Infektionen der Lunge
Eine Fortbildung für Lungeninteressierte Lungenzentrum
St. Gallen

Immunologische Abwehrmechanismen und Pathogenitätsfaktoren

Angriffsstrategien

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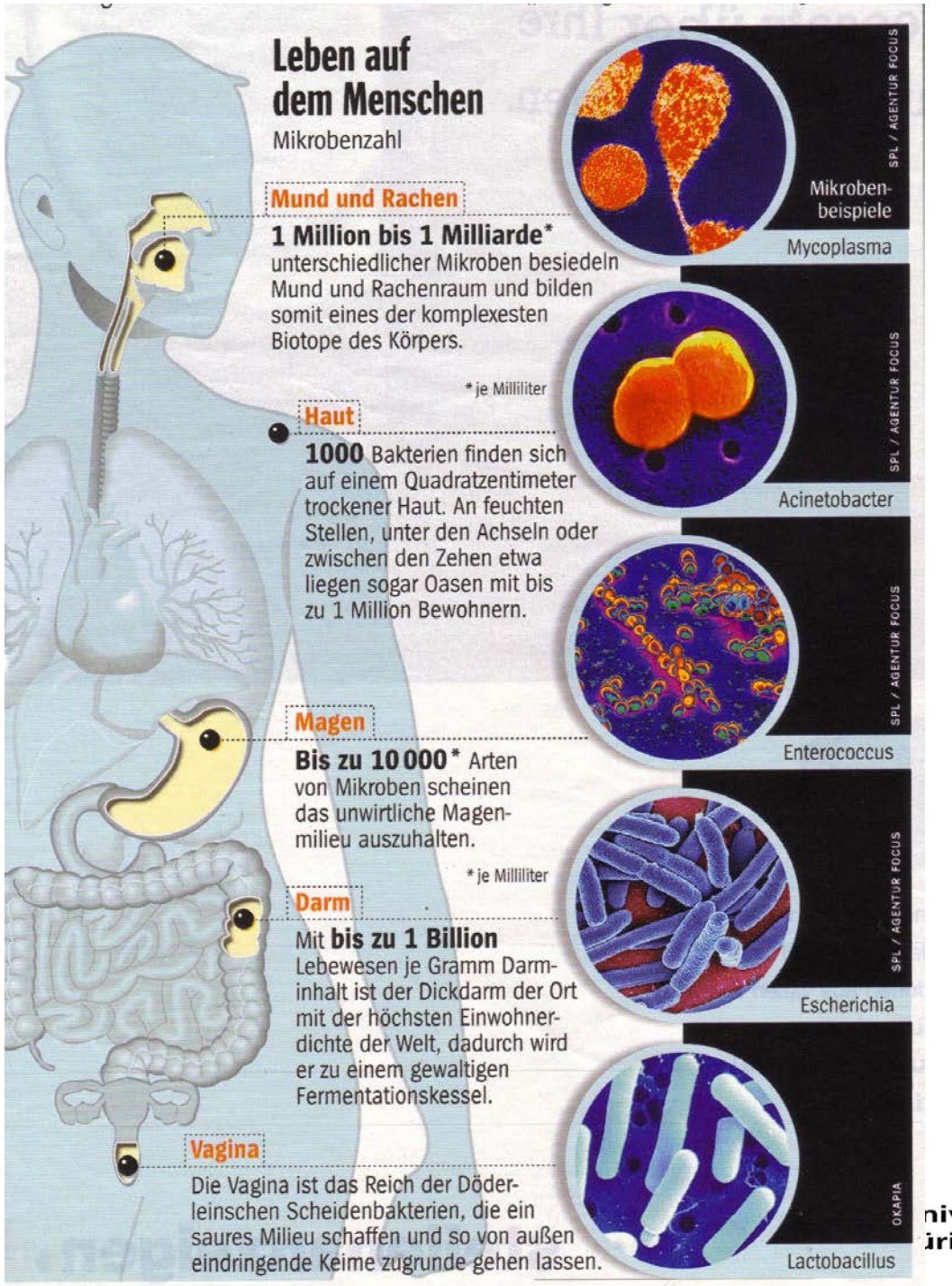
University of Zurich[UZH]

UniversityHospital Zurich

Die Bakterienflora des Menschen



Der Spiegel, 21/2007, p. 140

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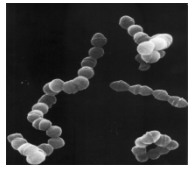
Commensals- Pathogens

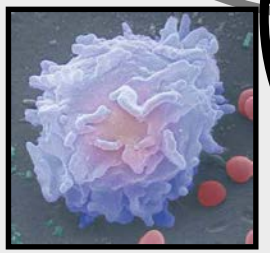
BACTERIA

Commensal wright place
Pathogen wrong place
virulence factors
commensals misbehaving

Commensals
- Digestion
- Vitamin production
- Space holder



HOST
Innate Resistance



Pathogens
- Virulence Factors

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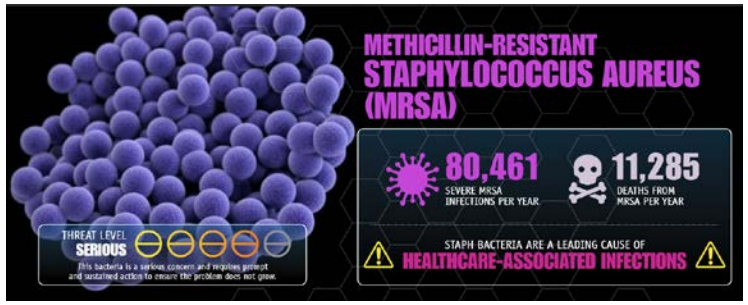
Angriffsstrategien



BACTERIA

Commensal wright place
Pathogen wrong place
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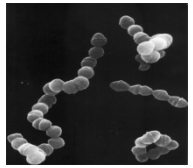
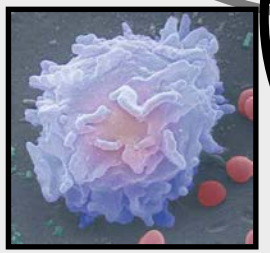
Commensals

- Digestion
- Vitamin production
- Space holder



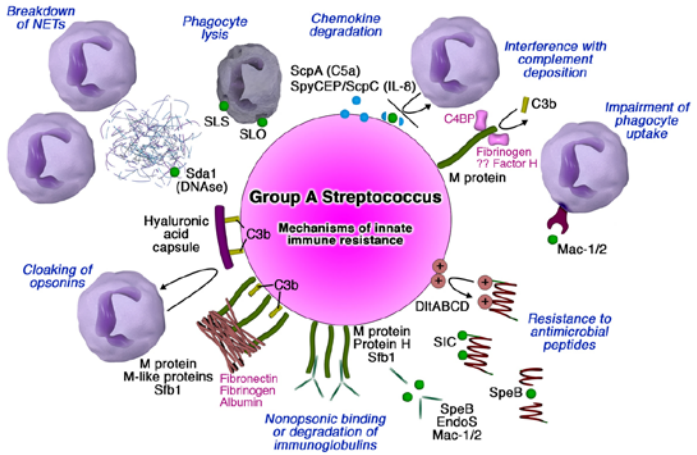
HOST

Innate Resistance



Pathogens

- Virulence Factors



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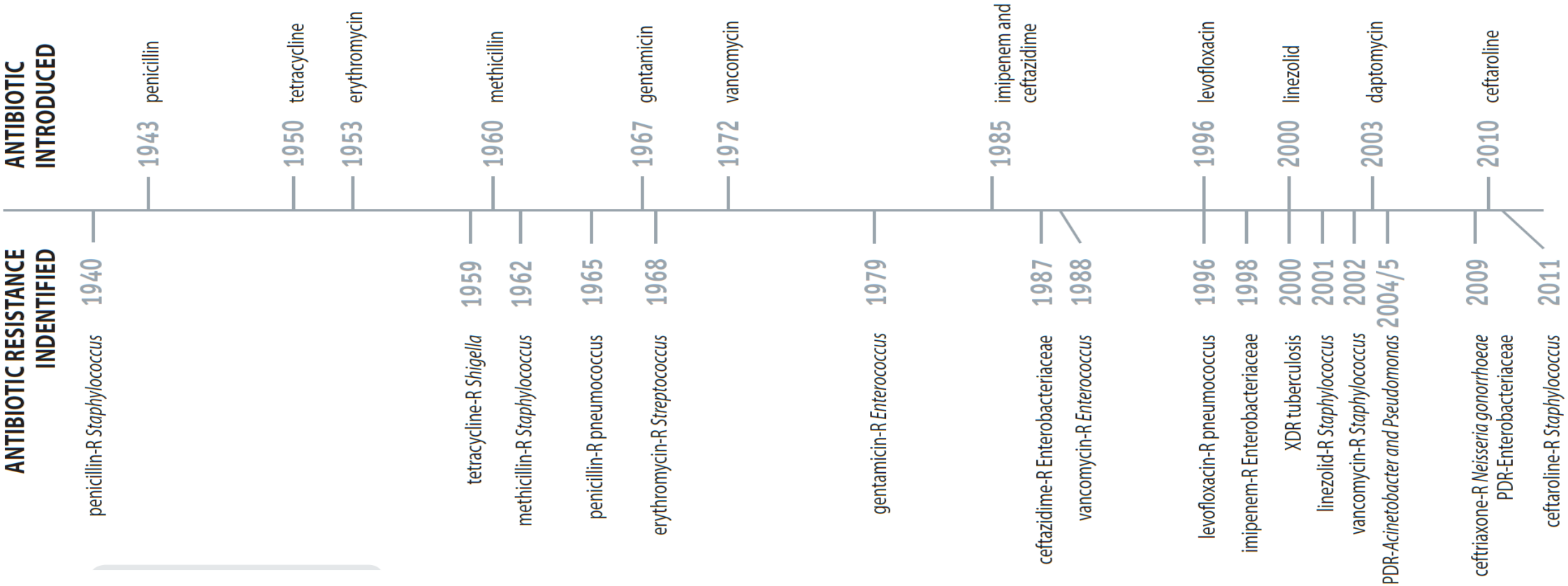
Evolution of Resistance



Antibiotic resistance threats, CDC 2013

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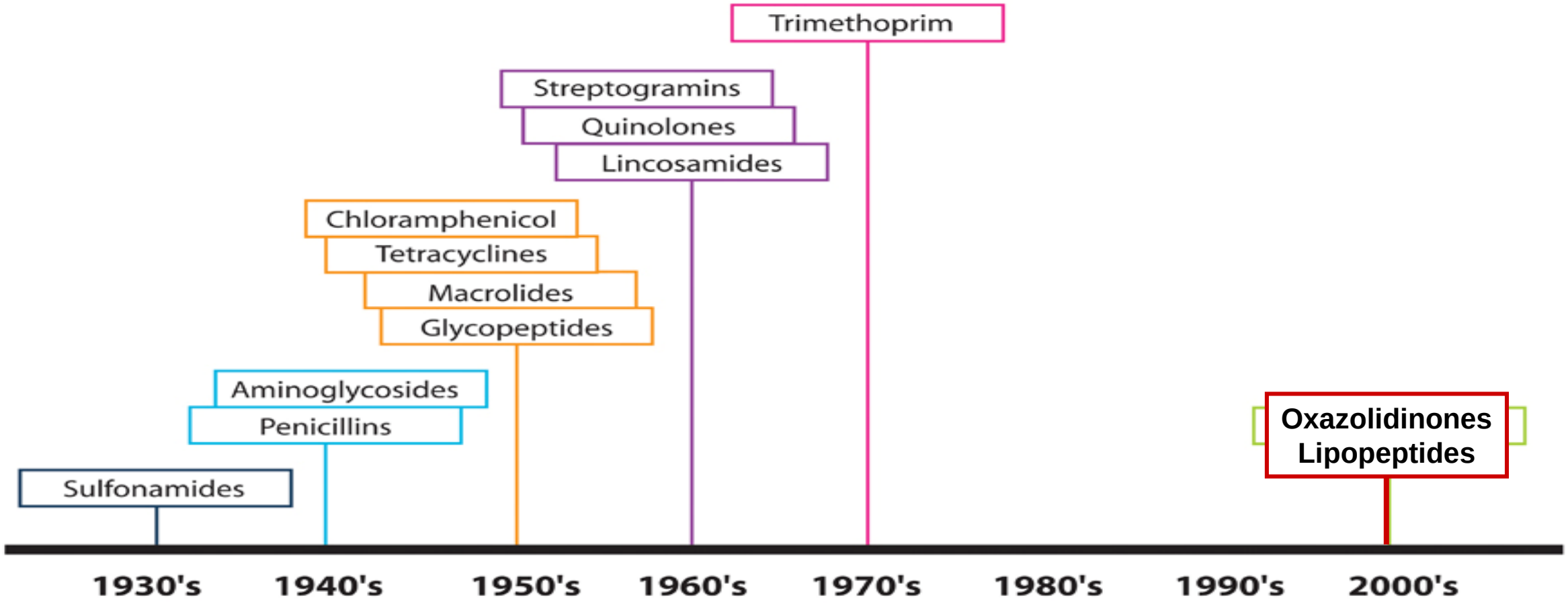
Evolution of new Antibiotics



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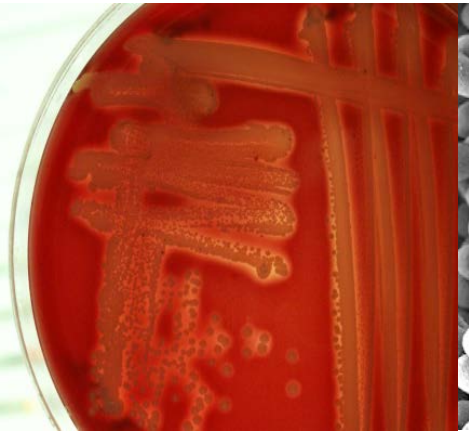
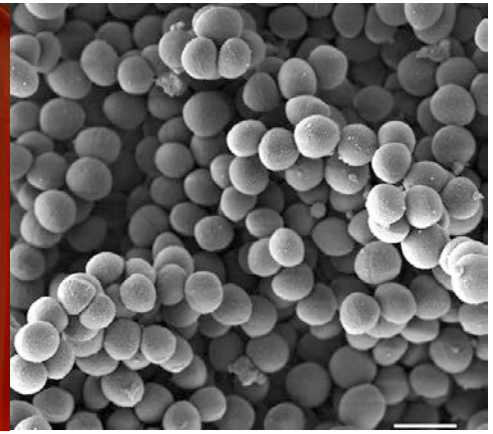
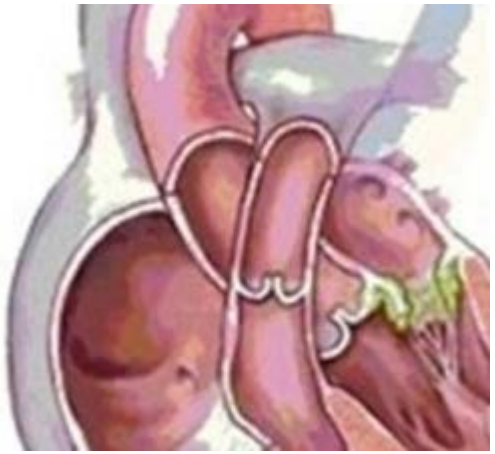
Staphylococcus aureus: a commensal misbehaving







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

(Fowler *et al*. 2005 JAMA; Cosgrove and Fowler. 2008 CID; Chang *et al*. 2003 Medicine)

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CHAPTER 24

Pulmonary Infections

Dennis M. Marchiori

Empyema

Background

Empyema is an intrapleural infection distinguished from simple parapneumonic effusions based on positive cultures. The most likely infectious agents are tuberculosis (TB) or *Staphylococcus*, although many others have been identified. Often other radiographic evidence accompanies empyema, including pneumonia, surgery, trauma, and abdominal infections.[2,21]

Imaging Findings

Lung Abscess

Background

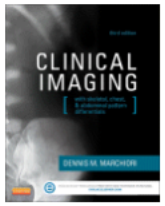
A lung abscess is a localized suppurative process marked by tissue necrosis. It commonly results from aspiration and bronchogenic spread of foreign material or infectious debris secondary to oropharyngeal surgery, sinobronchial infections, dental sepsis, and so on. Aspiration is common among patients with a suppression of the cough reflex caused by alcoholism, coma, general anesthesia, or narcotic use. Antecedent bacterial pneumonias (commonly *Staphylococcus aureus* and *Klebsiella pneumoniae*) may result in abscess formation.

Mosby

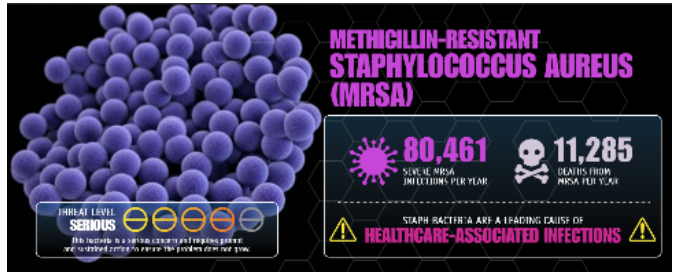
Clinical Imaging (Third Edition)

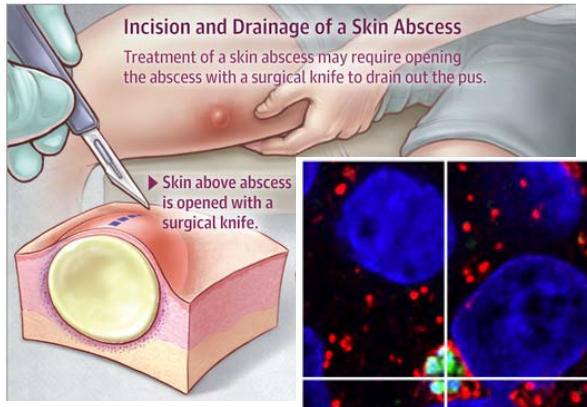
With Skeletal, Chest and Abdomen Pattern Differentials

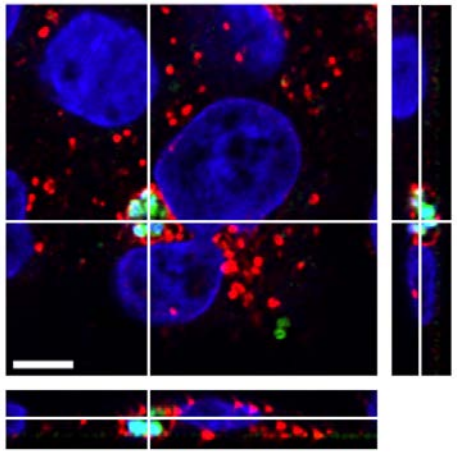
2014, Pages 1192-1208

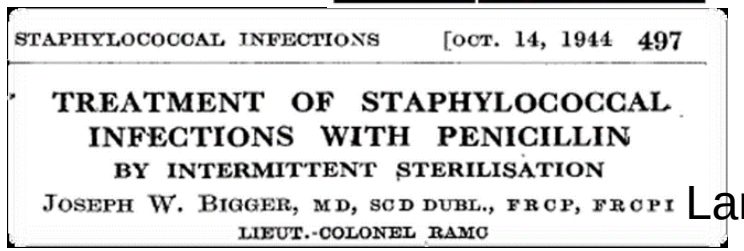


How do *S.aureus* withstand antibiotics?









STAPHYLOCOCCAL INFECTIONS [OCT. 14, 1944 497

TREATMENT OF STAPHYLOCOCCAL INFECTIONS WITH PENICILLIN BY INTERMITTENT STERILISATION

JOSEPH W. BIGGER, MD, SCD DUBL., FRCP, FRCPI
LIEUT.-COLONEL RAMC

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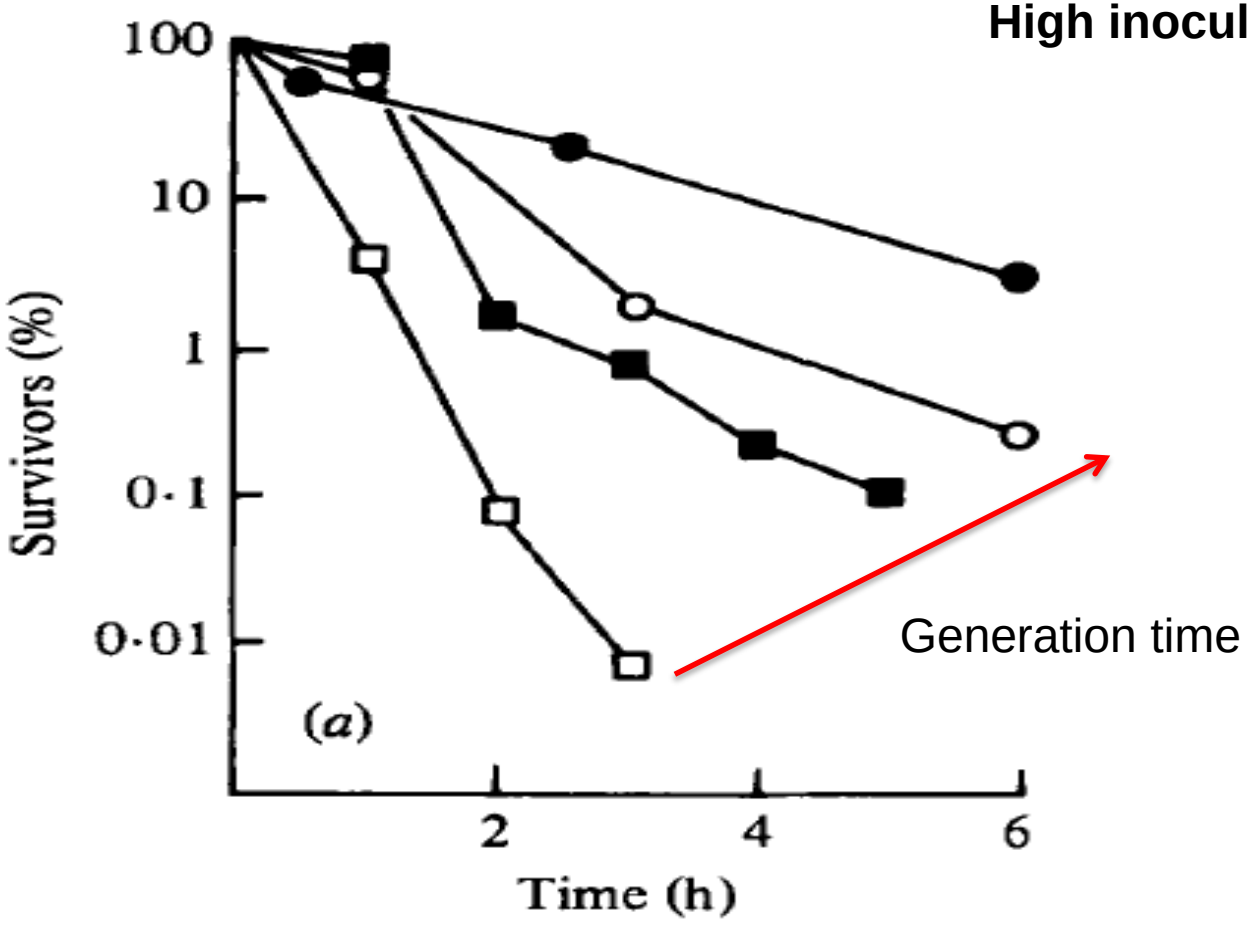
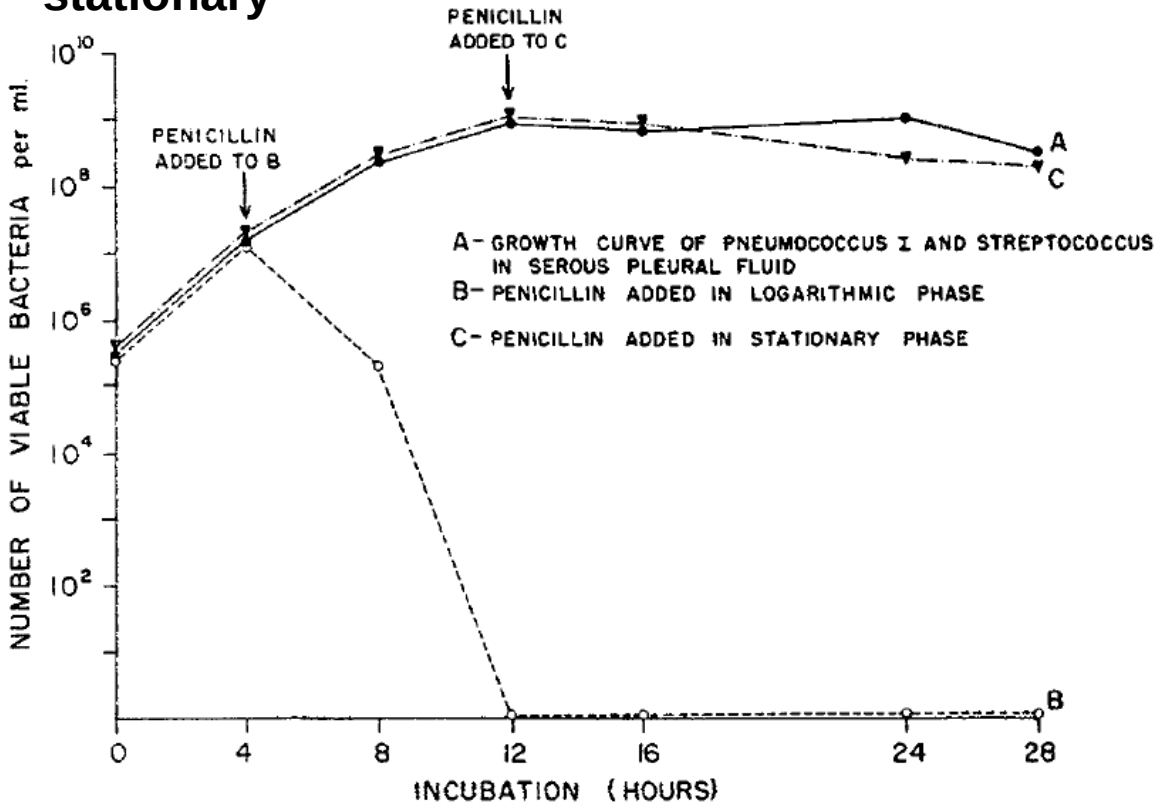
Rate of bacterial killing by beta-lactams is proportional to the bacterial growth rate



