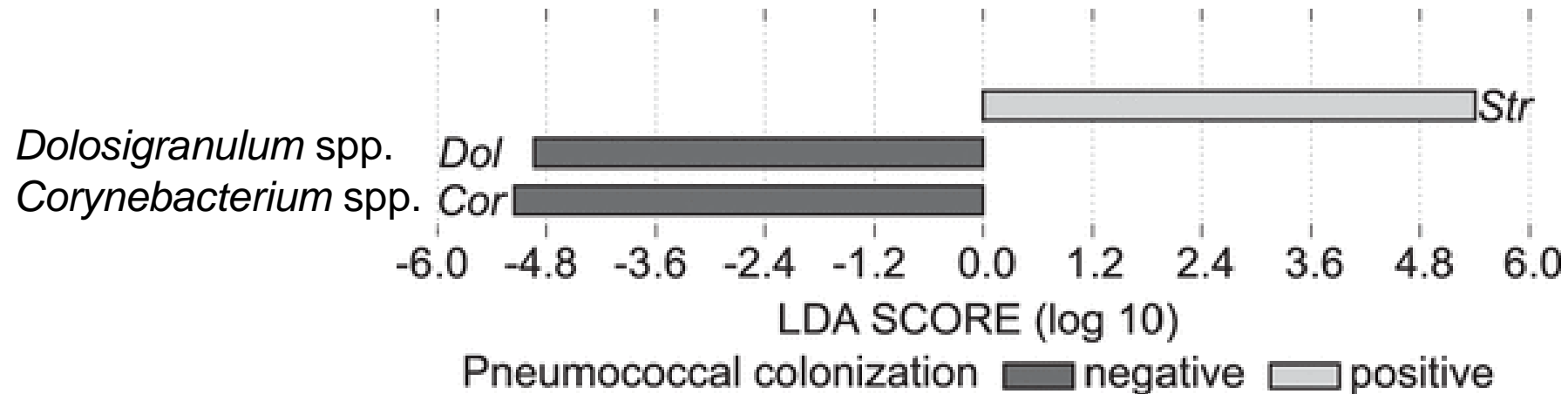
Brugger, Bomar, Lemon, PLoSPath, 2016



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# *Corynebacterium accolens* Releases Antipneumococcal Free Fatty Acids from Human Nostril and Skin Surface Triacylglycerols

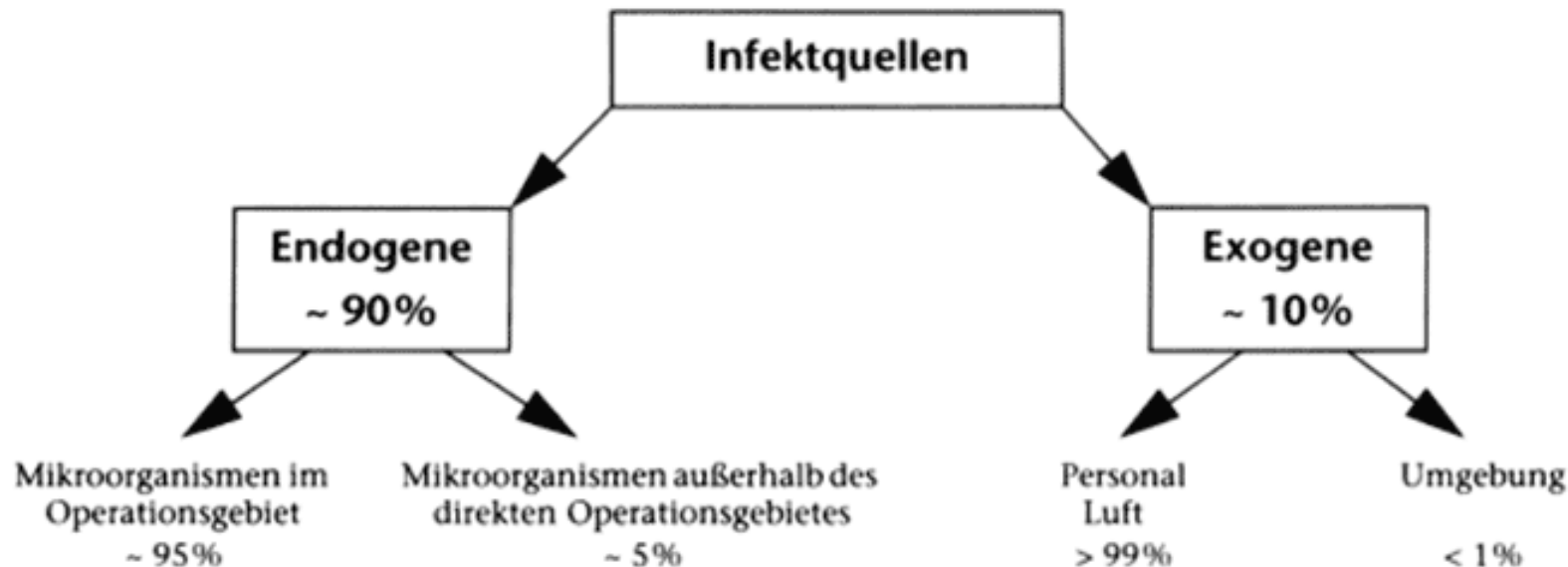
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# Commensales Misbehaving



Erreger	Total n = 28'451
Staphylococcus aureus	18 %
Staphylococcus, koagulase-negative	13 %
Enterococcus spp	12 %
Escherichia coli	9 %

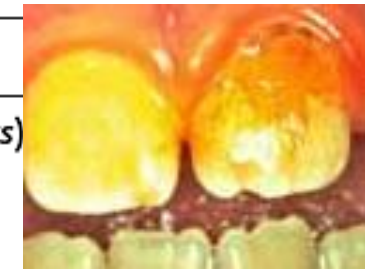


Susi, 8yr

**Table 1.** Partial list of human infections involving biofilms.

Plaque

Infection or disease	Common biofilm bacterial species
Dental caries	Acidogenic Gram-positive cocci (e.g., <i>Streptococcus</i> )
Periodontitis	Gram-negative anaerobic oral bacteria
Otitis media	Nontypable strains of <i>Haemophilus influenzae</i>
Musculoskeletal infections	Gram-positive cocci (e.g., staphylococci)
Necrotizing fasciitis	Group A streptococci
Biliary tract infection	Enteric bacteria (e.g., <i>Escherichia coli</i> )
Osteomyelitis	Various bacterial and fungal species—often mixed
Bacterial prostatitis	<i>E. coli</i> and other Gram-negative bacteria
Native valve endocarditis	Viridans group streptococci
Cystic fibrosis pneumonia	<i>P. aeruginosa</i> and <i>Burkholderia cepacia</i>
Meloidosis	<i>Pseudomonas pseudomallei</i>
Nosocomial infections	
ICU pneumonia	Gram-negative rods
Sutures	<i>Staphylococcus epidermidis</i> and <i>S. aureus</i>
Exit sites	<i>S. epidermidis</i> and <i>S. aureus</i>
Arteriovenous shunts	<i>S. epidermidis</i> and <i>S. aureus</i>
Scleral buckles	Gram-positive cocci
Contact lens	<i>P. aeruginosa</i> and Gram-positive cocci
Urinary catheter cystitis	<i>E. coli</i> and other Gram-negative rods
Peritoneal dialysis (CAPD) peritonitis	A variety of bacteria and fungi
IUDs	<i>Actinomyces israelii</i> and many others
Endotracheal tubes	A variety of bacteria and fungi
Hickman catheters	<i>S. epidermidis</i> and <i>C. albicans</i>
Central venous catheters	<i>S. epidermidis</i> and others
Mechanical heart valves	<i>S. aureus</i> and <i>S. epidermidis</i>
Vascular grafts	Gram-positive cocci
Biliary stent blockage	A variety of enteric bacteria and fungi
Orthopedic devices	<i>S. aureus</i> and <i>S. epidermidis</i>
Penile prostheses	<i>S. aureus</i> and <i>S. epidermidis</i>



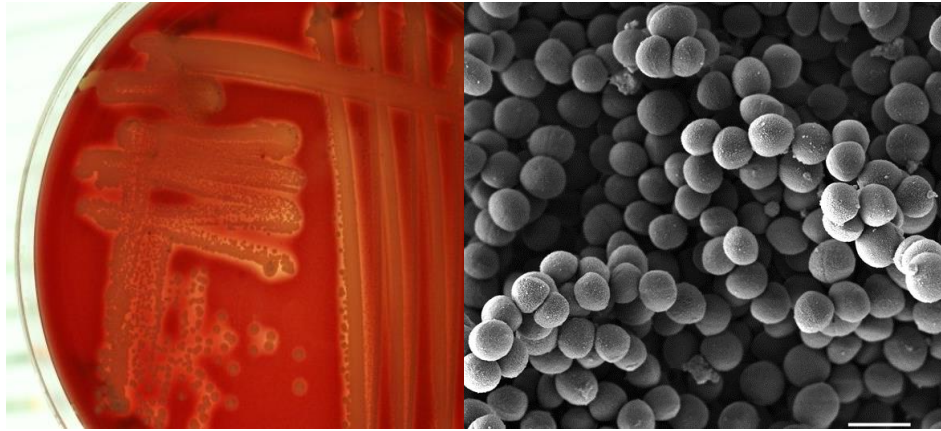
Science 1999;284:1318



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# ***Staphylococcus aureus*: a commensal**

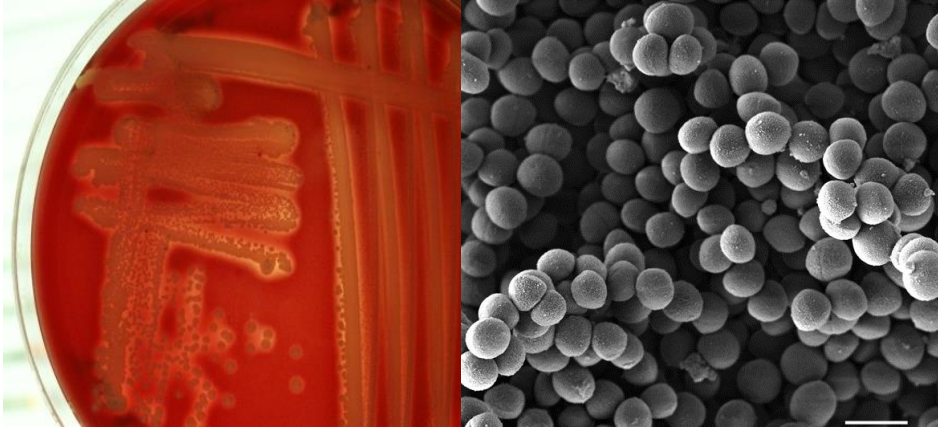
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- **Gram positive extracellular bacterium**
- **30% colonization**

# ***Staphylococcus aureus*: a commensal misbehaving**

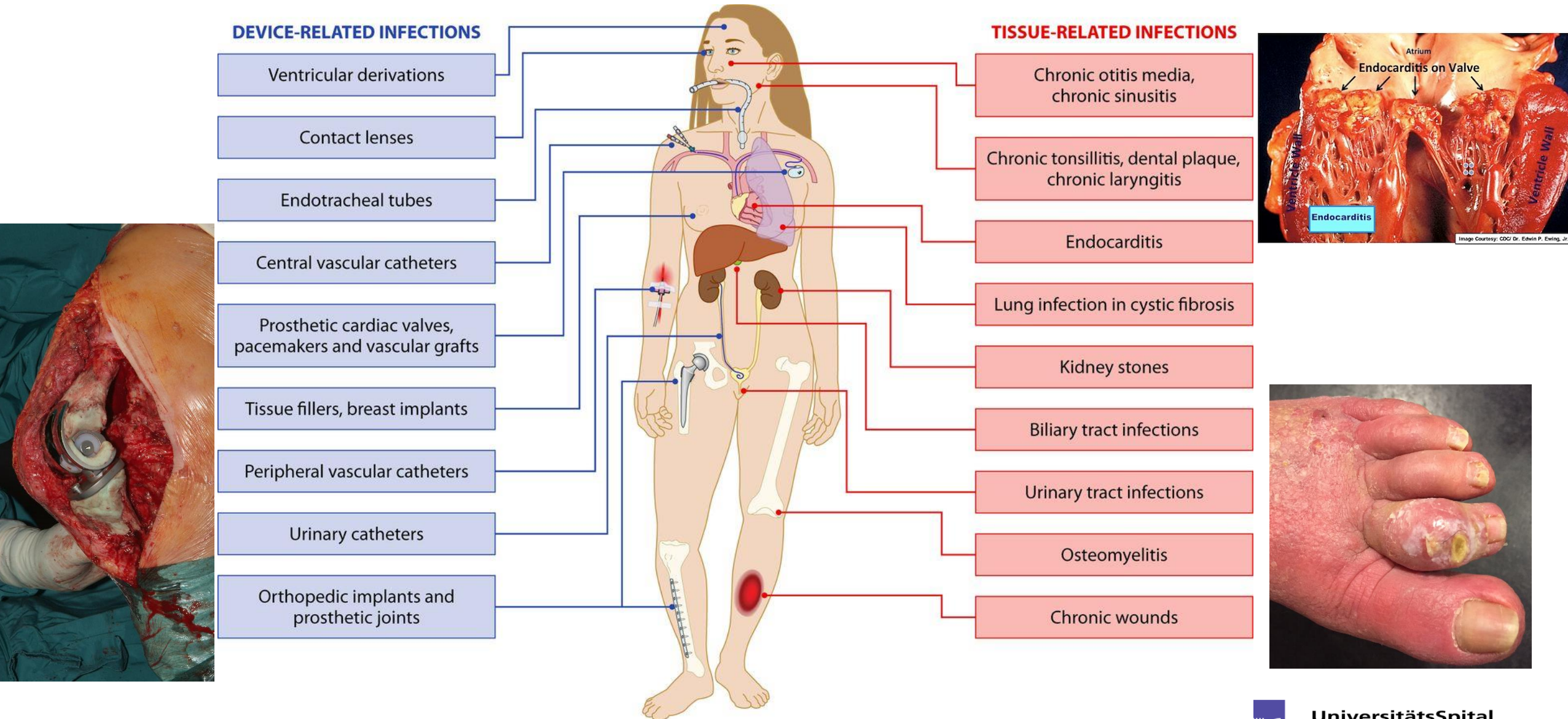
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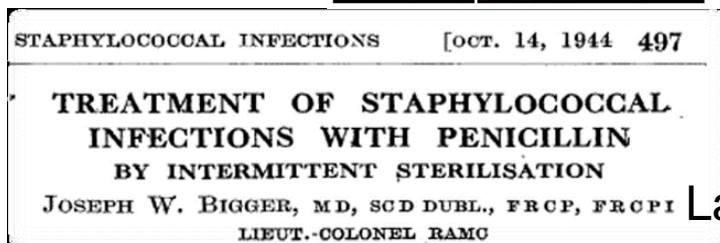
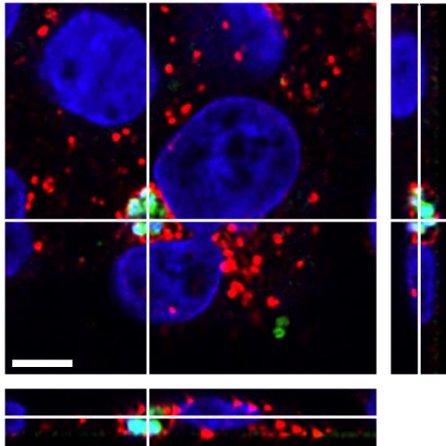
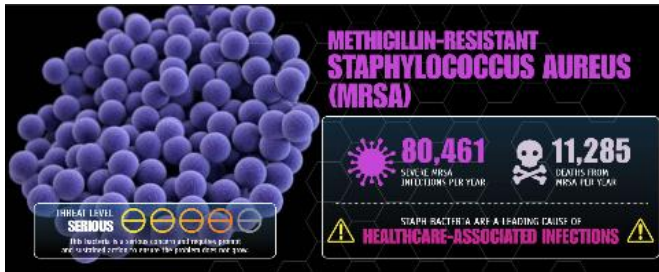
- **Gram positive extracellular bacterium**
- **30% colonization**
- **Increased risk for subsequent infection**
  - **Recurring skin infections – abscesses**
  - **Prosthetic joint infections**
  - **Endocarditis**



# Chronic –recurring infections - Biofilm-related infections



# How do bacteria withstand antibiotics?



Lancet

## Resistance - MRSA

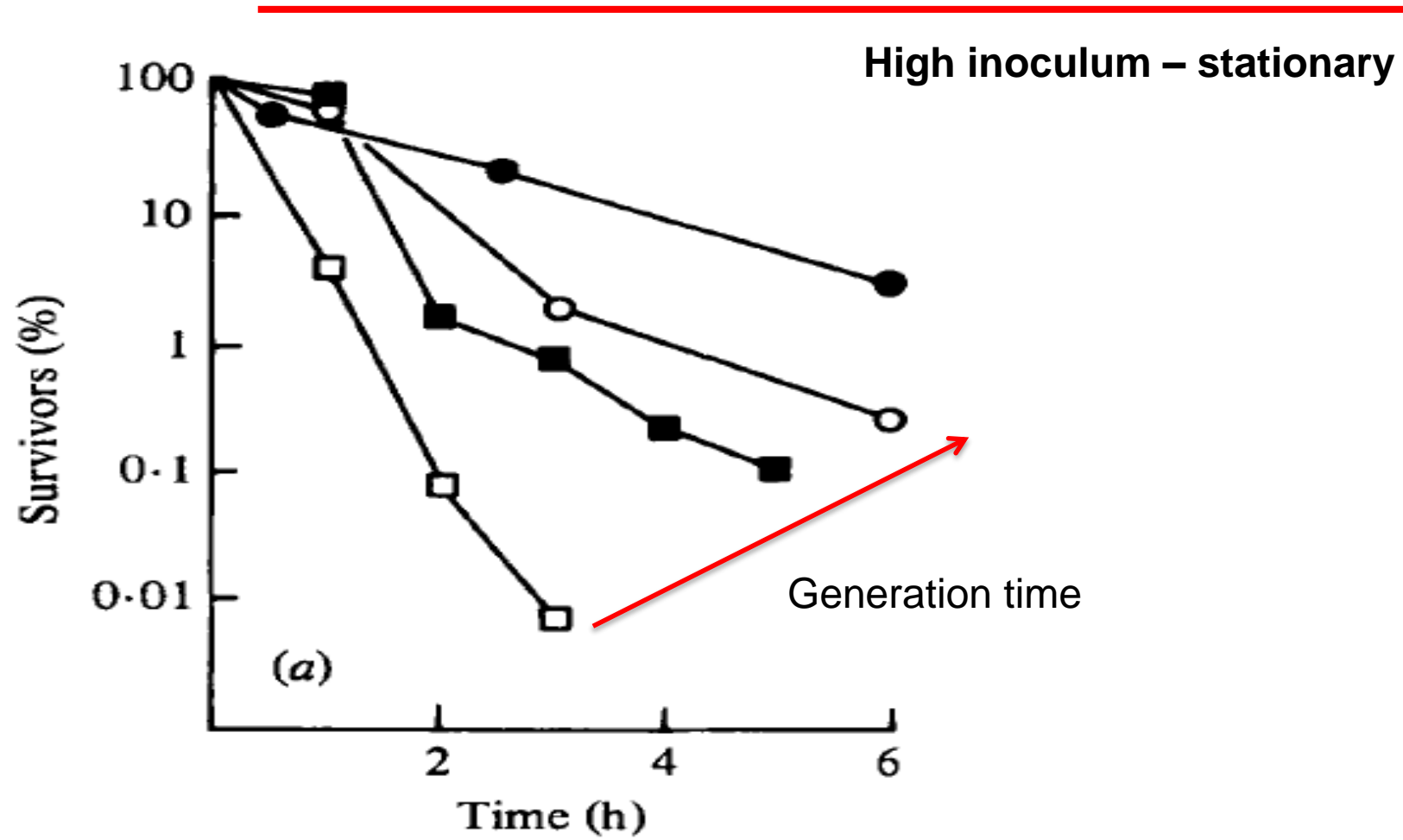
### Susceptible

- 1. 'Location':
  - in 'privileged' sites such as abscess, intracellular, biofilm
  - > AB do not reach bacteria, milieux
- 2. 'Growth'
  - Stationary bacteria
  - Persisters = metabolically inactive



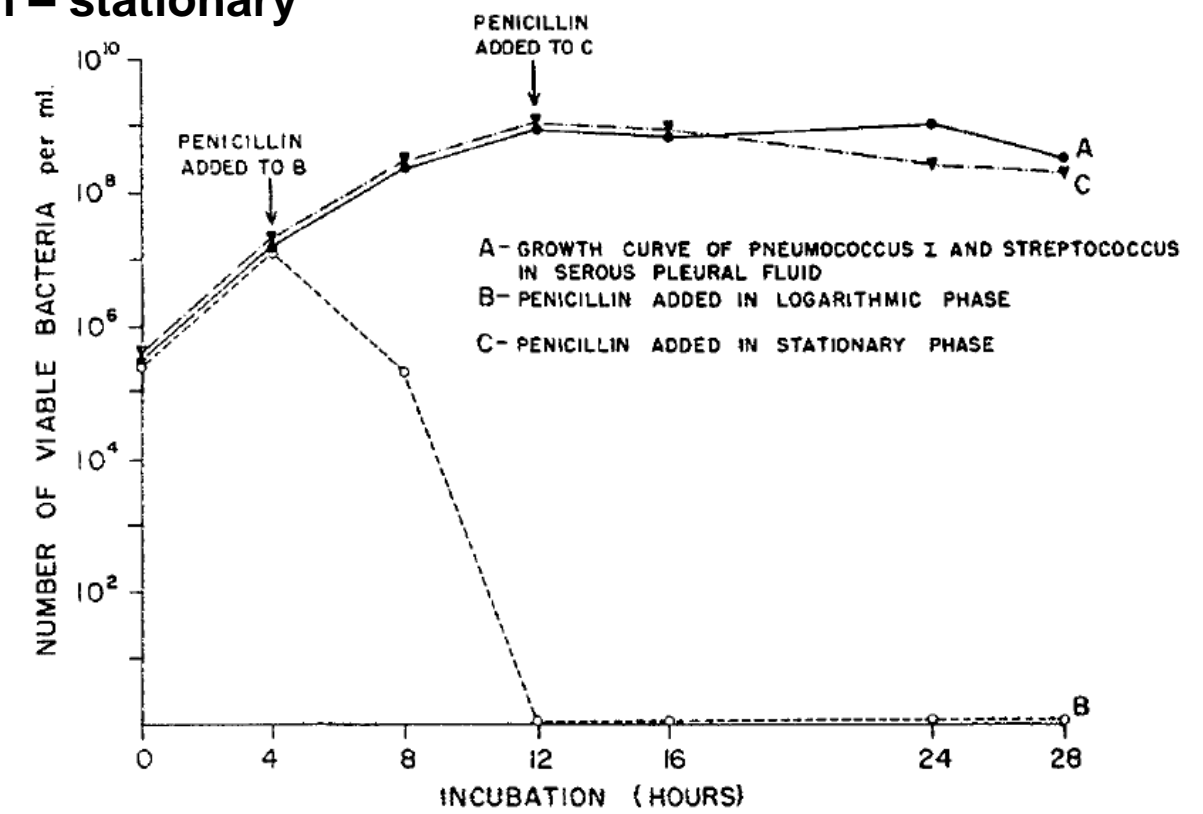
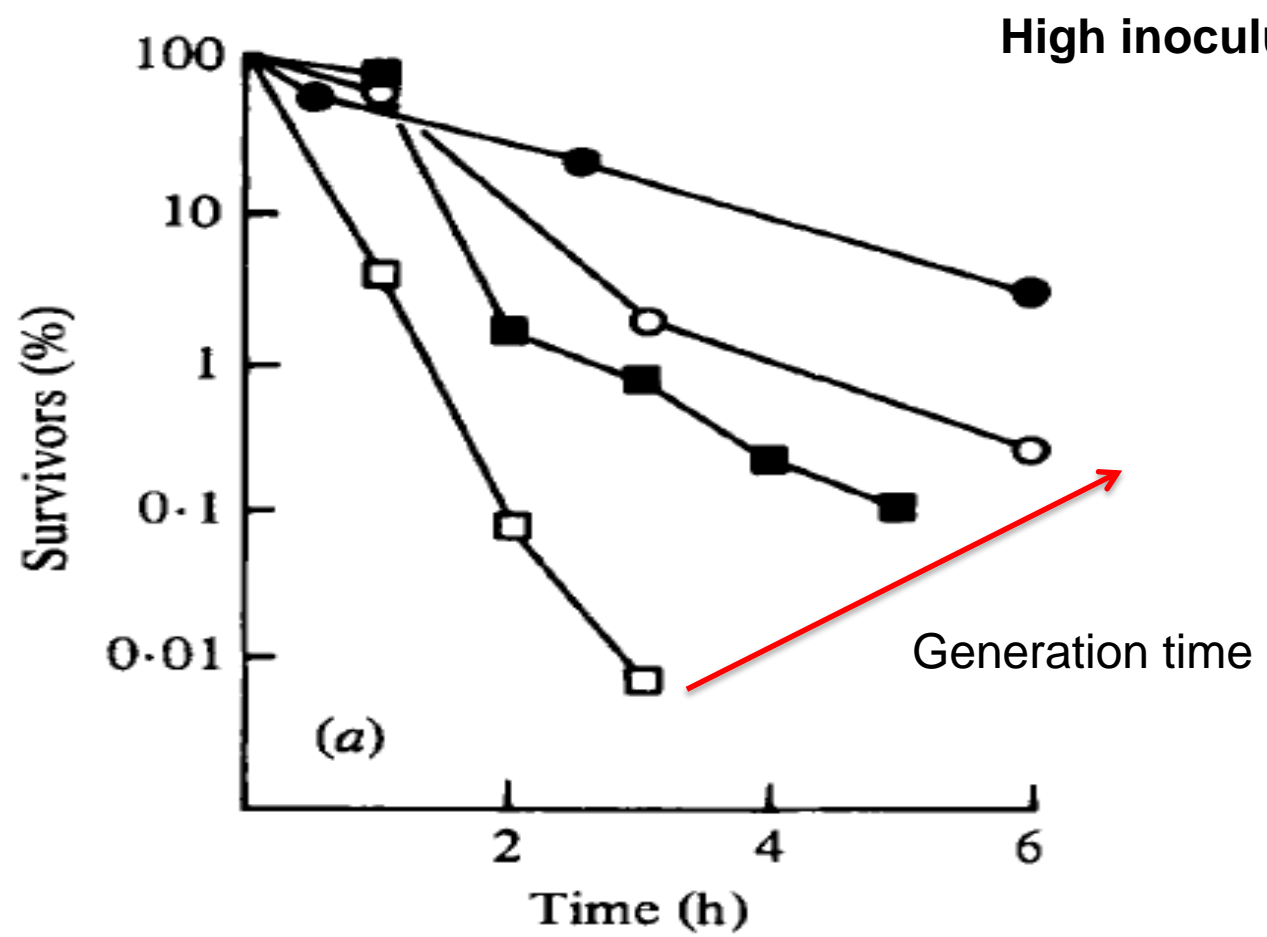
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# Rate of bacterial killing by beta-lactams is proportional to the bacterial growth rate



Tuomanen *et al*, J Gen Microbiology, 1986

# Rate of bacterial killing by beta-lactams is proportional to the bacterial growth rate



TEXT-FIG. 3. Action of penicillin on type I pneumococci and beta hemolytic streptococci contained in thin serous fluid collected from pleural cavities of rats with experimental streptococcal pneumonia. The pneumococci were added to the fluid at the start of each experiment.

