

27.11.2015

6. Balgrist Symposium zum Diabetischen Fuss: Infektionen

Antibiotika: Gewebeinpenetration und Biofilm-Aktivität

Annelies S Zinkernagel

Infektiologie, USZ

annelies.zinkernagel@usz.ch



**University of
Zurich** UZH

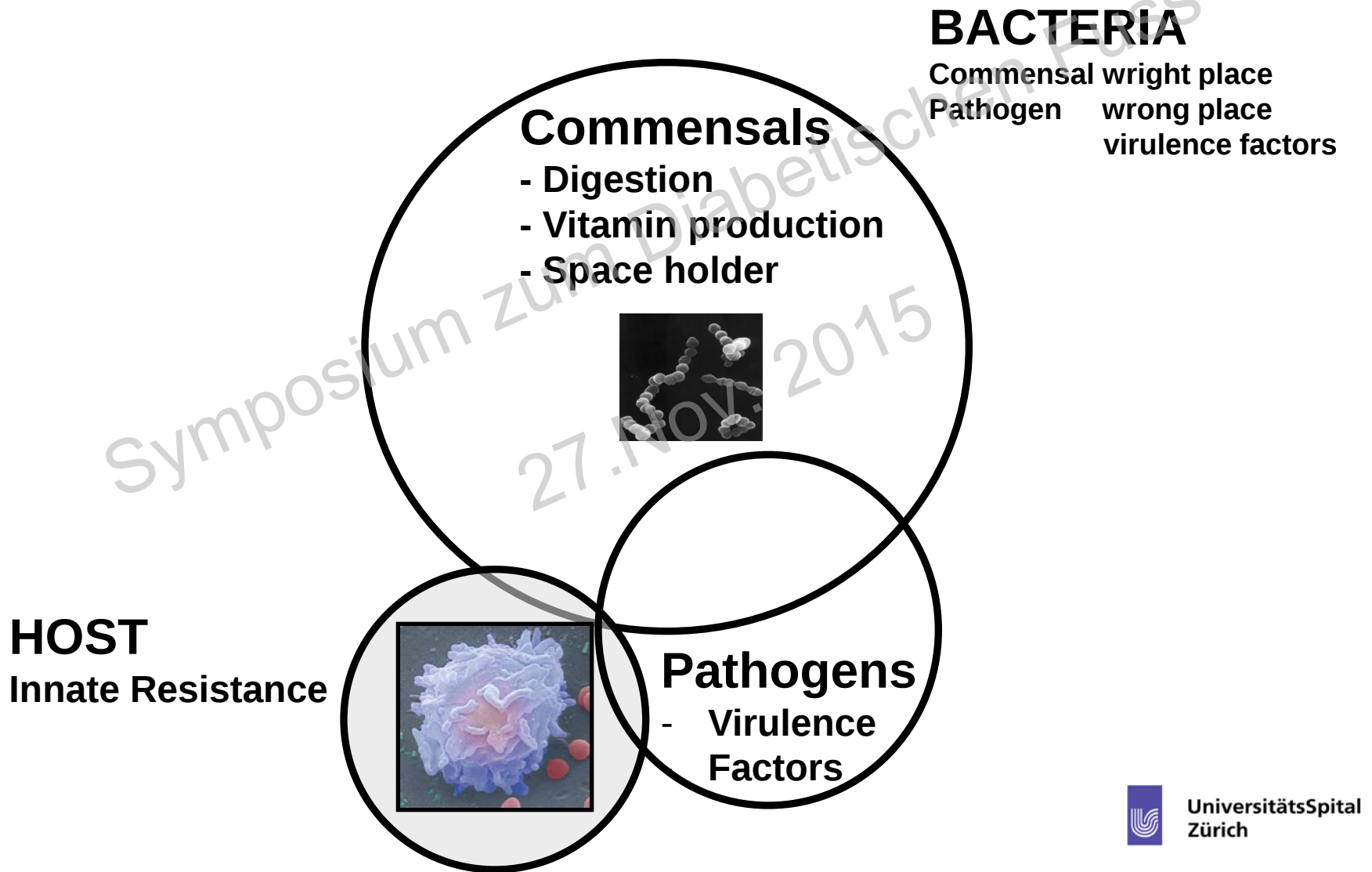


**UniversityHospital
Zurich**

Antibiotika beim diabetischen Fuss

- Konservative Therapie:
 - Gezielte, optimale Antibiotikatherapie
 - Mikrobiologie incl. Resistenzprüfungen (Knochen)
- Amputation verhindern