Tuomanen *et al*, J Gen Microbiology, 1986

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

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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_{50} (50 per cent of animals protected)	PD_{90} (90 per cent of animals protected)
	units/kg.						*per cent*	*units/kg.*	*units/kg.*
	16,000	6	0	6	0	6	100		
	8,000	6	0	5	0	6	100		
4 hrs.	4,000	6	0	4	0	6	100	1,500	3,500
	2,000	6	2	2	0	4	67 (71)		
	1,000	4	3	1	0	3	25 (17)		
	16,000	5	0	4	0	5	100		
	8,000	5	0	5	0	5	100		
4 days	4,000	6	0	5	0	6	100	2,000	3,500
	2,000	5	3	2	0	2	40		
	1,000	4	4	—	—	0	0		
	64,000	6	0	6	0	6	100		
	32,000	6	3	3	0	3	50 (77)		
2 wks.	16,000	5	1	4	0	4	80 (64)	14,000	50,000
	8,000	6	4	2	0	2	33 (20)		
	4,000	5	4	1	1‡	0	0		
	160,000	5	1	3	0	4	80 (89)		
	80,000	5	0	5	1	4	80 (67)		
6 wks.§	40,000	5	1	4	4	0	0	65,000	160,000
	20,000	5	2	3	3	0	0		
	10,000	6	0	6	6	0	0		

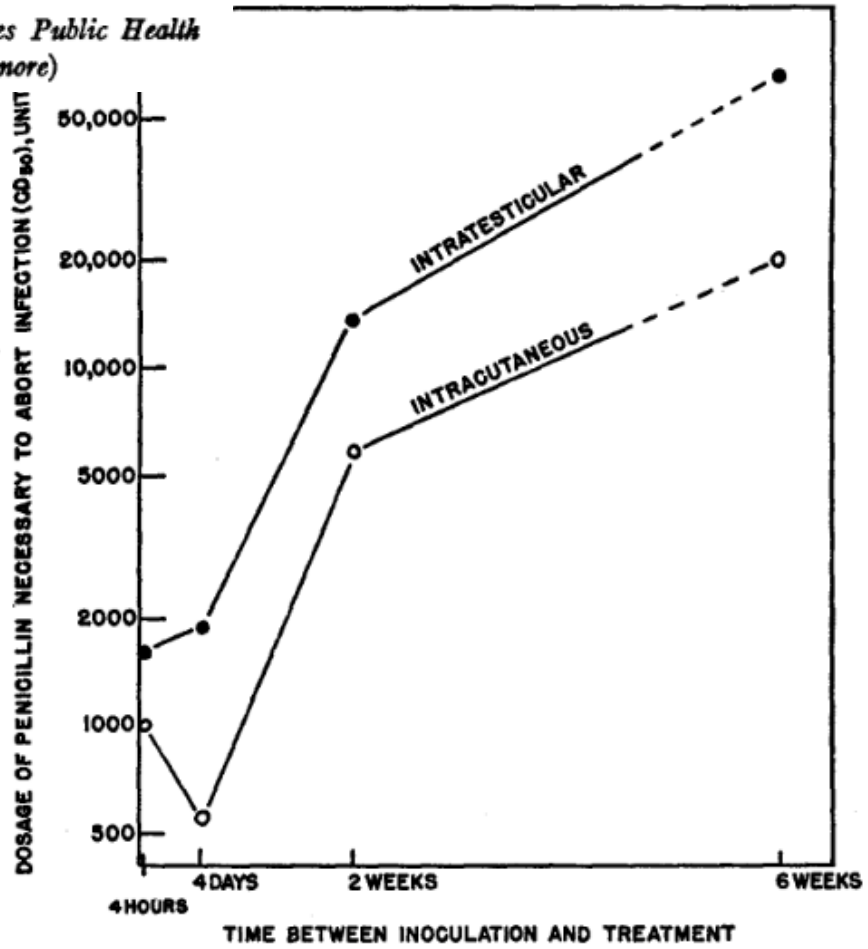

Fig. 2. Relation of the age of the infection to the curative (abortive) dose of penicilli Rabbits were inoculated intracutaneously or intratesticularly with 2 × 10^3 organisms. varying periods after inoculation, penicillin was administered as a single intramuscular injecti of a suspension in peanut oil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

1947

JEM Journal of Experimental Medicine

TABLE II

*Effect of the Size of the Inoculum on the Curative Dose of Penicillin G in White Mice Infected with a Group B β-Hemolytic Streptococcus**

	No. of organisms inoculated‡	Penicillin	Survived	Died	Curative dose (CD_{50}) of penicillin G, ± standard error§, ‖
		mg./kg.			*mg./kg.*
Group 1	2,235,000	2,048	20	0	424±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±45
		512	14	6	
		256	5	15	
		128	0	20	
		64	1	19	
		32	1	19	
		0	0	10	
Group 3	1,750 (estimated)	256	19	1	139±51**,§
		128	7	13	
		64	2	18	
		32	1	19	
		16	5	15	
		0	1	9	
Group 4	17¶	64	19	1	2.8±1.1
		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)

UBI PUS IBI EVACUA

J Ex Med 1949

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Chronic –recurring infections

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?

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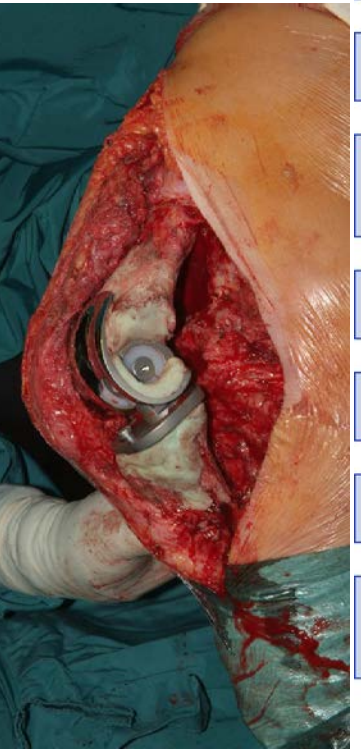
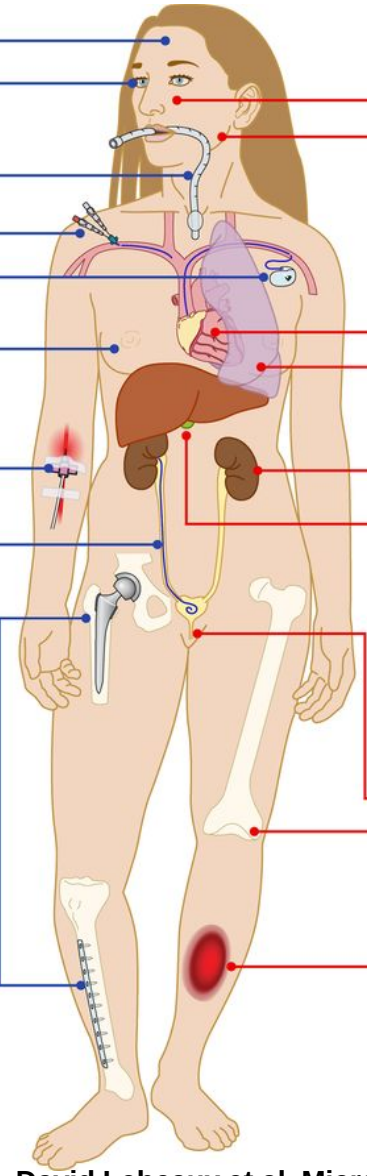
Chronic –recurring infections - Biofilm-related infections



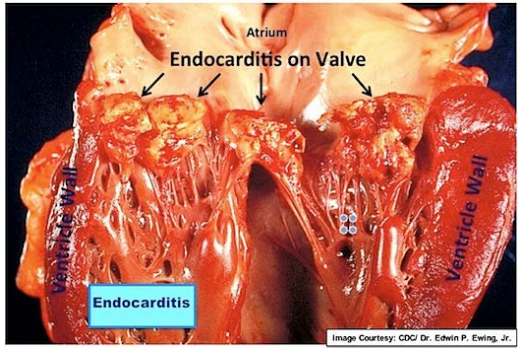
DEVICE-RELATED INFECTIONS

- Ventricular derivations
- Contact lenses
- Endotracheal tubes
- Central vascular catheters
- Prosthetic cardiac valves, pacemakers and vascular grafts
- Tissue fillers, breast implants
- Peripheral vascular catheters
- Urinary catheters
- Orthopedic implants and prosthetic joints



TISSUE-RELATED 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
- Osteomyelitis
- Chronic wounds



David Lebeaux et al. Microbiol. Mol. Biol. Rev. 2014;78:510-543

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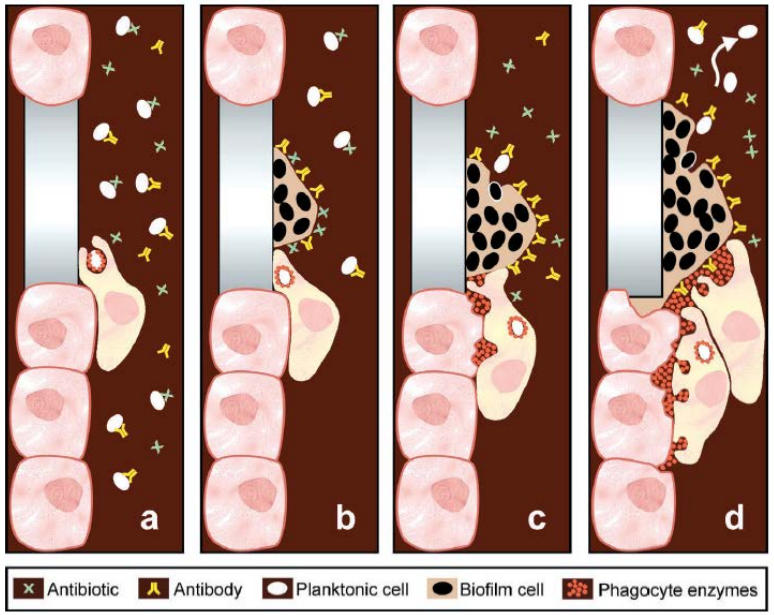
Besonderheiten von Bakterien im Biofilm



Science 1999;284:1318 (May 21)

- Verminderte Exposition gegenüber Abwehrmechanismen des Wirtsorganismus
- Unterschiedliche Mikrohabitate durch chemische und physikalische Gradienten, verschiedene biologische Eigenschaften (unterschiedliche Phänotypen): HETEROGENITÄT
- Interaktionen zwischen Bakterien (Signalaustausch [*quorum sensing*], Nutrition)
- Verminderte Empfindlichkeit gegenüber Antibiotika
- Persistenz im Biofilm

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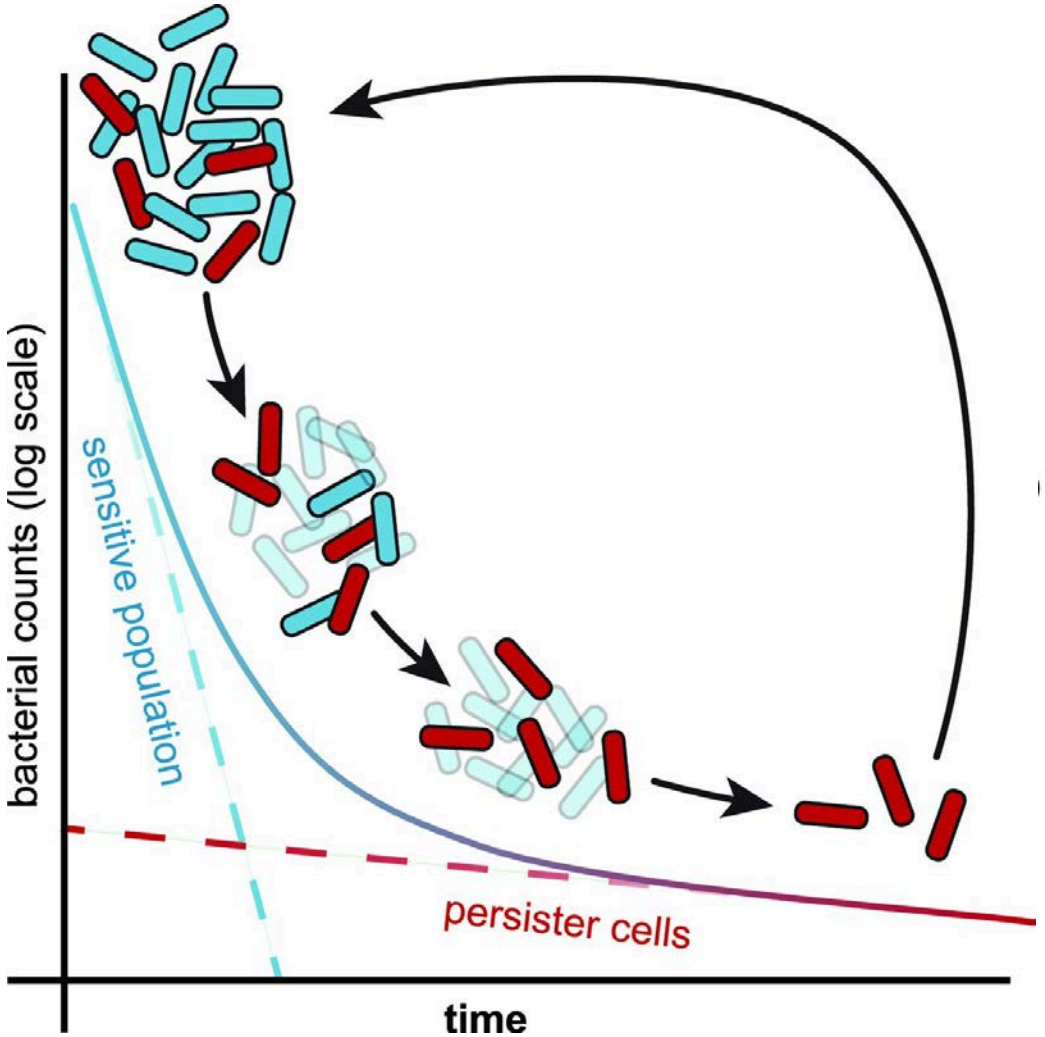
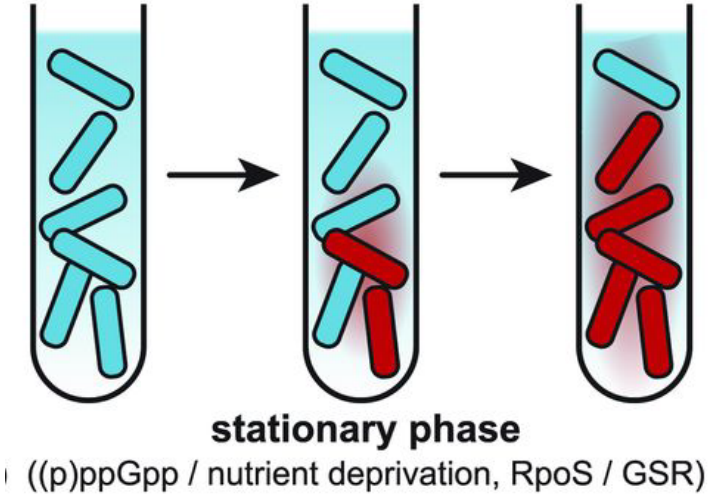
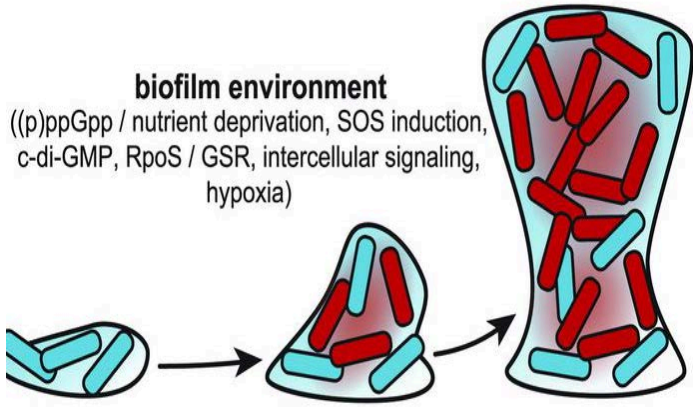
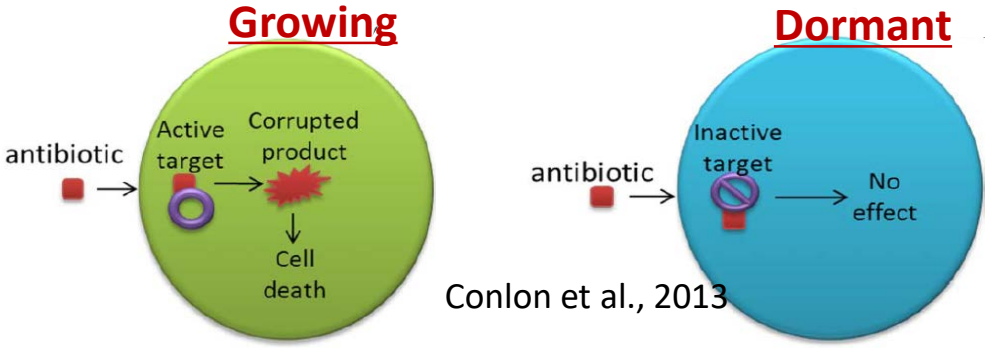
Persisters = metabolically inactive bacteria



Biphasic killing kinetics of bactericidal antibiotic treatment.







Conlon et al., 2013

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

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

Science AAAS

Alexander Harms et al. Science 2016;354:aaf4268

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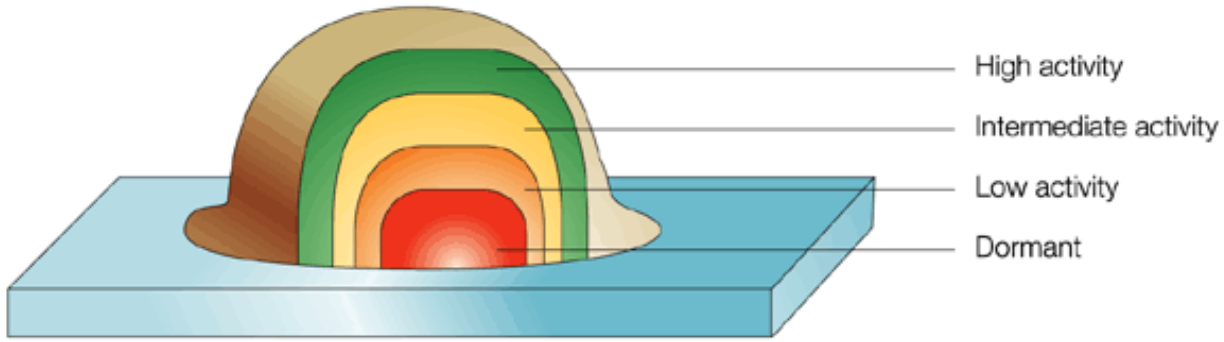
Persisters



Nature Reviews | Drug Discovery

Phänotypische Resistenz

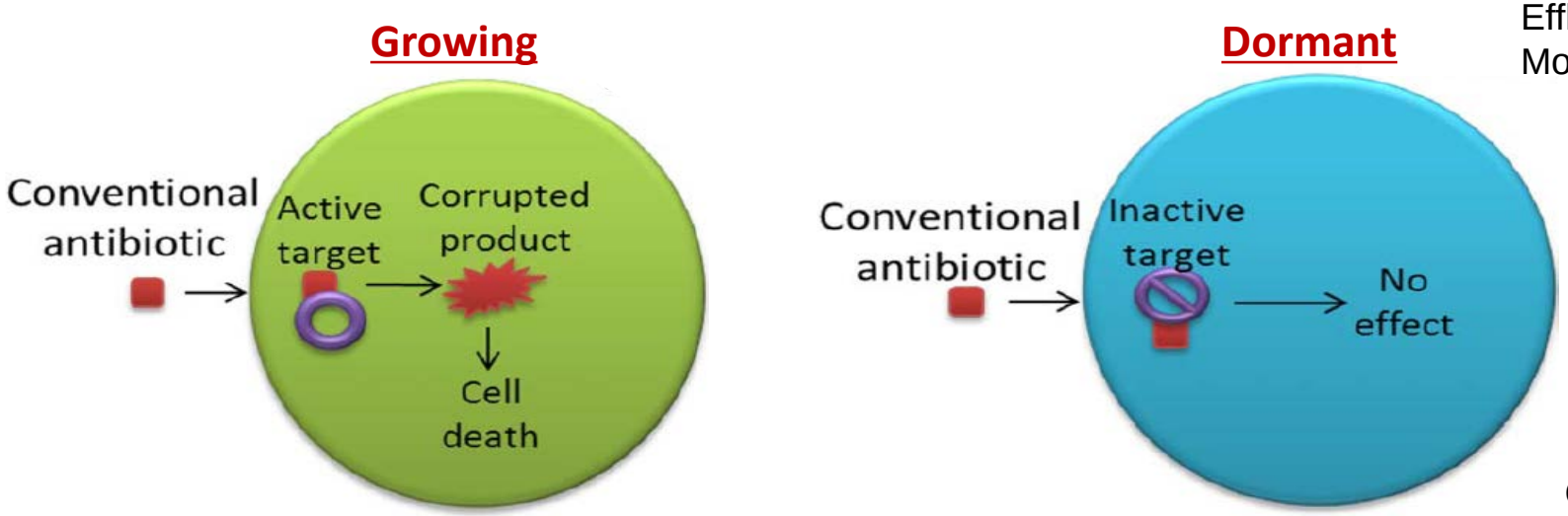
Persistenz, Toleranz

Natürliche Resistenz

Zielstruktur nicht vorhanden
Permeabilitätsbarriere
(z.B. äussere Membran bei Gram-)