Tuomanen *et al*, J Gen Microbiology, 1986

# RELATION OF THE SIZE OF THE INOCULUM AND THE AGE OF THE INFECTION TO THE CURATIVE DOSE OF PENICILLIN IN EXPERIMENTAL SYPHILIS, WITH PARTICULAR REFERENCE TO THE FEASIBILITY OF ITS PROPHYLACTIC USE

By HARRY EAGLE, M.D., H. J. MAGNUSON, M.D., AND RALPH FLEISCHMAN

(From the Laboratory of Experimental Therapeutics of the United States Public Health Service and The Johns Hopkins School of Hygiene, Baltimore)

Rabbits were inoculated intratesticularly with 2,000 spirochetes. thereafter, penicillin was given as a single intramuscular injection of a oil and beeswax.

Time when penicillin was administered after inoculation	Penicillin dosage	No. rabbits tested	Developed syphilitic lesion despite penicillin	Results of lymph node transfer on animals apparently protected		No. of animals protected	Animals protected*	Protective dose of penicillin	
				No. tested	Infectious			PD <sub>50</sub> (50 per cent of animals protected)	PD <sub>90</sub> (90 per cent of animals protected)
	units/kg.						per cent	units/kg.	units/kg.
4 hrs.	16,000	6	0	6	0	6	100	1,500	3,500
	8,000	6	0	5	0	6	100		
	4,000	6	0	4	0	6	100		
	2,000	6	2	2	0	4	67 (71)		
	1,000	4	3	1	0	3	25 (17)		
4 days	16,000	5	0	4	0	5	100	2,000	3,500
	8,000	5	0	5	0	5	100		
	4,000	6	0	5	0	6	100		
	2,000	5	3	2	0	2	40		
	1,000	4	4	—	—	0	0		
2 wks.	64,000	6	0	6	0	6	100	14,000	50,000
	32,000	6	3	3	0	3	50 (77)		
	16,000	5	1	4	0	4	80 (64)		
	8,000	6	4	2	0	2	33 (20)		
	4,000	5	4	1	1‡	0	0		
6 wks.§	160,000	5	1	3	0	4	80 (89)	65,000	160,000
	80,000	5	0	5	1	4	80 (67)		
	40,000	5	1	4	4	0	0		
	20,000	5	2	3	3	0	0		
	10,000	6	0	6	6	0	0		

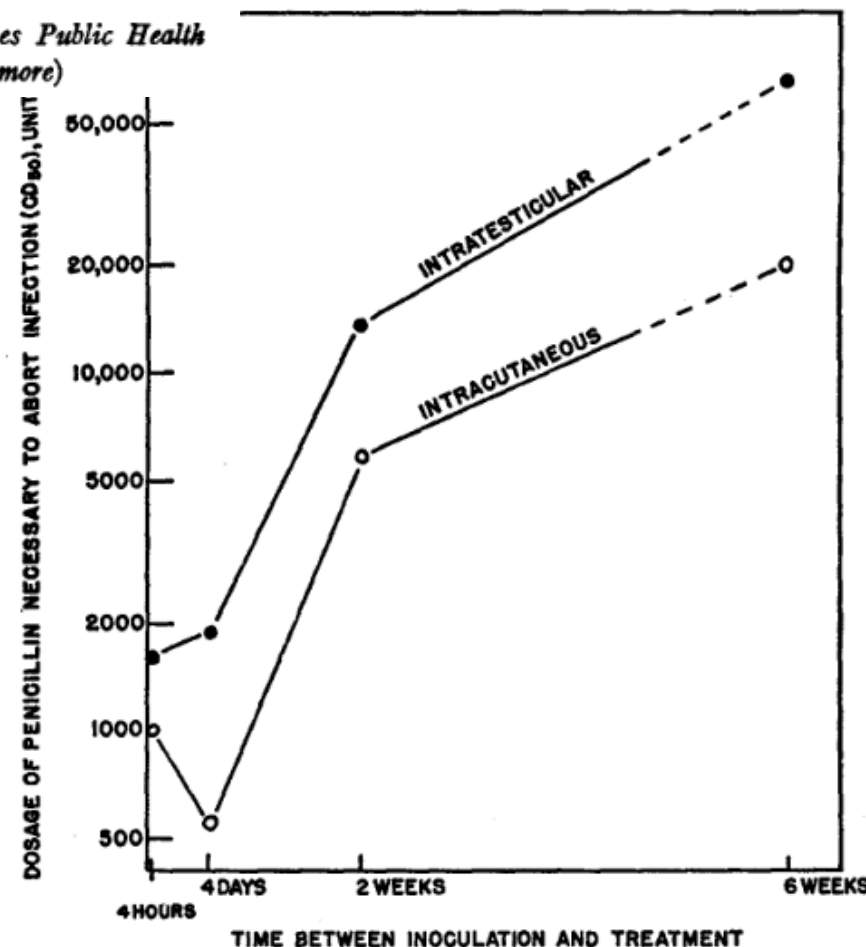


FIG. 2. Relation of the age of the infection to the curative (abortive) dose of penicillin. Rabbits were inoculated intracutaneously or intratesticularly with  $2 \times 10^3$  organisms. At varying periods after inoculation, penicillin was administered as a single intramuscular injection of a suspension in peanut oil and beeswax. The dashed portion of the curves indicates that the animals had developed darkfield-positive lesions by the 6th week, and that treatment at that time was curative rather than abortive.

1947

TABLE II  
Effect of the Size of the Inoculum on the Curative Dose of Penicillin G in White Mice Infected with a Group B  $\beta$ -Hemolytic Streptococcus\*

	No. of organisms inoculated†	Penicillin	Survived	Died	Curative dose (CD <sub>50</sub> ) of penicillin G, $\pm$ standard error‡, §
		mg./kg.			mg./kg.
Group 1	2,235,000	2,048	20	0	424 $\pm$ 52
		1,024	18	2	
		512	9	11	
		256	7	13	
		128	1	19	
		0	0	10	
Group 2	180,000	1,024	20	0	339 $\pm$ 45
		512	14	6	
		256	5	15	
		128	0	20	
		64	1	19	
		32	1	19	
Group 3	1,750 (estimated)	0	0	10	139 $\pm$ 51**§
		256	19	1	
		128	7	13	
		64	2	18	
		32	1	19	
		16	5	15	
Group 4	17¶	0	1	9	2.8 $\pm$ 1.1
		64	19	1	
		32	15	5	
		16	18	2	
		8	11	9	
		4	14	6	
		2	11	9	
		1	7	13	
		0	1	9	

The mice (CFW strain) were inoculated intraperitoneally with an appropriate dilution of a 3 hour culture in blood-broth, and treated immediately with a single intramuscular injection of penicillin G in aqueous solution. The number of organisms indicated in the table is actually the number of bacterial clumps, determined by plate counts. The number of organisms per clump in the original culture averaged 2.0.



Fotonin

Curative dose of penicillin increases with the size of the inoculum, and increases also with the age of the infection (paradoxical more-drug-kills-less Eagle effect)

UBI PUS IBI EVACUA

# Chronic –recurring infections

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## **Surgery: Scars- morbidity -mortality**

- **Removal of infected tissue -foreign body**

» Therapy of *Staphylococcus aureus* Bacteremia Associated with a Removable Focus of Infection, PAUL B. IANNINI, M.D.; KENT CROSSLEY, M.D. 1976

## **Antibiotics: Long treatment duration, i.v.**

- **Endocarditis: >4 weeks**

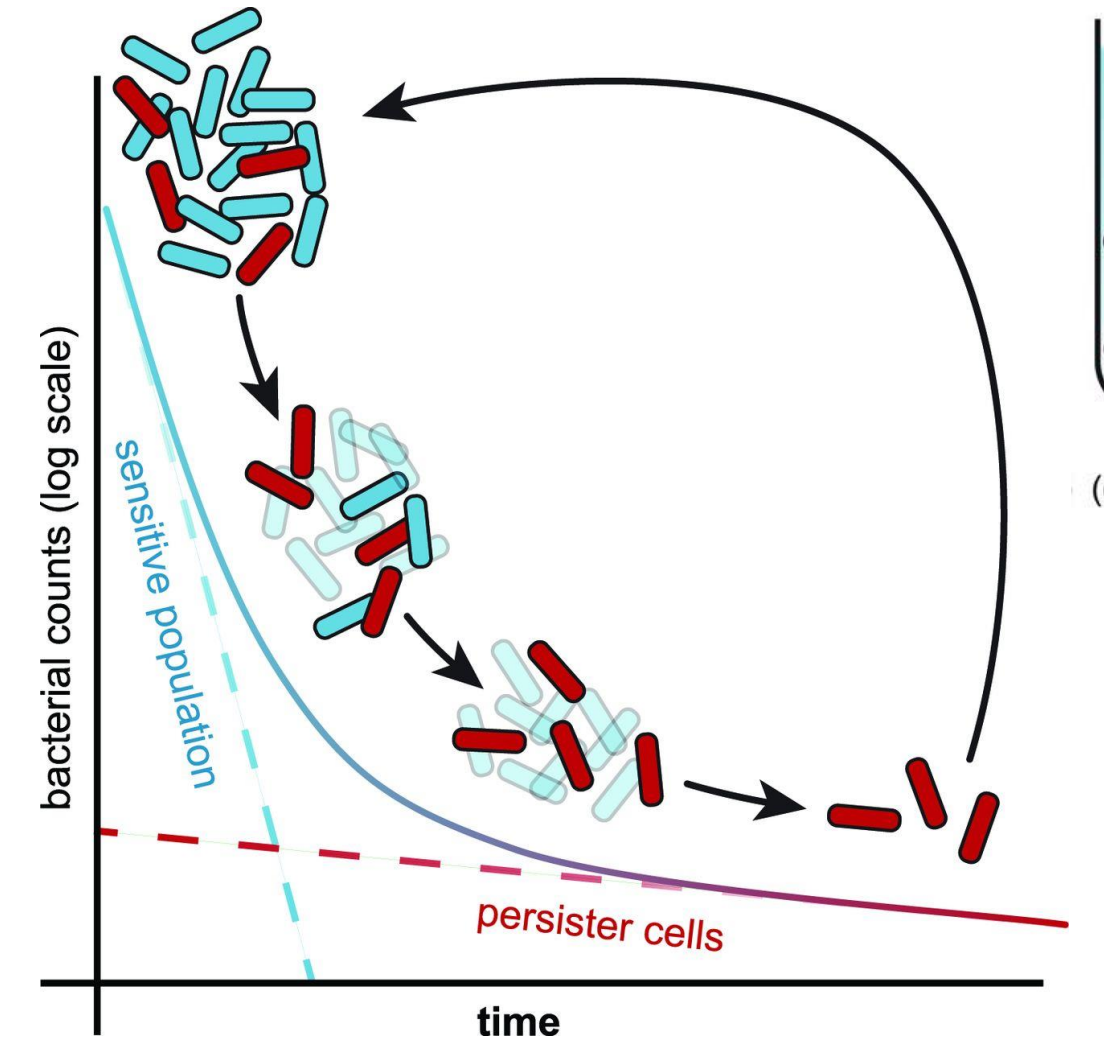
- 1943: Mortality 100% -now with antibiotic therapy 30%

- **Osteomyelitis/ Orthopedic implant associated infections: 6-12 weeks**

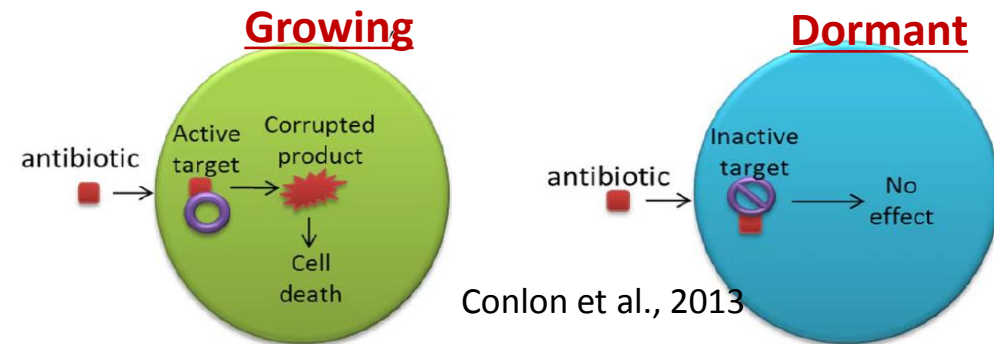
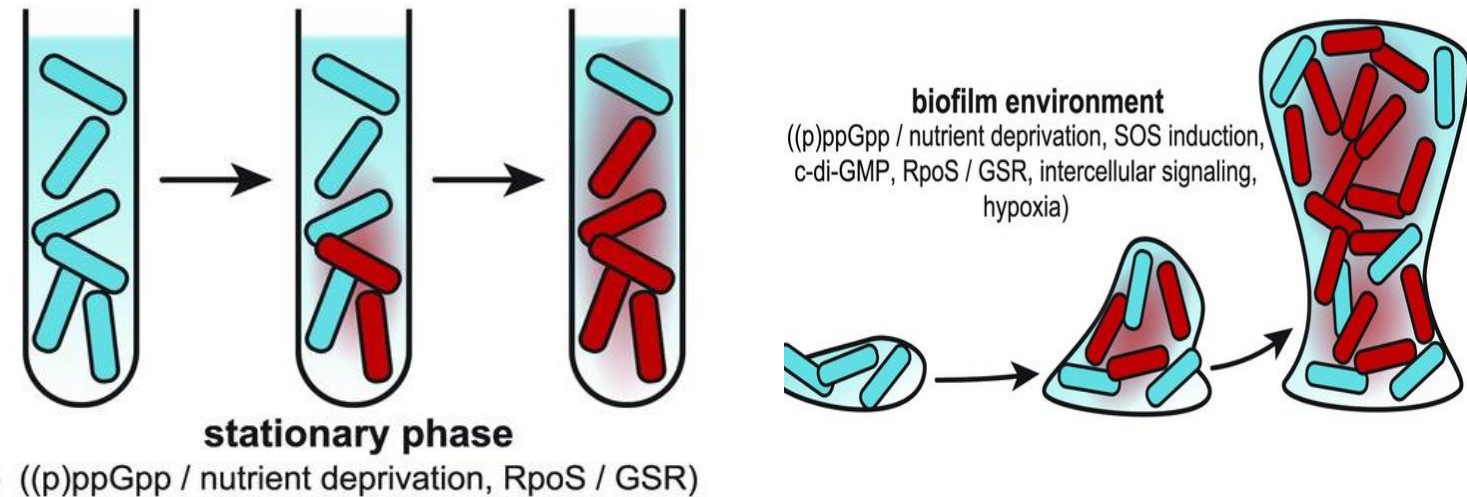
**? How long is long enough? What is most effective?**



# Persisters = metabolically inactive bacteria



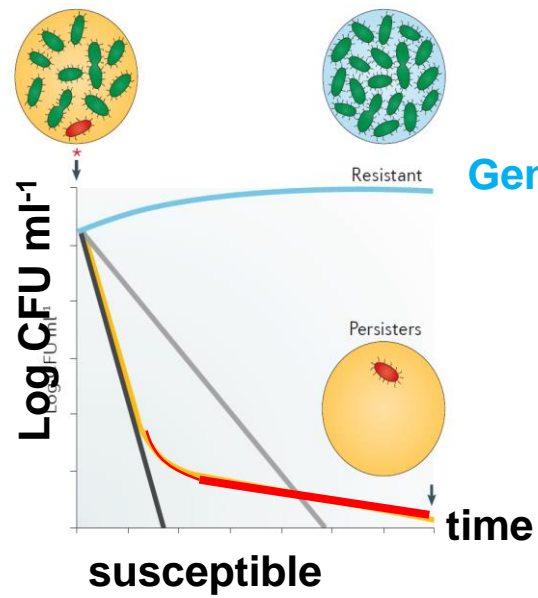
Biphasic killing kinetics of bactericidal antibiotic treatment.



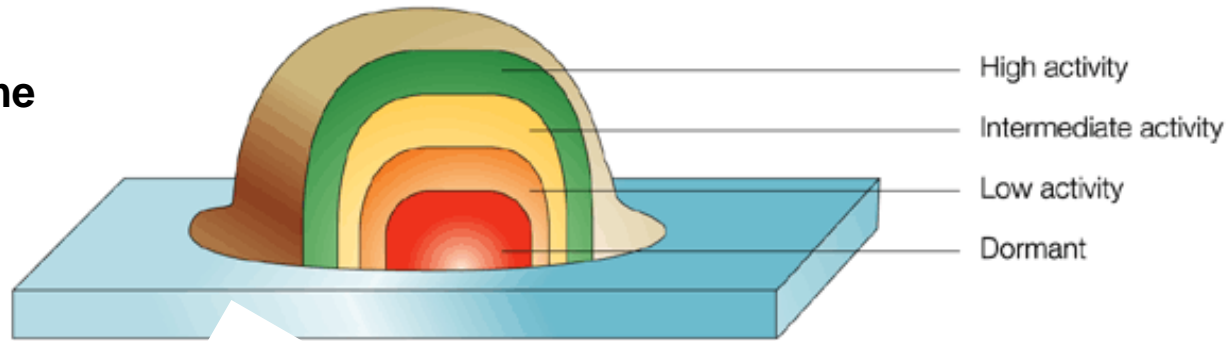
*E.coli*, *Tbc*, *S.aureus*, *Salmonella* ssp.

**Stressors:** reactive oxygen species (ROS), lack in nutrients, low pH, antibiotics

# Persisters



Genetic Resistant



Phenotypic Resistance

Persistenz, Toleranz

Natural Resistance

outer Membrane Gram-

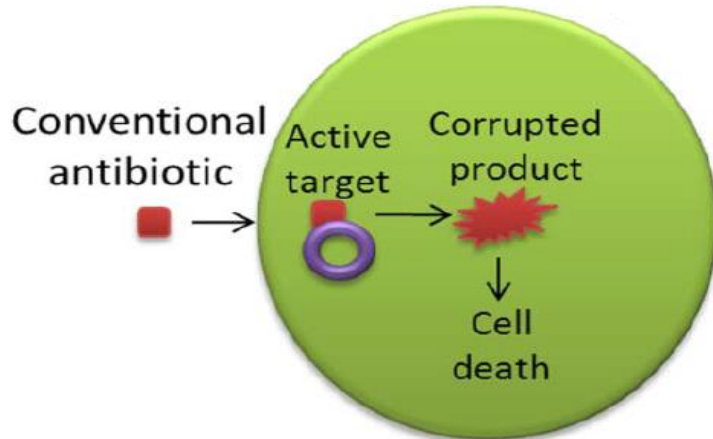
Genetic Resistance

Mutations

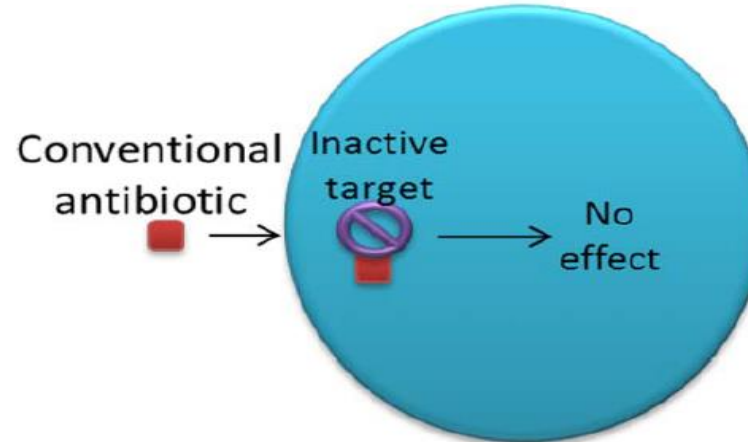
Effluxpumps upregulated

Modified enzymes

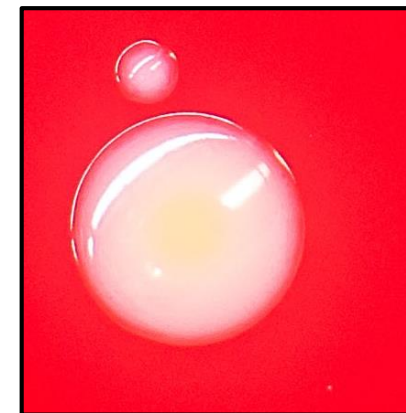
Growing



Dormant



**Growth – No Growth**

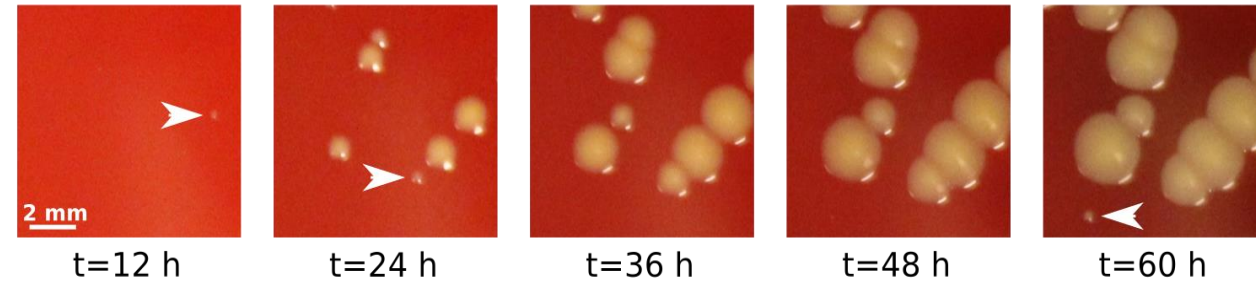
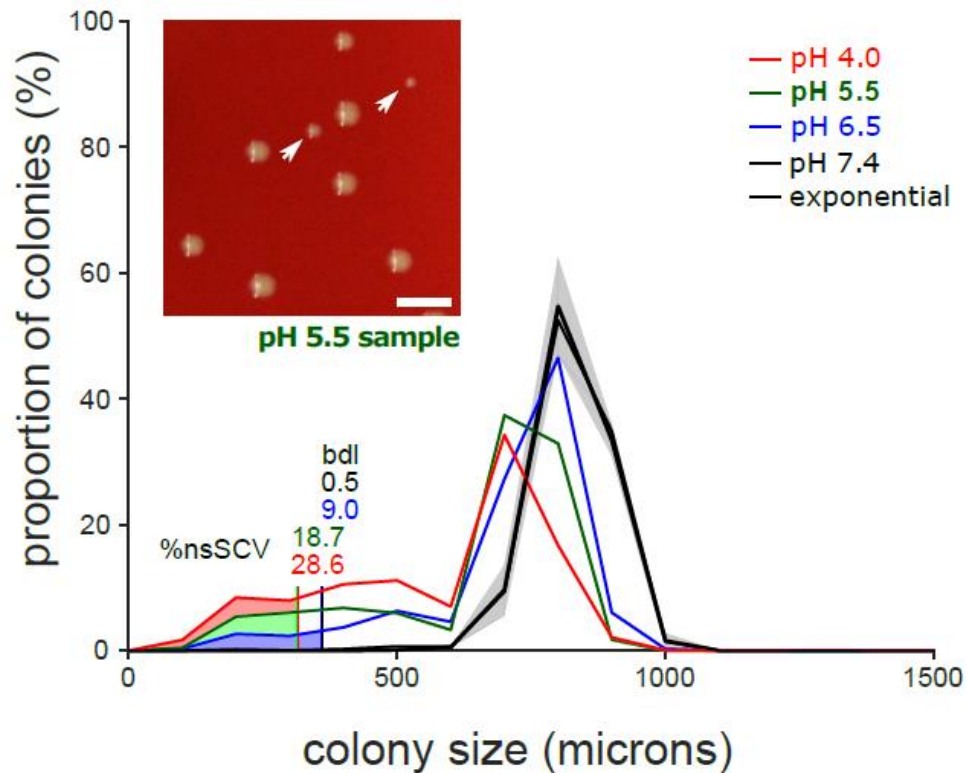
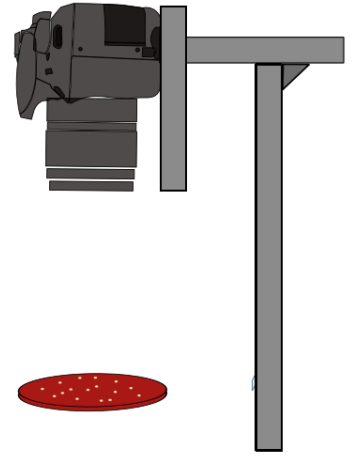


- **Indispensable feature for recurrent infections**

- In contrast to stable SCV - genetically determined

- Electron transport-defective SCVs, auxotroph for hemin, menadione, thymidine (hemB, menD, thyD)

# Heterogenous colony size



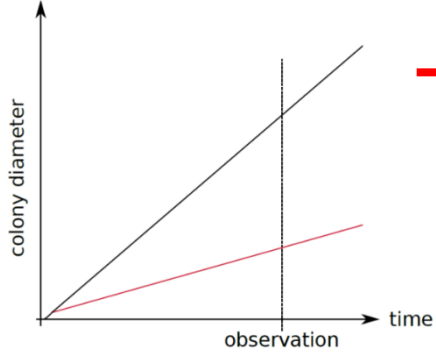
Long tailed size distribution

- semi-automated colony growth analysis
- analyzing time lapse movies with MATLAB for growth rate and lag-time

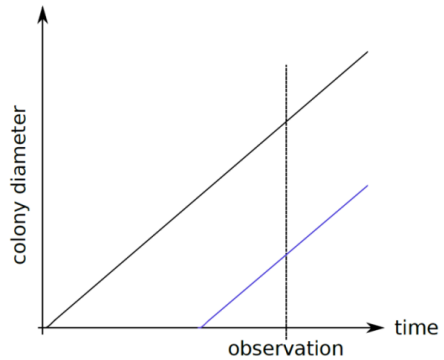


# Heterogeneity?

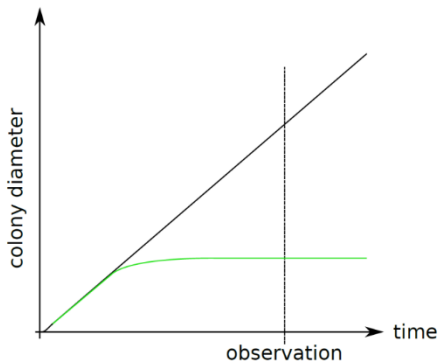
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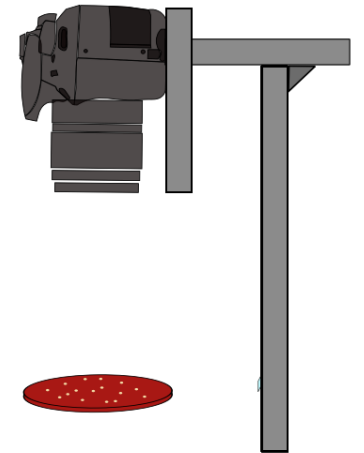
1. Difference in **growth rate**? Do they grow slower?