Commensals- Pathogens



Diabetischer Fuss

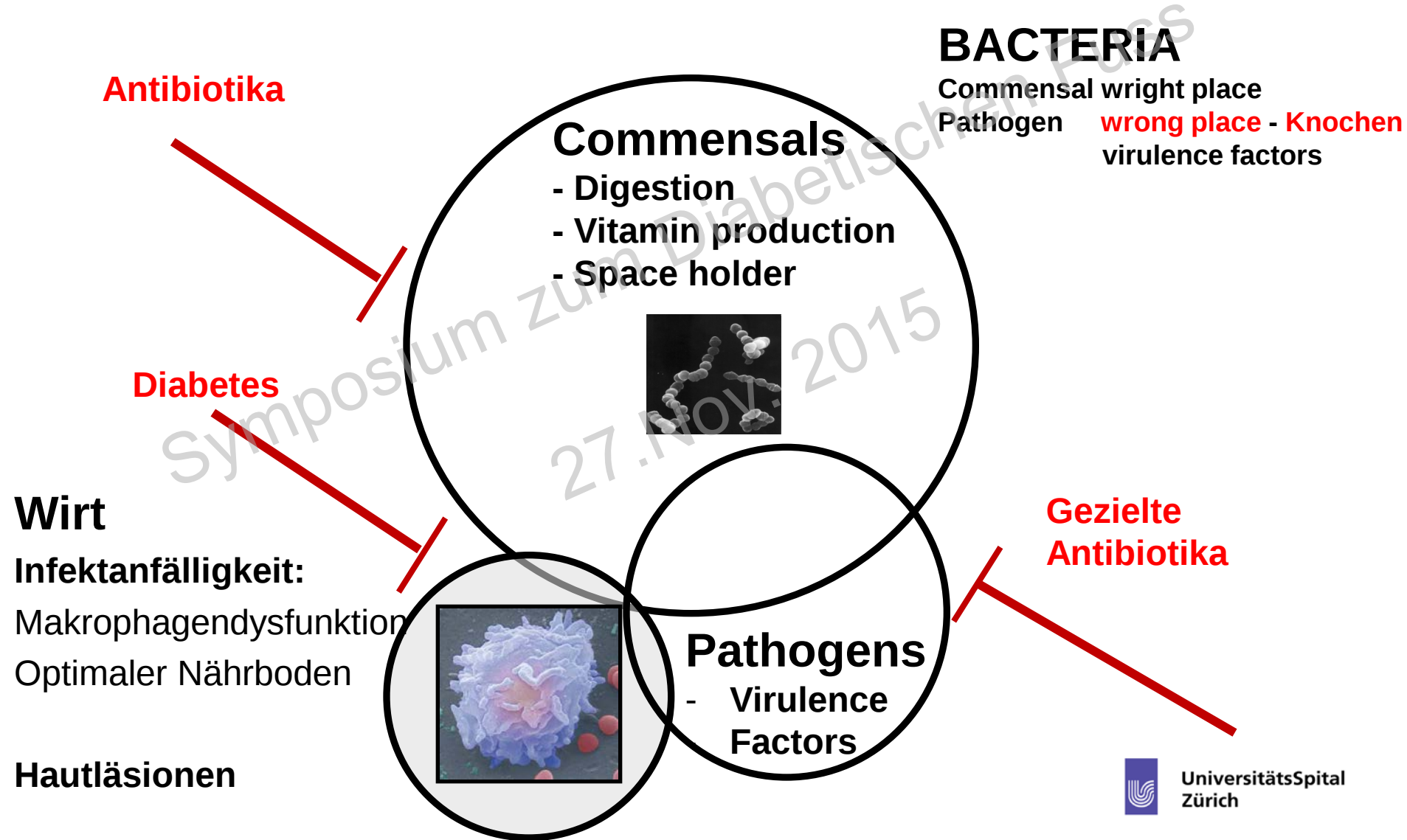


TABLE 2. Bacteria found in 80 bone biopsies from the 80 patients included in the present study