Genetische Resistenz

Zielstruktur mutiert
Effluxpumpe hochreguliert
Modifizierende Enzyme



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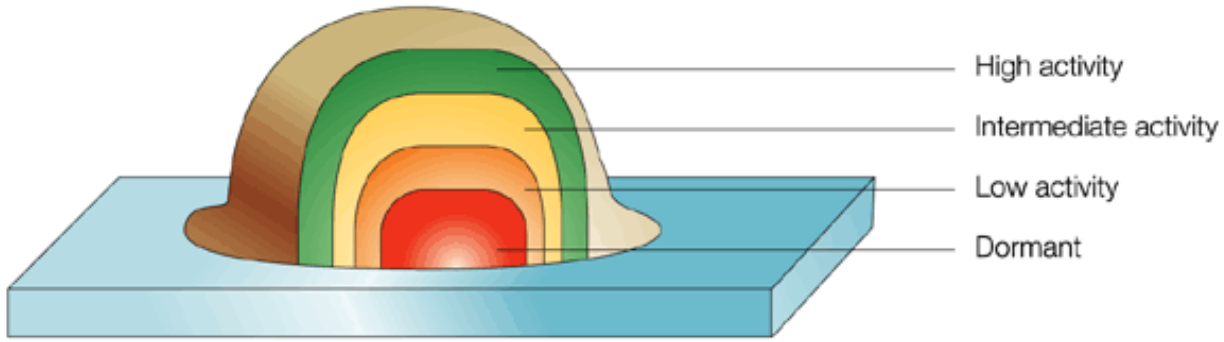
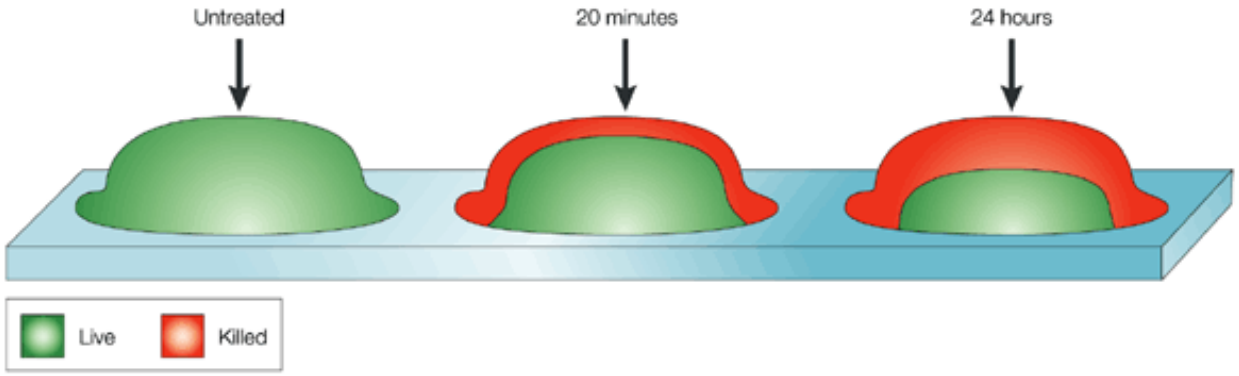
Conlon et al., 2013

Biofilm-Aktivität



Nature Reviews | Drug Discovery

- **Extrazelluläre Matrix**: Schlechte Penetration/ Diffusion



Nature Reviews | Drug Discovery

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Antibiotika Empfindlichkeit “planktonisch” vs. Biofilm

TABLE 4. Susceptibility of planktonic and biofilm bacteria to selected antibiotics

Organism	Antibiotic	MIC or MBC of planktonic phenotype (μg/ml)	Concn effective against biofilm phenotype (μg/ml)
S. aureus NCTC 8325-4	Vancomycin	2 (MBC)	20[a]
Pseudomonas aeruginosa ATCC 27853	Imipenem	1 (MIC)	>1,024[b]
E. coli ATCC 25922	Ampicillin	2 (MIC)	512[b]
P. pseudomallei	Ceftazidime	8 (MBC)	800[c]
Streptococcus sanguis 804	Doxycycline	0.063 (MIC)	3.15[d]

[a] Concentration required for 99% reduction.
[b] Minimal biofilm eradication concentration.
[c] Concentration required for ~99% reduction.
[d] Concentration required for >99.9% reduction.

MIC, minimal inhibitory concentration [minimale Hemmkonzentration]
MBC, minimal bactericidal concentration

Clin Microbiol Rev 2002;15:167–193

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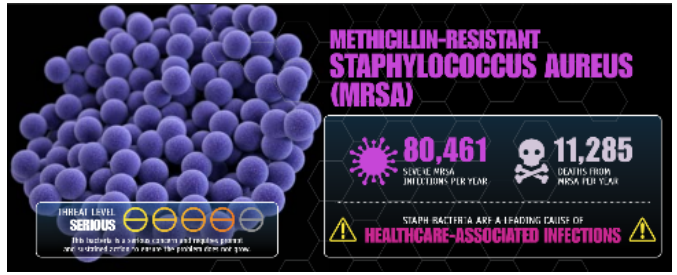
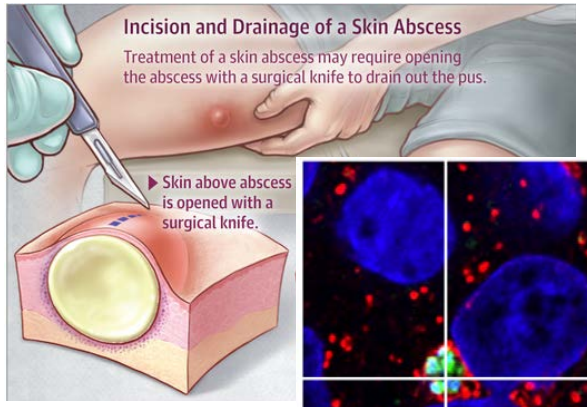
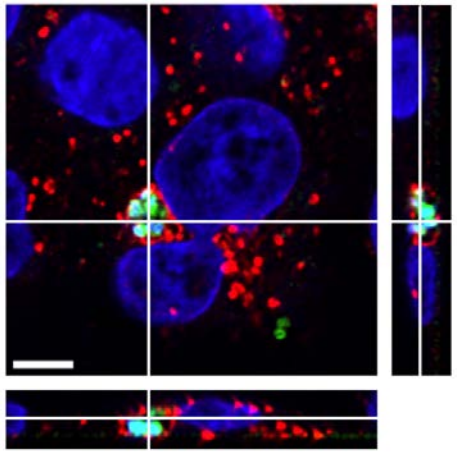
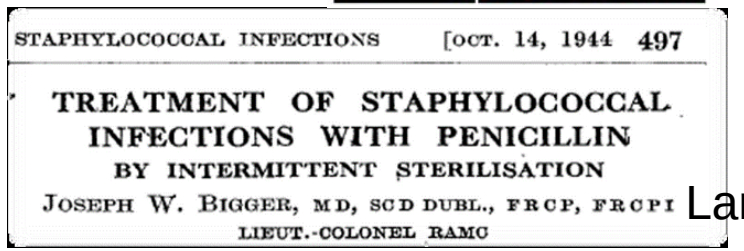
How do *S.aureus* withstand antibiotics?









STAPHYLOCOCCAL INFECTIONS [OCT. 14, 1944 497

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JOSEPH W. BIGGER, MD, SCD DUBL., FRCP, FRCPI
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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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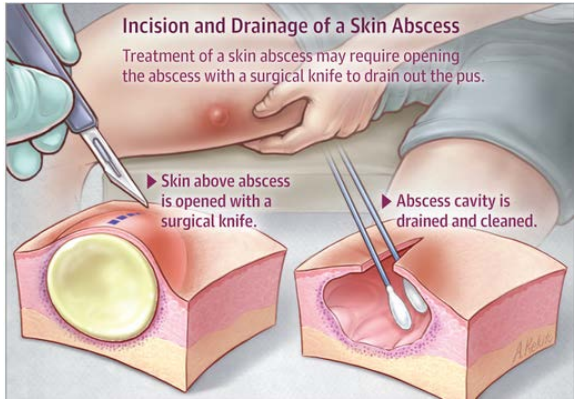
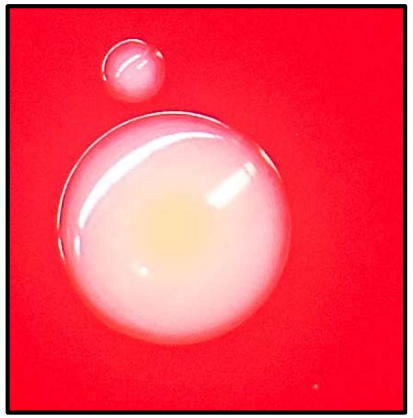
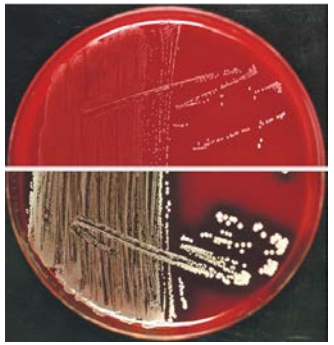
Small Colony Variants (SCV) in clinical isolates:





- Challenging detection – reduced growth



ko

wt

von Eiff, 2006

- Phenotype switching, revert to normal colony phenotyp
- **Indispensable feature for recurrent infections**
- In contrast to stable SCV - genetically determined
  - Electron transport-defective SCVs, auxotroph for hemin, menadione, thymidine (hemB, menD, thyD)

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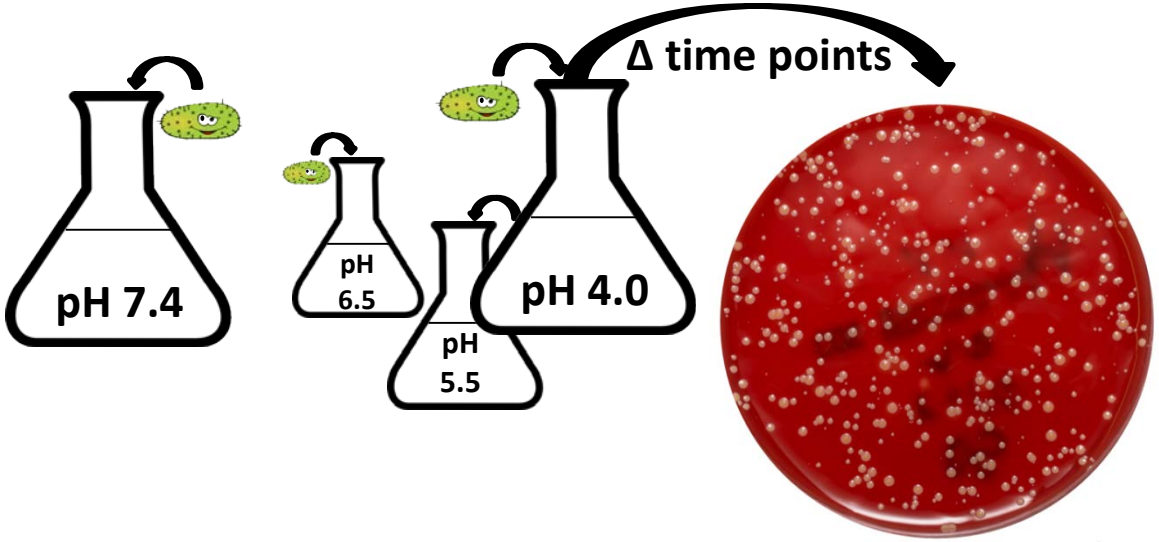
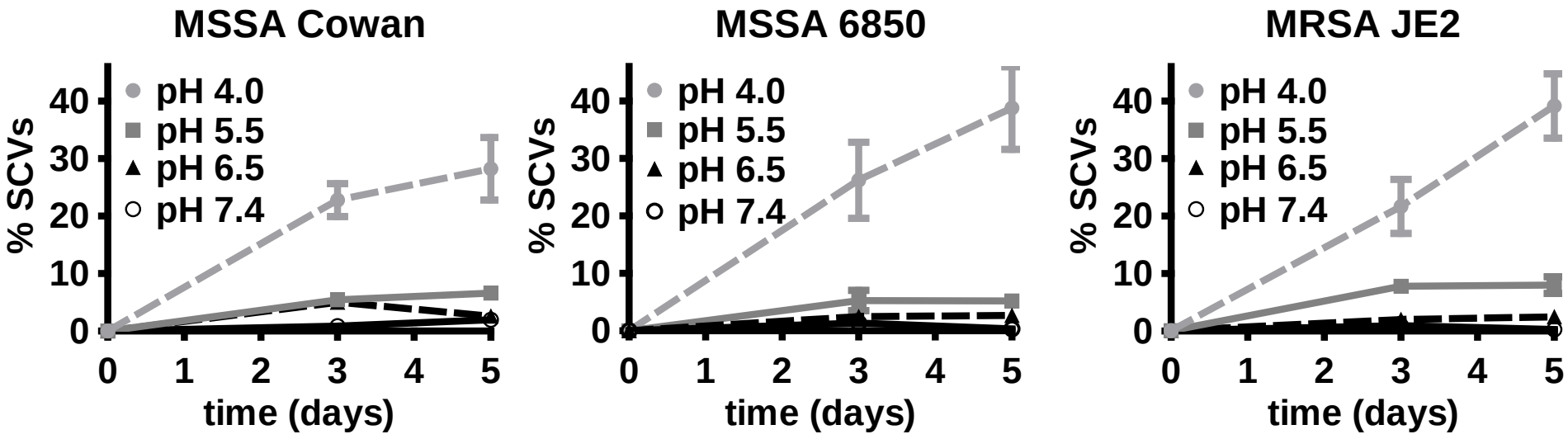
Low pH - > more SCV





Leimer, N. *et al.*, J Infect Dis. 2016

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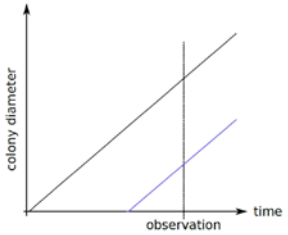
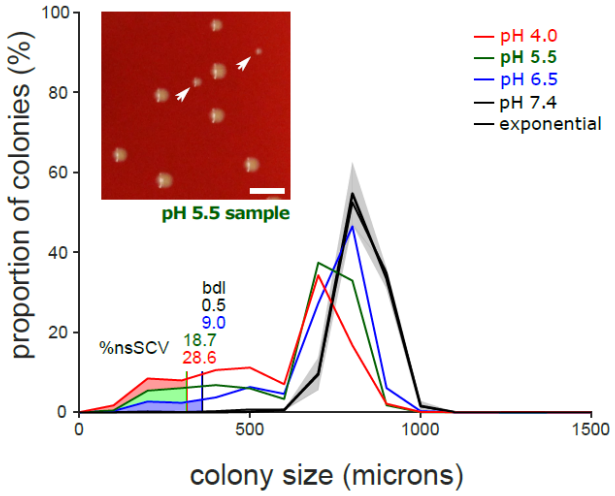
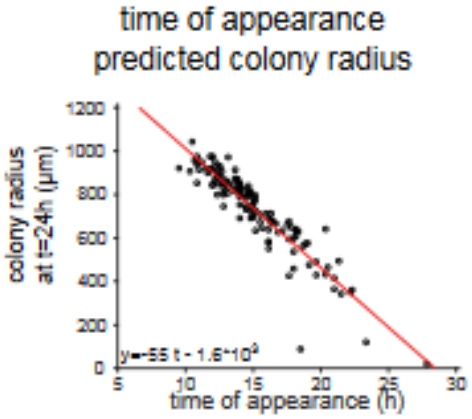
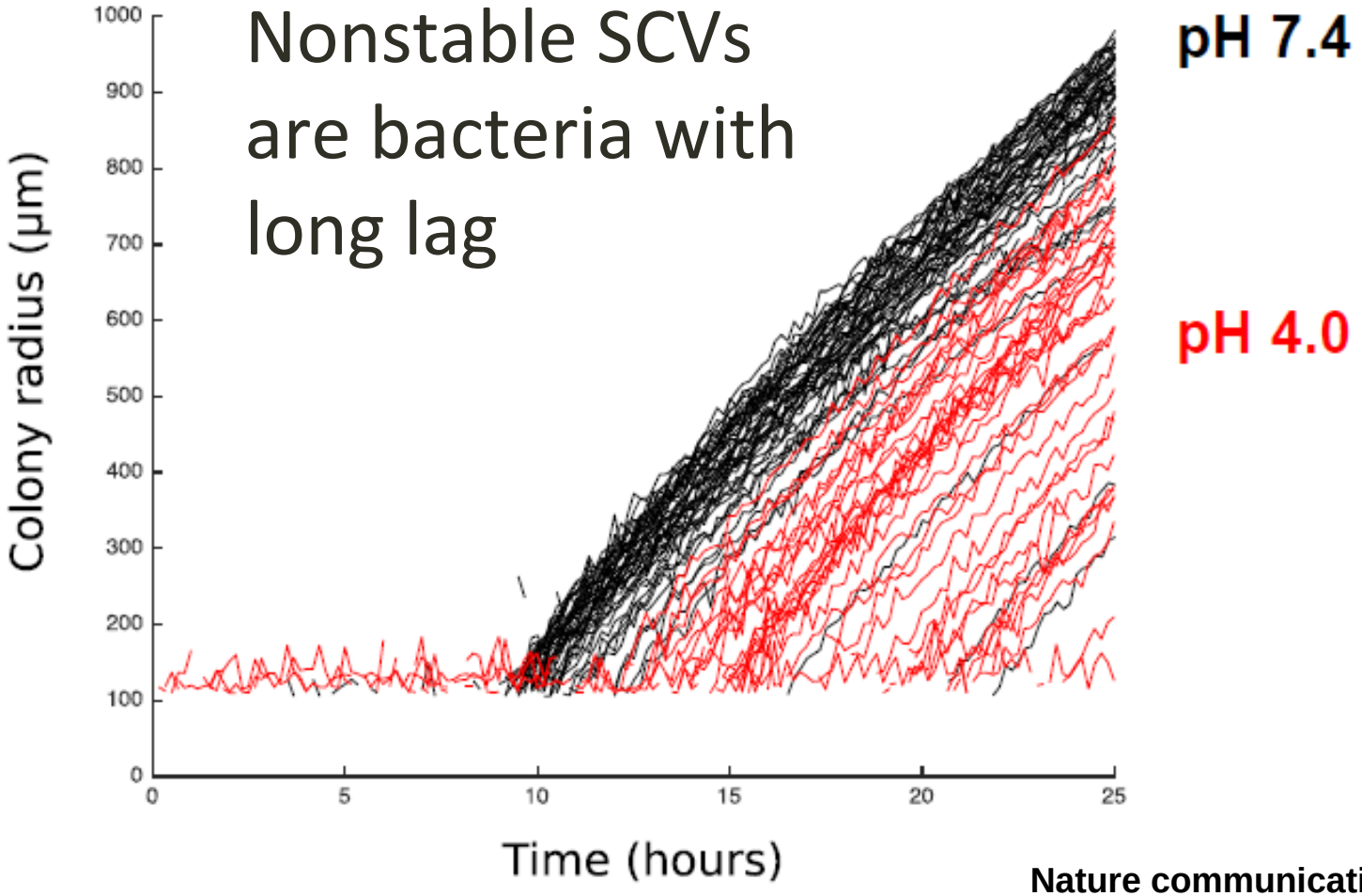


SCV formation is a consequence of a late emergence of colonies







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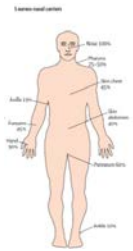
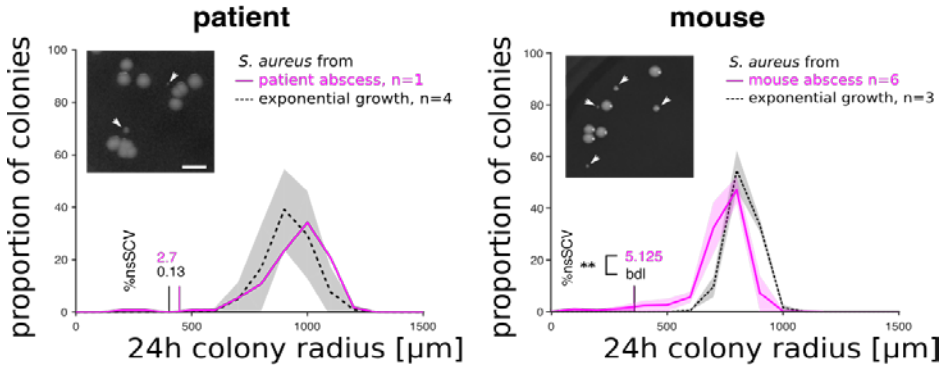
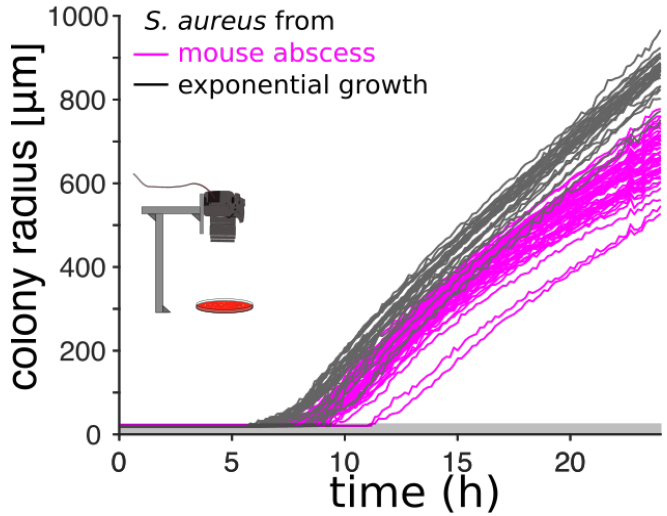