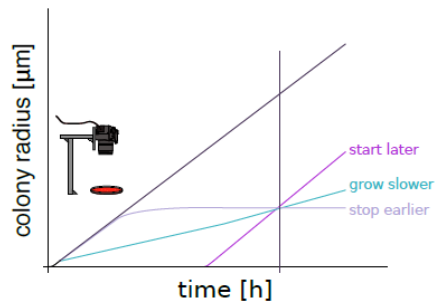
2. Difference in **growth start**? Do they start later?



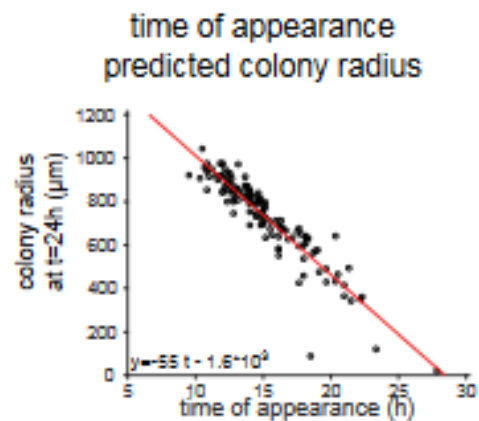
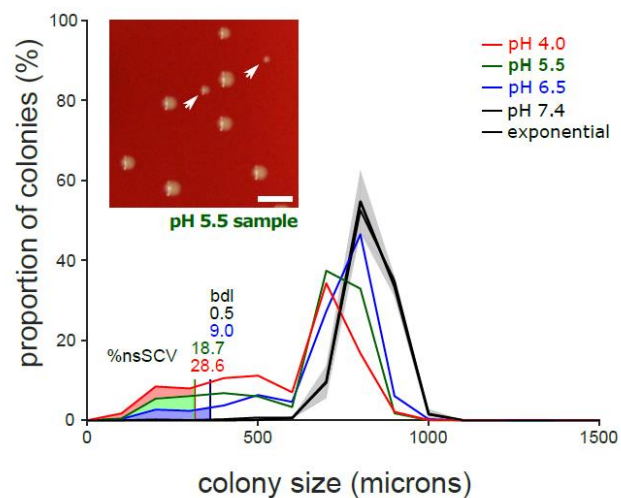
3. Difference in **growth end**? Do they stop growing?



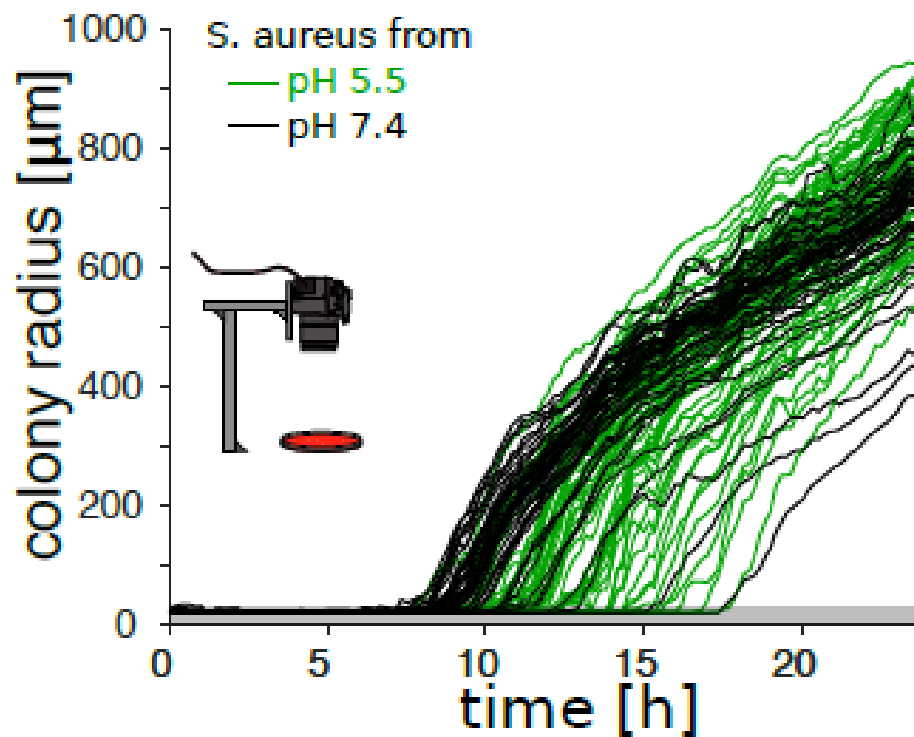
A

kinetic models for  
nsSCV phenotype

# SCV formation is a consequence of a late emergence of colonies



colony growth curves  
from liquid culture



Nonstable SCVs  
are bacteria with  
long lag

Nature Communications 2018

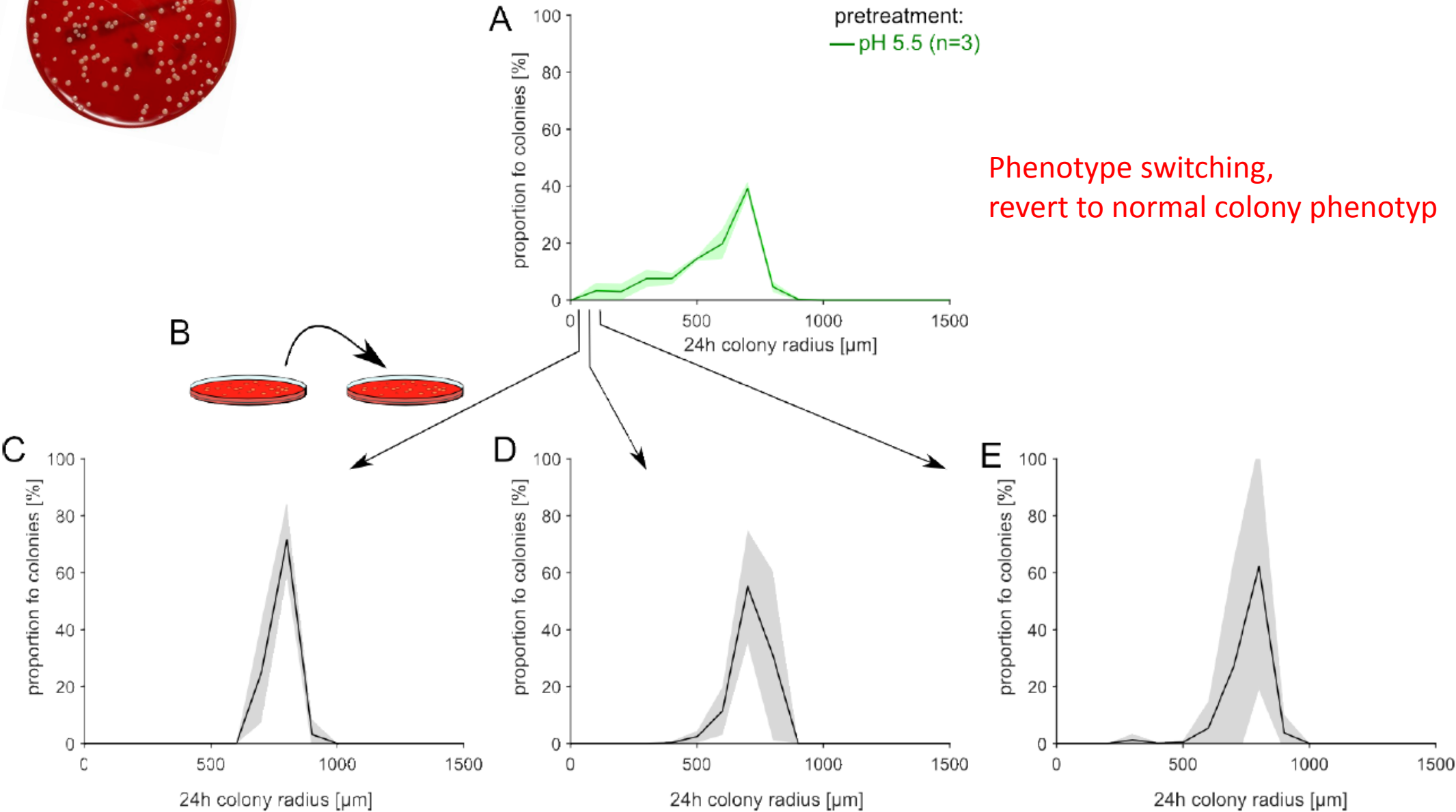


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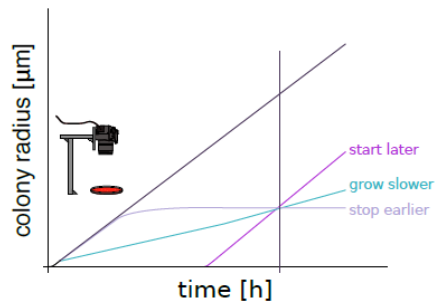


# Replating small colonies on agar plates

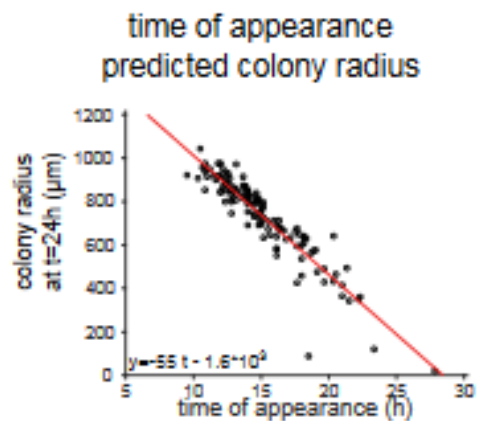
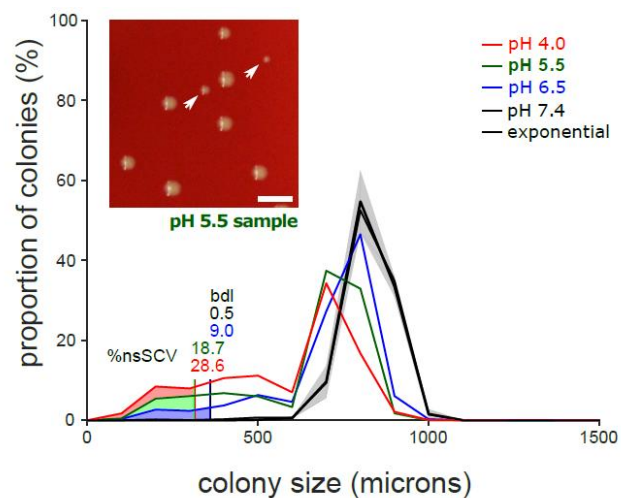
Phenotype switching,  
revert to normal colony phenotyp



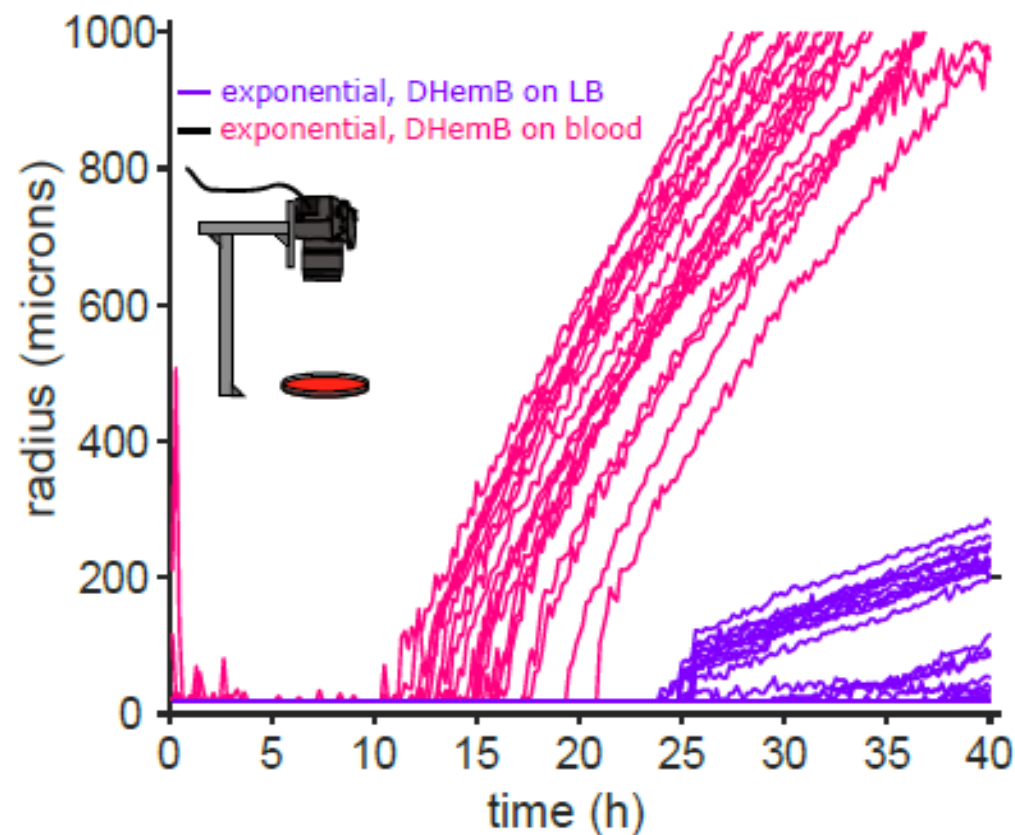
A

kinetic models for  
nsSCV phenotype

# SCV formation is a consequence of a late emergence of colonies

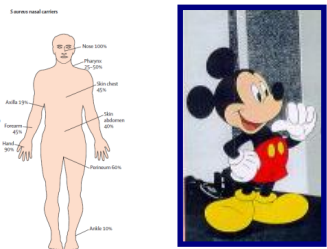


auxotroph for hemin

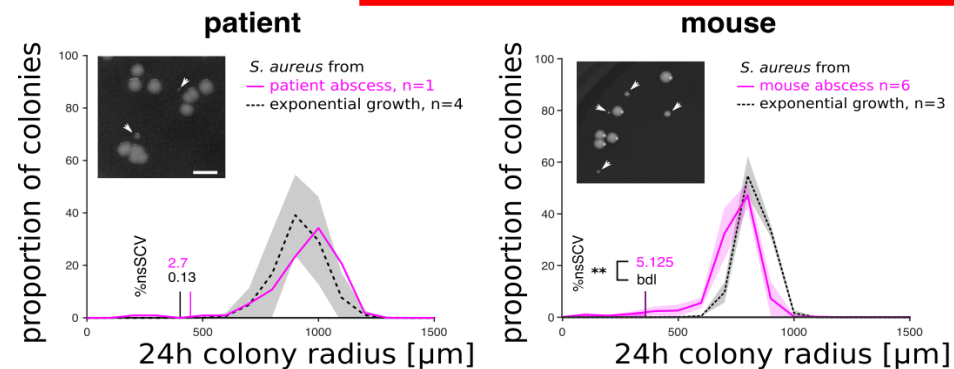


Stable SCVs  
are bacteria  
which  
grow slower

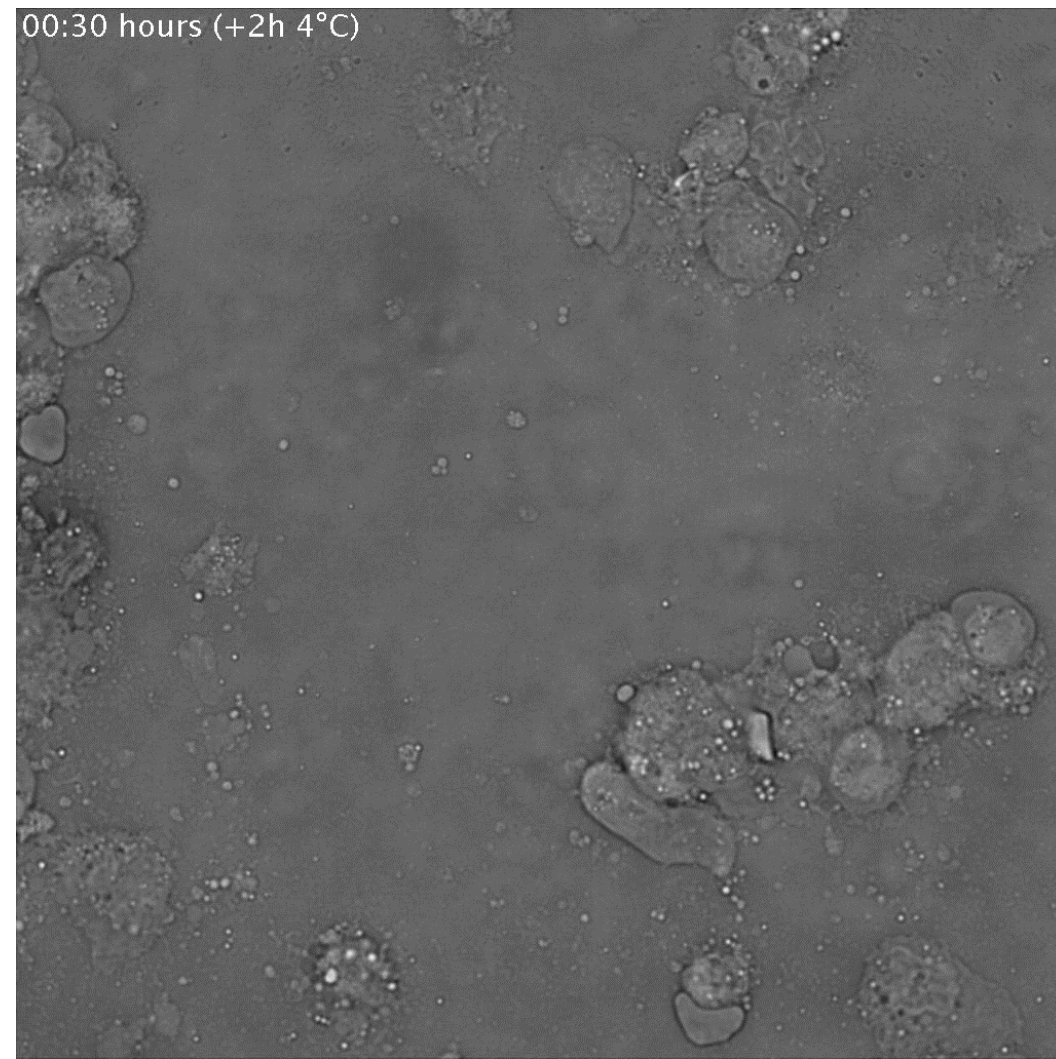




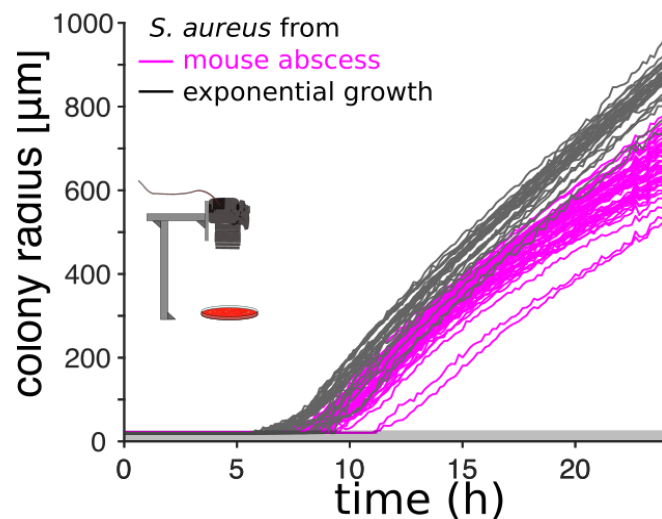
***S. aureus* recovered from  
human and murine abscesses**



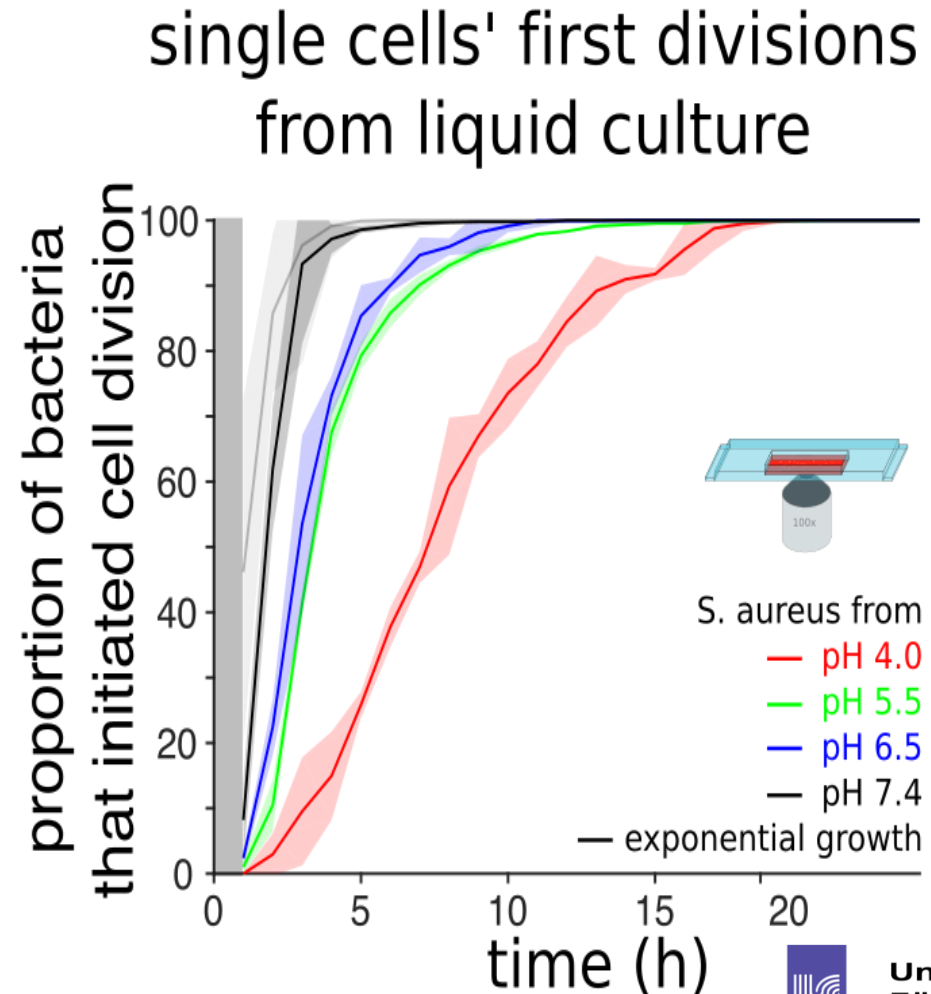
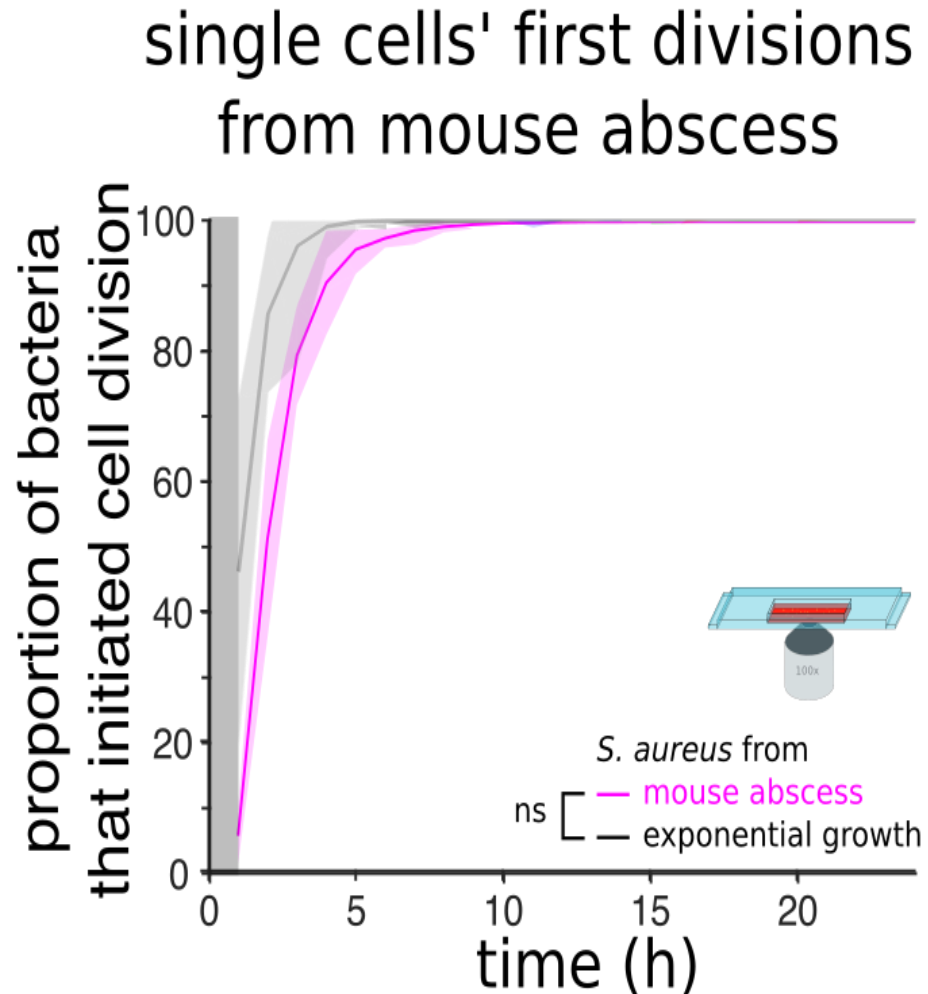
## Microscopic timelapse

colony growth curves  
from mouse abscess

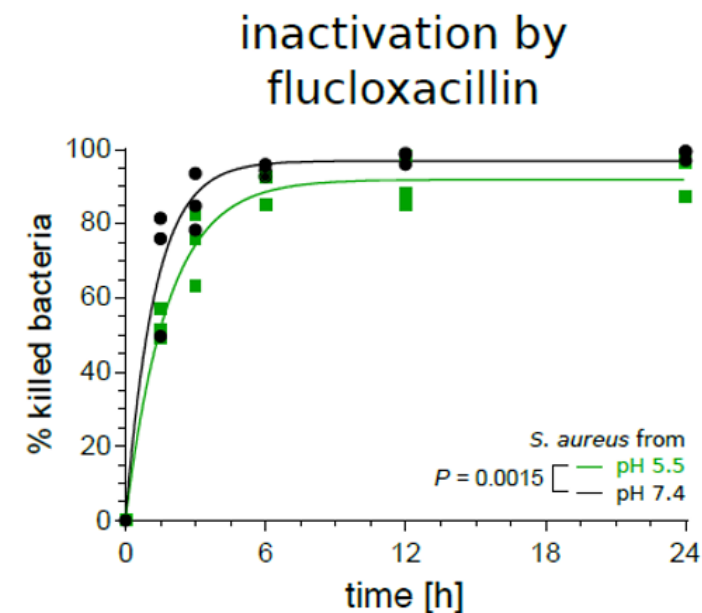
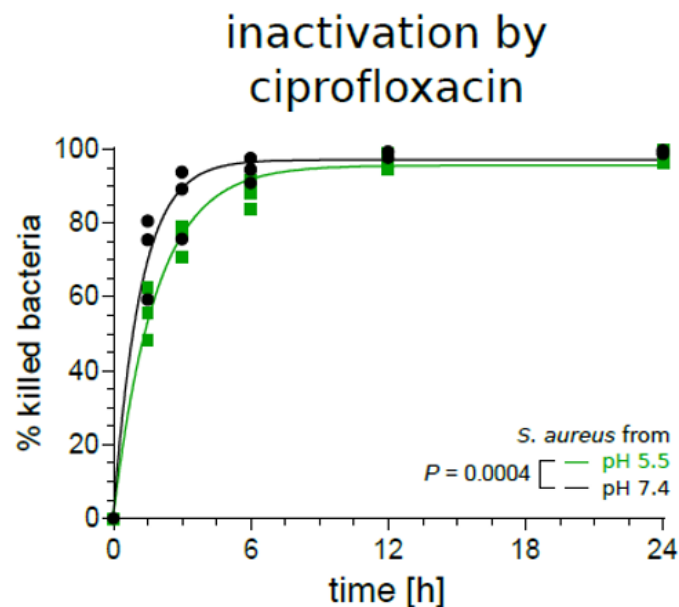
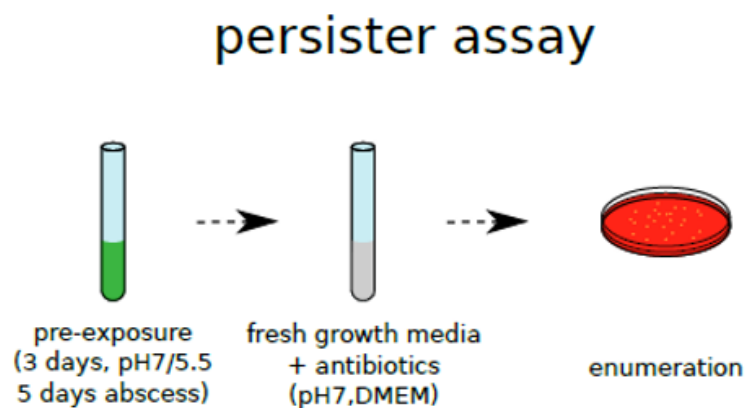
## Heterogeneous and lag