Variables	Present study	Senneville et al. [14]	Aragon-Sanchez et al. [11]
Number of samples	80	76	176
Number of isolates	129	125	204
Mean number of isolates per sample	1.6 ± 1	1.54	—
Number of culture negative samples (%)	2 (2.5%)	2 ^a	20 (11%)
Number (%) of isolates, by pathogen			
Gram-positive			
Staphylococci	61 (47%)	65 (52%)	117 (57%)
Staphylococcus aureus	43 (33%)	33 (26%)	95 (47%)
MRSA	24 (19%)	12 (10%)	35 (17%)
Central nervous system	18 (14%)	32 (26%)	22 (11%)
Streptococci	12 (9%)	15 (12%)	7 (3%)
Enterococci	15 (12%)	10 (8%)	2 (1%)
Corynebacteriae	5 (4%)	3 (2%)	—
Gram-negative bacilli	26 (20%)	23 (18%)	59 (29%)
Pseudomonas aeruginosa	10 (8%)	3 (2%)	18 (9%)
Anaerobes	5 (4%)	6 (5%)	—

^aNot included in the present study.

It's all about location

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"I go south in the winter. I have a condo in Hades."

Bakterien

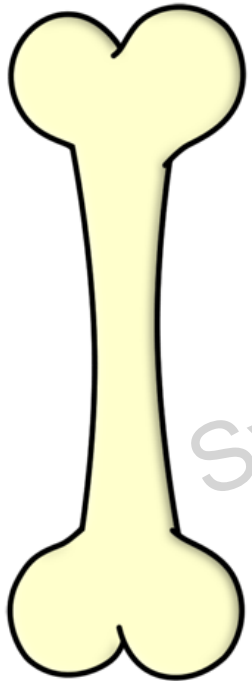
- Knochen
- Biofilm
- Intrazellulär

Antibiotika

Gewebepenetration

Biofilmaktivität

Gewebepenetration -Knochen



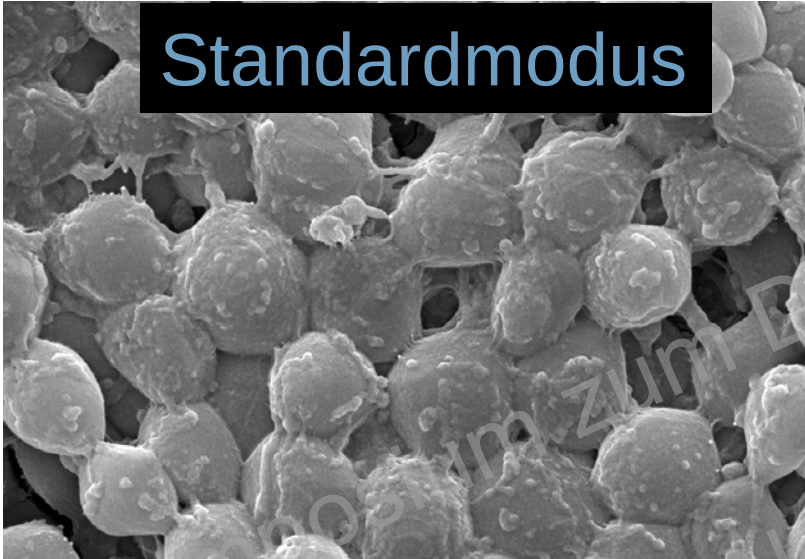
- Beta-laktame und Glycopeptide:
 - im Knochen 5% - 20% Serumspiegel (i.v.>>>>>p.o.)
- Fluoroquinolones, Linezolid, Trimethoprim, Clindamycin:
 - im Knochen 50% Serumspiegel (i.v. = p.o.)
- Doxycycline: 2% (Skelet), 86% Mandibula (i.v. = p.o.)
- Metronidazol, Rifampicin: Knochen = Serum
- Fusidin Säure und Fosfomycin: Knochen > Serum
- Rifampicin und Fusidin Säure : **nie solo**