S. aureus recovered from human and murine abscesses

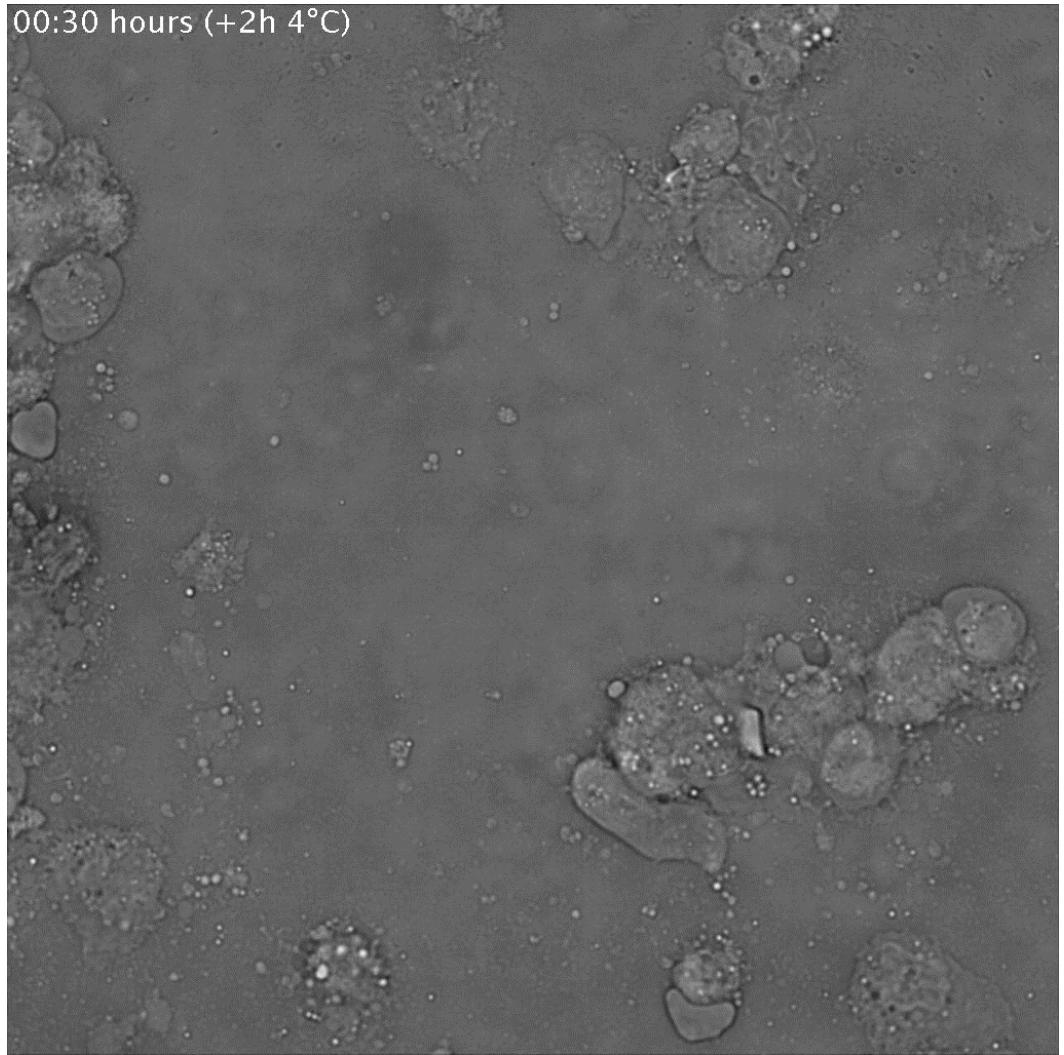


colony growth curves from mouse abscess

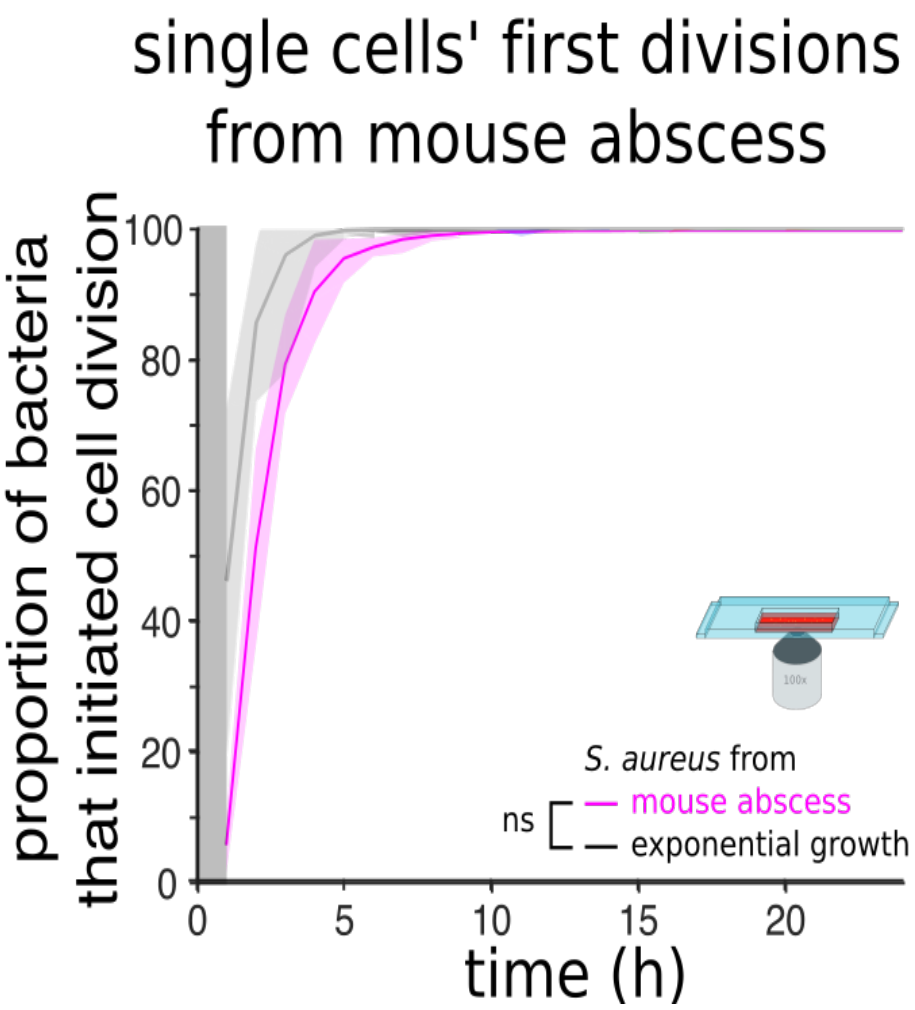
Heterogeneous and lag

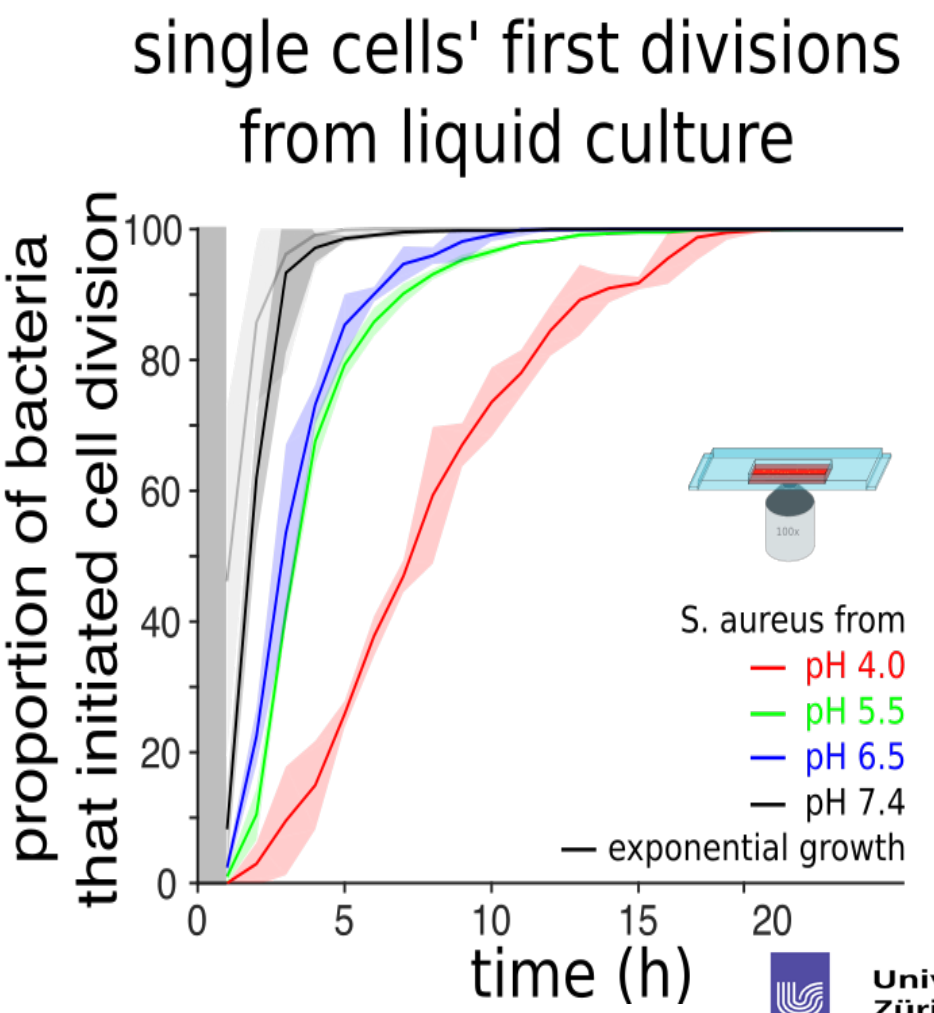


Microscopic timelapse



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





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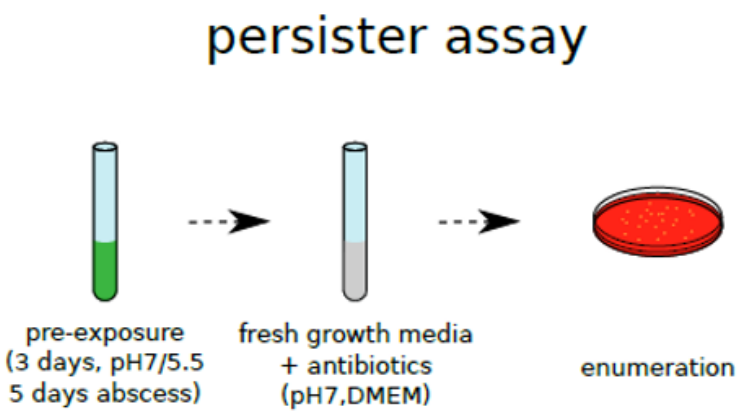
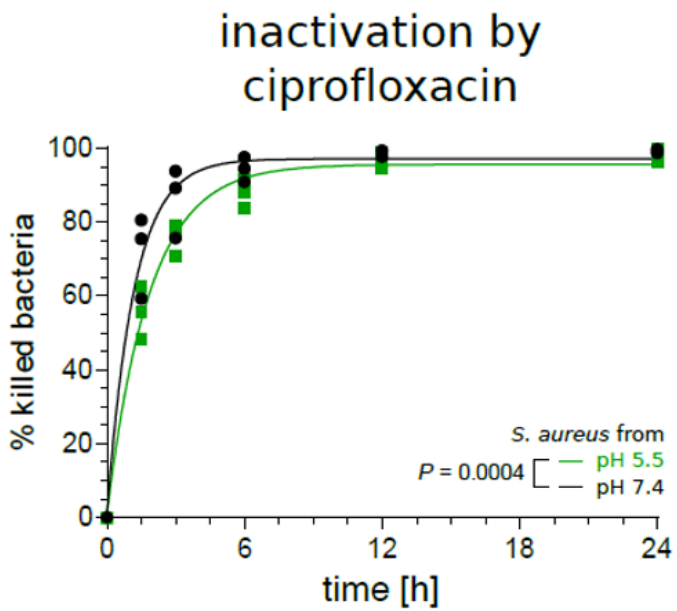
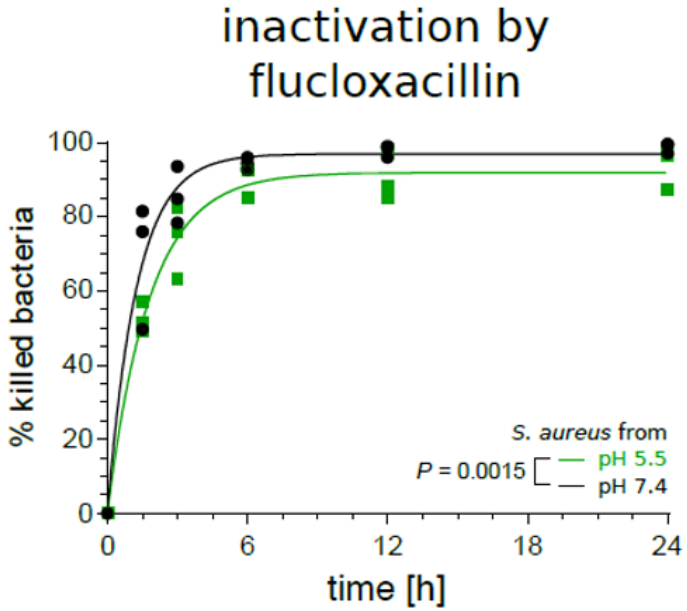
Bakterien, die sich nicht teilen, widerstehen Antibiotika besser







Nature communications 2018

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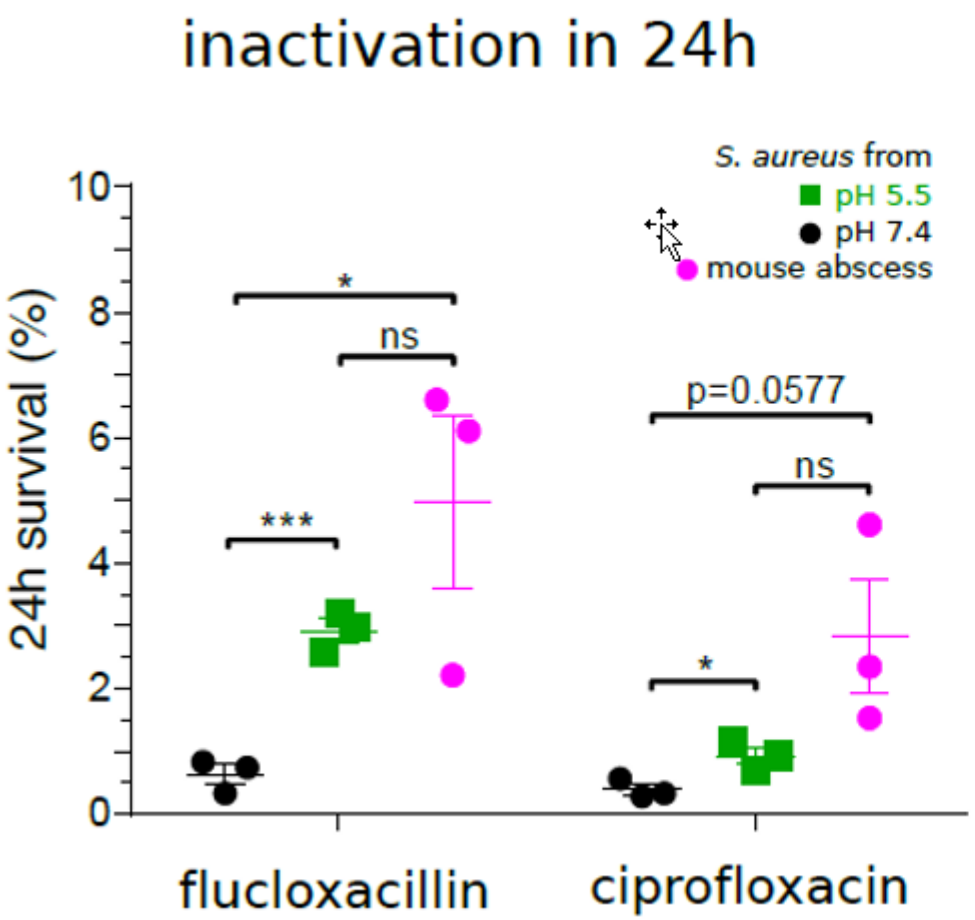

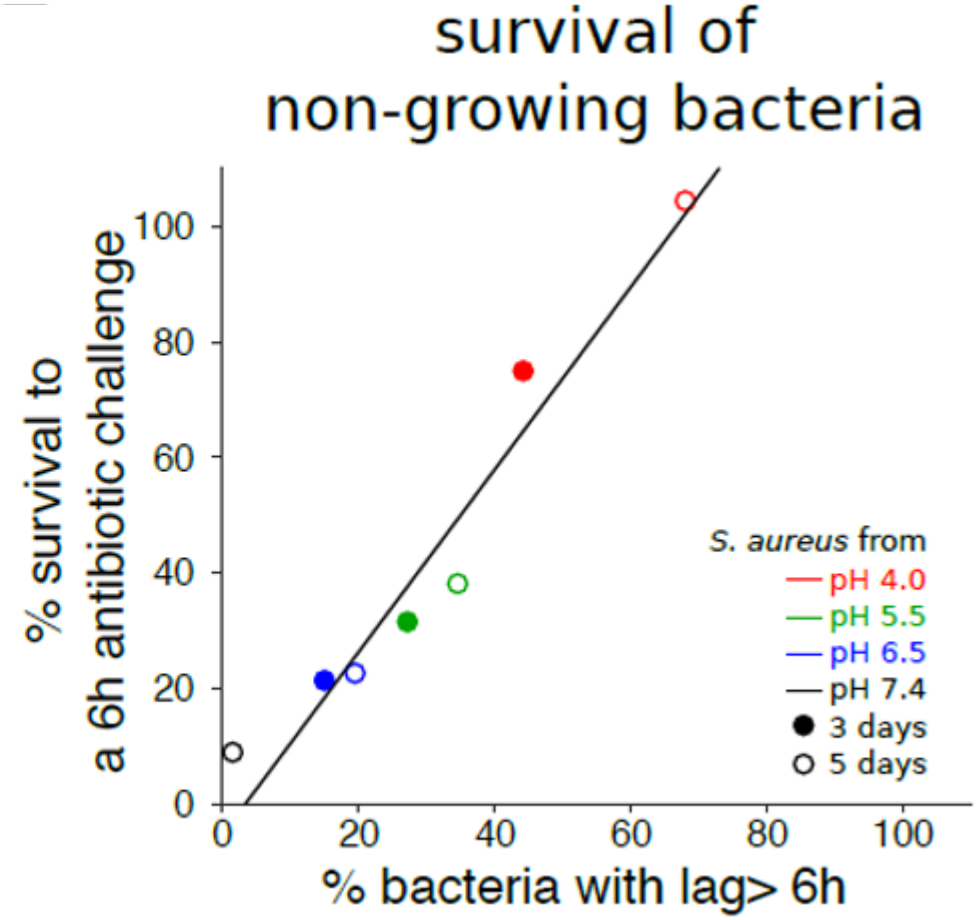

Nature communications 2018

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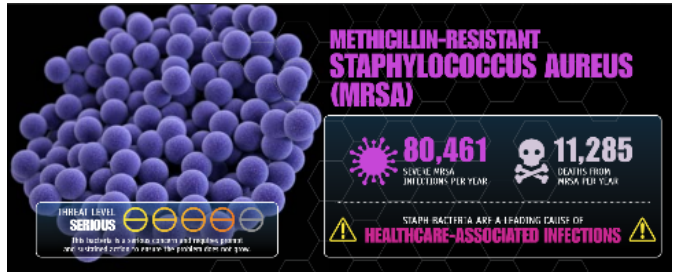
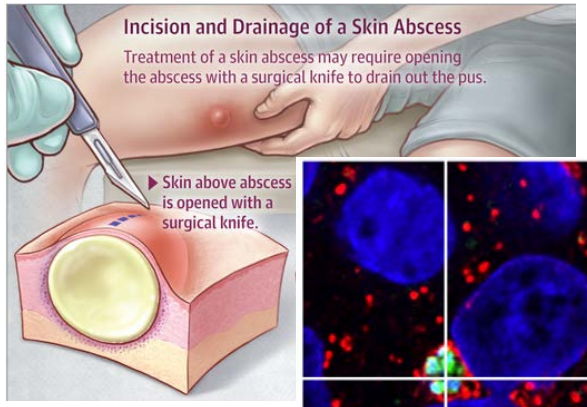
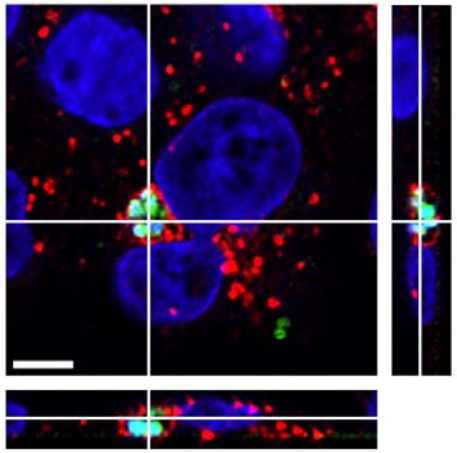
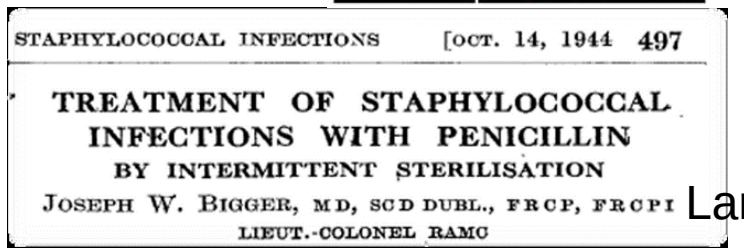
How do *S.aureus* withstand antibiotics?