# heterogeneous colony size - delay in the first division at the level of single cells

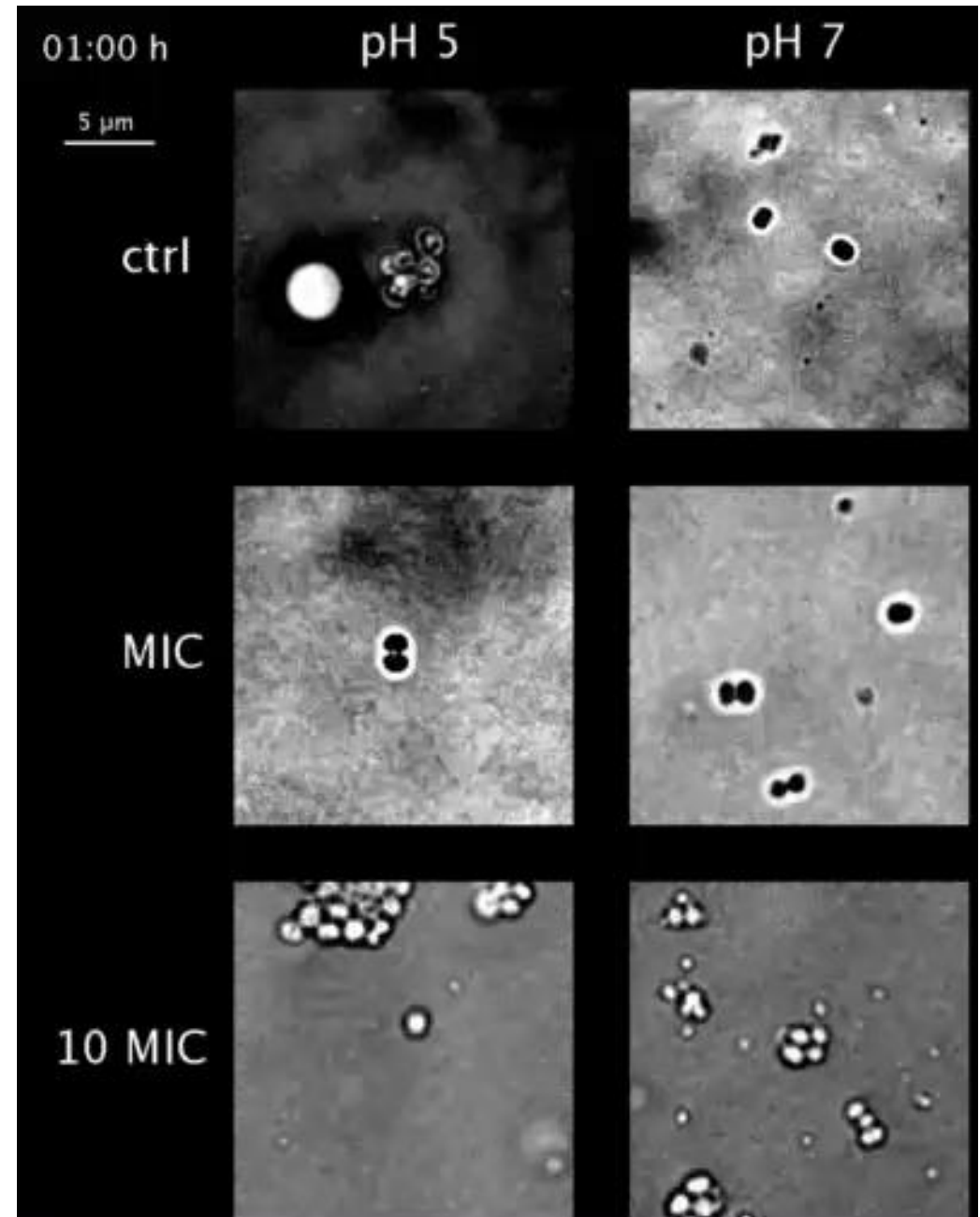
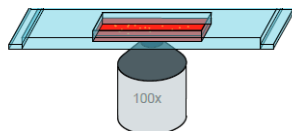
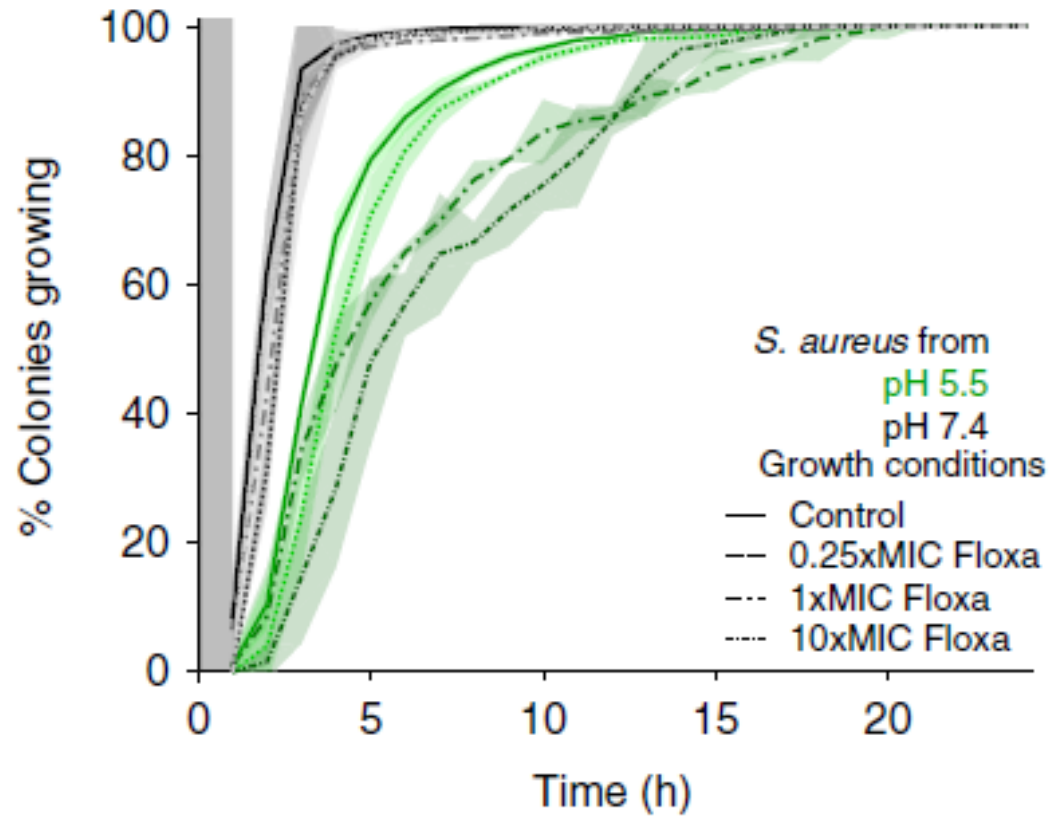


# Non dividing bacteria withstand antibiotics

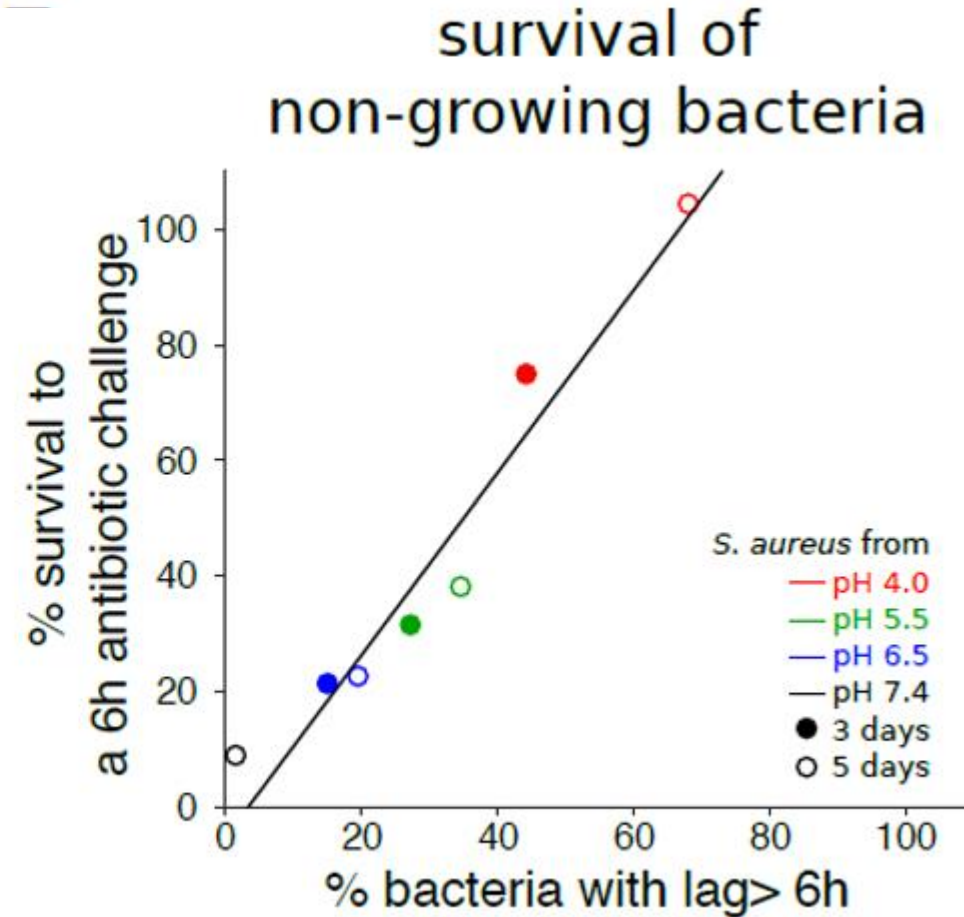
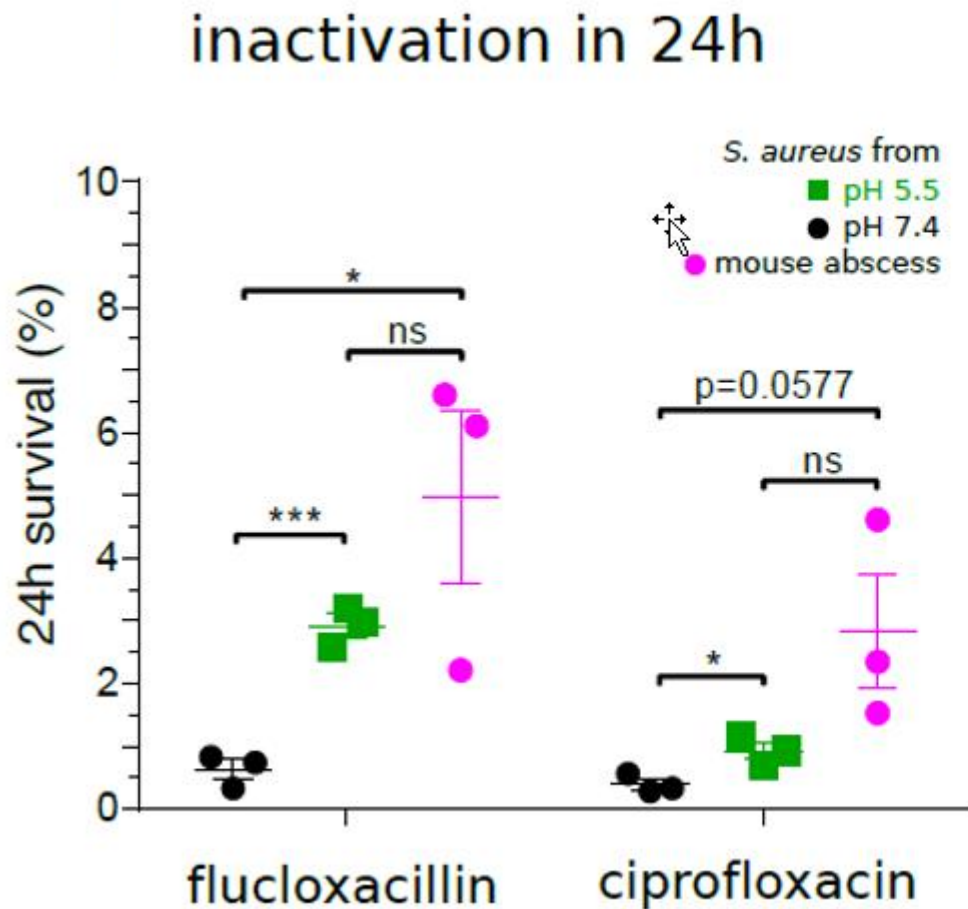


# lag time of individual bacterial cells

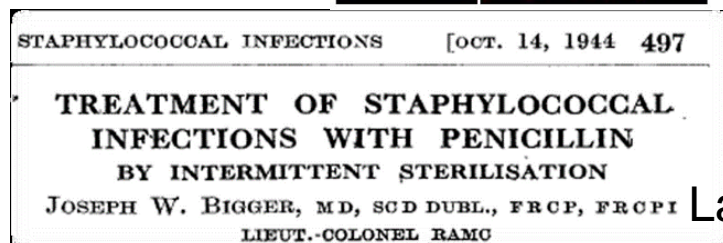
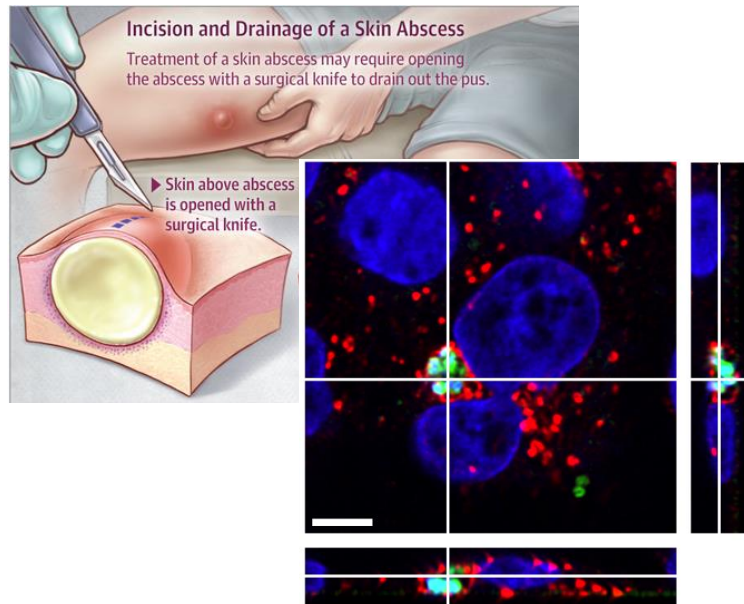
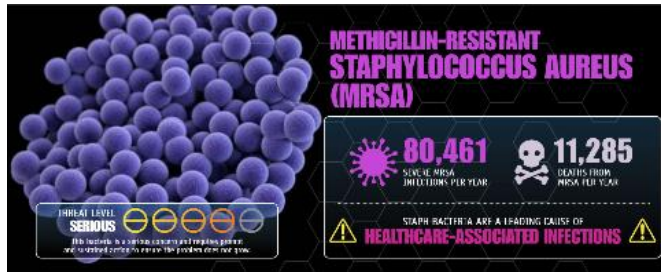
Single cells' first divisions from liquid culture



# The proportion of bacteria in lag phase correlates with the proportion of bacteria surviving antibiotics



# How do *S.aureus* withstand antibiotics?



## Resistance - MRSA

## Susceptible

- 1. 'Location':
  - in 'privileged' sites such as abscess, intracellular, biofilm
  - > AB do not reach bacteria, milieux
- 2. 'Growth'
  - Stationary bacteria
  - Persisters = metabolically inactive



# Recurrence: Weeks - years after apparent cure

## CORRESPONDENCE

### *Staphylococcus aureus* Reactivation Osteomyelitis after 75 Years

N Engl J Med 2012; 366:481-482 | February 2, 2012 | DOI: 10.1056/NEJMc1111493

### Daptomycin versus Standard Therapy for Bacteremia and Endocarditis Caused by *Staphylococcus aureus* -open-label, randomized trial

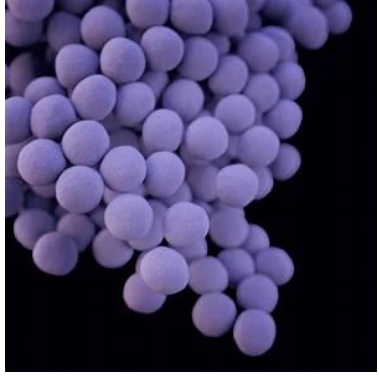
**Table 3.** Reasons for Treatment Failure According to the Adjudication Committee.\*

Reason for Failure	Daptomycin (N=120)	Standard Therapy (N=115)	P Value†
	no. (%)	no. (%)	
Overall	67 (55.8)	67 (58.3)	
Microbiologic failure, clinical failure, or both	23 (19.2)	15 (13.0)	0.22
Microbiologic failure‡	19 (15.8)	11 (9.6)	0.17
Clinical failure without microbiologic failure§	4 (3.3)	4 (3.5)	1.00
Adverse event	8 (6.7)	17 (14.8)	0.06
Receipt of nonstudy antibiotics that could have influenced outcome	20 (16.7)¶	16 (13.9)¶	0.59
Death	13 (10.8)	13 (11.3)	1.00
No blood obtained for culture**	9 (7.5)	12 (10.4)	0.50
Patient could not be evaluated (e.g., withdrew consent, left hospital against medical advice)	9 (7.5)	14 (12.2)	0.27

Fowler VG Jr et al.  
2006;355:653-665

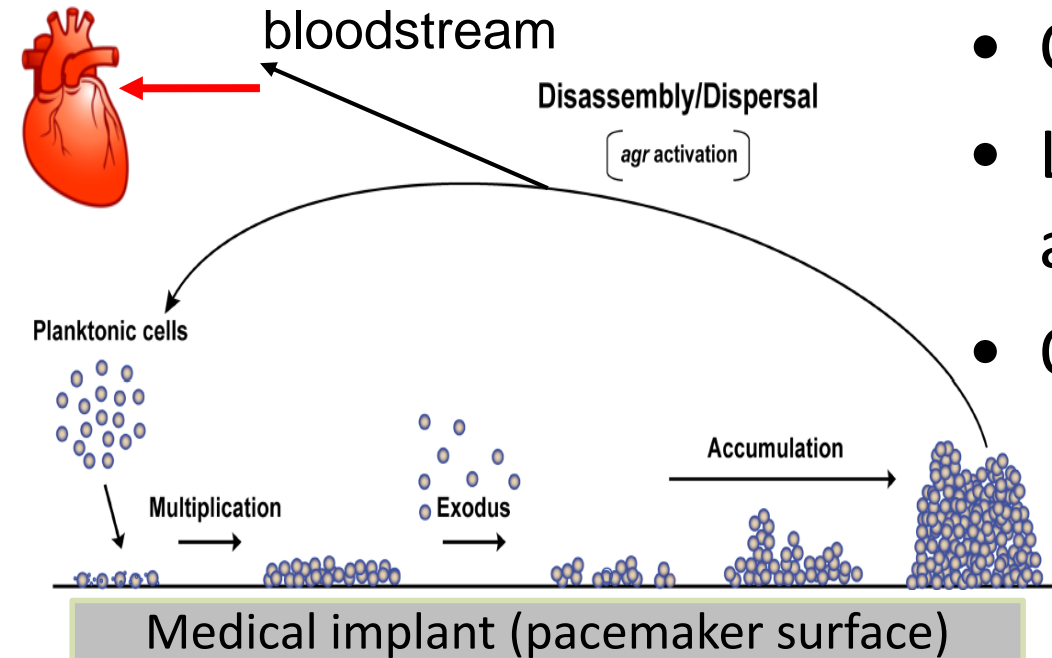


The NEW ENGLAND  
JOURNAL of MEDICINE



# *Staphylococcus epidermidis*

## Endocarditis



Adapted from Paharik and Horswill Microbiol Spectr. 4(2) 2016

- Gram-positive coagulase-negative staphylococcus
- Commensal: skin and mucosa
- Less virulent than *S. aureus* (less toxins and aggressive exoenzymes)
- Opportunistic pathogen:
  - Nosocomial bacteraemia
  - Biofilm associated infections
- Major cause of medical device associated infections
  - Prosthetic valve infections
  - Pacemaker associated infections

# ***Staphylococcus epidermidis***

## **pacemaker pocket infection**

---

Augmentin



Week

1



**Pacemaker pocket infection in 40 year old male**, debridement of the pacemaker pocket, vacuum assisted closure therapy (VAC), antibiotics

# ***Staphylococcus epidermidis***

## **pacemaker pocket infection**

---

Augmentin



Erreger

Total  
n = 28'451

Staphylococcus aureus

18 %

Staphylococcus, koagulase-negative

13 %

Enterococcus spp

12 %

Escherichia coli

9 %

Week

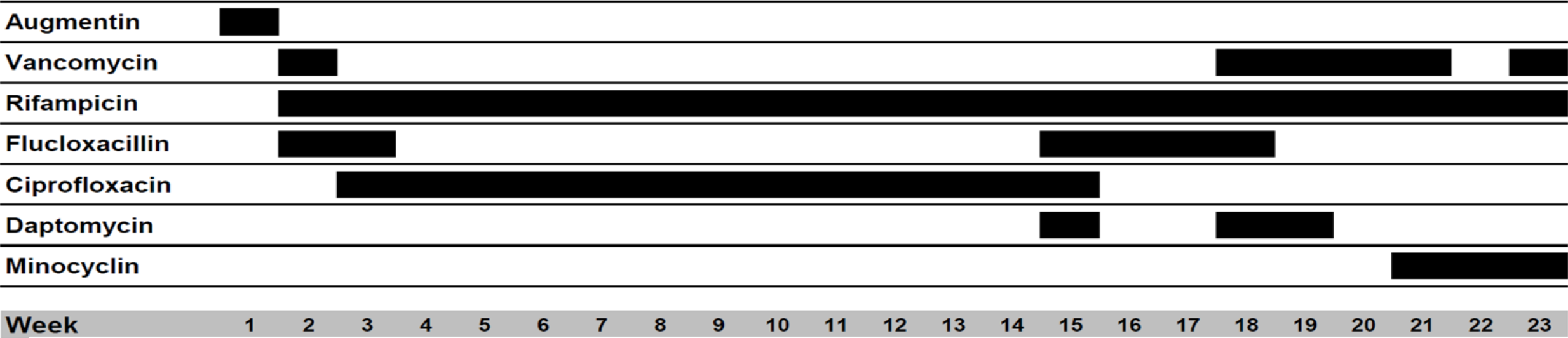
1



**Pacemaker pocket infection in 40 year old male**, debridement of the pacemaker pocket, vacuum assisted closure therapy (VAC), antibiotics