Table 2. Bone Penetration of Antibiotics With High Oral Bioavailability: Data From Clinical Studies

Drug	Patients, No.	Dose	Route	Serum Level, Mean (Range), µg/mL	Bone Level, Mean (Range), µg/g	Serum-Bone Ratio, %	Reference
Ciprofloxacin	7	500 mg	Oral	1.4 (0.4–2)	0.4 (0.2–0.9)	30	[50]
	7	750 mg	Oral	2.6 (0.9–4)	0.7 (0.2–1.4)	27	
	6	500 mg	Oral	2.0 (0.9–3)	0.7 (0.2–1.4) ^a	35	
	4	750 mg	Oral	2.9 (1–6)	1.4 (0.6–2.7) ^a	48	
Ciprofloxacin	20	200 mg	Intravenous	NA	2 (medullary)	66	[51]
					1.4 (cortical)	47 ^c	
Ciprofloxacin	15	200 mg	Intravenous	NA	0.1–0.9	3–30 ^b	[52]
Levofloxacin	9	500 mg	Intravenous	8	6 (medullary)	75	[53]
					3 (cortical)	38	
Levofloxacin	12	500 mg	Intravenous	7.5	7.4 (medullary)	99	[54]
					3.9 (cortical)	50	
Enoxacin	24	400 mg	Oral or	2.4	0.9	37.5	[55]
	7		Intravenous		1.3 ^b	55	
Moxifloxacin	10	400 mg	Intravenous	4.9	1.9 (medullary)	39	[56]
					1.3 (cortical)	27	
	10	400 mg	Oral	3.7	1.8 (medullary)	49	
					1.6 (cortical)	43	
Linezolid	13	600 mg	Oral	NA	4	40 ^c	[57]
Linezolid	12	600 mg	Oral		9	51 ^c	[58]
Linezolid	10	600 mg	Oral	23	8.5	37	[59]
TMP-SMX	14	1 DS tablet twice daily for 2 d	Oral	7.4/143	3.7/19	50/15	[60]
Doxycycline	6	200 mg	Intravenous	NA	2.6	86 ^a	[61]
Doxycycline	25	200 mg	Intravenous	NA	0.2	6 ^a	[62]
Doxycycline	34	200 mg	Intravenous	6	0.13	2	[63]
Clindamycin	13	600 mg	Intravenous or intramuscular	NA	5	67 ^a	[64]
Clindamycin	27	300 mg	Intramuscular	7.33	2.63	40	[65]
Clindamycin	23	600 mg	Intravenous	8.5	3.8	45	[66]
Metronidazole	16	500 mg	Intravenous	NA	14	100 ^a	[67]
Metronidazole	17	1500 mg	Intravenous	34	27	79	[68]
Rifampin	32	300 mg	Intravenous	2	5 (1.4–8.8)	>100 ^c	[69]
Fusidic acid in infected bone ^d	15	500 mg 3 times daily	Oral	NA	7.3 (1.7–14.9)		[70]
	14	1 g 3 times daily	Oral	NA	9.8 (3.4–14.8)		
Fusidic acid in uninfected bone	9	500 mg 3 times daily for 5 d	Oral	27 (2–109)	12 (1–40)	44	[70]
	15	500 mg 3 times daily for 6–10 d	Oral	45 (5–166)	21 (2–75)	47	