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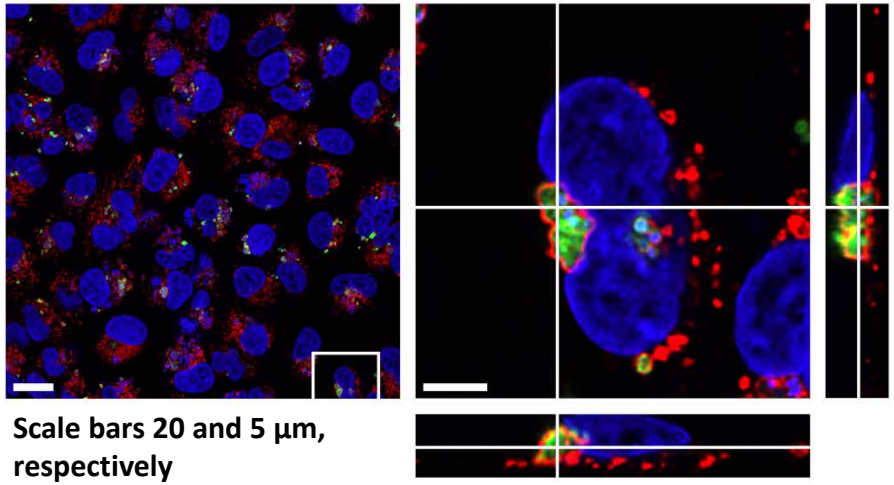
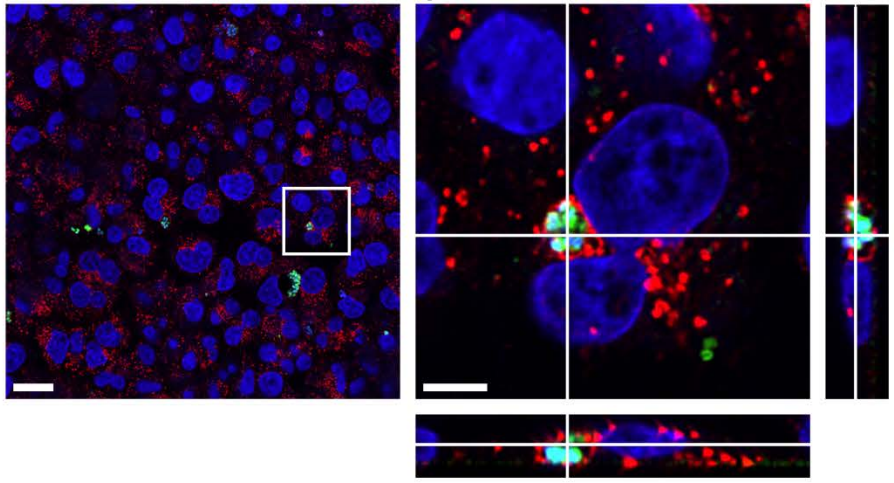
Intracellular localization

Day 0

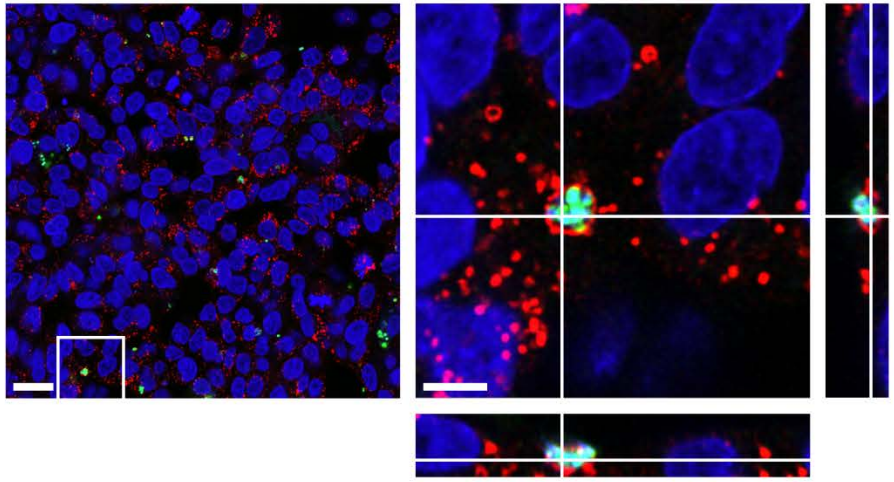


Scale bars 20 and 5 µm, respectively

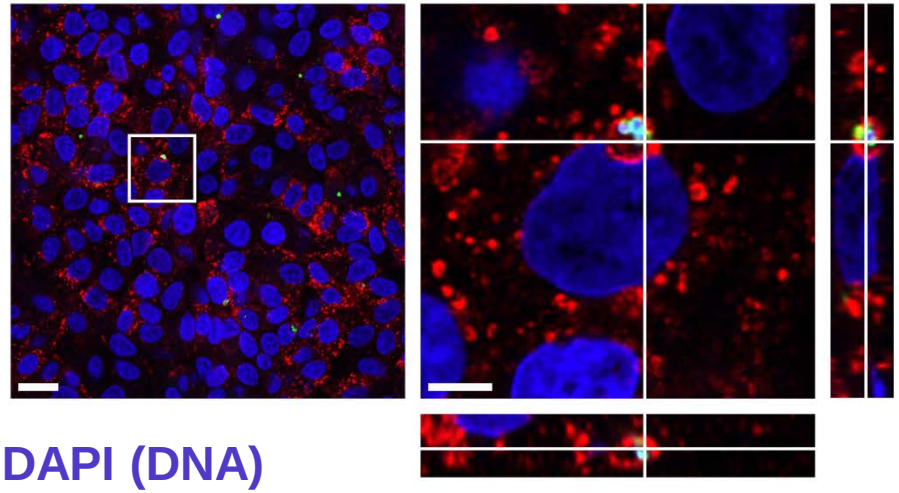
Day 3



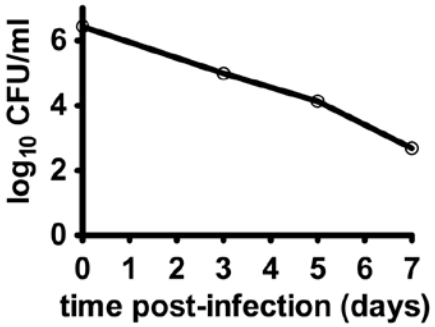
Day 5

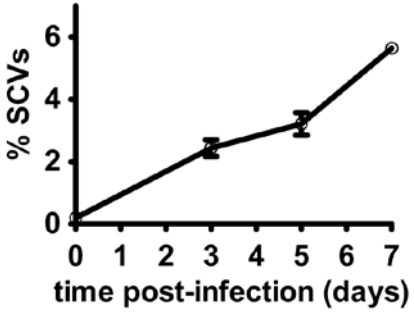


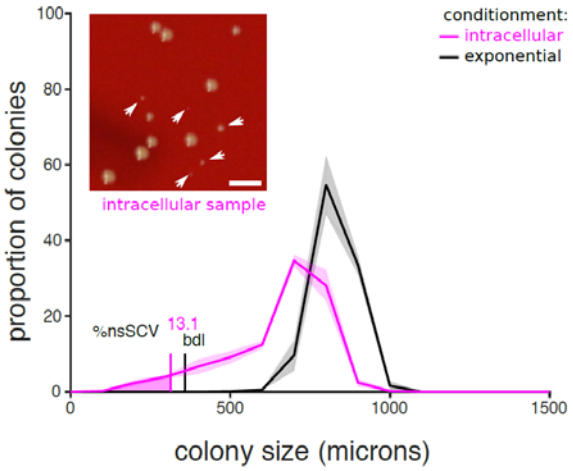
Day 7



DAPI (DNA)
LAMP-2 (lysosome)
CFSE (bacteria)







Leimer, N. *et al.*, J Infect Dis. 2016

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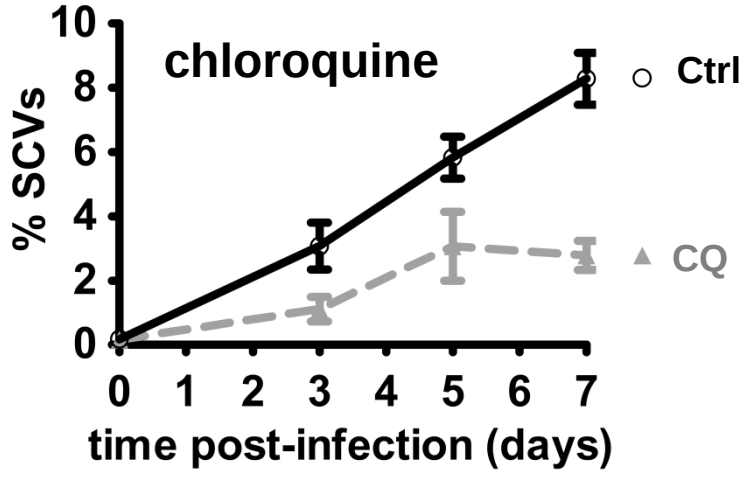
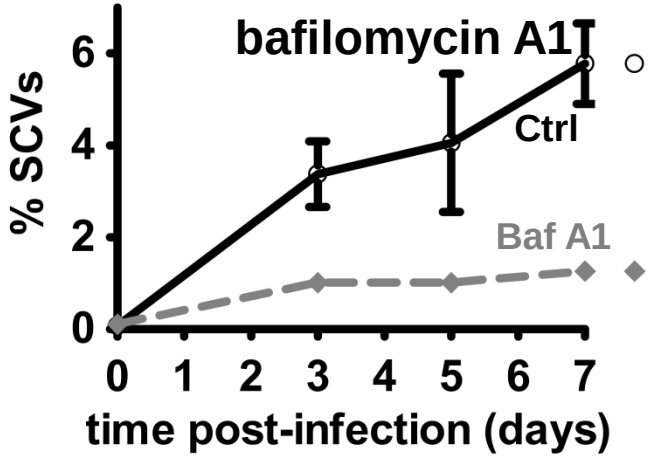
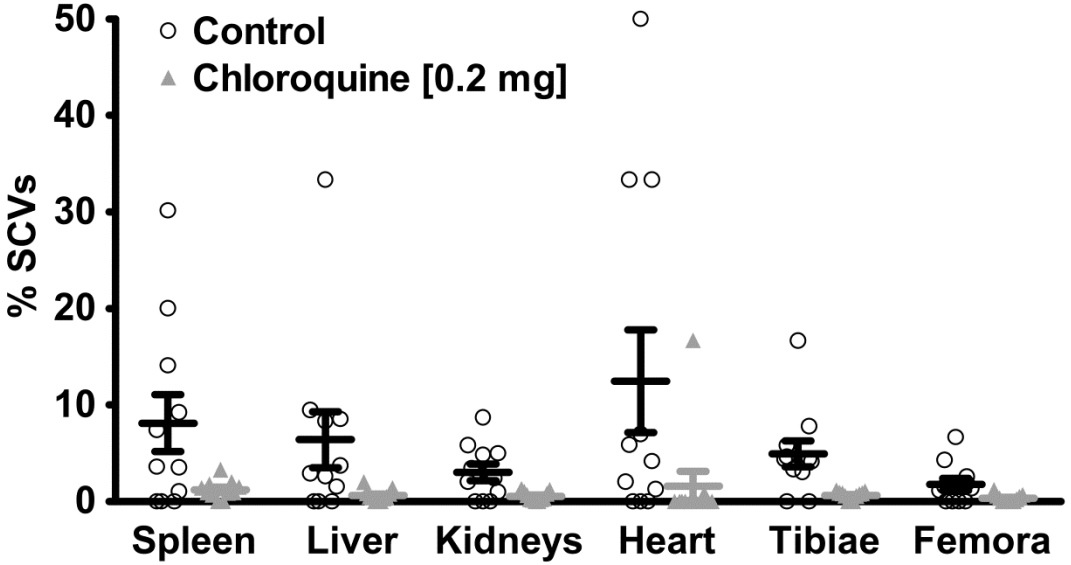
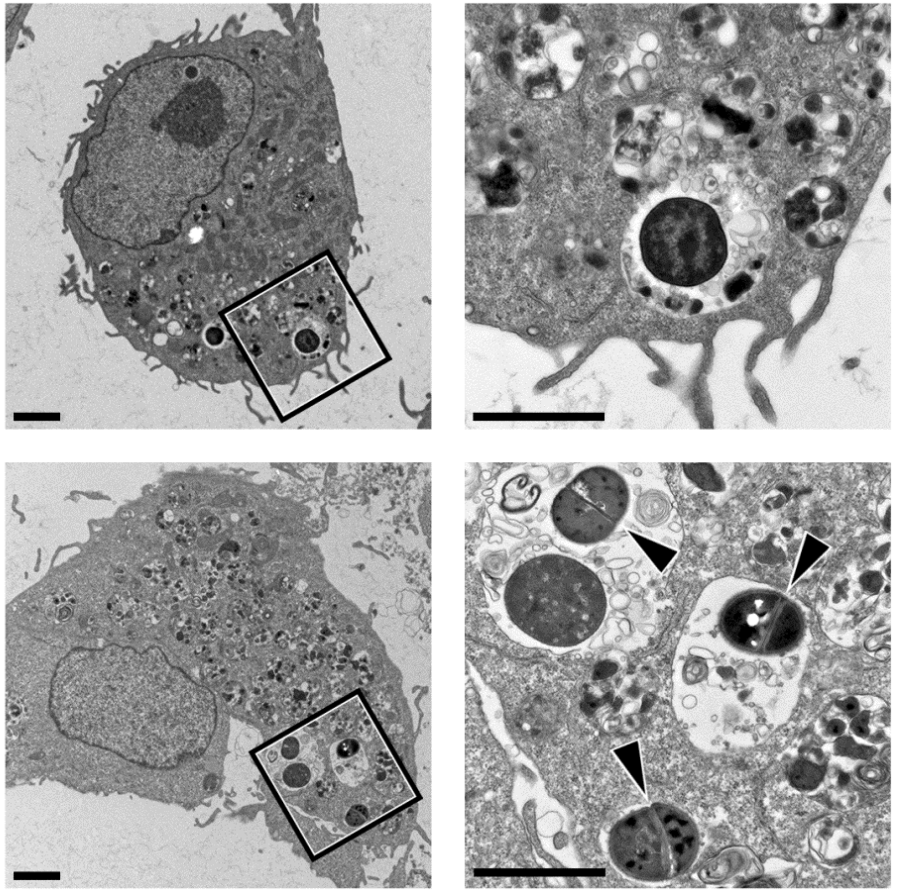
Reduction of SCV frequency by phagolysosomal alkalinization *in vitro* and *in vivo*









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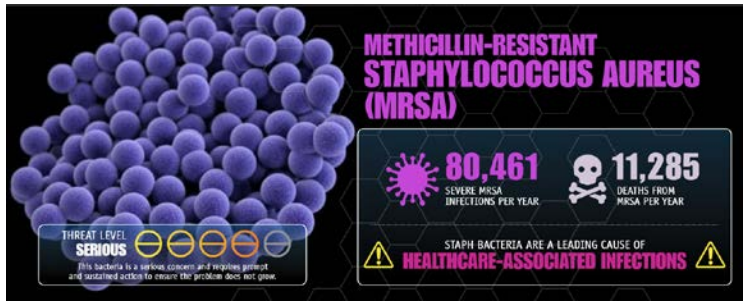
Angriffsstrategien

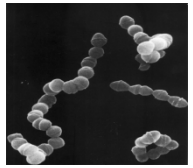


BACTERIA

Commensal wright place
Pathogen wrong place
virulence factors
commensals misbehaving

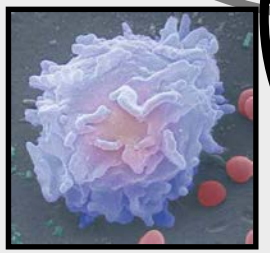
Commensals

- Digestion
- Vitamin production
- Space holder



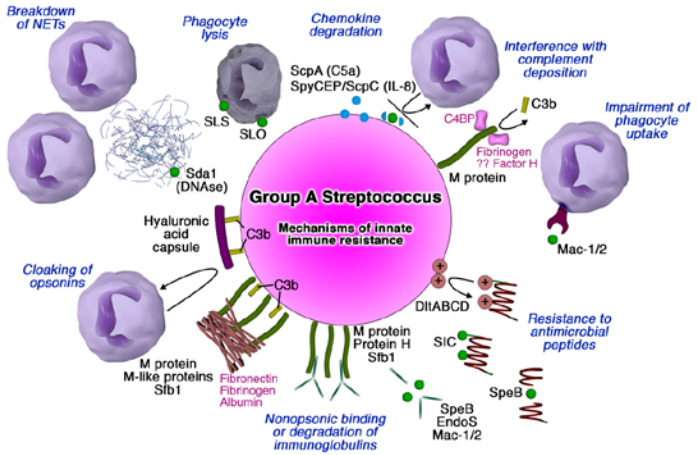
HOST

Innate Resistance



Pathogens

- Virulence Factors



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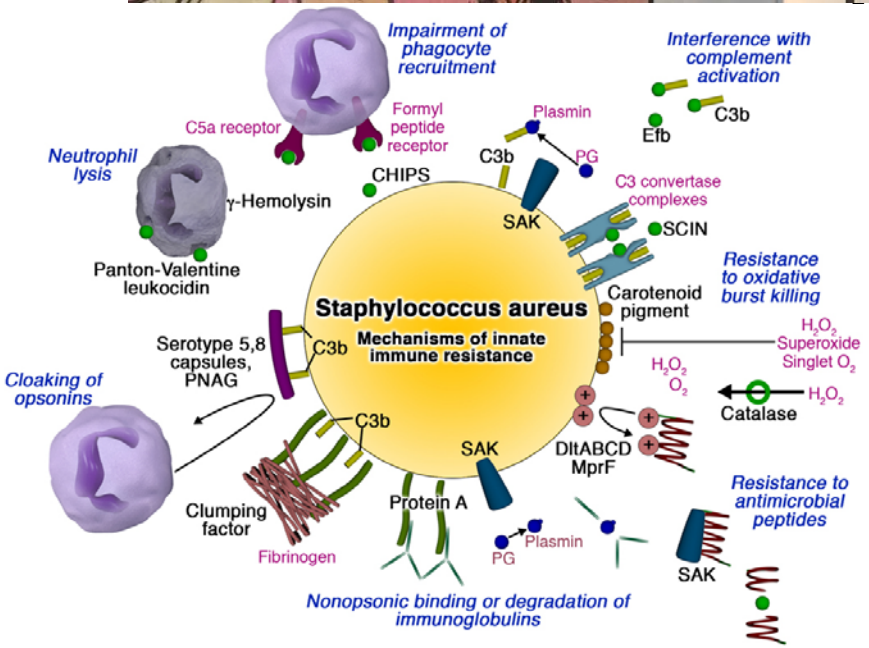
Living with the enemy

There's another big problem with antibiotics: indiscriminately killing bugs is making us sick. It may be time to call a truce, says **Drew Smith**

YOU are home to 10,000 species of bacteria. The vast majority, more than 90 per cent, cause you no harm. Indeed

infectious diseases fell by more than 90 per cent, contributing to a broader transformation of society. We take for granted our long lives

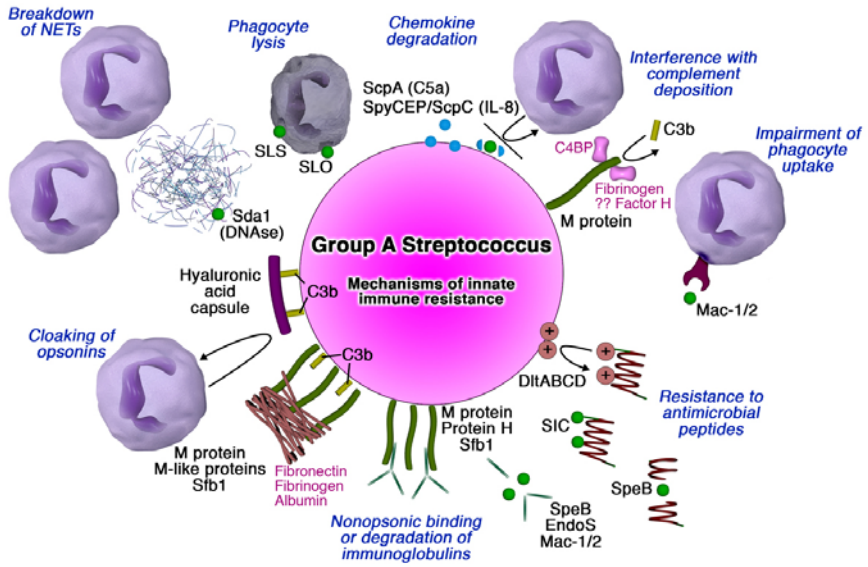
So how can we do it? The ide bacteria behaving badly. Befo an infection, bad bugs first ne



Commensals

Pathogens

- **Virulence**
- **Factors**



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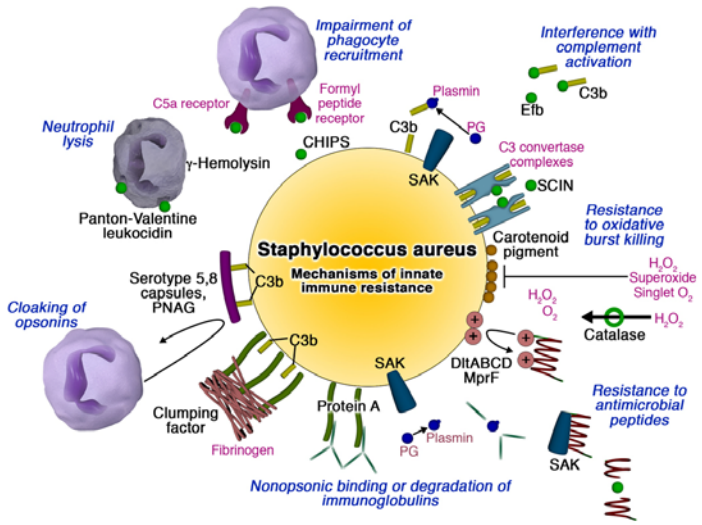
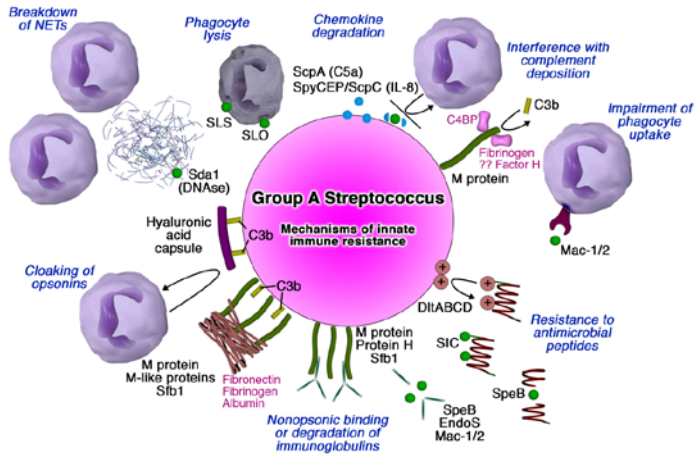
Pathogenesis
S.aureus and Streptococci





- Phages – virulence factors
- **Adhesion & Invasion**
  - M protein, protein A
- **Immunmodulation**
  - Cytolysis (PVL, α-toxin, PSM, SLO, SLS)
  - Proteases (IL-8)
  - DNases
- **Immunstimulation** Superantigens
- Extracellular bacteria – Intracellular persistence

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Therapeutic options

HOST

‘SiecheHuus’ / Sanatorium

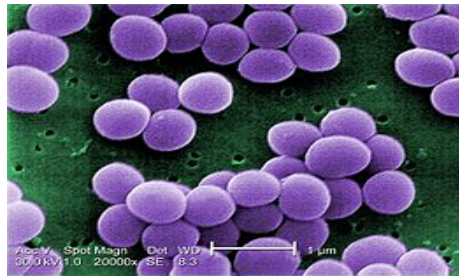
Surgery

Decolonise

Lower immunsuppression

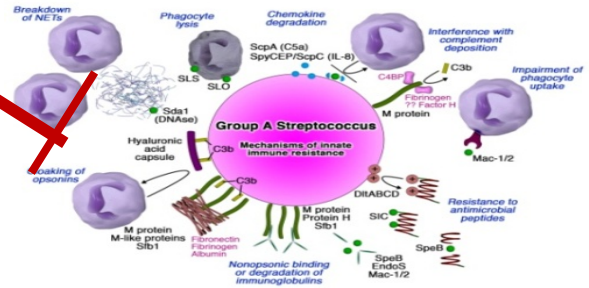
Vaccination

PATHOGEN



Kill:
Antibiotics

Disarm the pathogens:
Inhibition of bacterial virulence factors



Disarm the pathogens: Inhibition of bacterial virulence factors

Emil von Behring (1854-1917)

- **Diphtheria:** caused by a polypeptide exotoxin of *Corynebacterium diphtheria*
  - Diphtheria antitoxin 1890s: in horses hyperimmunised with diphtheria toxoid
  - diphtheria toxoid containing vaccines
  - Endemic countries: Afghanistan, Bangladesh, Cambodia, China, India, Indonesia, Malaysia, Nepal, Pakistan, Papua New Guinea, the Philippines, Thailand, Vietnam and the Pacific Islands

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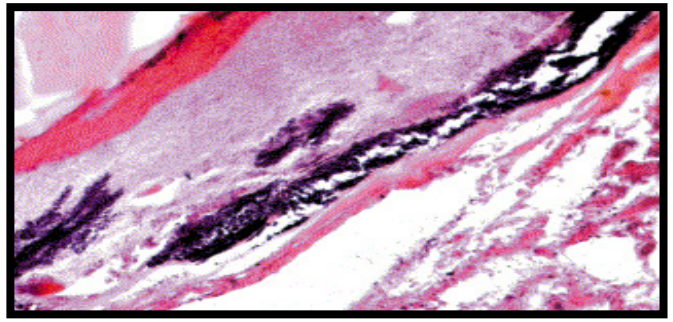
Pathogen is key

- **Disadvantages:** Pathogen is not killed
- **Advantages:**
  - Very specific, no collateral damage, i.e. diversity, microbe–microbe interactions
    - Skin/Mucosa: human skin/nasal commensals affect *S. aureus* behavior and fitness
      - Corynebacteria control *S.aureus,* pneumococci
        - » Ramsey MM et al, 2016, Frontiers in Microbiology
    - Gastrointestinal: ***Clostridium difficile*-associated diarrhea**
      - pathology induced by two exotoxins: toxin A and toxin B, antibodies specific for TcdA and TcdB