# Staphylococcus epidermidis

## pacemaker associated endocarditis

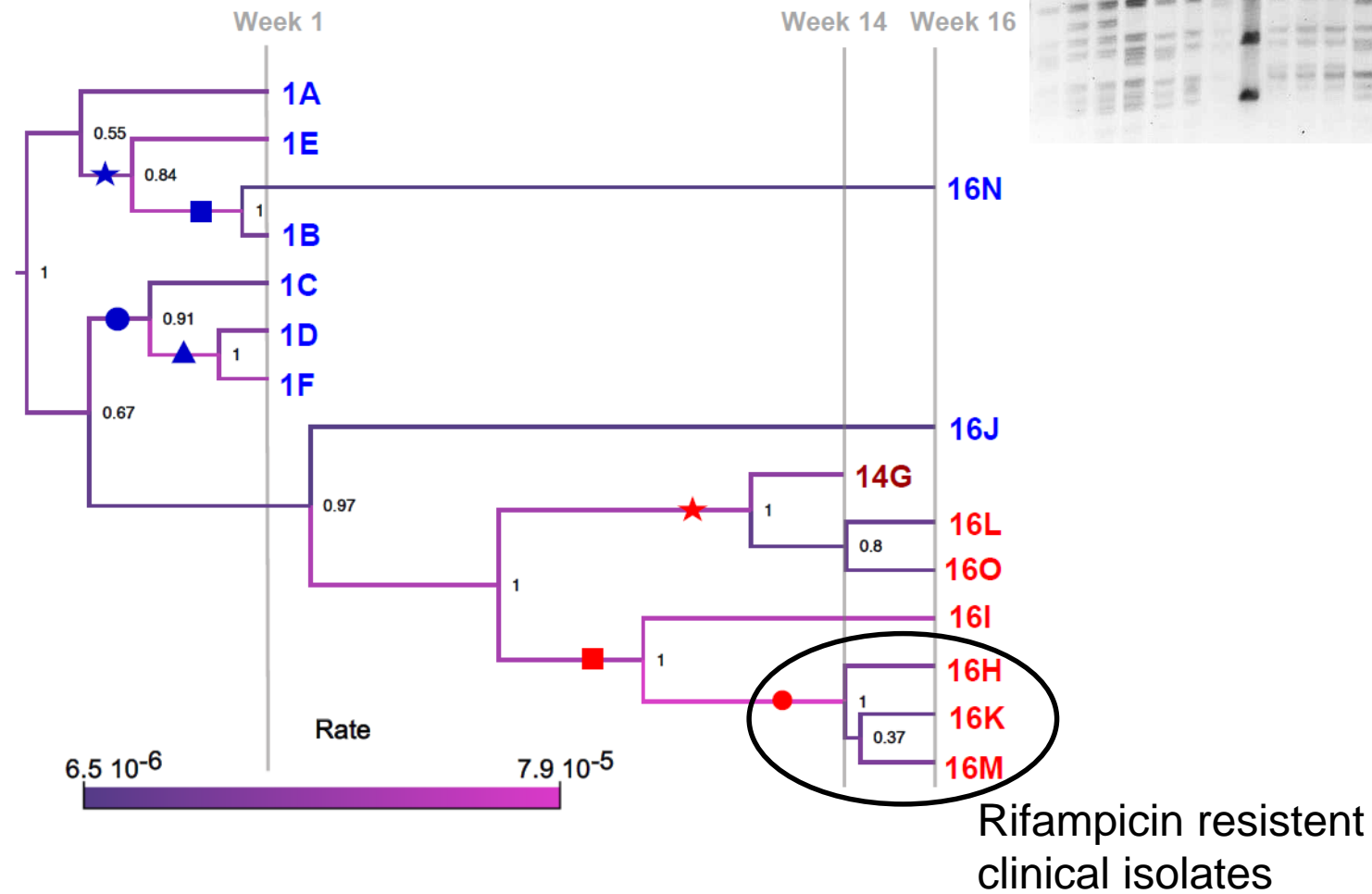
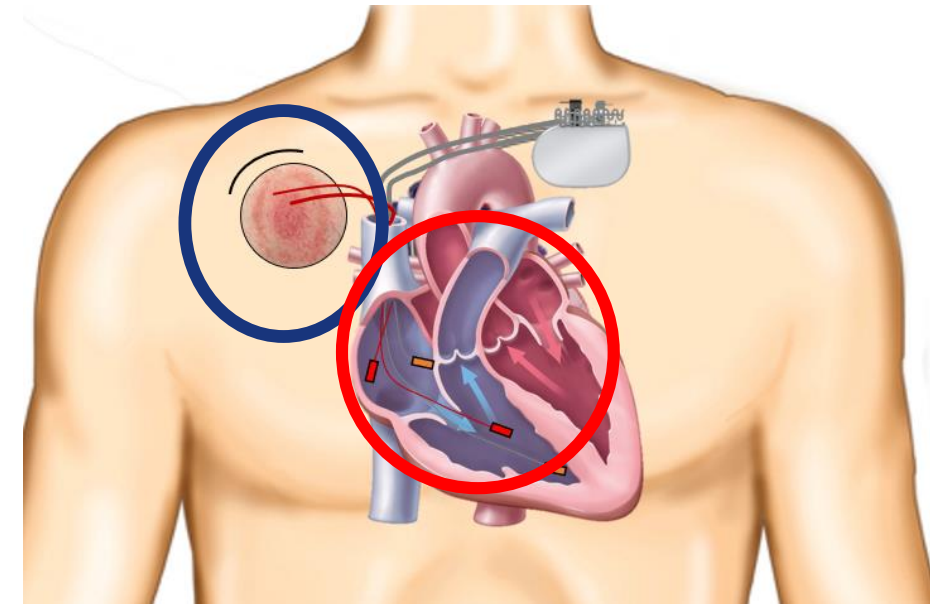
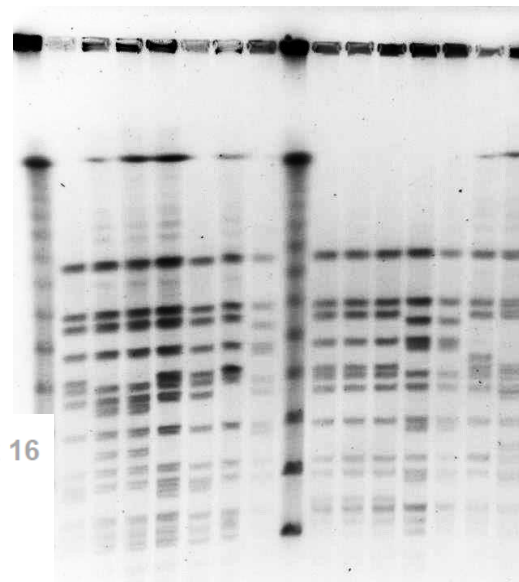


↑  
**Pacemaker pocket infection in 40 year old male**, debridement of the pacemaker pocket, vacuum assisted closure therapy (VAC), antibiotics

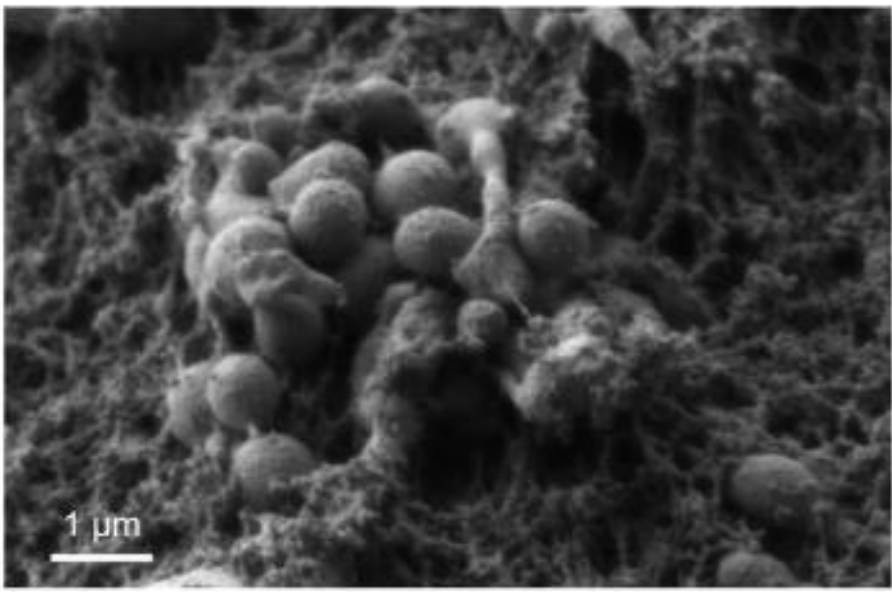
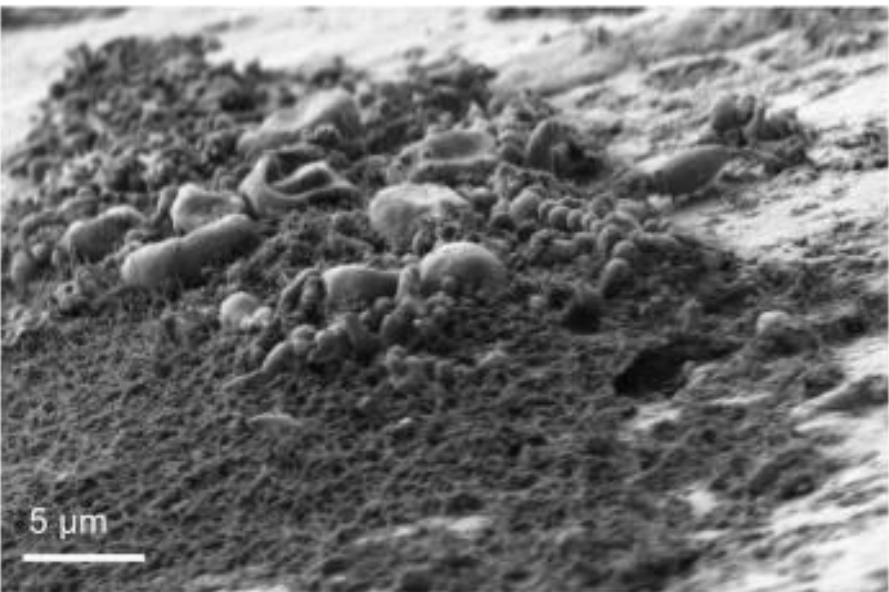
↑  
**Bacteremia**, vegetation in echocardiography

↑  
**Explantation of pacemaker**  
**The 'Endocarditis Team'**

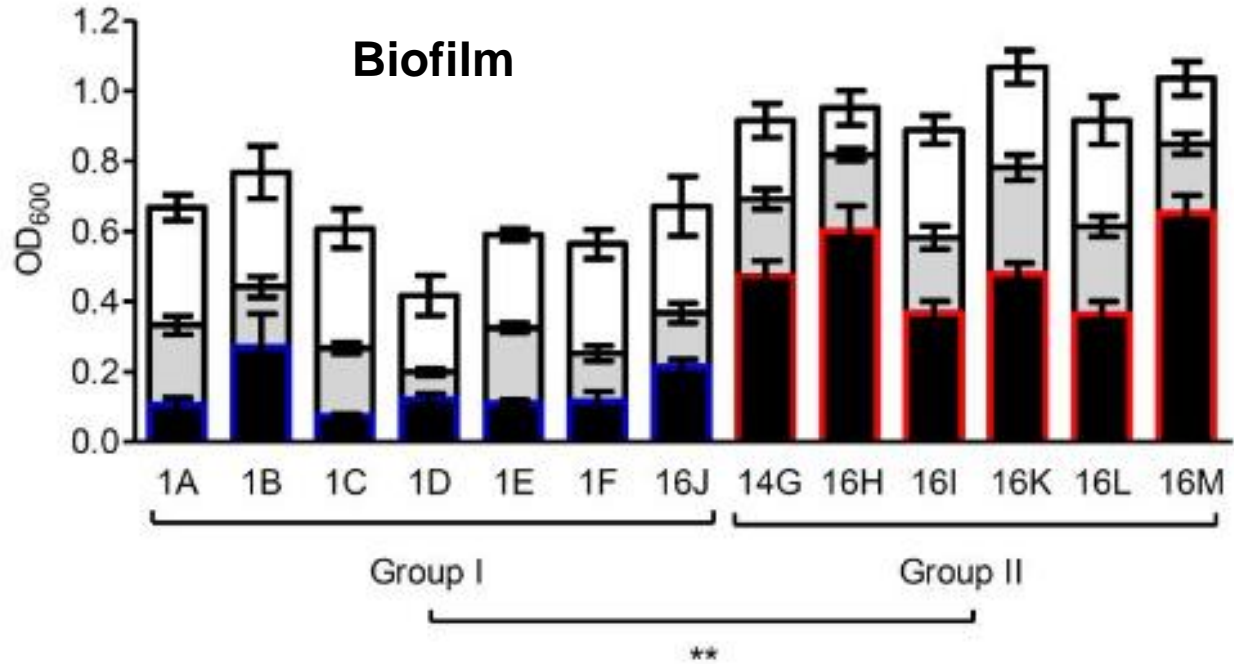
# Phylogenetic tree of the clinical *S. epidermidis* isolates



Bacterial isolate	Isolation week	Laboratory internal CI number	Isolation origin	Pacemaker area	Sequence Type
1A	1	835	Wound extraction of PM pocket	Old	378
1B	1	836	Wound extraction of PM pocket	Old	378
1C	1	837	Tissue	Old	378
1D	1	838	Tissue	Old	378
1E	1	839	Electrode	Old	378
1F	1	841	Electrode	Old	378
14G	14	753	Blood	-	378
16H	16	842	n.d	New	378
16I	16	848	Aggregate	New	378
16J	16	849	Aggregate	New	378
16K	16	788	Ventricle electrode	New	378
16L	16	792	Atrial electrode	New	378
16L	16	792	Atrial electrode	New	378
16M	16	797	Ventricle electrode	New	378

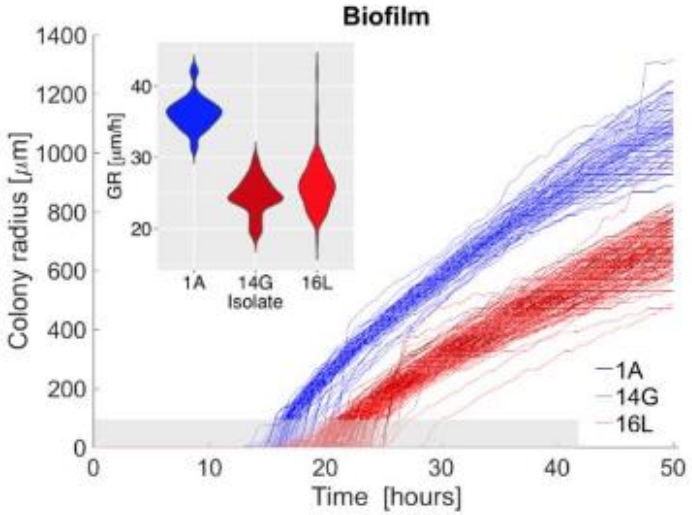
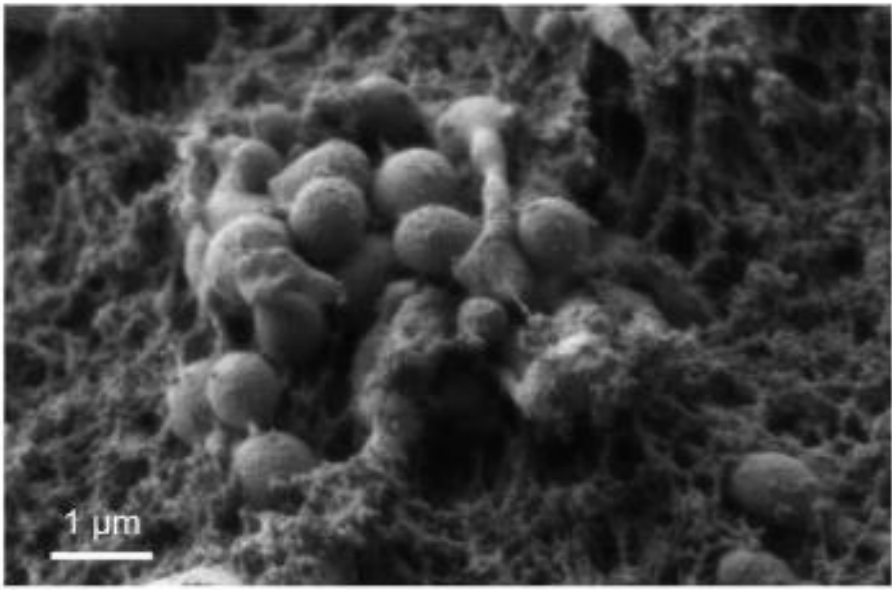
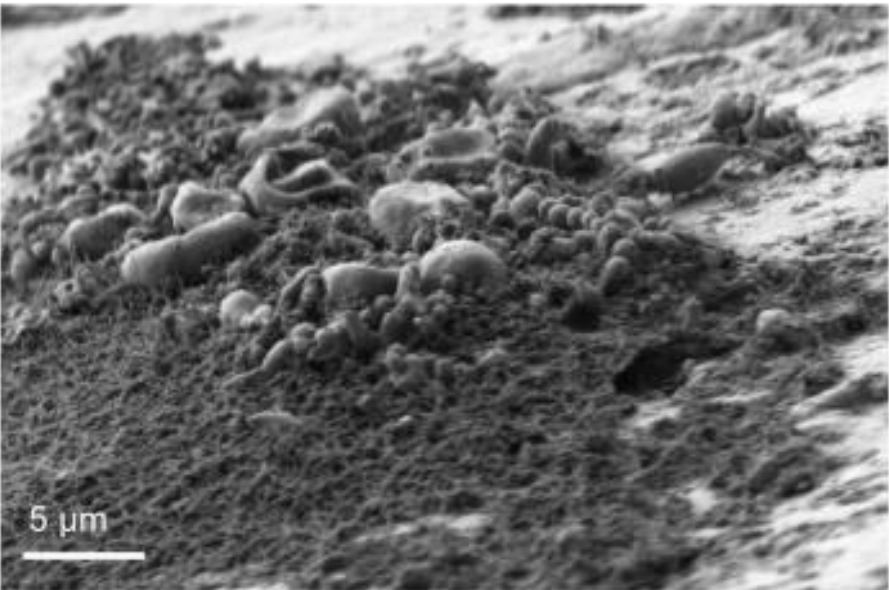


Scanning Electron Microscopy



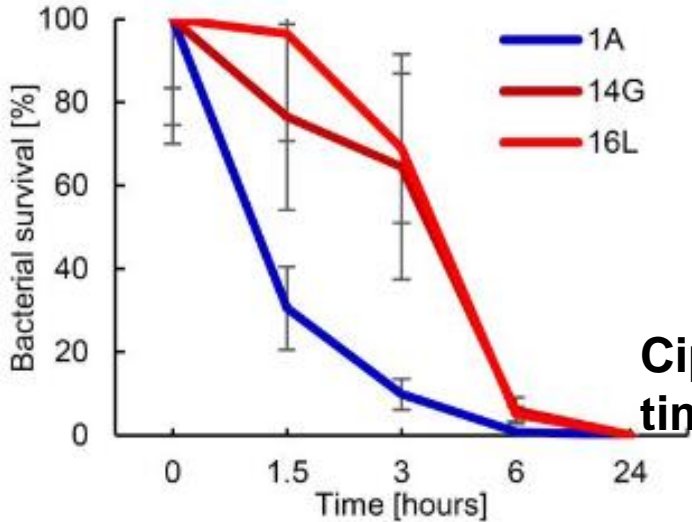
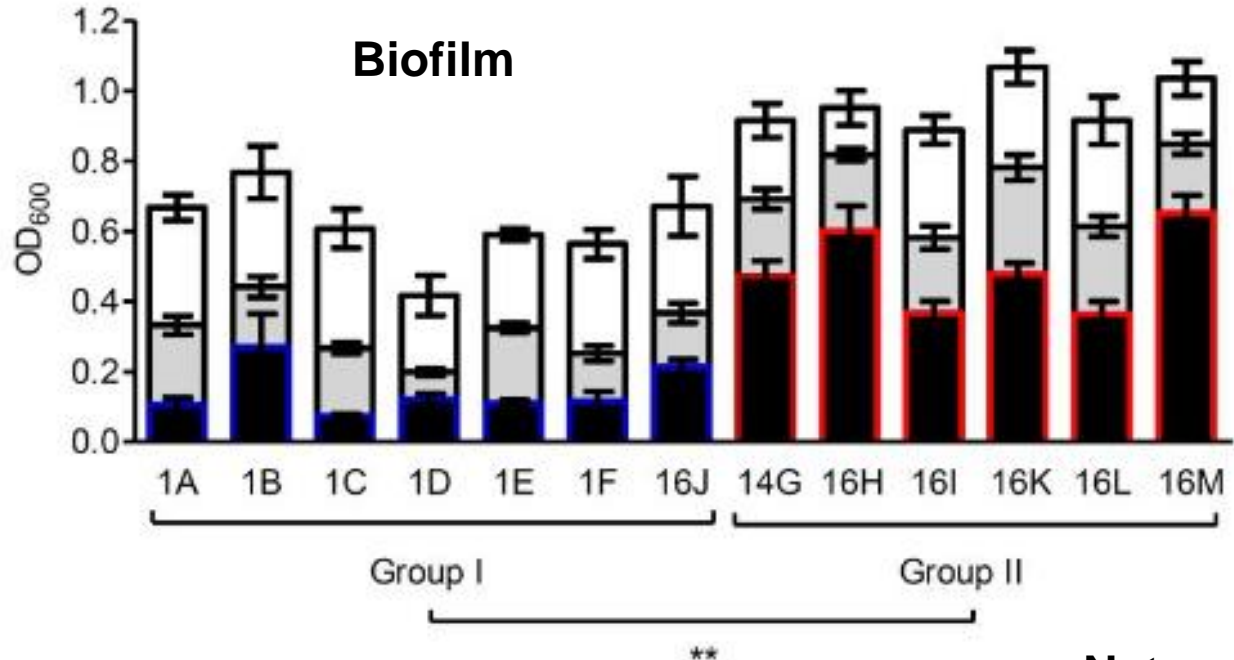
Nature communications 2019





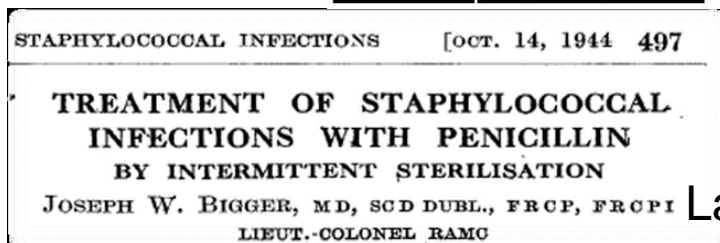
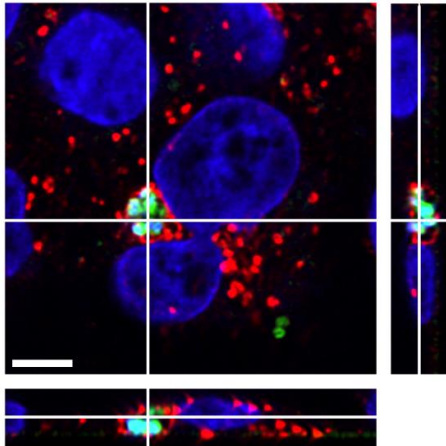
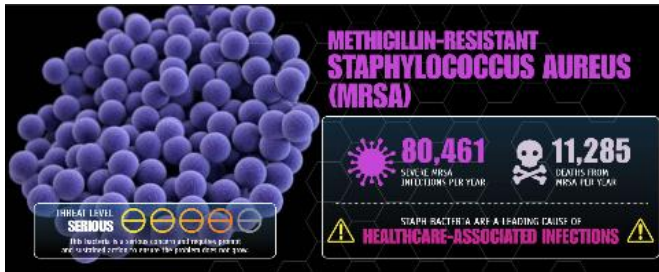
Scanning Electron Microscopy

Lag phase



Ciprofloxacin MIC 40 x time kill curves

# How do *S.epidermidis* withstand antibiotics?



Lancet

## Resistance - rifampicin Susceptible

- 1. 'Location':
  - in 'privileged' sites such as abscess, intracellular, **biofilm**
  - > AB do not reach bacteria, milieu
- 2. 'Growth'
  - **Stationary bacteria**
  - **Persisters = metabolically inactive**
  - **in host evolution**

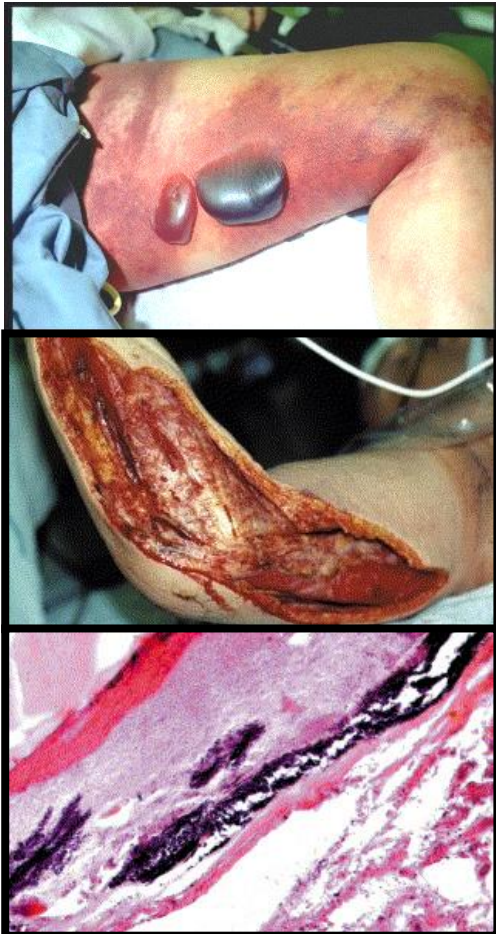


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# Invasive Group A Streptococcal Disease

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**Necrotizing fasciitis**



**Global burden of disease / year:**

**663,000 invasive infections**

- 163,000 deaths
- streptococcal toxic shock syndrome
- necrotizing fasciitis

**Rapidly-progressive, destructive infection of the soft tissues.**

**Requires extensive surgical debridement, intravenous antibiotics, and ICU care.**

**High lethality (~35%)**

# Why does treatment fail?

## Rapidly progressive disease

### Doc:

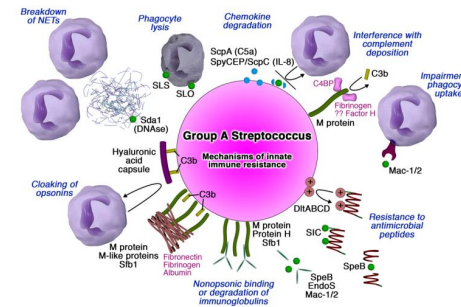
- quick diagnosis, surgery + 'wright' antimicrobials

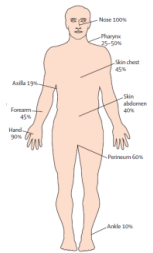
### Patient:

- Delay in presentation, necrotic tissue

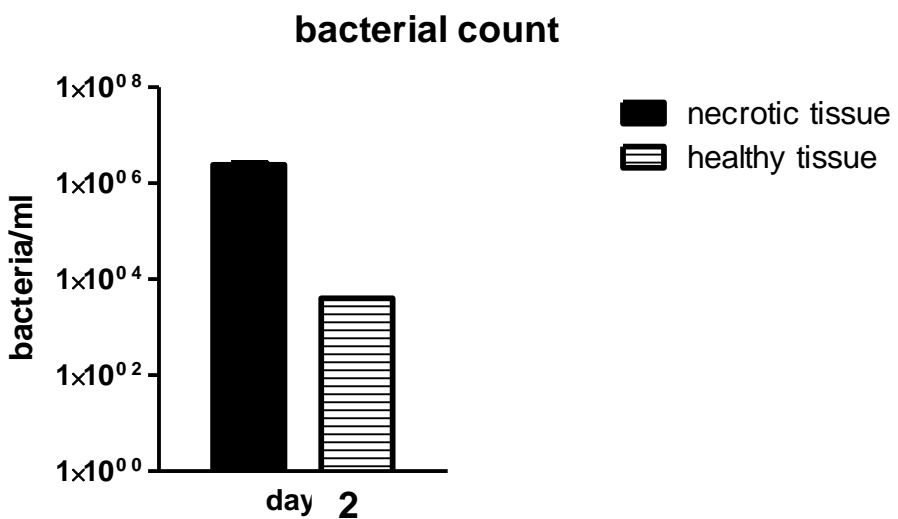
### Bug:

- Extracellular bacterium, 100% peni susceptible
- Many bacteria
- Virulence factors
- Biofilms

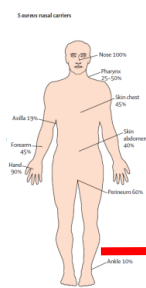




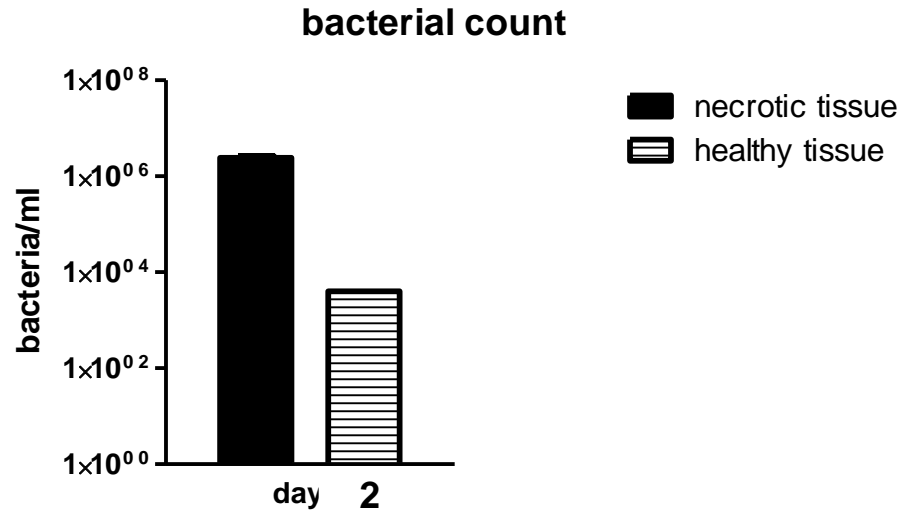
# Necrotizing fasciitis - 2 days ceftriaxone