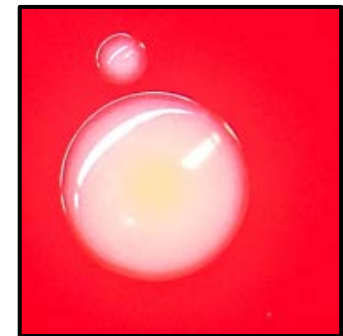
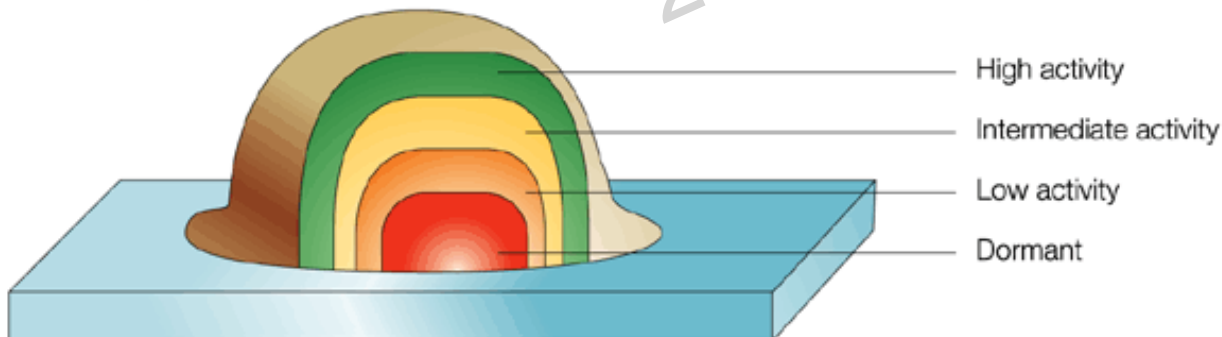
Biofilm