Antibiotics

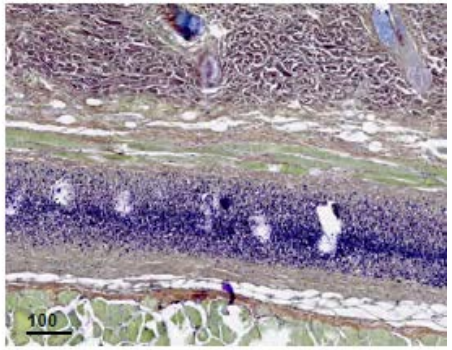
- **Cell wall active antibiotic:**
  - Penicillin: 100% susceptibility



- **Protein synthesis inhibitor:**
  - Clindamycin
    - clindamycin is not affected by the inoculum size or stage of bacterial growth

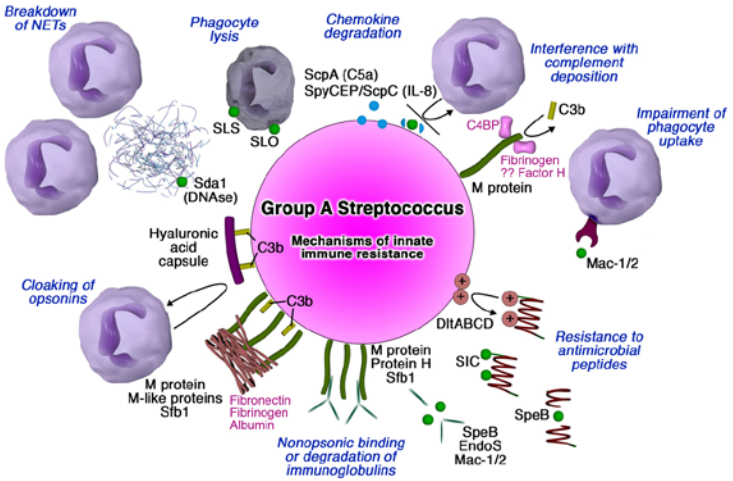
IDSA Guidelines, CID 2005

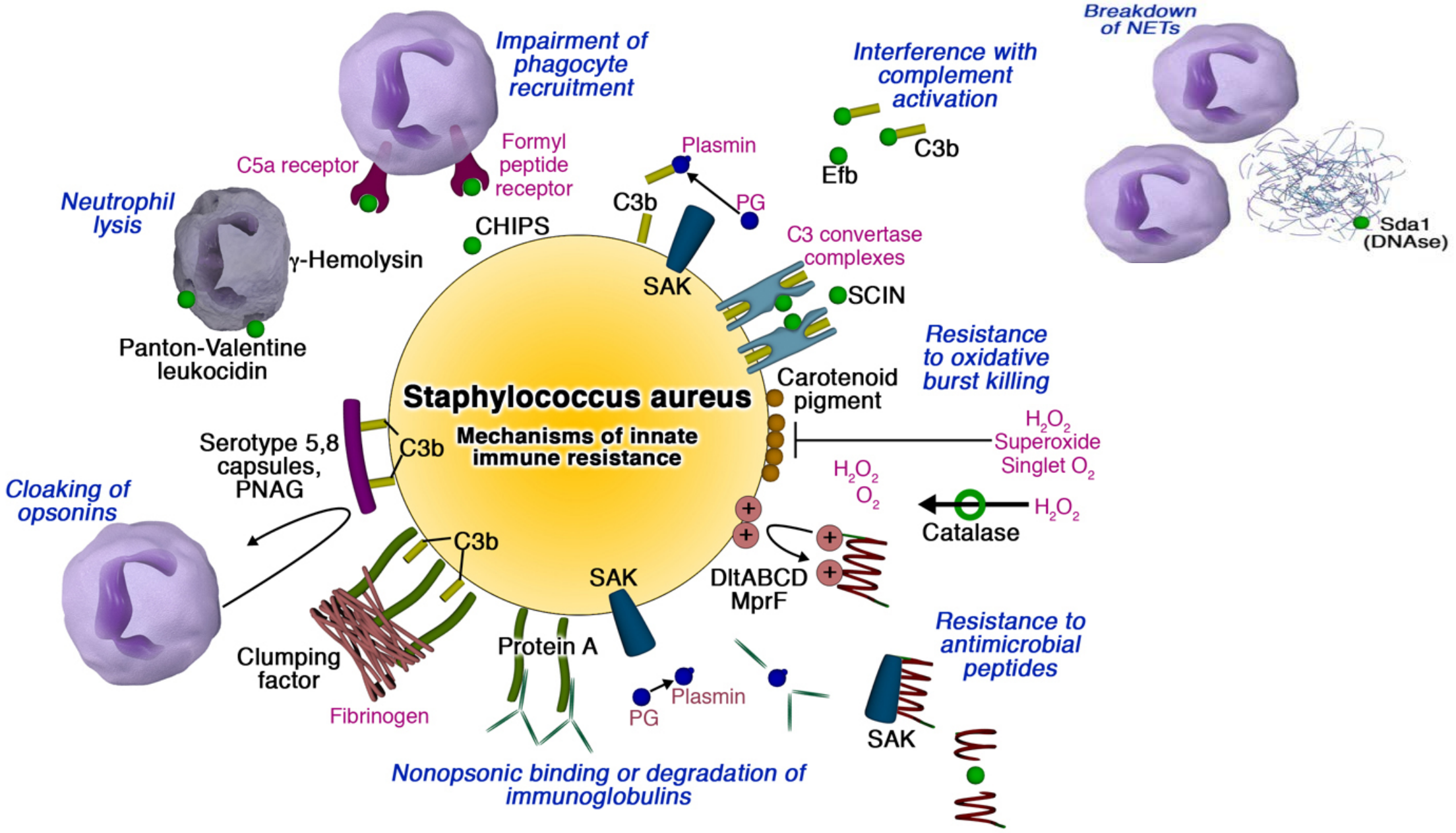
Eliminate + Disarm



- **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 Antimicrib Agents 2001
    - Goscinski G et al., Scand J Infect Dis 2006
- **Immunglobuline**

IDSA Guidelines, CID 2005





Nizet, J Allergy Clin Immunol 2007

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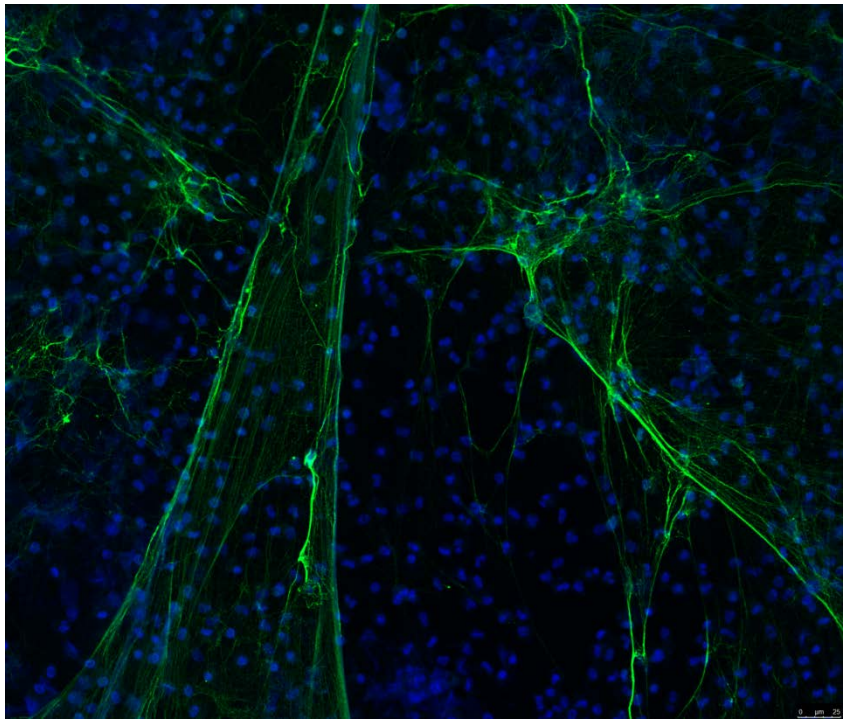
Neutrophil Extracellular Traps

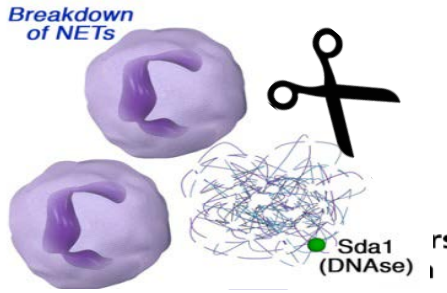


Katrin Schilcher

Released from neutrophils

Trap and kill pathogens

DNA backbone with embedded cationic histones, antimicrobial peptides and granule proteases with antibacterial properties



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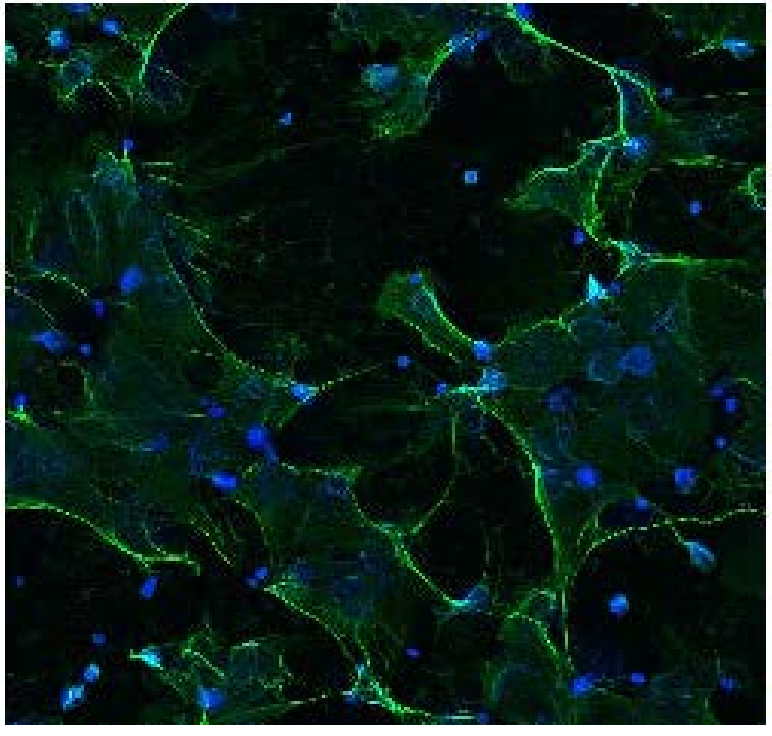
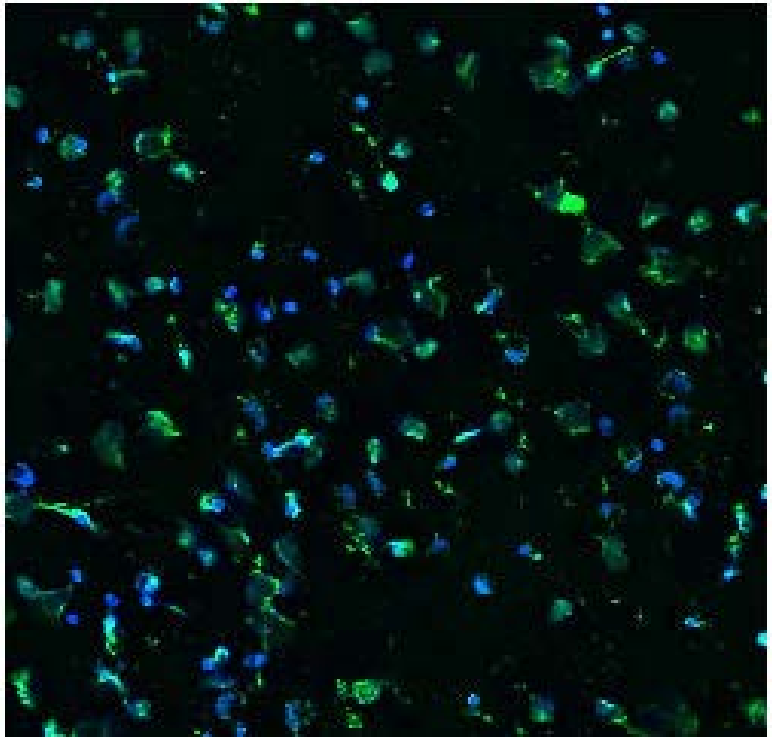
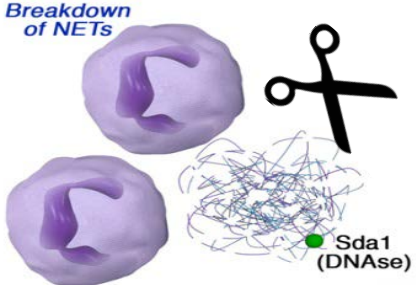
Bacterial nucleases degrade NETs



DNase ko



WT



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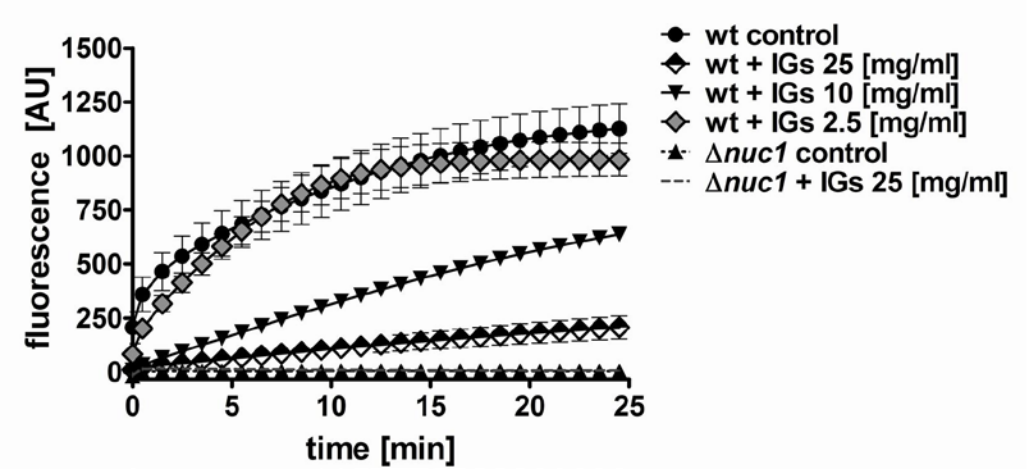
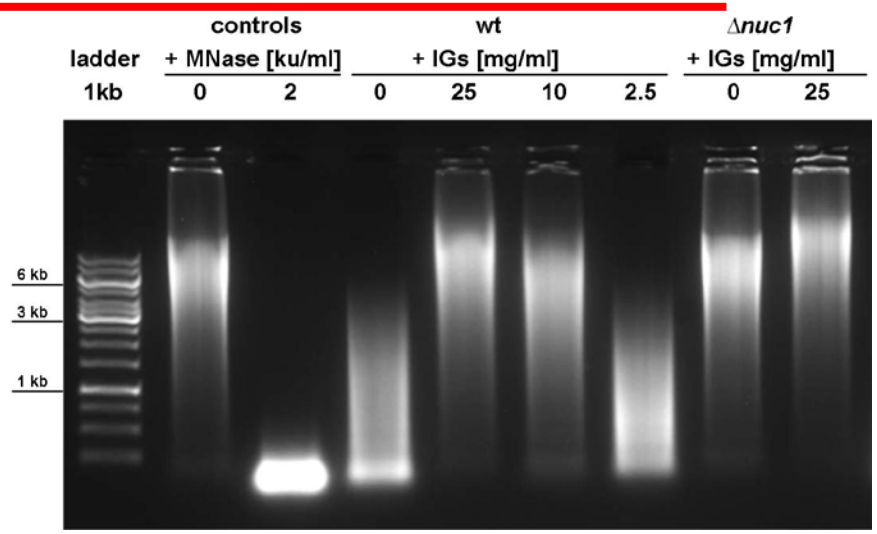
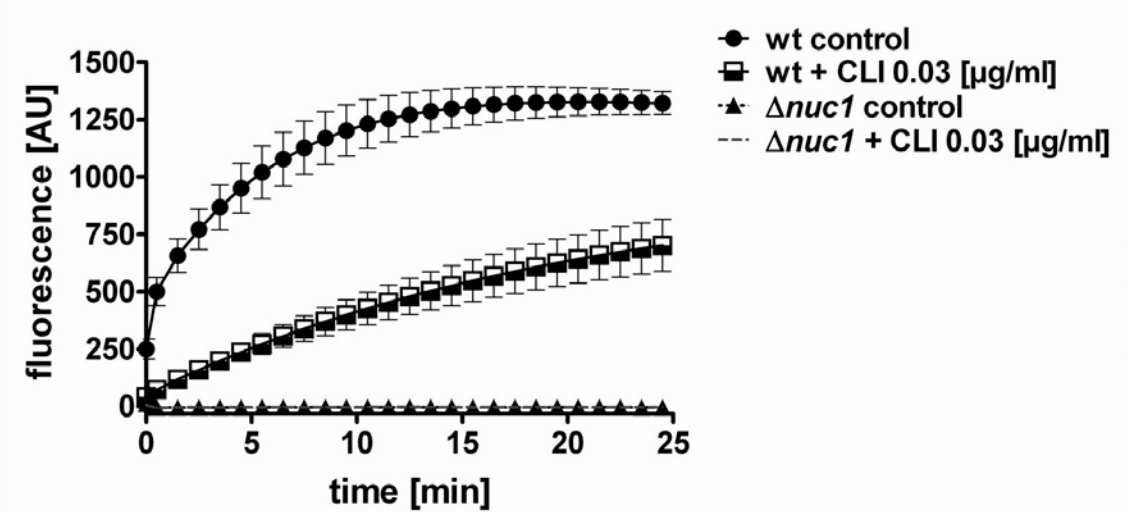
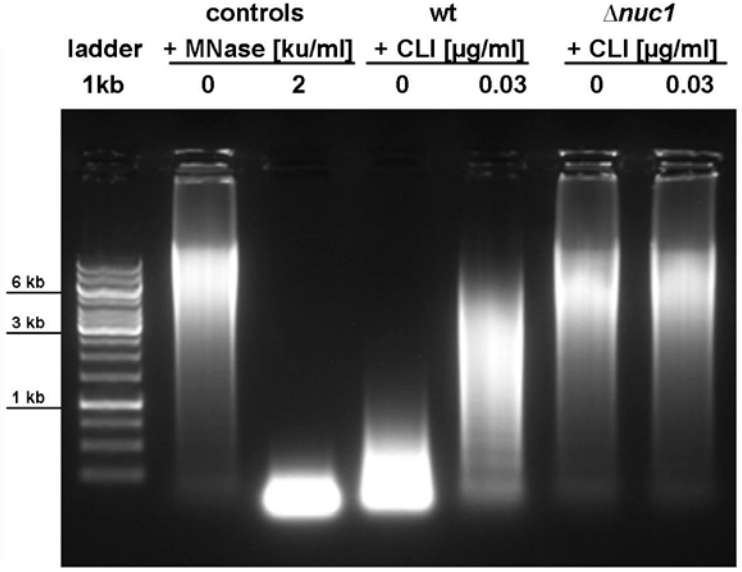
Inhibition of *S. aureus* Nuc1 activity CLI and by IG









Schilcher K *et al*., Journal Infectious Diseases 2014

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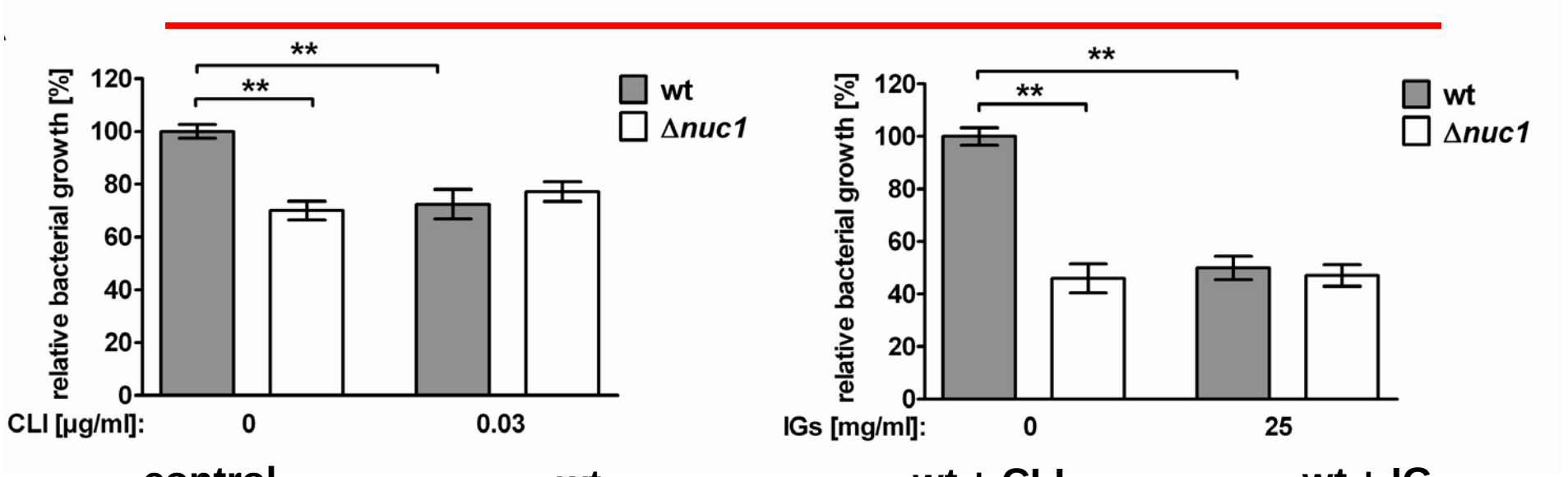
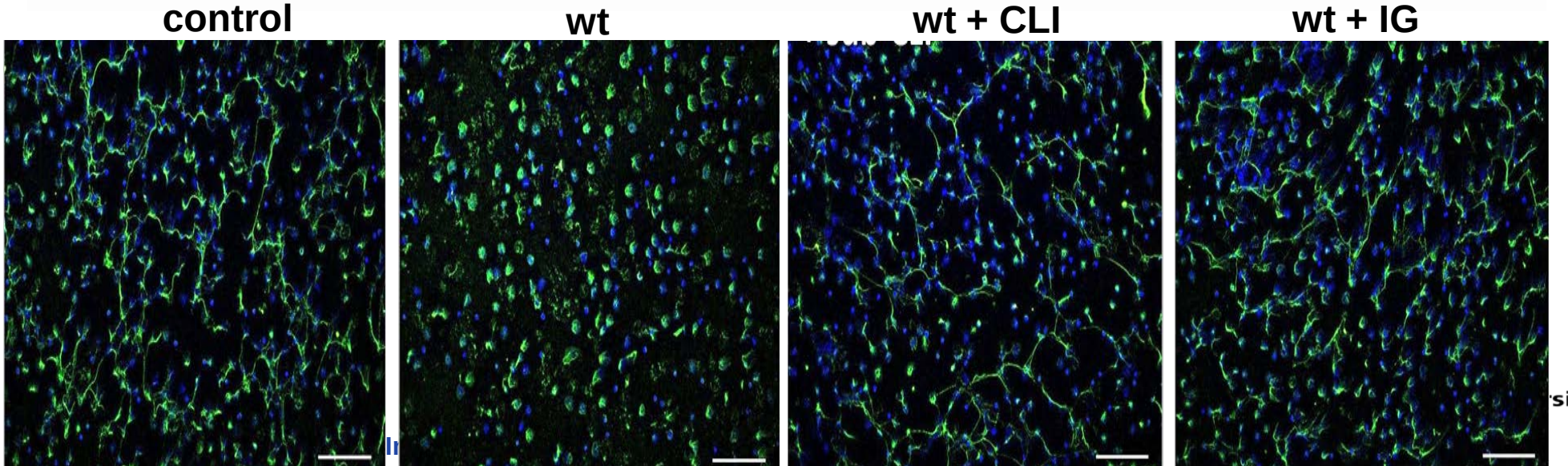
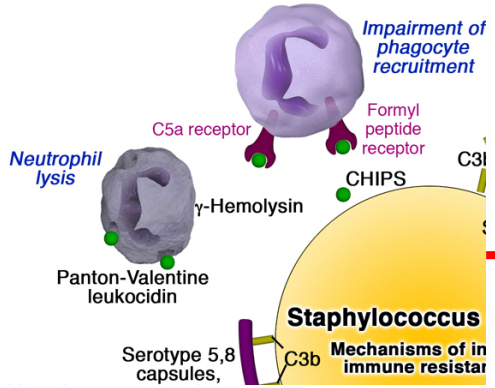
Enhanced bacterial clearance in presence of CLI and IGs