Ceftriaxone



# Group A Streptococcus - *Streptococcus pyogenes*



Ceftriaxone

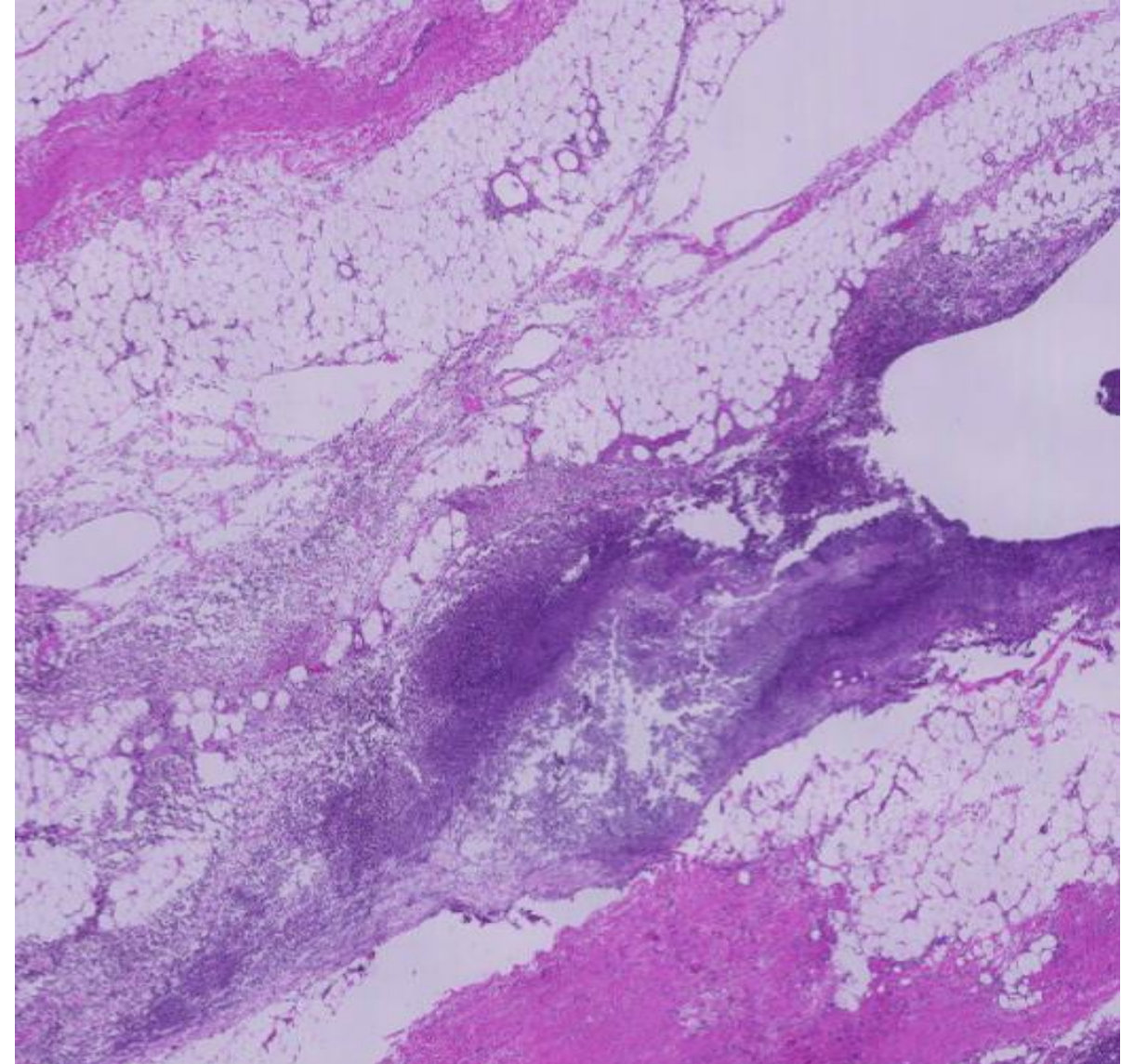
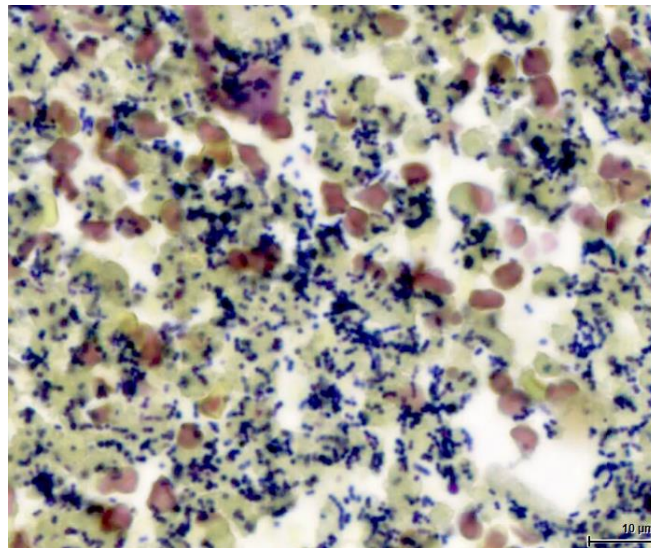


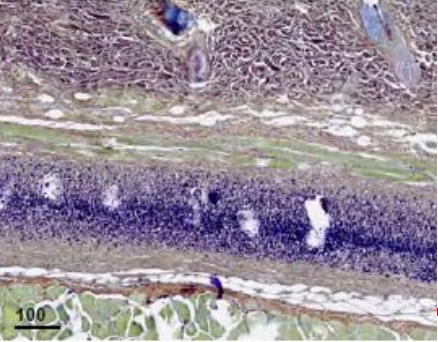
TABLE II  
Effect of the Size of the Inoculum on the Curative Dose of Penicillin G in White Mice Infected with a Group B  $\beta$ -Hemolytic Streptococcus\*

	No. of organisms inoculated†	Penicillin	Survived	Died	Curative dose (CD <sub>50</sub> ) of penicillin G, $\pm$ standard error‡, §
		mg./kg.			mg./kg.
Group 1	2,235,000	2,048	20	0	424 $\pm$ 52
		1,024	18	2	
		512	9	11	
		256	7	13	
		128	1	19	
		0	0	10	
Group 2	180,000	1,024	20	0	339 $\pm$ 45
		512	14	6	
		256	5	15	
		128	0	20	
		64	1	19	
		32	1	19	
Group 3	1,750 (estimated)	0	0	10	139 $\pm$ 51**§
		256	19	1	
		128	7	13	
		64	2	18	
		32	1	19	
		16	5	15	
Group 4	17¶	0	1	9	2.8 $\pm$ 1.1
		64	19	1	
		32	15	5	
		16	18	2	
		8	11	9	
		4	14	6	
		2	11	9	
		1	7	13	
		0	1	9	

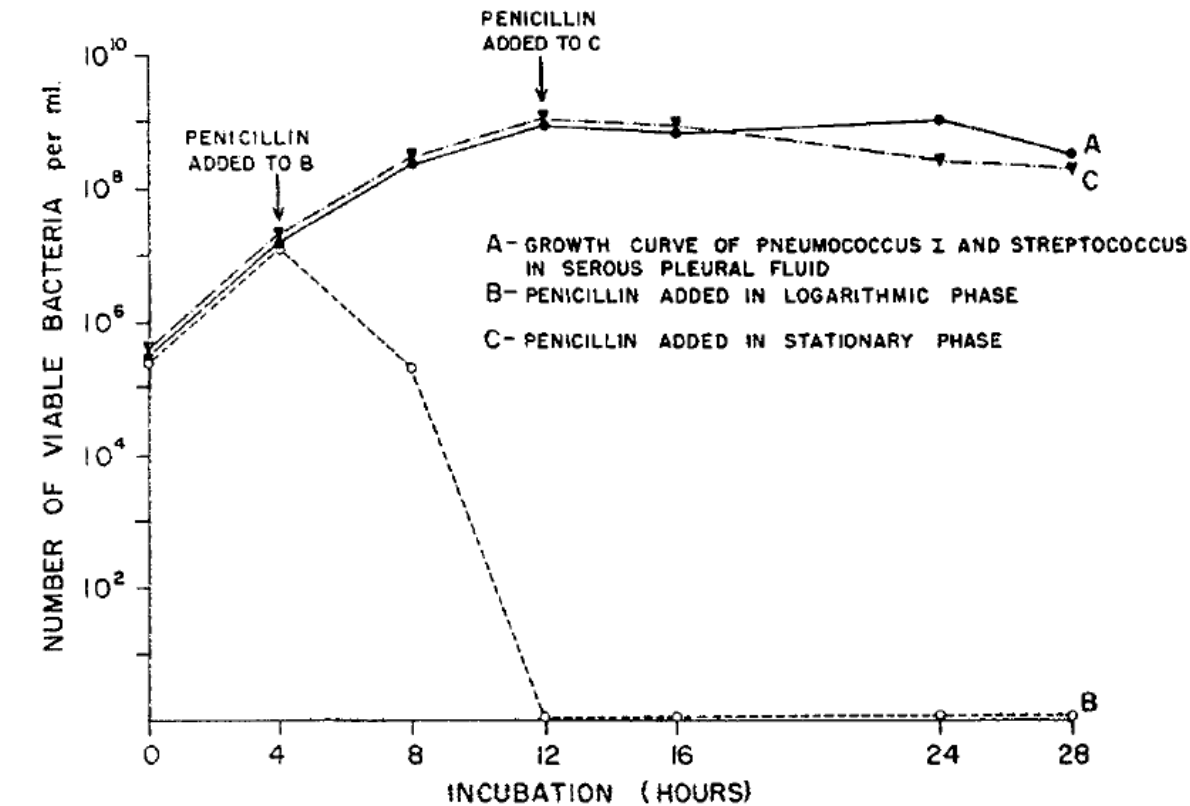
The mice (CFW strain) were inoculated intraperitoneally with an appropriate dilution of a 3 hour culture in blood-broth, and treated immediately with a single intramuscular injection of penicillin G in aqueous solution. The number of organisms indicated in the table is actually the number of bacterial clumps, determined by plate counts. The number of organisms per clump in the original culture averaged 2.0.



Curative dose of penicillin increases with the size of the inoculum, and increases also with the age of the infection (paradoxical more-drug-kills-less Eagle effect)



# High inoculum – stationary



TEXT-FIG. 3. Action of penicillin on type I pneumococci and beta hemolytic streptococci contained in thin serous fluid collected from pleural cavities of rats with experimental streptococcal pneumonia. The pneumococci were added to the fluid at the start of each experiment.

J Ex Med 1956

*J Infect Dis.* 1993 Jun;167(6):1401-5.

Penicillin-binding protein expression at different growth stages determines penicillin efficacy in vitro and in vivo: an explanation for the inoculum effect.

Stevens DL<sup>1</sup>, Yan S, Bryant AE.

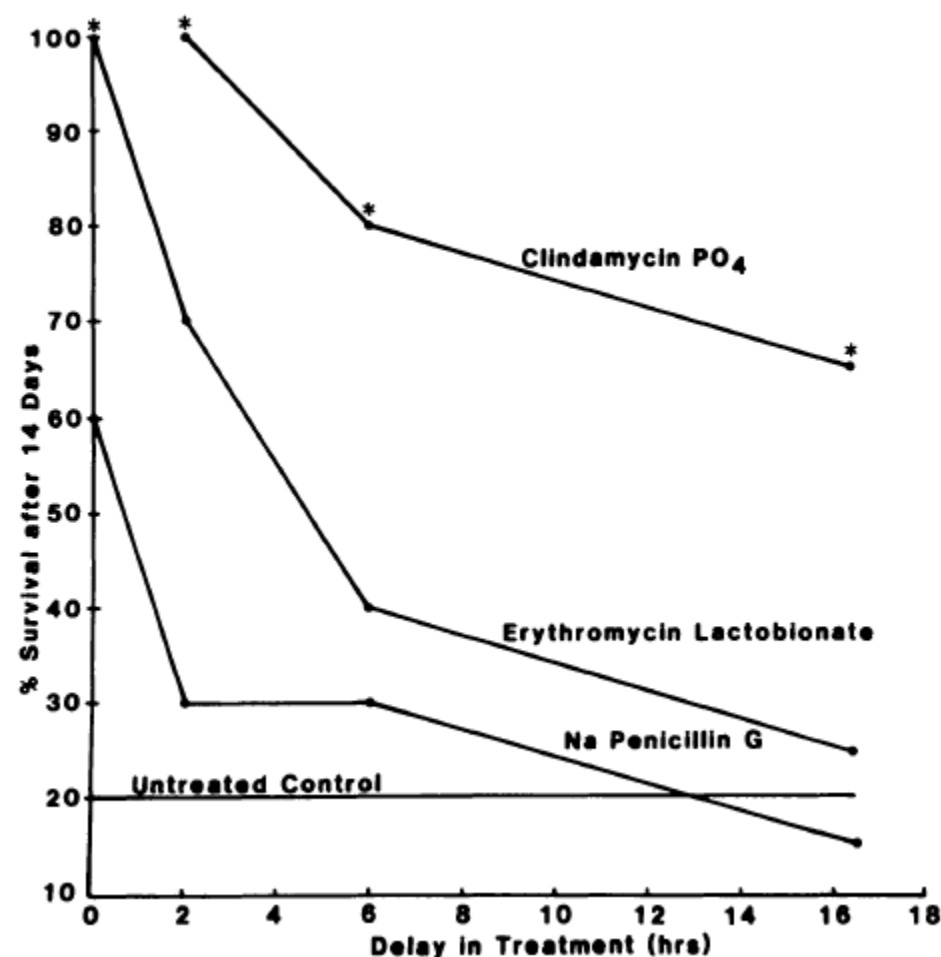
Once there is a high inoculum of Strep and it reaches the stationary phase of growth, Strep does not express penicillin-binding proteins and thus is less susceptible to beta-lactams.



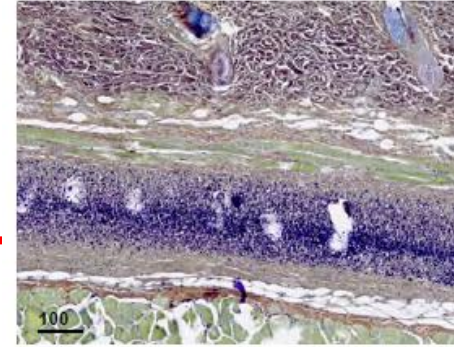
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Zürich

## The Eagle effect revisited: efficacy of clindamycin, erythromycin, and penicillin in the treatment of streptococcal myositis.

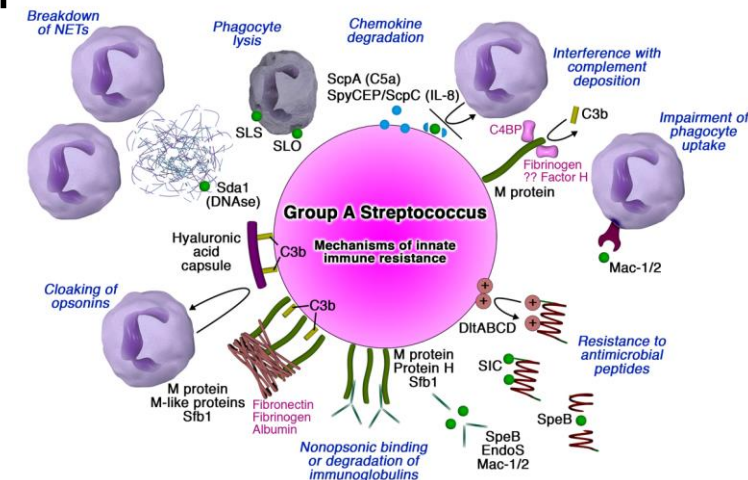
Stevens DL<sup>1</sup>, Gibbons AE, Bergstrom R, Winn V.



# Antibiotics: **Eliminate + Disarm**



- **Cell wall active antibiotic:**
  - Penicillin: 100% susceptibility
  - Penicillin should be given as quickly as possible
- **Protein synthesis inhibitor:**
  - Clindamycin
    - clindamycin is not affected by the inoculum size or stage of bacterial growth
  - suppressor of bacterial toxin synthesis
    - Sriskandan et al., J Antimicrob Chemother. 1997
    - Mascini et al., Int J Antimicrob Agents 2001
    - Goscinski G et al., Scand J Infect Dis 2006



# Reality

Observational prospective surveillance of iGAS in Victoria, Australia (4.9mio), 3/2002-8/2004.

**Table 2. Clinical Manifestations and Treatment of 84 Patients With Severe Invasive Group A Streptococcal Disease**

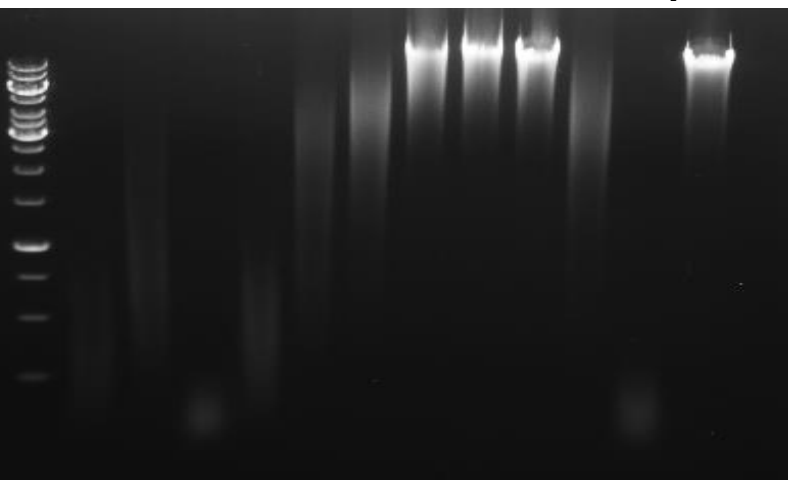
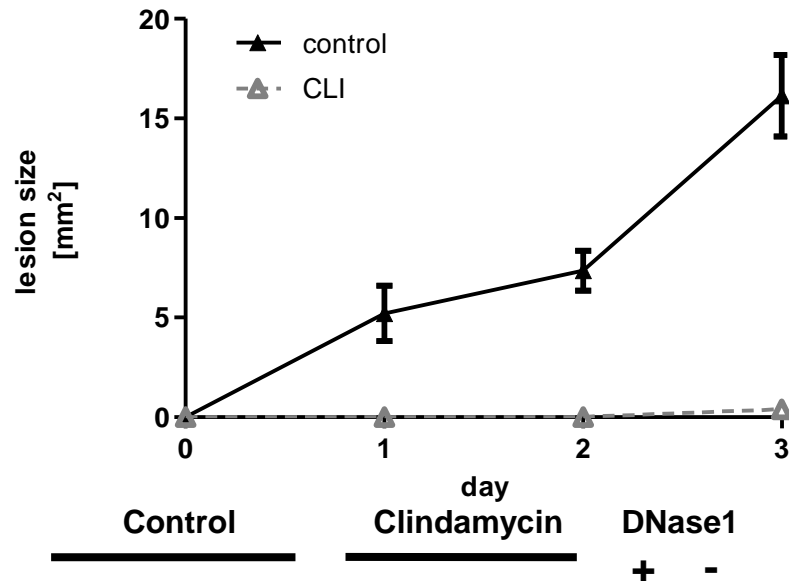
Manifestation	No. of Patients	No. (%) Treated With Clindamycin	No (%) Treated With IVIG <sup>a</sup>
NF + STSS	20	20 (100)	7 (35)
NF + septic shock	1	1 (100)	0
NF alone	8	7 (88)	1 (13)
STSS alone	29	17 (59)	6 (21)
Septic shock alone	16	6 (38)	0
Severe cellulitis	10	2 (20)	0
Total severe iGAS	84	53 (63)	14 (17)

Invasive Group A Streptococcal Infections • CID 2014:59 (1 August)

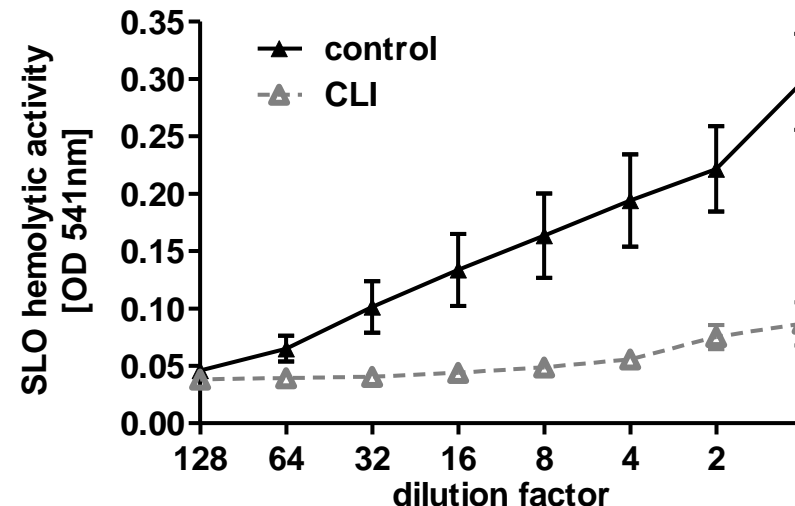
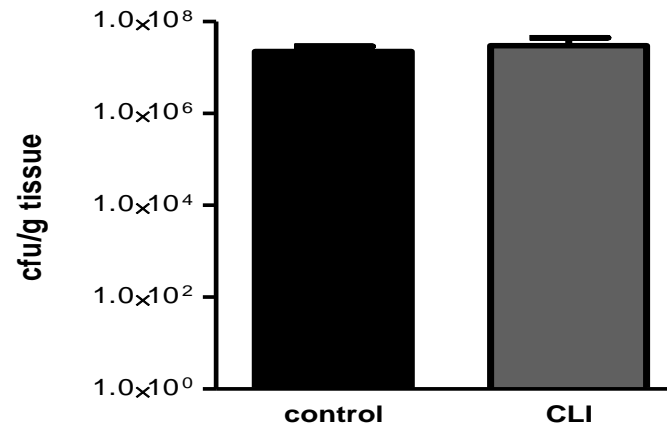


# Reduced virulence ... *in vivo*

Lesion size



CI529



## NEJM Journal Watch

HOME SPECIALTIES & TOPICS NEWS BLOGS CME

SUMMARY AND COMMENT | INFECTIOUS DISEASES, HOSPITAL MEDICINE

INFORMING PRACTICE

February 15, 2017

### Why Adding Clindamycin Is Important in Treating Group A Streptococcal Necrotizing Fasciitis

Neil M. Ampel, MD reviewing Andreoni F et al. J Infect Dis 2017 Jan 15.

In an experimental model using mice and human tissue, therapeutic doses of clindamycin were found to inhibit key virulence factors in group A streptococci.

High-dose penicillin plus surgical debridement has been the standard therapy for necrotizing fasciitis caused by group A streptococci (GAS). Although adding clindamycin (CLI) is strongly recommended because of its ability to inhibit bacterial protein synthesis, this approach has never been tested in a prospective clinical trial, nor is such a trial feasible.

Now, Swiss and French investigators report the possible benefits of clindamycin therapy against GAS invasive infections with both CLI-susceptible and CLI-resistant isolates in an in vivo murine model and in tissue from a patient with GAS necrotizing fasciitis. In the mouse model, addition of therapeutic doses of clindamycin was associated with decreases in the virulence factors DNase and streptolysin O, and in the size of areas of skin necrosis, despite minimal reductions in bacterial concentrations. These effects were seen even when the infecting strain of GAS was clindamycin resistant. When subtherapeutic doses of clindamycin were given, other virulence factors that mimicked the more virulent animal-passaged GAS phenotype were seen to increase. In the human tissue samples, DNase activity was completely abolished after 2 days of adjunctive clindamycin treatment, although high concentrations of bacteria persisted.

#### COMMENT

This interesting study provides evidence favoring addition of clindamycin to the standard treatment of GAS-associated necrotizing fasciitis. The authors note that the results support the use of early, high-dose clindamycin in combination with a  $\beta$ -lactam antibiotic and surgical debridement and caution that subtherapeutic clindamycin doses may actually result in a more virulent organism.