Standardmodus

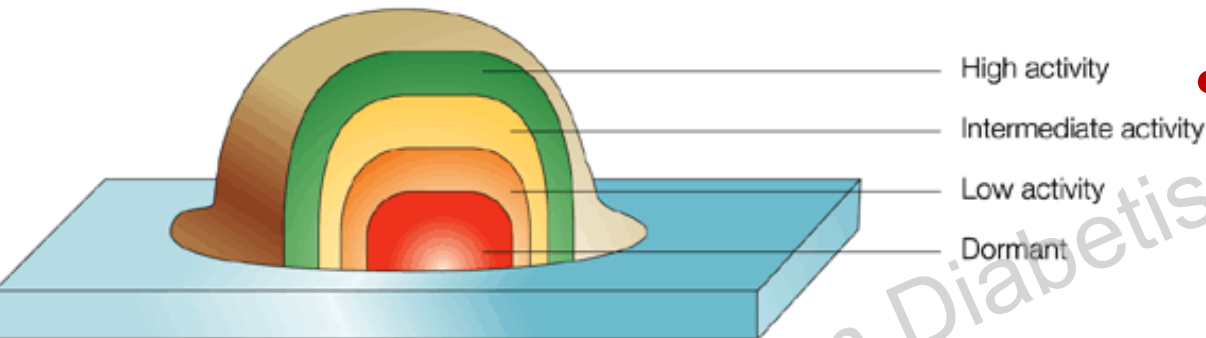


- **Extrazelluläre Matrix:**
Schlechte Penetration/
Diffusion

- **Persister Bakterien**

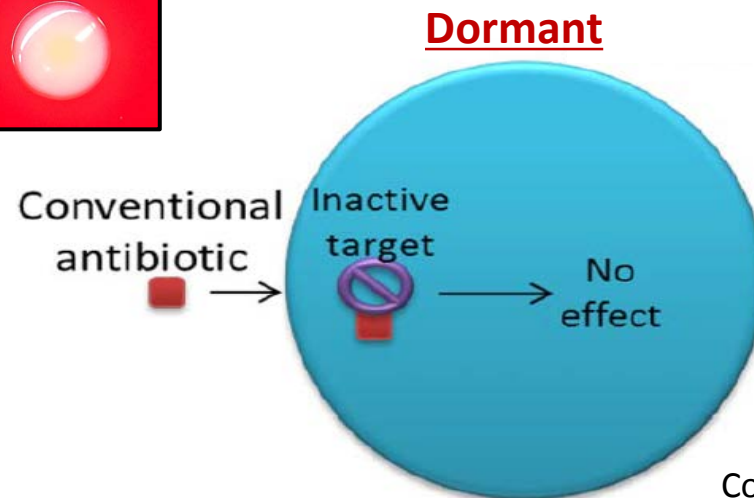
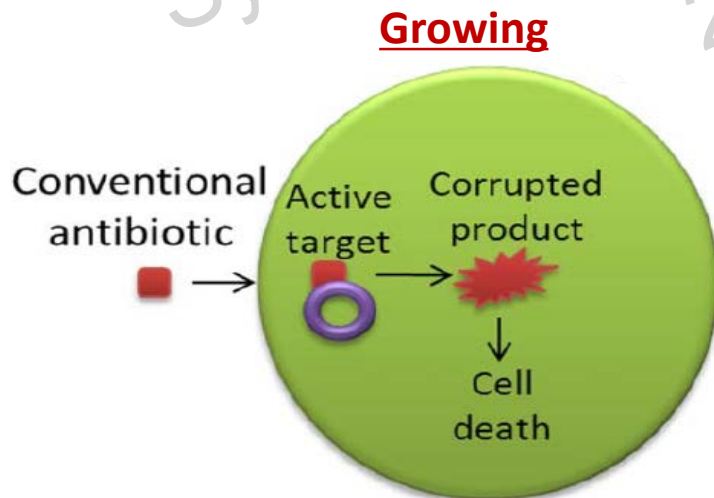