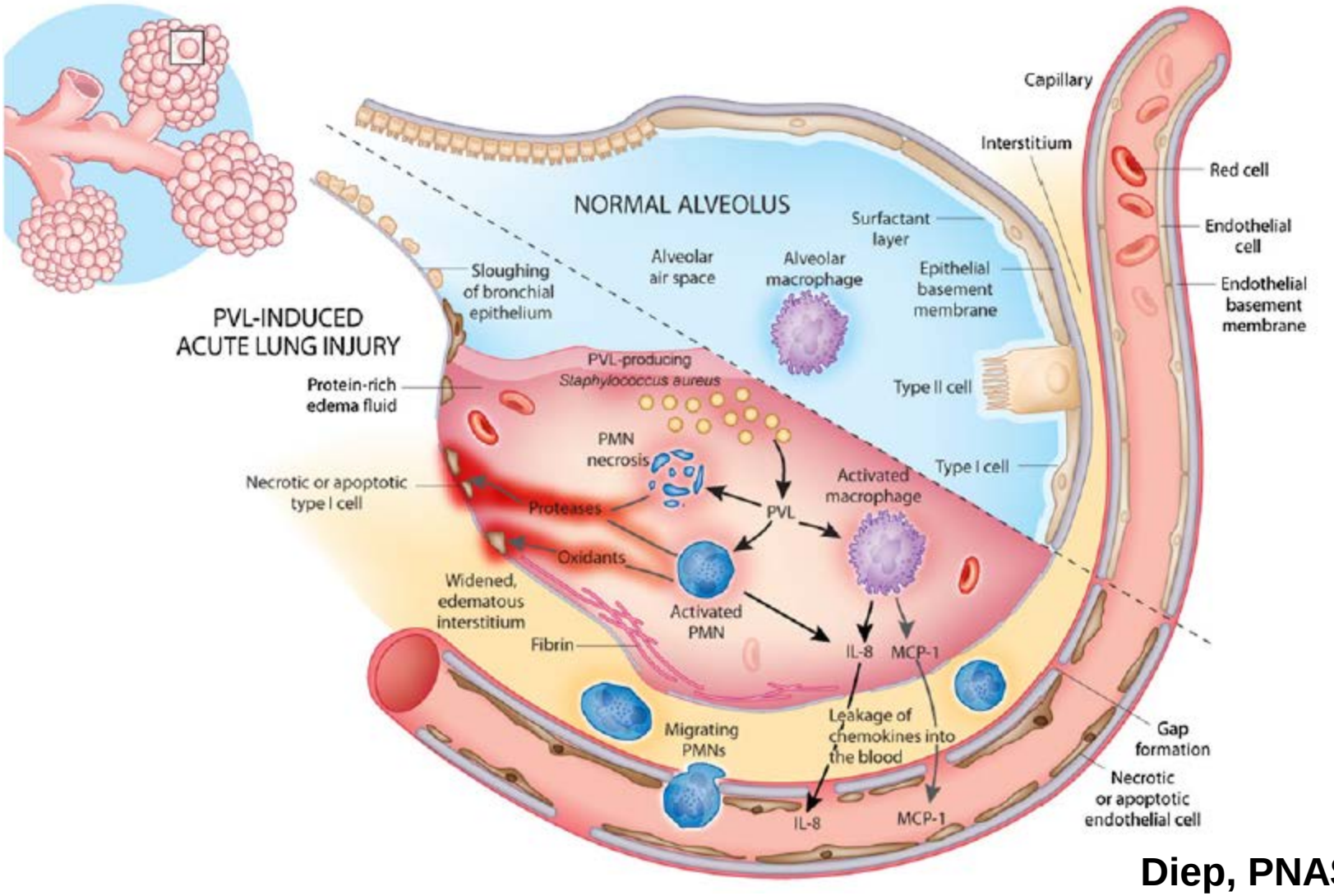




PVL

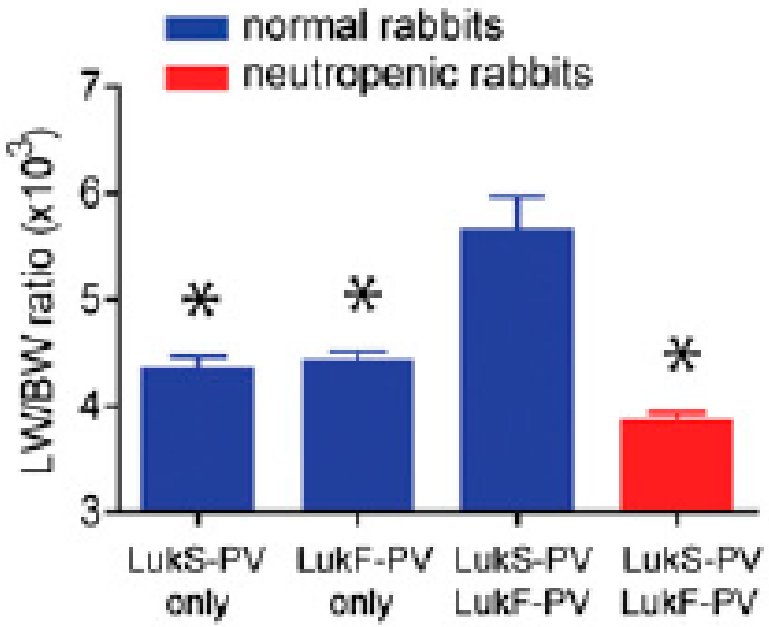
- Panton-Valentin leucocidin
- 2 component pore forming toxin
  - LukS-PV und LukF-PV
- Erstbeschreibung 1932 Panton und Valentine
- 2- 3% *S.aureus*
  - USA 300 – CA MRSA

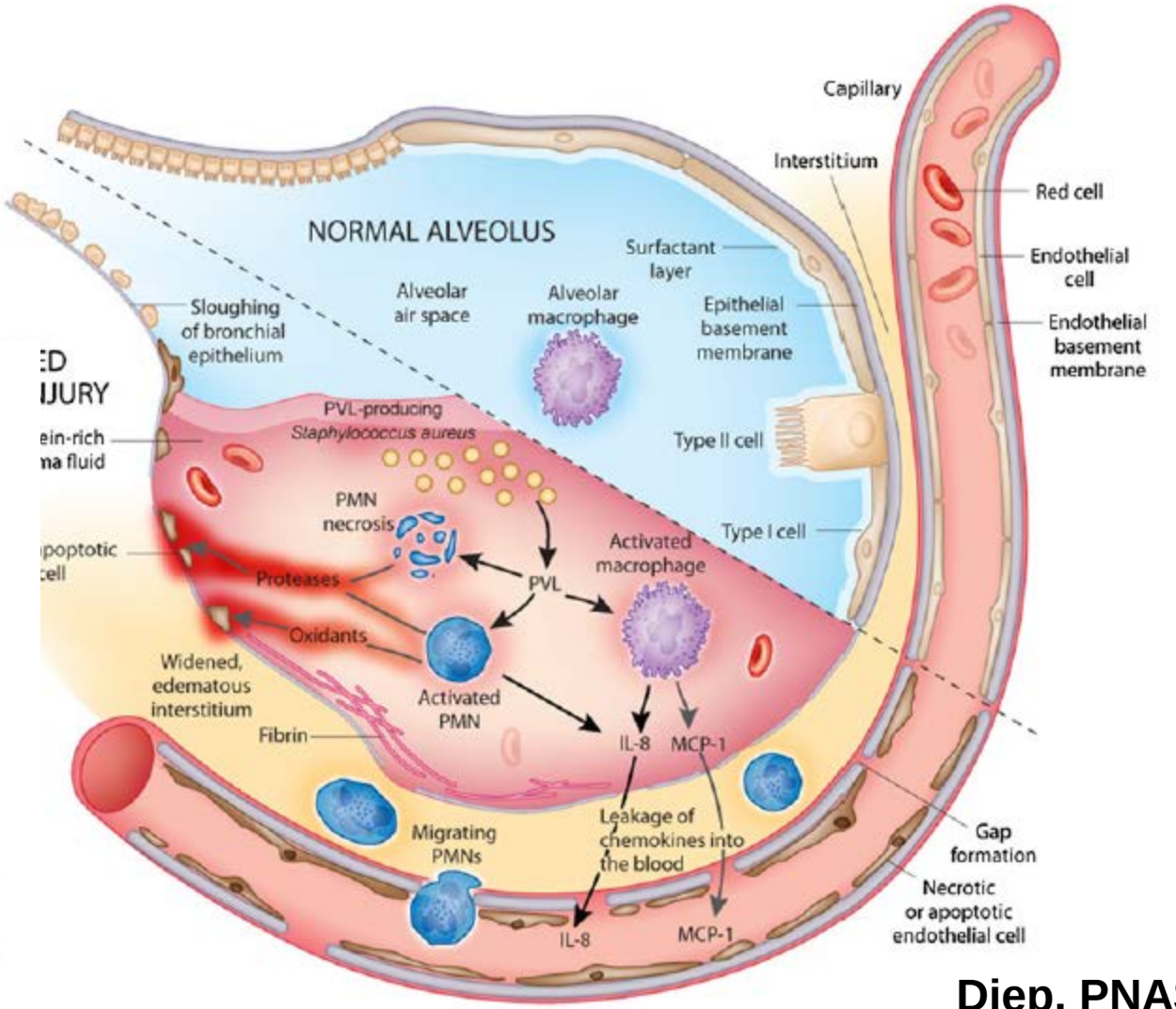
PVL- Neutrophile



Diep, PNAS 2010

PVL- Neutrophile





Diep, PNAS 2010

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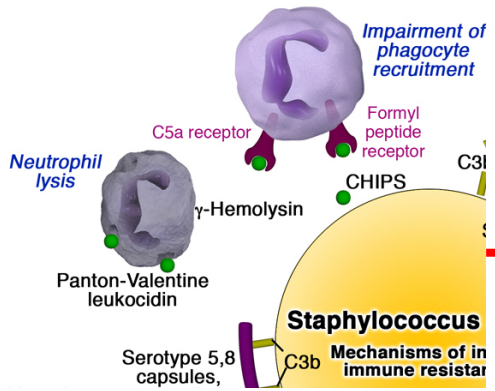
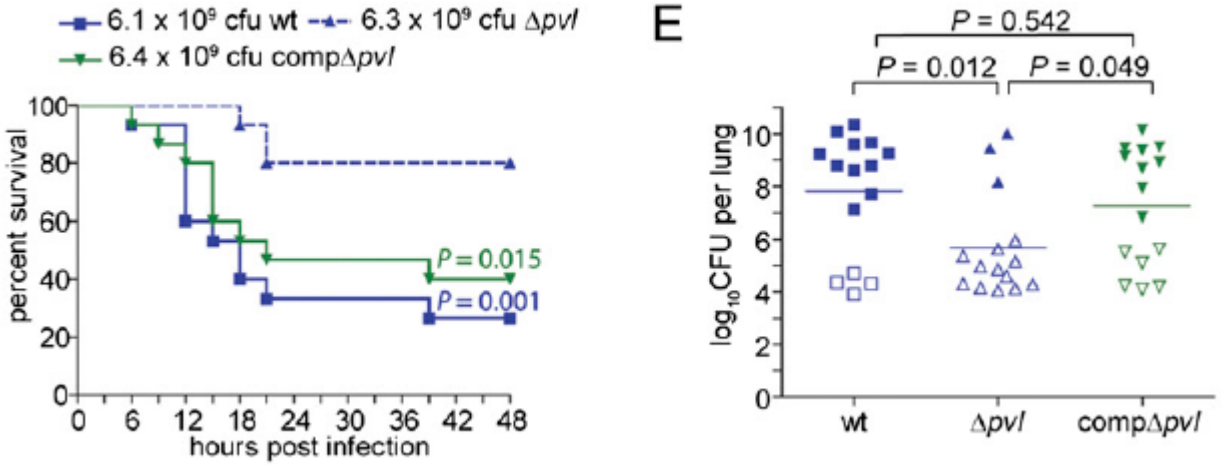


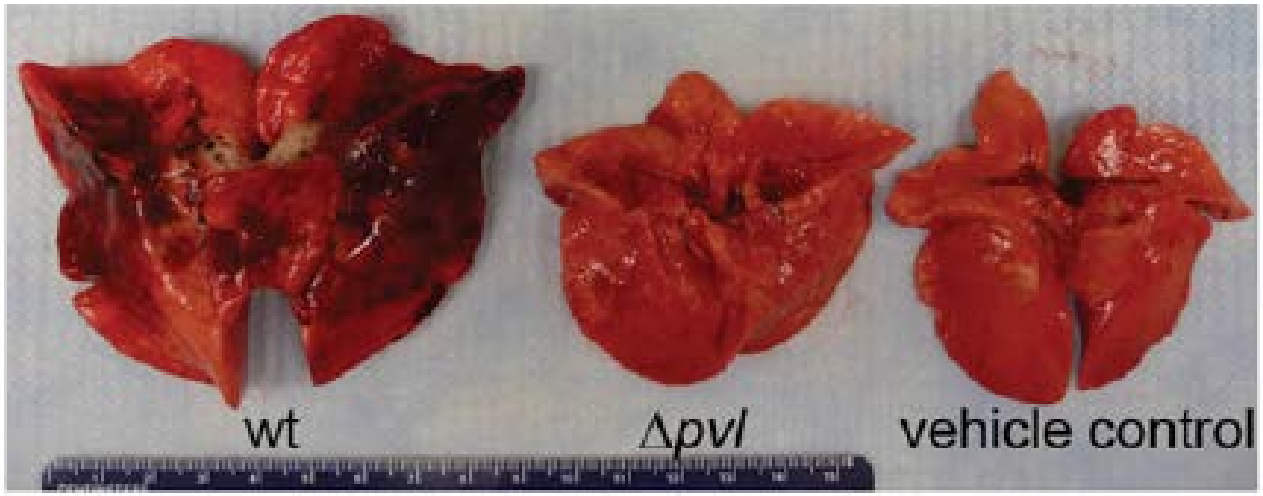
PVL - Necrotizing Pneumonia

Genetic approach

WT vs Δpvl *S.aureus* endotracheal



Diep, PNAS 2010



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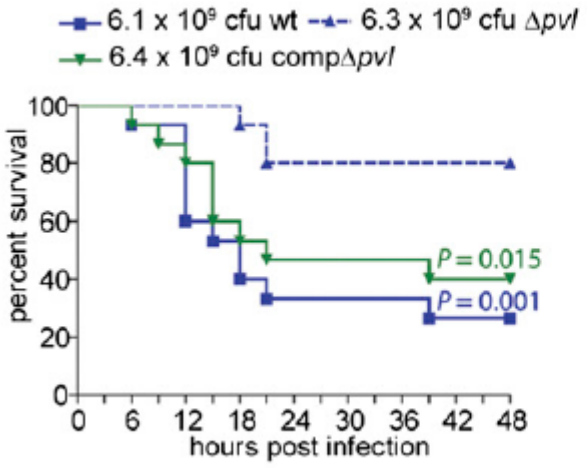
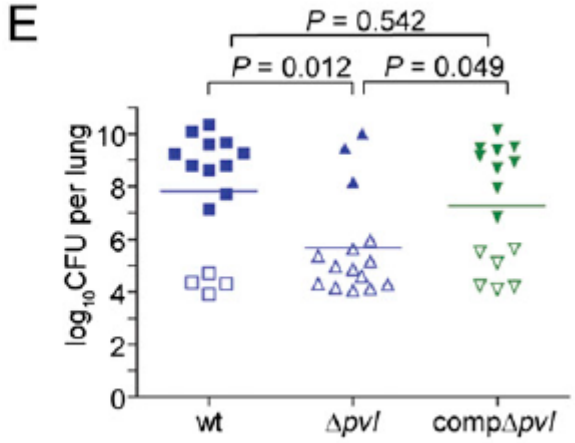
PVL - Necrotizing Pneumonia

<u>Genetic</u> approach

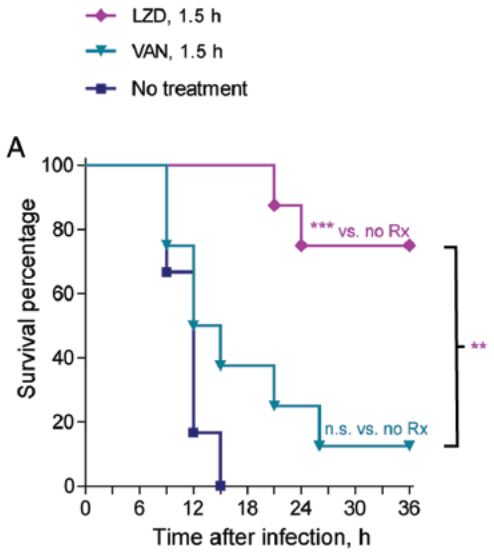
WT vs Δpvl *S.aureus* endotracheal

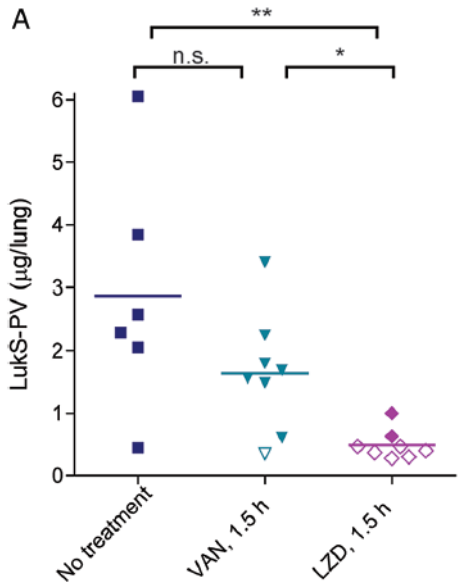




<u>Pharmacological</u> approach

Protein synthesis inhibitor





Diep, PNAS 2010

Diep, JID 2013

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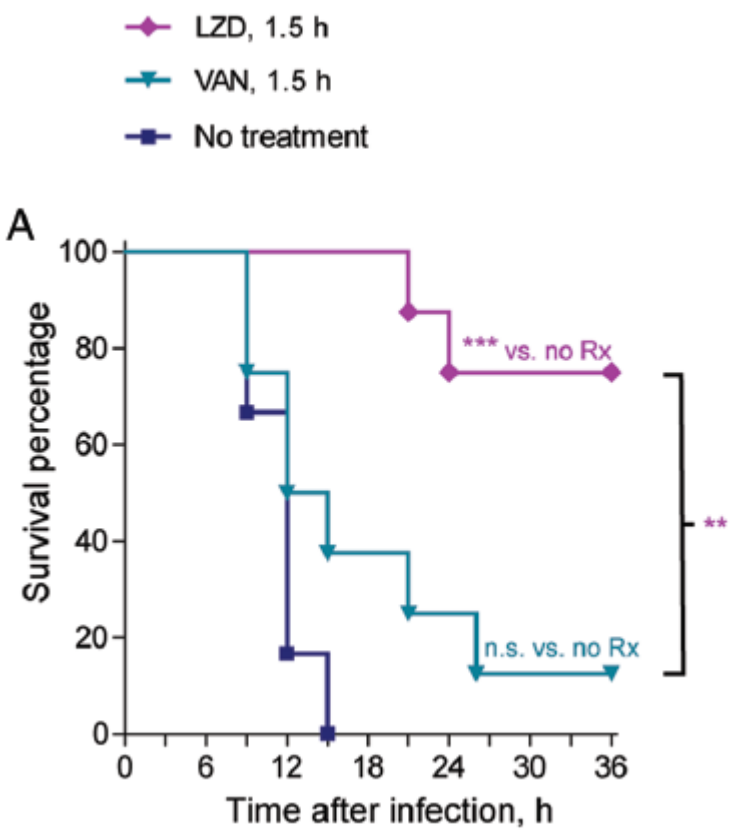
Disarming the pathogen
Proteinsynthesis inhibition



MRSA Pneumonia

- Vanco or Linezolid or Clindamycin

IDSA Guidelines, CID 2011

MSSA Pneumonia

- BetaLactam plus Clindamycin

Gillet Y et al, Int Journal of Antimicrobial Agents 2011

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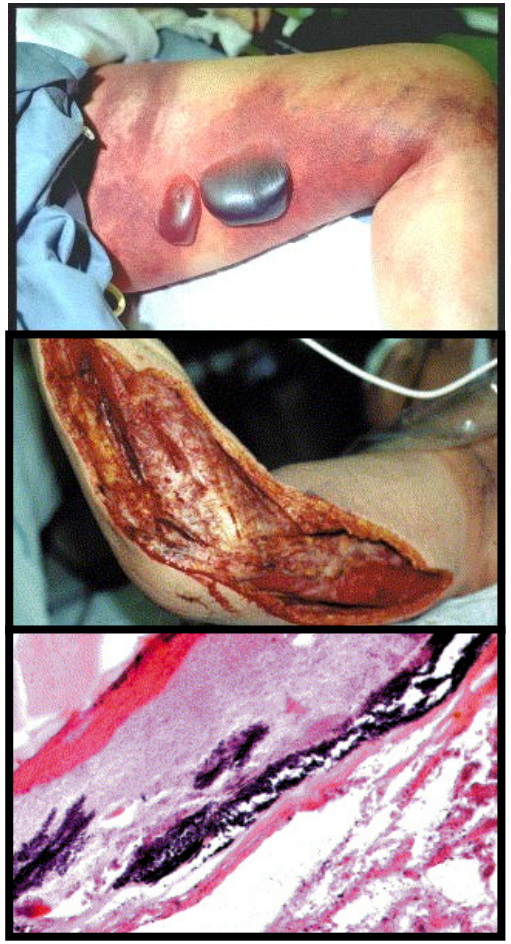
Invasive GAS Disease

Necrotizing fasciitis



Global burden of disease / year:

663,000 <u>invasive infections</u>

- **163,000 deaths**
- **streptococal 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%)