Andreoni F. et al., J Infect Dis. 2016

# Reality

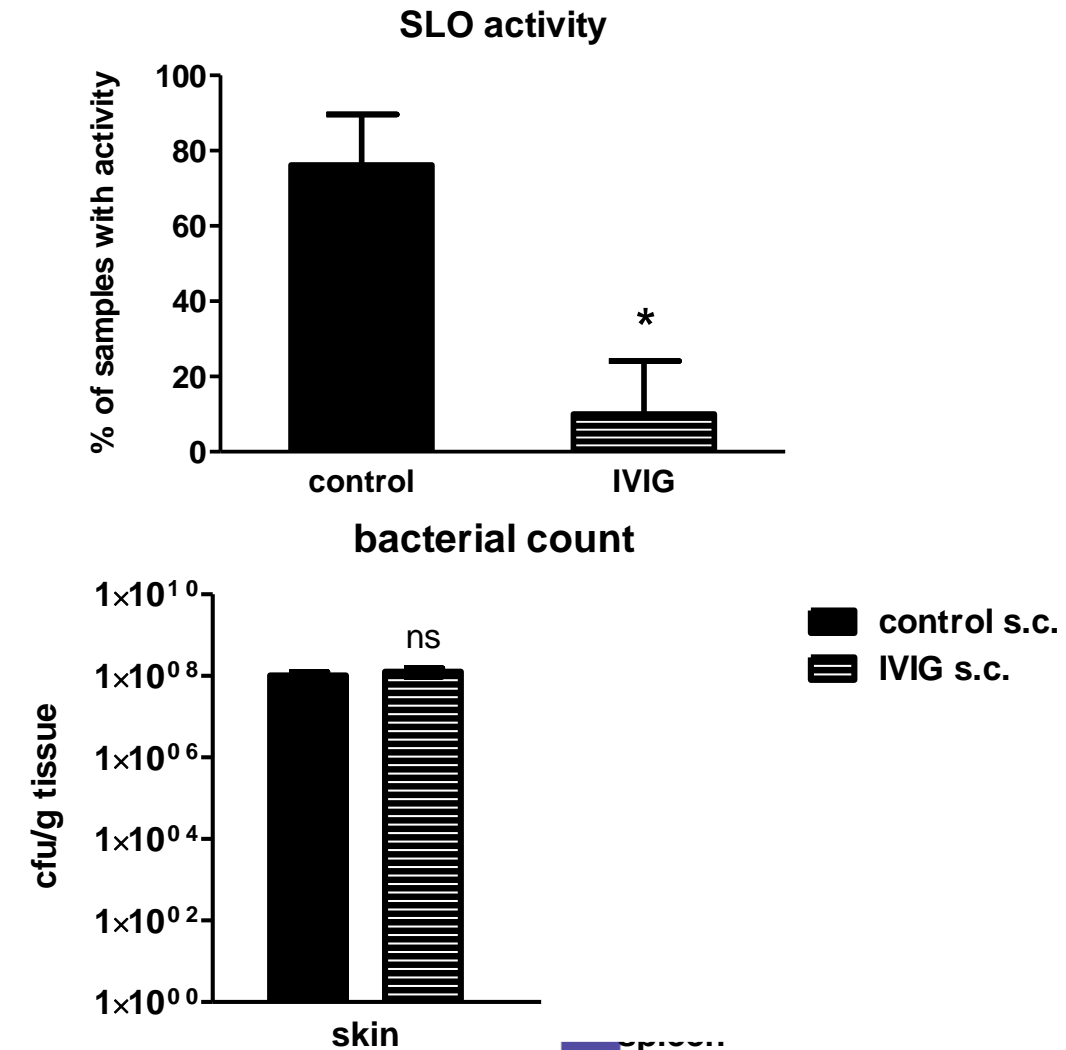
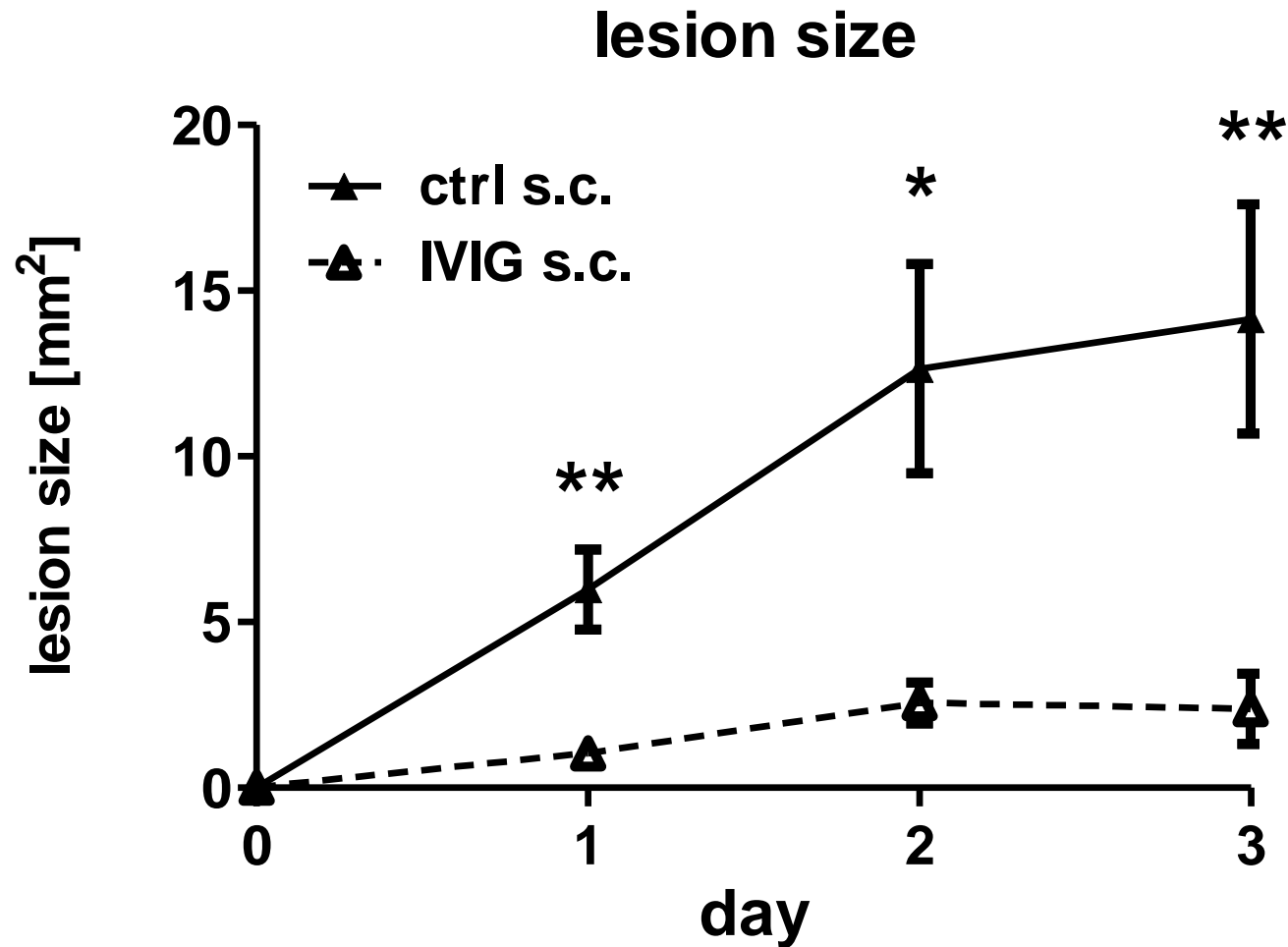
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Total severe iGAS	84	53 (63)	14 (17)



# IVIg inhibit VF activity + reduce lesion size





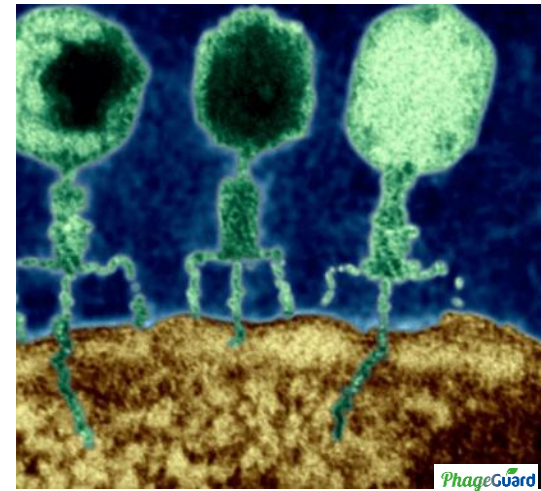
Bacteriophages could be a resource for fighting drug-resistant bacterial infections.

MICROBIOLOGY

# Phage therapy gets revitalized

The rise of antibiotic resistance rekindles interest in a century-old virus treatment.

5 JUNE 2014 | VOL 510 | NATURE



- Virus
- Lytic
- Resistances - Cocktails
- FDA approved for treatment of *Listeria monocytogenes* contamination in food industry (poultry & cattle)
- Aquaculture & sewage treatment

# PHAGE THERAPY BACK TO THE FUTURE!

Name of companies	Country	Web	Notes
AmpliPhi BioSciences Corporation	Australia	<a href="http://www.ampliphio.com">http://www.ampliphio.com</a>	Clinical trials against infections of the group « ESKAPE » on humans and among pets and livestock animals for MRSA and PYO
Biophage Pharma Inc	Canada	<a href="http://www.biophagepharma.net/index.php/en/">http://www.biophagepharma.net/index.php/en/</a>	Biosensor division : dev. & commercialization of simple, accurate, highly sensitive biosensors based on phages / Therapeutic division dev. Phage therapies for human health.
Pherecydes Pharma	France	<a href="http://www.pherecydes-pharma.com">www.pherecydes-pharma.com</a>	Development of phagetherapies for human health. EU funded PHAGOBURN clinical trial
Gangagen Inc.	India	<a href="http://www.gangagen.com">www.gangagen.com</a>	Developments of products against MRSA and PYO infections
Biotech Laboratories	Israel	<a href="http://www.biotech.com/index.asp">www.biotech.com/index.asp</a>	Rapid detection of rifampicine resistance in sputum positive for M.tb / Rapid detection of BK in human sputum
Micreos Food Safety	Netherlands	<a href="http://www.ebifoodsafety.com">www.ebifoodsafety.com</a>	Protection against LISTER in food preparation
CheilJedang Corp.	South Korea	<a href="http://www.cjj.co.kr">www.cjj.co.kr</a>	To protect chicken feed from Salmonella gallinarum et pullorum
Phico Therapeutics	UK	<a href="http://www.phicotherapeutics.co.uk">www.phicotherapeutics.co.uk</a>	Bacteriophages for several bacteria : Listeria monocytogenes , M. tuberculosis), MRSA, MSSA
Novolytics	UK	<a href="http://www.novolytics.co.uk">www.novolytics.co.uk</a>	Gels /MRSA / C. Difficile and products to decrease nasal portage of MRSA /gels for skin infections and medical devices
Biocontrol	UK	<a href="http://www.biocontrol-ltd.com">www.biocontrol-ltd.com</a>	Cinical trials on otitis to treat PYO infections
Omnilytics	USA	<a href="http://www.phage.com">www.phage.com</a>	Development and use of lytic bacteriophages against tomato wilt disease
Intralytix	USA	<a href="http://www.intralytix.com">www.intralytix.com</a>	Decontamination and food additive against Escherichia coli O157:H7 in food preparation /Food additive against contamination by LISTER of uncooked food
Viridax Inc.	USA	<a href="http://www.viridax.com">www.viridax.com</a>	Development of products against staphylococcal infections.
New Horizons Diagnostics Corporation	USA	<a href="http://www.nhdiag.com/phage.shtml">http://www.nhdiag.com/phage.shtml</a>	Enzybiotics: Phage Associated Enzymes (PAE) that act as antibiotics

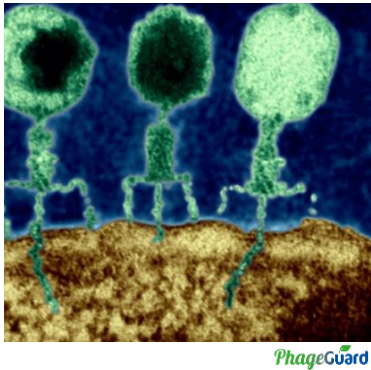
AMR CONTROL  
2015



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# PHAGE THERAPY BACK TO THE FUTURE!

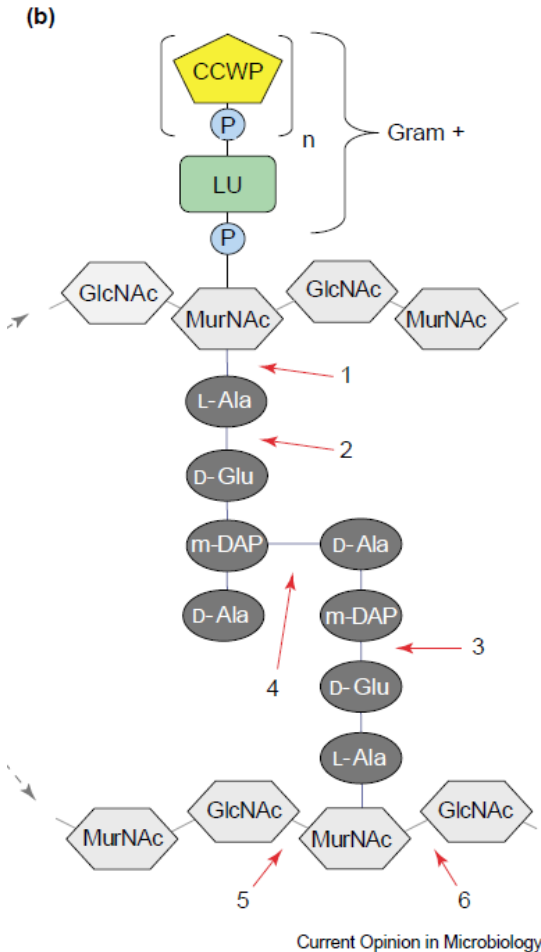
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PhageGuard

- Phages used to transport and target antibiotics into bacteria
  - » Yacoby et al, 2006, AAC Targeting Antibacterial Agents by Using Drug-Carrying Filamentous Bacteriophages
- Rapid resistance development
- Phage lysins = cell wall hydrolases, bind to peptidoglycans  
-> disrupt cell walls of Gram pos bacteria

# Endolysins

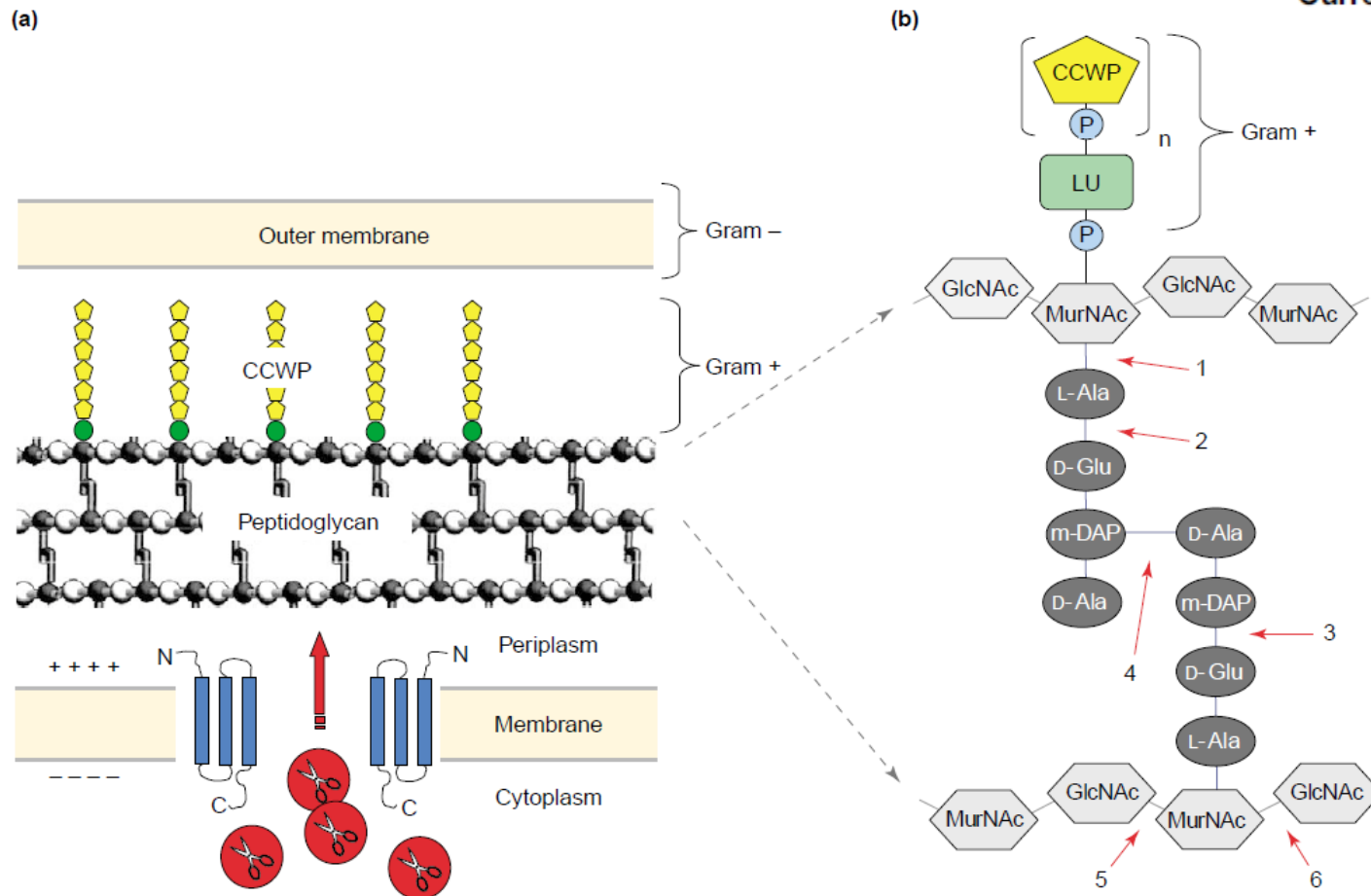


- Bacteriophage endolysins (=peptidoglycan hydrolase enzymes) are enzymes which cleave essential bonds in the peptidoglycans of bacterial cell wall for phage progeny release
- can cause “lysis from without.”
- Endolysins can act synergistically with antibiotics by resensitizing bacteria to non-susceptible antibiotics
- No strains resistant to phage endolysin

# Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme

Daniel Nelson<sup>\*†</sup>, Lawrence Loomis<sup>‡</sup>, and Vincent A. Fischetti<sup>\*</sup>

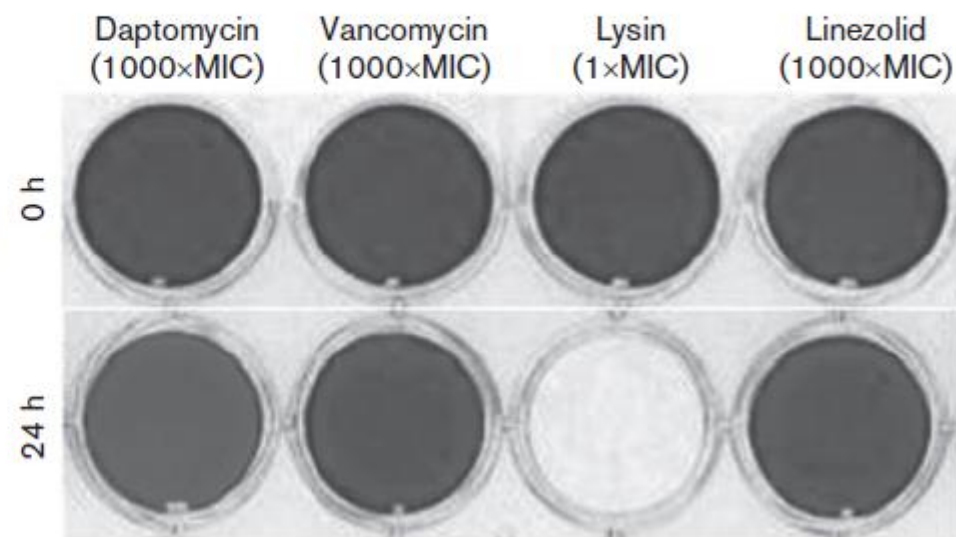
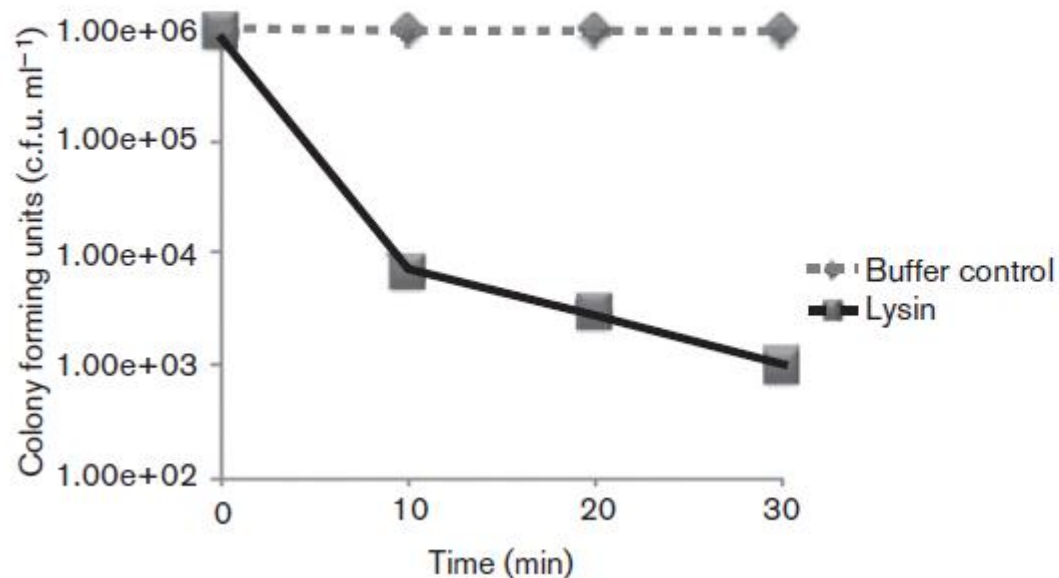
Current Opinion in Microbiology 2005, 8:480–487



Current Opinion in Microbiology



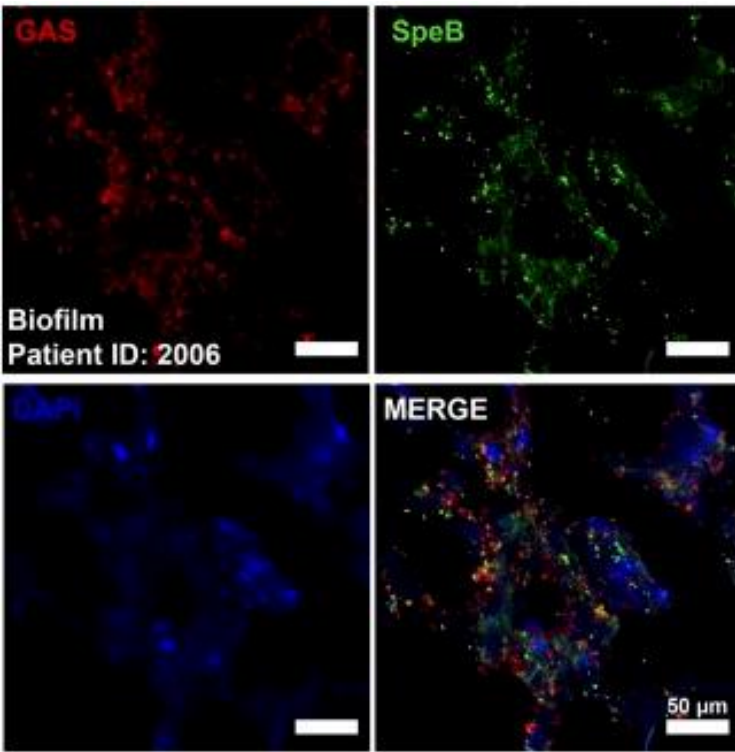
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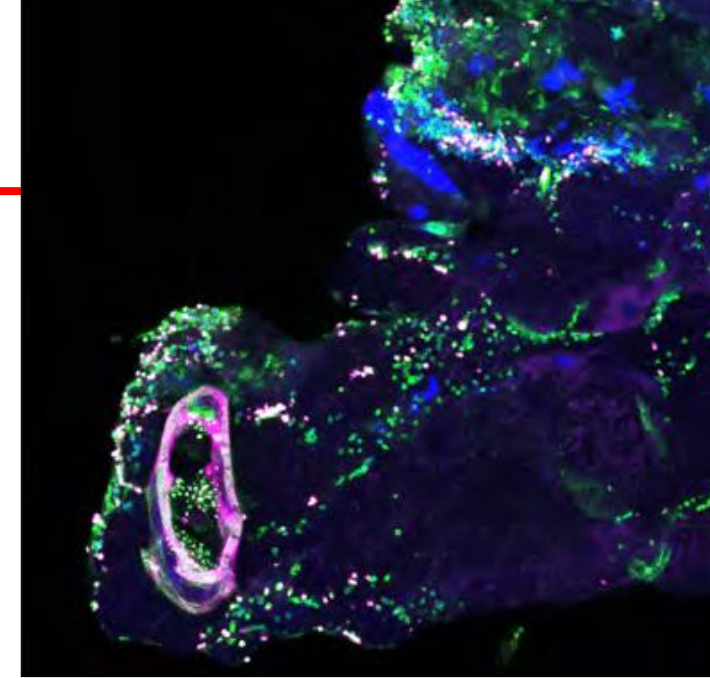
**Table 1.** Characteristics of lysins effective against antibiotic-resistant Gram-positive bacteria

Antibiotic-resistant bacteria	Lysin	Efficacy model	Unique characteristics	Reference
<i>C. difficile</i> (B1/NAP1 strain)	CD27L	<i>In vitro</i>	Can be expressed in <i>Lactococcus lactis</i> for gastrointestinal delivery	Mayer <i>et al.</i> (2008)
<i>E. faecalis</i> , <i>E. faecium</i> [vancomycin-resistant (VRE)]	PlyV12	<i>In vitro</i>	Broad-spectrum lysin; also active against groups A and B streptococci	Yoong <i>et al.</i> (2004)
<i>Staphylococcus aureus</i> [meticillin- (MRSA), vancomycin- (VRSA) and vancomycin intermediate- (VISA) resistant]; biofilm-forming <i>Staphylococcus epidermidis</i> , strain RP62A	ClyS, LysK	Murine sepsis (ClyS), murine nasal (ClyS, LysK) and murine skin decolonization (ClyS)	<u>ClyS</u> : bioengineered chimeric lysin; resensitizes MRSA to oxacillin <u>LysK</u> ; catalytic domain alone is active	Becker <i>et al.</i> (2008); Horgan <i>et al.</i> (2009); Daniel <i>et al.</i> (2010); Fenton <i>et al.</i> (2010a); Pastagia <i>et al.</i> (2011)
<i>Streptococcus agalactiae</i> (streptomycin-resistant)	PlyGBS	Murine pharyngeal and murine vaginal decolonization	DNA mutagenesis increases lysin activity	Cheng <i>et al.</i> (2005); Cheng & Fischetti (2007)
<i>Streptococcus pneumoniae</i> (penicillin-resistant)	Cpl-1, Pal	Rat endocarditis and rat meningitis (Cpl-1); murine sepsis (Cpl-1, Pal), murine pneumonia (Cpl-1) and murine nasal (Cpl-1) decolonization	Dimerization increases half-life	Loeffler <i>et al.</i> (2001, 2003); Jado <i>et al.</i> (2003); Entenza <i>et al.</i> (2005); McCullers <i>et al.</i> (2007); Grandgirard <i>et al.</i> (2008); Witzentrath <i>et al.</i> (2009); Resch <i>et al.</i> (2011)
<i>Streptococcus pyogenes</i> (streptomycin-resistant)	PlyC	Murine pharyngeal decolonization	Pre-infection dose prevents subsequent colonization	Nelson <i>et al.</i> (2001)

# GAS make biofilm

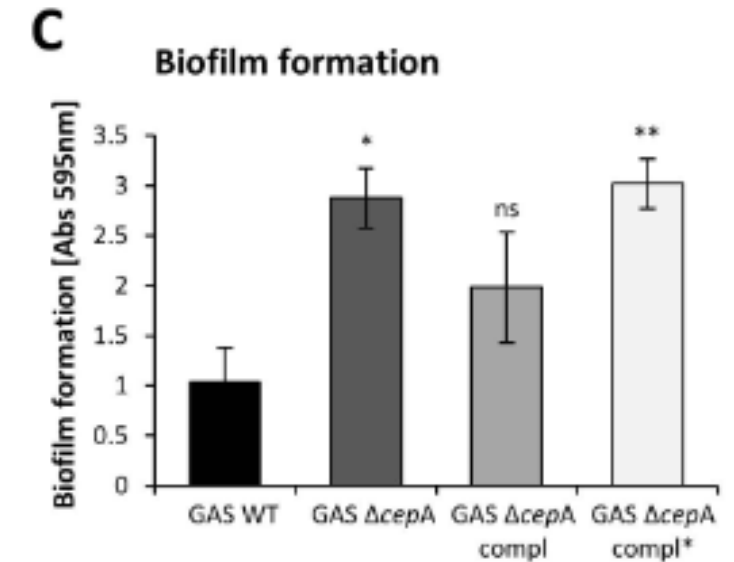
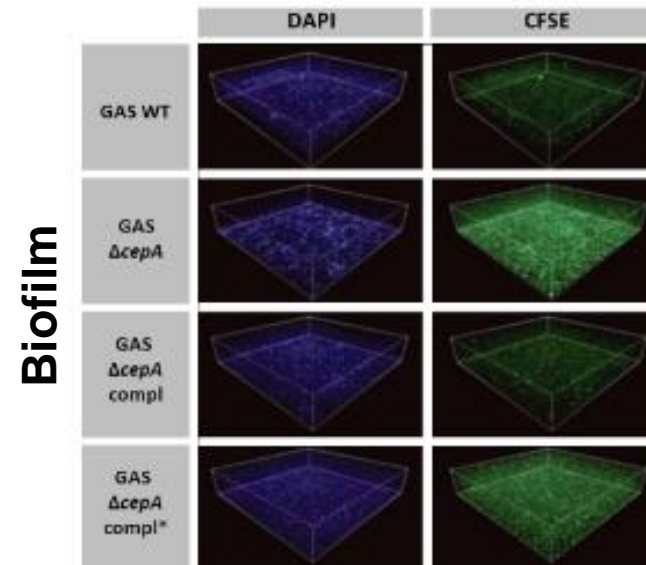


JCI Insight. 2016  
Jul 7; 1(10): e87882.