Persister Bakterien



- Vermindertes/kein Wachstum – kein Metabolismus → Antibiotika wirken nicht

Nature Reviews | Drug Discovery



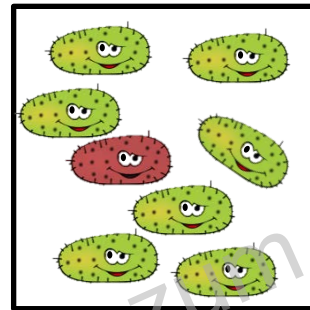
Persistenz

Persister Bakterien

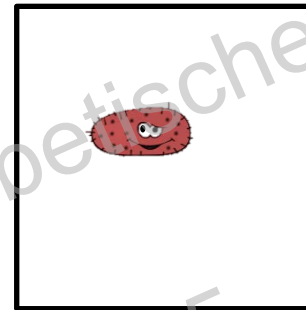
Resistance

≠

Persistence

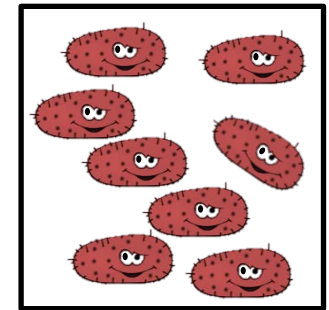


+AB
→

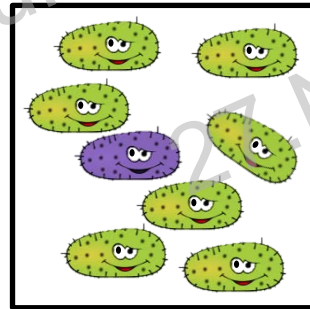


Resistant cell

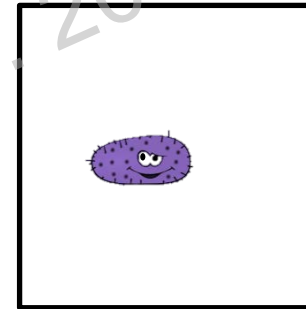
+/-AB
...→



Resistant population

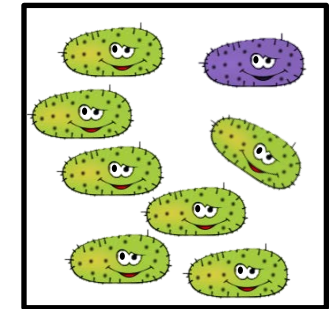


+AB
→



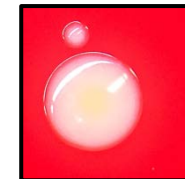
Persister cell

-AB
...→



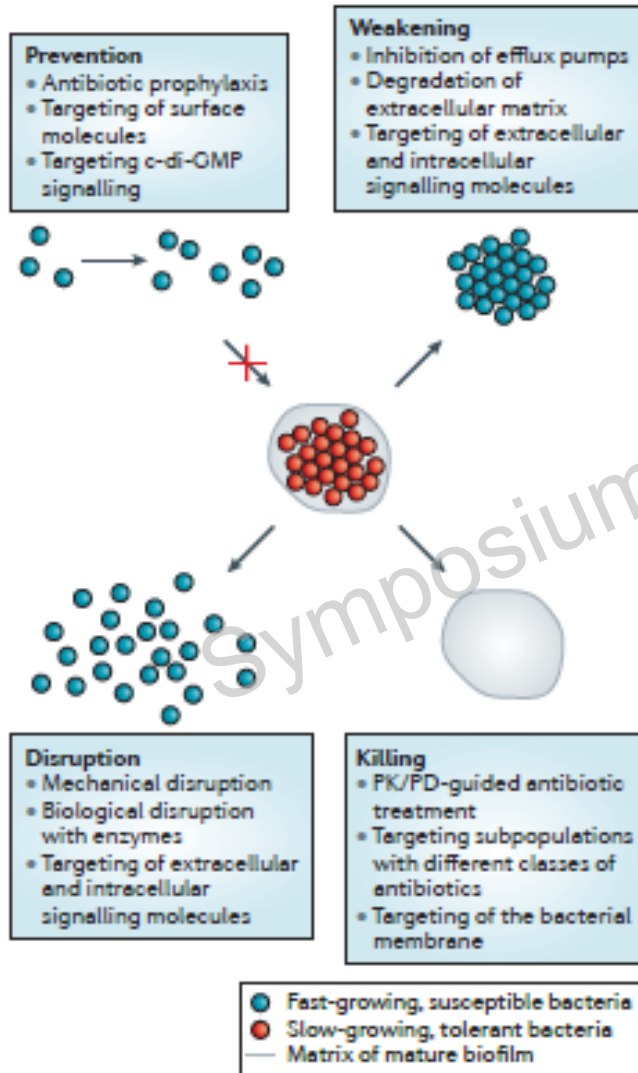
Sensitive population

Phenotype switching



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Therapeutische Optionen?



Zukunft - Impfen

Ag im Biofilm hochregulierte Proteine

Extrazelluläre Matrix:

- mechanisch: Sonikation, Chirurgie
- enzymatisch: DNase, Dispersin B

Bakterien:

- † : Antibiotika

Figure 4 | The four anti-biofilm strategies. The blue circles represent susceptible bacteria and the red circles represent tolerant bacteria. The large light

Zerkleinern der extrazellulären Matrix

Mechanisches Zerkleinern

- Zähneputzen
- Chirurgie -Debridement
- Vortex/Sonication (Nierenstein)

Biologisches Zerkleinern mit Enzymen

- DNase (DNA) p.i.
- Dispersin B (Polysacharide)