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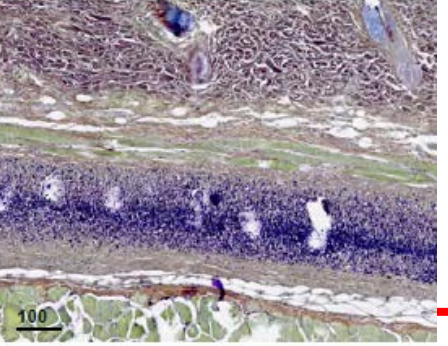
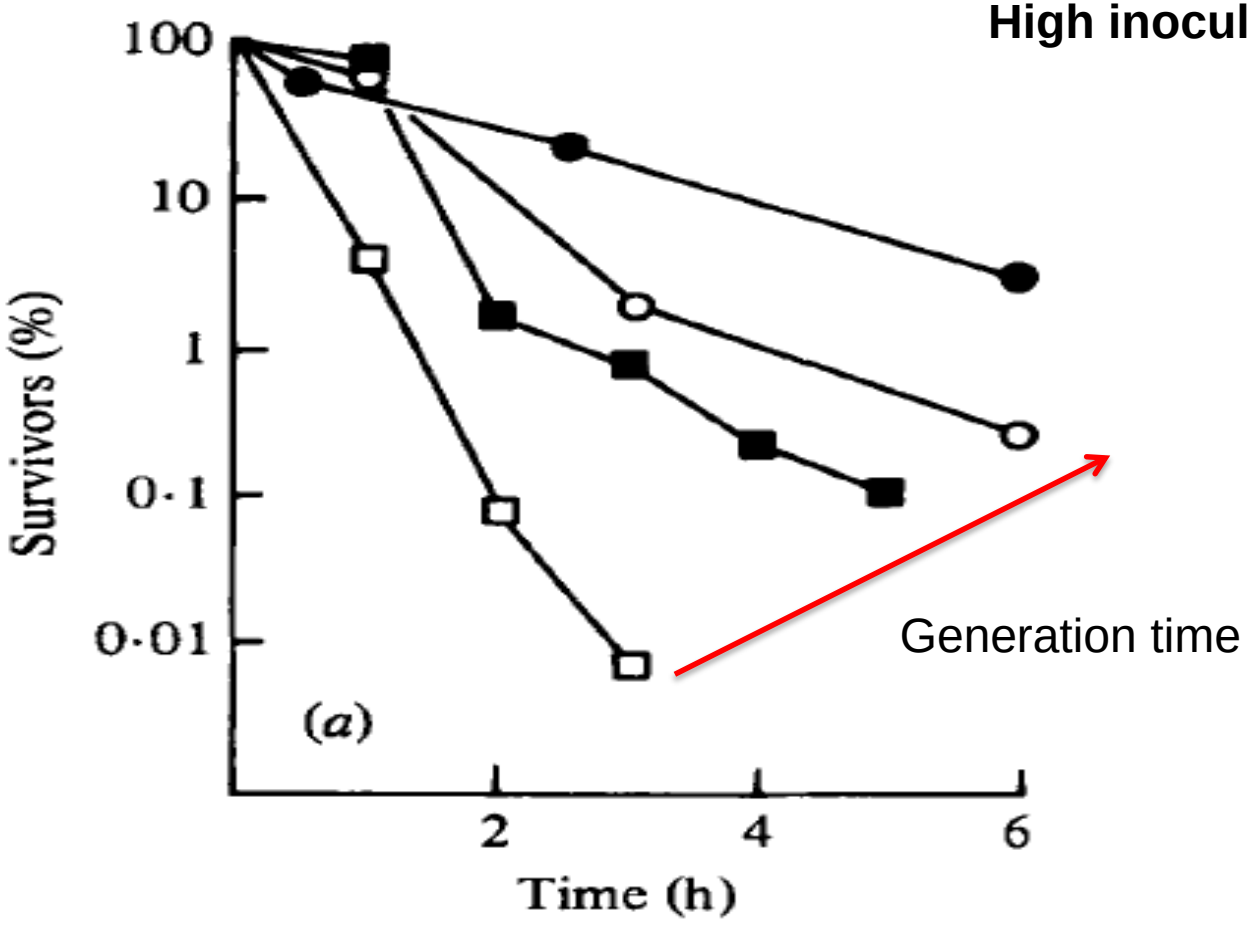
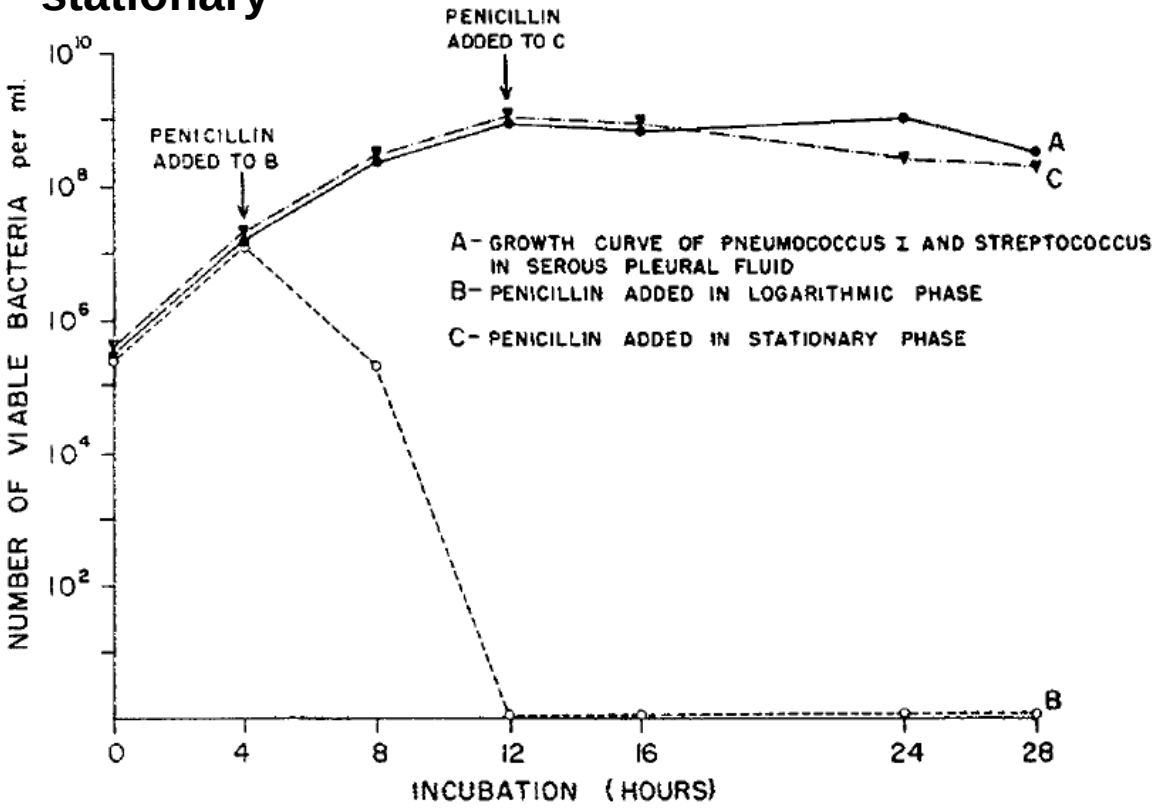


High inoculum – stationary



Tuomanen *et al*, J Gen Microbiology, 1986



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

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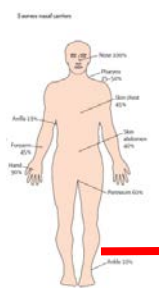
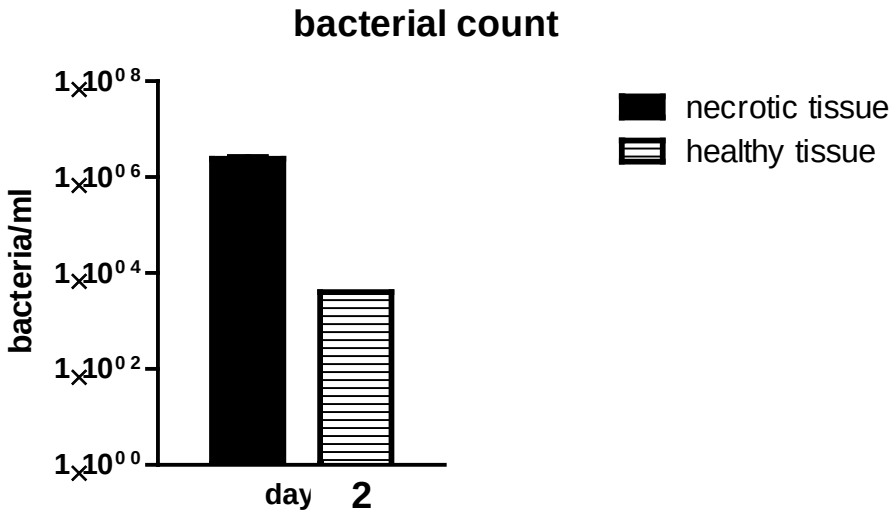
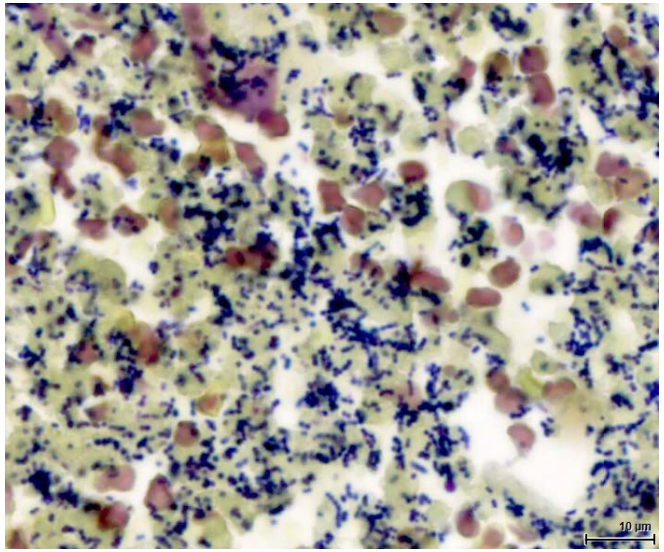
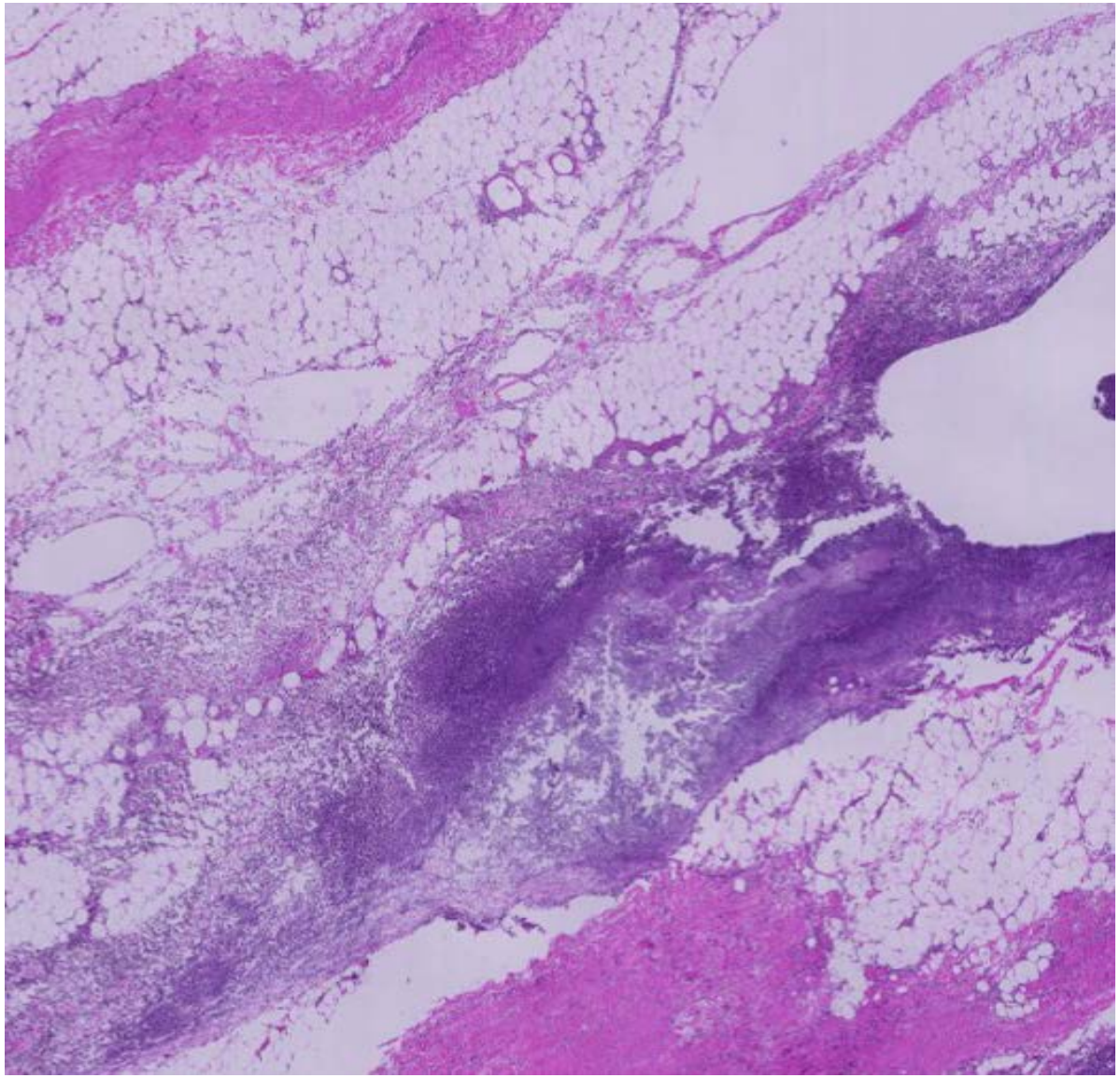


Group A Streptococcus - *Streptococcus pyogenes*

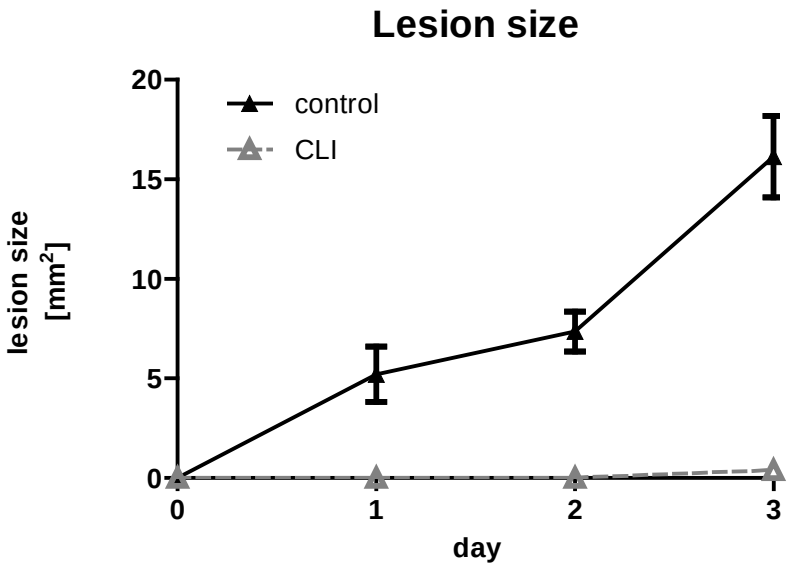


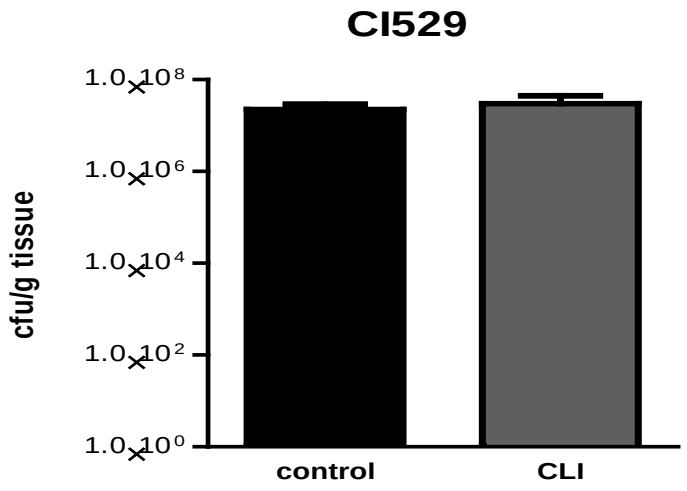
Ceftriaxone

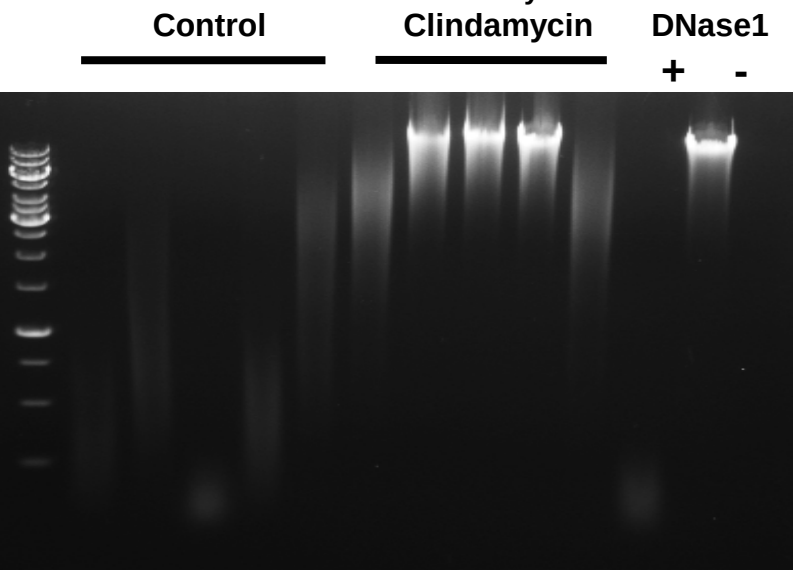


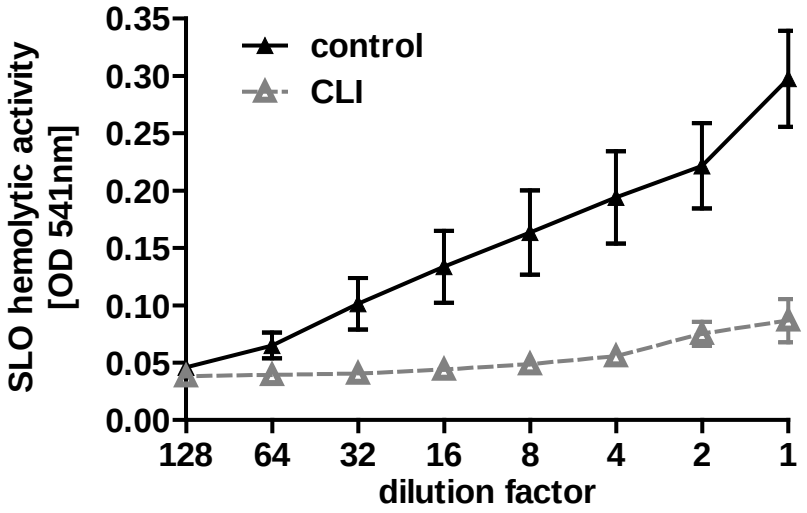


Reduced virulence … *in vivo*









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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.

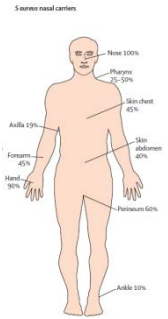
High-dose penicillin plus surgical debridement has been the standard therapy for necrotizing fasciitis caused by group A streptococci (GAS). Although adding clindamycin (CLI) is strongly recommended because of its ability to inhibit bacterial protein synthesis, this approach has never been tested in 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.

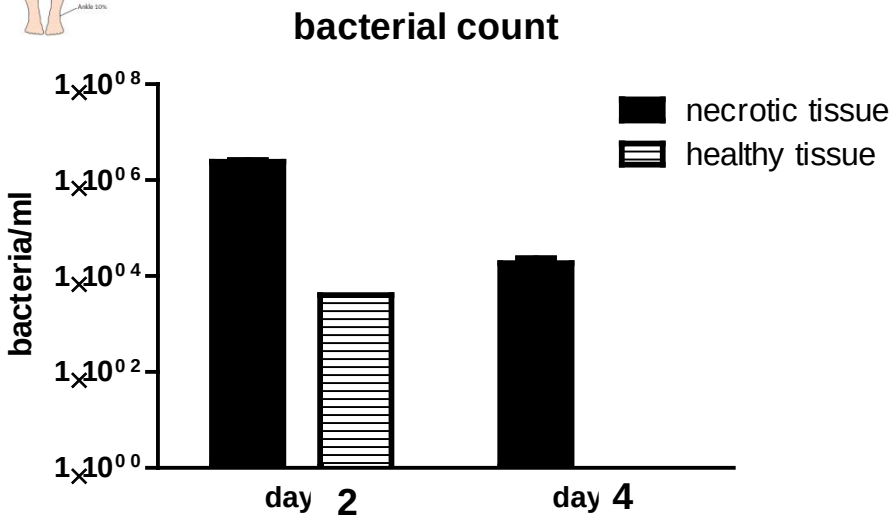
COMMENT

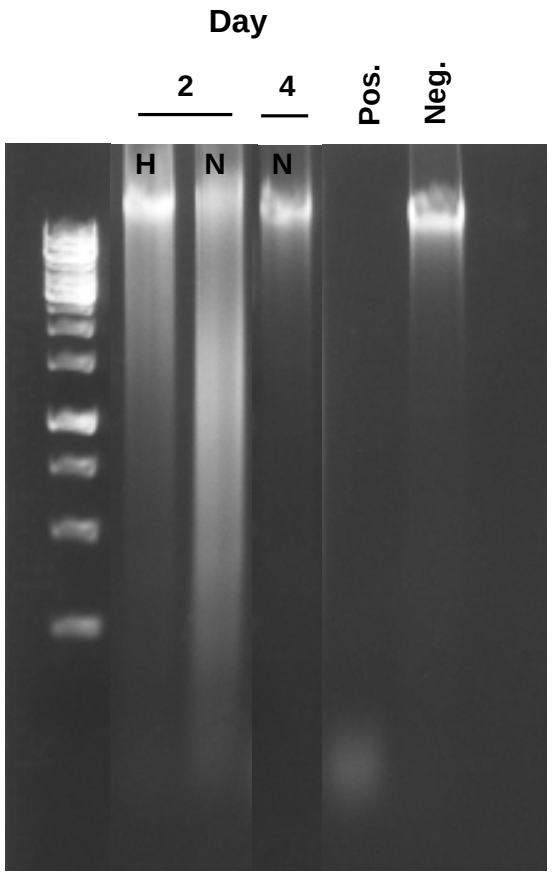
This interesting study provides evidence favoring addition of clindamycin to the standard treatment of GAS-associated necrotizing fasciitis. The authors note that the results support the use of early, high-dose clindamycin in combination with a β-lactam antibiotic and surgical debridement and caution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

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

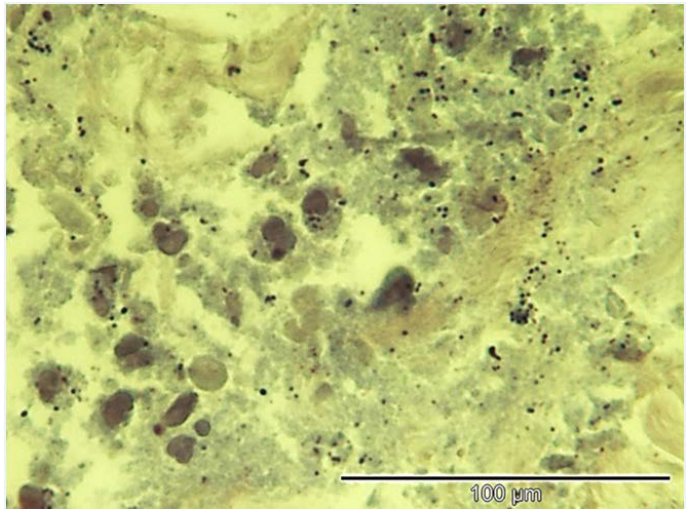


Reduced virulence … and man





Ceftriaxone
Clindamycin



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

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Treating invasive bacterial infections

- **Pathogen directed therapy**
  - Killing – beta lactams, phages, endolysine
  - Anti- toxine strategies
    - Protein synthesis inhibitors
    - IVIG (Lack of antibodies - > fulminant NF)
  - Anti- biofilm strategies

- **Host directed therapy**

Many thanks

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Klinik für Infektionskrankheiten & Spitalhygiene

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