## SpyCEP reduces biofilm

Confocal fluorescence microscopy



Andreoni et al. 2014

frontiers in  
MICROBIOLOGY

# Antibiotic activity against planktonic and biofilm-embedded *Streptococcus pyogenes*

**Table 1.** Antimicrobial susceptibility of *Streptococcus* spp. by Etest and microcalorimetry in planktonic and biofilm

Antimicrobial	MIC (mg/L)					
	<i>S. agalactiae</i> (ATCC 13813)			<i>S. pyogenes</i> (ATCC 19615)		
	Etest	MHIC/MBBC		Etest	MHIC/MBBC	
		planktonic	biofilm		planktonic	biofilm
Fosfomycin	64	64	>1024	64	128	>1024
Rifampicin	0.064	0.128	1024	0.023	0.064	256
Benzylpenicillin	0.047	0.064	64	0.016	0.016	32
Daptomycin	0.25	0.5	64	0.23	0.125	16
Gentamicin	3	4	8	1	4	4
Levofloxacin	0.75	1	1024	0.38	0.5	1024

**Table 3.** MBEC and FICI<sub>MBEC</sub> of antibiotic combinations against *Streptococcus* spp. biofilms evaluated by sonication

Antimicrobial	<i>S. agalactiae</i>		<i>S. pyogenes</i>	
	(ATCC 13813)		(ATCC 19615)	
	MBEC (mg/L)	FICI <sub>MBEC</sub>	MBEC (mg/L)	FICI <sub>MBEC</sub>
Rifampicin	2048		512	
Benzylpenicillin	2048		512	
Gentamicin	8		4	
Rifampicin + gentamicin	8+1	0.129 (S)	≤4+1	≤0.258 (S)
Benzylpenicillin + gentamicin	4+0.5	0.064 (S)	≤2+0.25	≤0.066 (S)

## APPLE CIDER VINEGAR



1. Mix 1-3 tsp ACV in 1 glass of warm water.
2. Gargle with this solution several times a day for 2 to 3 days.

## GARLIC



Peel a fresh garlic clove, slice it in half and suck on a piece like a candy.

## SALT WATER

1. Add ½ tsp salt to 1 cup of water.
2. Gargle with this solution for a few seconds, then spit it out.



## CAYENNE PEPPER



## LEMON JUICE AND HONEY



## CINNAMON



## ONION

# HOME REMEDIES FOR STREP THROAT



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# Fresh Garlic Extract Enhances the Antimicrobial Activities

---



- Ajoene, a sulfur-rich molecule from garlic
- prevents bacteria from secreting the toxin rhamnolipid which destroys neutrophils
  - inhibits genes controlled by quorum sensing
    - promotes rapid clearing of pulmonary *Pseudomonas aeruginosa* infections.
    - renders *P. aeruginosa* sensitive to tobramycin, respiratory burst and phagocytosis by PMNs

**ScienceDaily**

[Microbiology](#). 2005 Dec;151(Pt 12):3873-80.

[Antimicrob Agents Chemother](#). 2012 May;56(5):2314-25. doi: 10.1128/AAC.05919-11.

[Jundishapur J Microbiol](#). 2015 May 31;8(5):e14814. doi: 10.5812/jjm.14814.

[Anc Sci Life](#). 2013 Oct;33(2):114-8. doi: 10.4103/0257-7941.139053.



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# Fresh Garlic Extract Enhances the Antimicrobial Activities



Garlic contains so little ajoene that you would need to eat around 50 a day to achieve the desired effect.

Credit: © Stefano Pareschi / Fotolia

ScienceDaily

[Microbiology](#). 2005 Dec;151(Pt 12):3873-80.

[Antimicrob Agents Chemother](#). 2012 May;56(5):2314-25. doi: 10.1128/AAC.05919-11.

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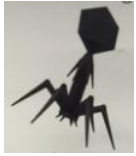
[Jundishapur J Microbiol](#). 2015 May 31;8(5):e14814. doi: 10.5812/jjm.14814.

[Anc Sci Life](#). 2013 Oct;33(2):114-8. doi: 10.4103/0257-7941.139053.



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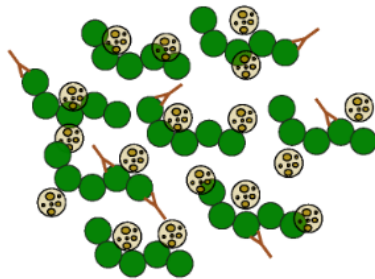
# pathogen directed therapy



Antibiotics, antimicrobials  
and  
antibodies



direct killing of pathogen  
disarming bacterial virulence factors



pathogen clearance

Adapted from  
Nadia Keller



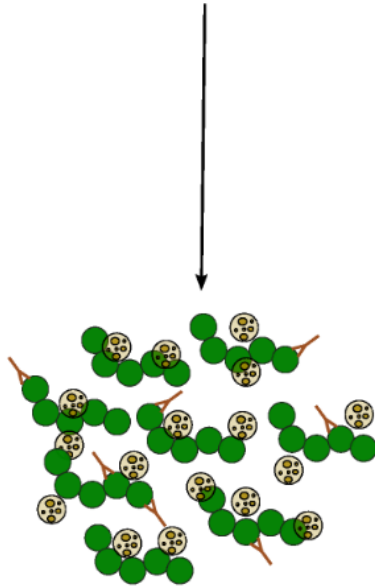
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Antibiotics, antimicrobials  
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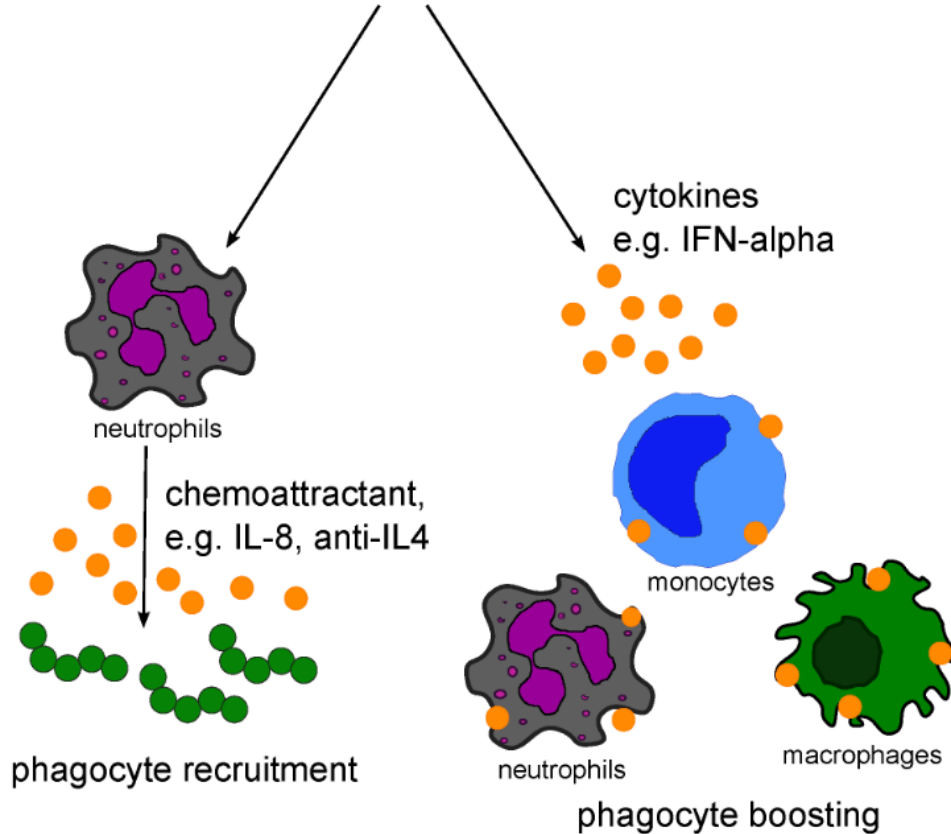
pathogen clearance

# host directed therapy

Cytokines  
and  
chemokines



direct effect on immune response



Adapted from  
Nadia Keller



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# host directed therapy

---

- Activation/boosting of eukaryotic effector mechanisms
    - increase neutrophil numbers
    - antimicrobial peptides,
    - nitric oxide,
    - reactive oxygen species
  - Vitamin D, HIF-1 $\alpha$  inducers, IL-4, Interferon  $\alpha$
- kill the bacteria

# Vitamin D

- potent inducer of antimicrobial peptides
- link between vitamin D deficiency and the recurrence of GAS tonsillopharyngitis.

**Table 2**  
Results of multiple logistic regression analysis of recurrent GAS tonsillopharyngitis

Variable	OR (95% CI)	p-Value
Male gender	1.15 (0.21–6.38)	0.86
Age	0.97 (0.91–1.03)	0.35
CRP >3 mg/l	1.57 (1.13–2.19)	0.007
Serum 25(OH) vitamin D <20 ng/ml	1.62 (1.51–1.76)	0.001

GAS, group A Streptococcus; OR, odds ratio; CI, confidence interval; CRP, C-reactive protein



The association between vitamin D levels and recurrent group A streptococcal tonsillopharyngitis in adults

William Nseir<sup>a,b,e,\*</sup>, Julnar Mograbi<sup>a,b</sup>, Zuhair Abu-Rahmeh<sup>c</sup>, Mahmud Mahamid<sup>a</sup>, Omar Abu-Elheja<sup>a</sup>, Adel Shalata<sup>d</sup>

# Atopic Dermatitis

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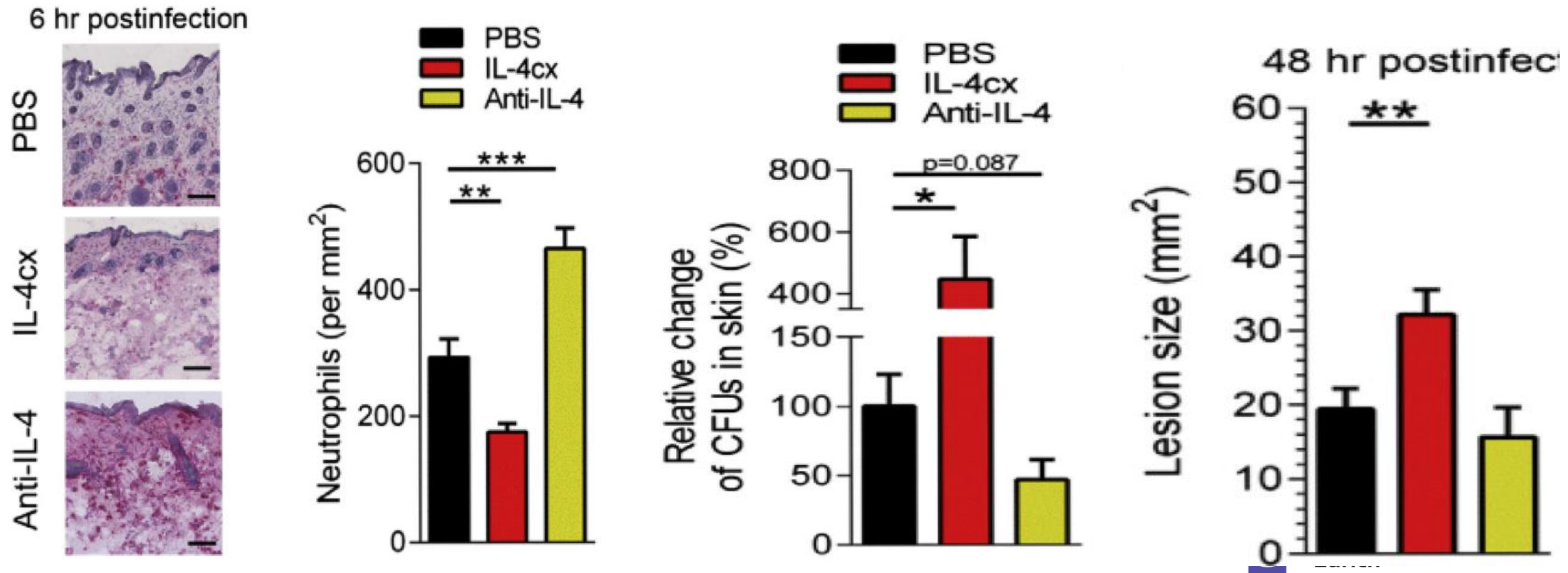


- Allergic 'type-2' inflammation (IL4)-> hampers neutrophil expansion and migration
- **Why are atopic individuals more prone to infections with Gram positive bacteria?**

# Atopic Dermatitis

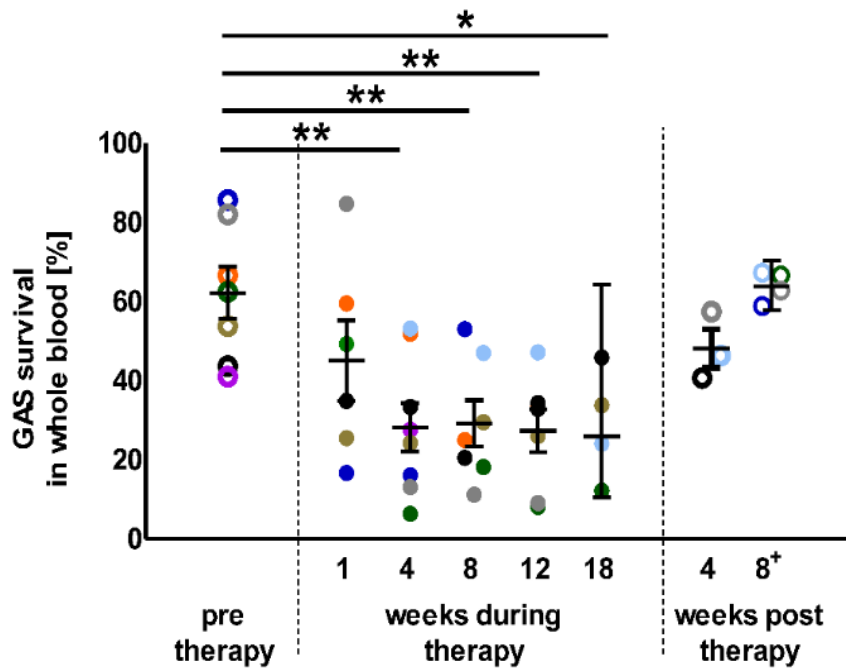
IL4-complex = fewer neutrophils, anti-IL4 = more neutrophils

J. Woytschak et al., *Immunity* 2016

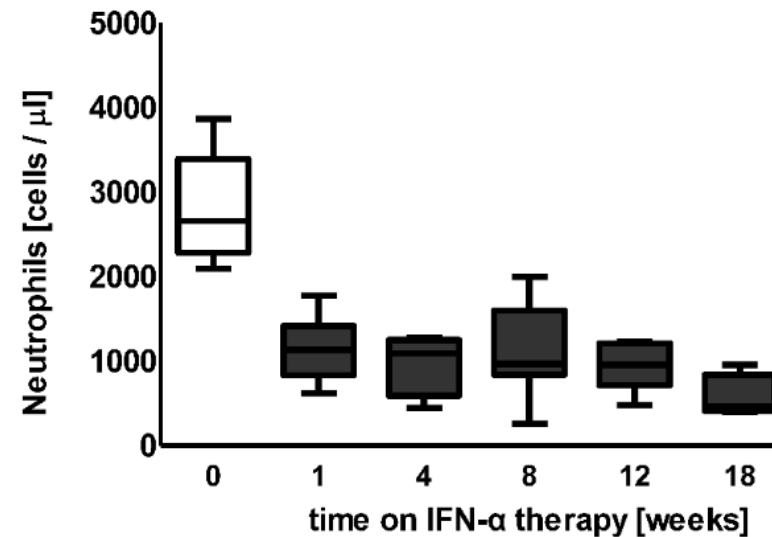


# Exogenous IFN- $\alpha$ boots GAS killing

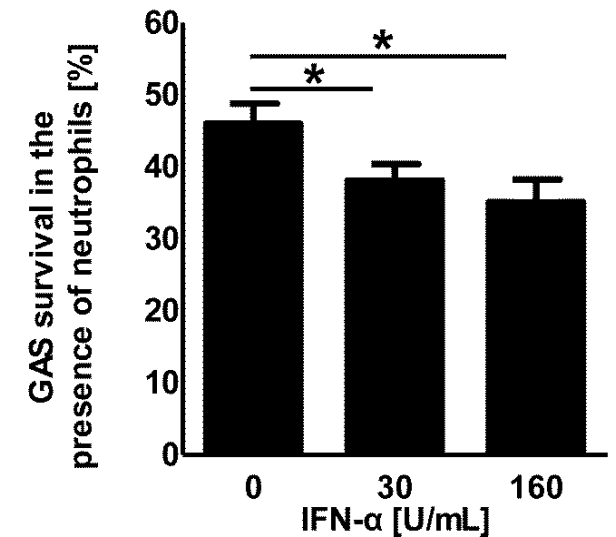
## GAS survival in patient whole blood



## Neutrophil counts over time of therapy

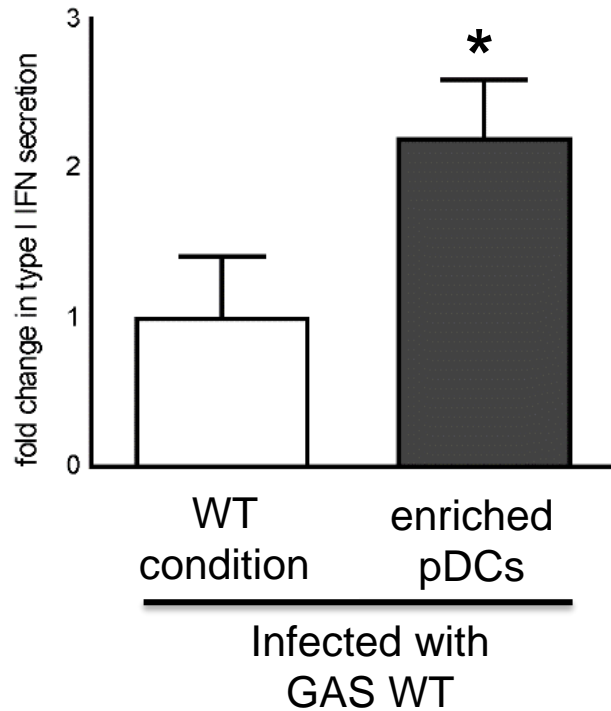


## GAS survival in IFN- $\alpha$ stimulated neutrophils

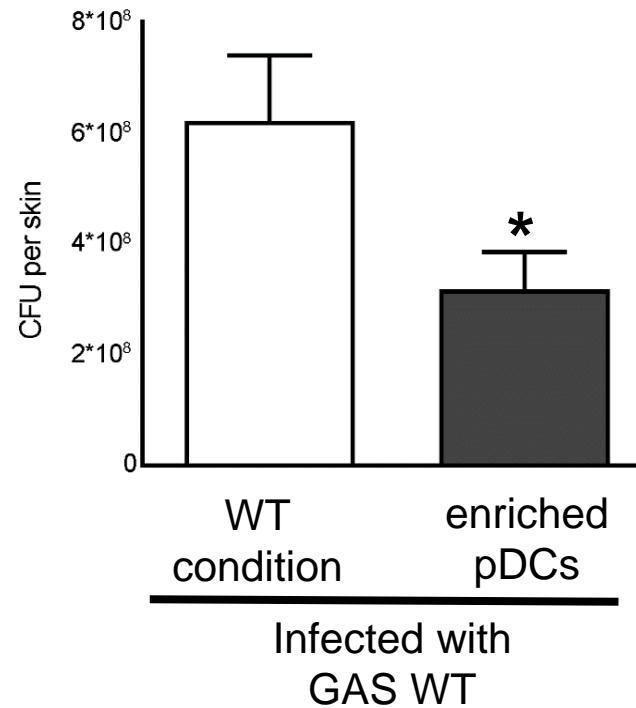


# Endogenous IFN- $\alpha$ improves outcome

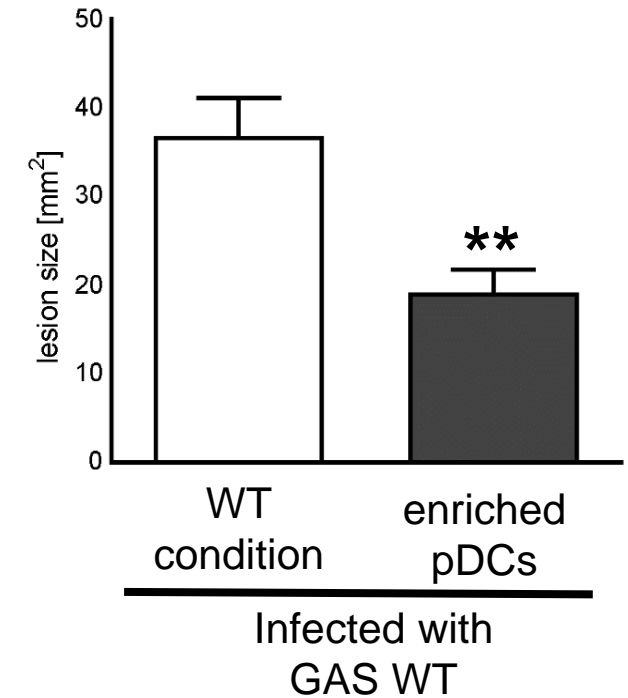
## Type I interferon levels



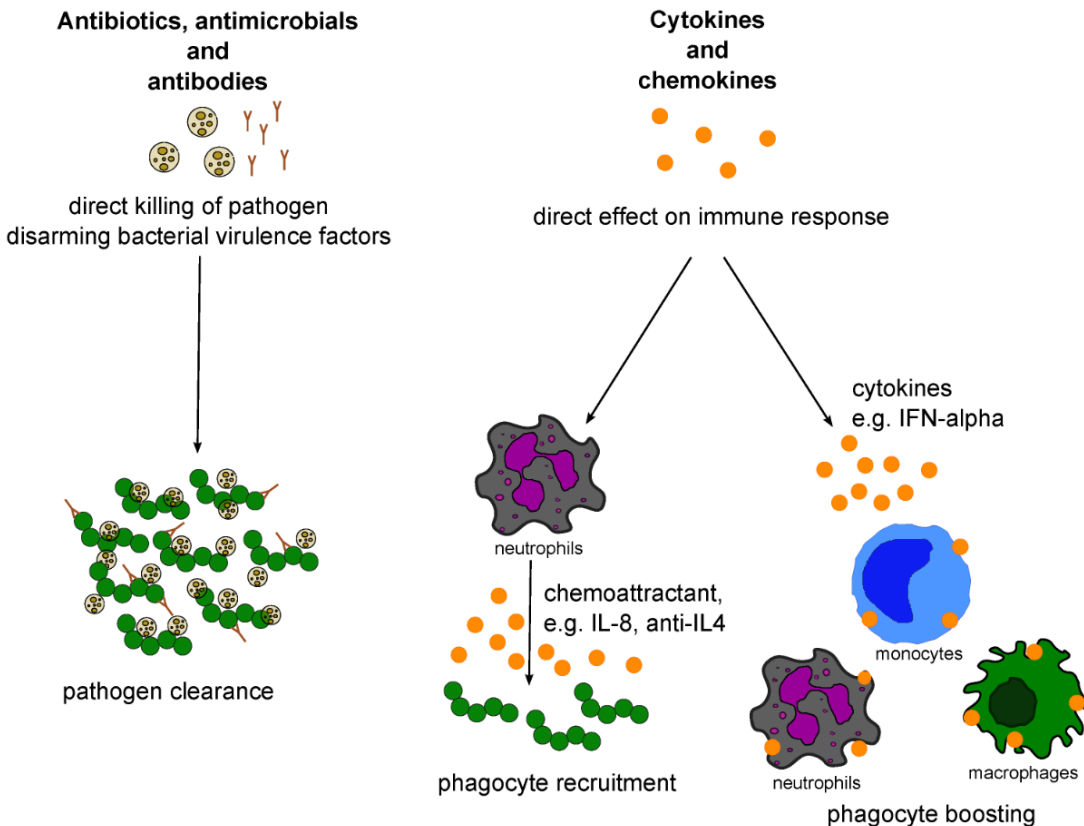
## Bacterial load



## Lesion sizes



# Antibiotics and beyond for treating invasive GAS infections



- **Pathogen directed therapy**
  - Killing
    - beta lactams, phages, endolysine
  - Anti- toxine strategies
    - Protein synthesis inhibitors
    - IVIG
  - Anti- biofilm strategies
- **Host directed therapy**
  - Vitamin D, HIF-1 $\alpha$  inducers, IL-4, Interferon  $\alpha$

# Pathogenesis of Gram positive Bacterial Infections



- Commensals Misbehaving
- Susceptible *Staphylococci* spp.

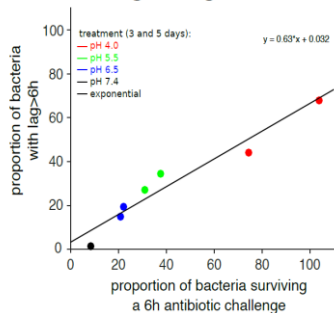
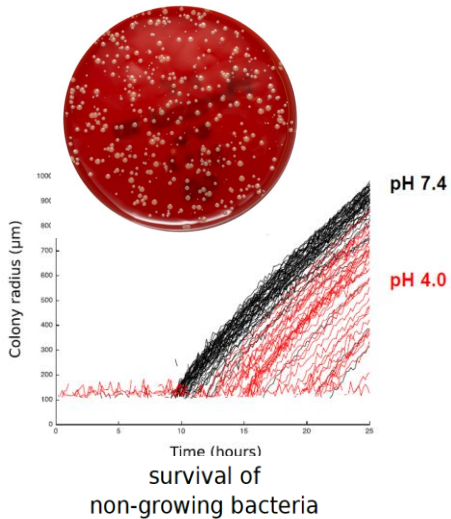
1. 'Location': abscess, intracellular, biofilm

2. 'Growth': stationary bacteria, persisters

-> Removal of infectious source crucial

-> Antibiotics: The proportion of bacteria in lag phase correlates with the proportion of bacteria surviving antibiotics

- **Group A Streptococci**: high inoculum, many virulence factors – combination therapy penicillin, clinda plus IVIG



# TEAMWORK

## 1+1=3

Klinik für Infektionskrankheiten &  
Spitalhygiene

Kardiologie und Herzchirurgie USZ

**Eawag**

Prof. M. Ackermann and  
C. Vulin, PhD



University of  
Zurich<sup>UZH</sup>



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# Many thanks



UniversitätsSpital  
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Federica Andreoni  
Andrea Tarnutzer  
Markus Huemner  
Nicola Häffner  
Vanina Hauenreiter Dengler  
Yvonne Achermann  
Mathilde Boumasmoud  
Srikanth MairpadyShambat  
Alejandro Gomez Mejia  
Tizian Schweizer  
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Katrin Schilcher  
Rey Gaffner



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