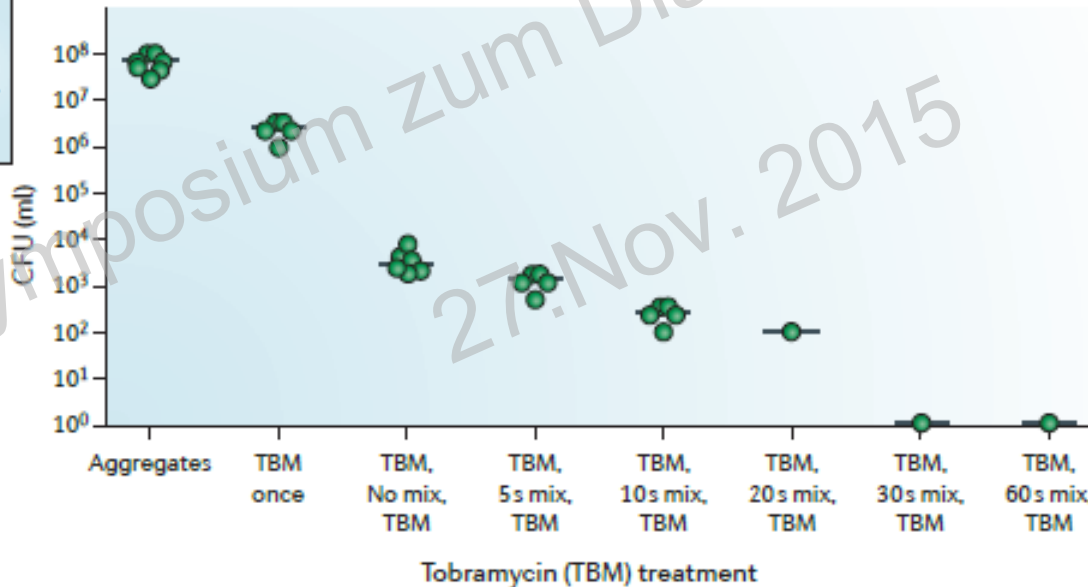


Figure 3 | Sensitivity of mechanically disrupted *Pseudomonas aeruginosa* biofilms to tobramycin. Aggregates were grown for 48 hours and subsequently treated with

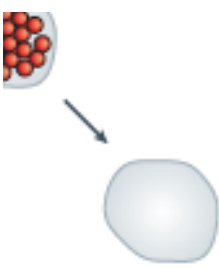
NatureReview 2013



Welches AB wirkt?

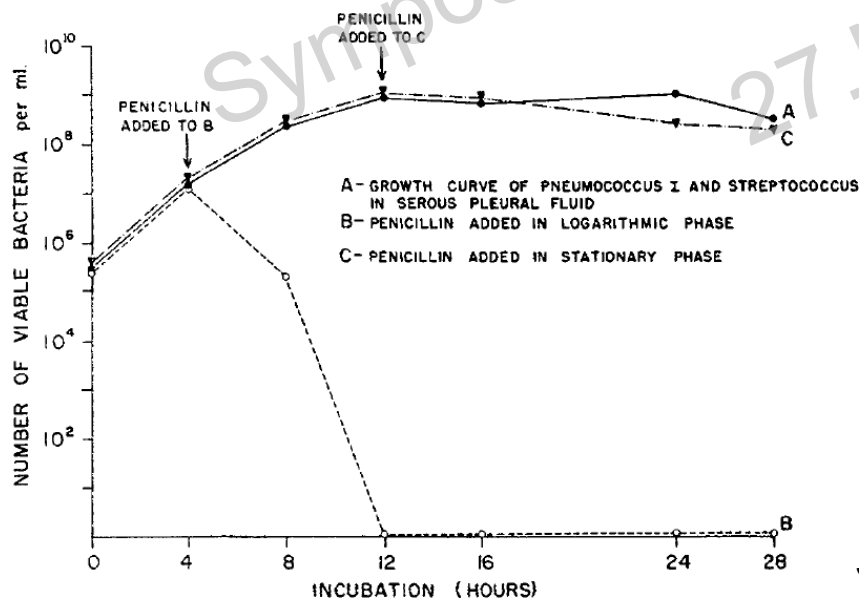
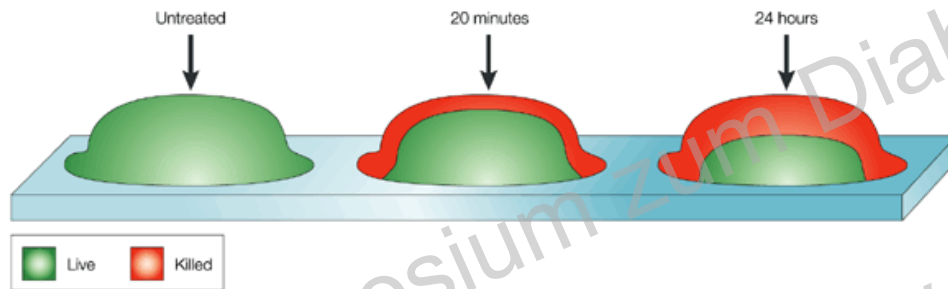
Aktivität im Biofilm und auf stationäre, nicht teilende Bakterien

- Extrazelluläre Matrix:
Schlechte
Penetration/ Diffusion
- Persister Bakterien

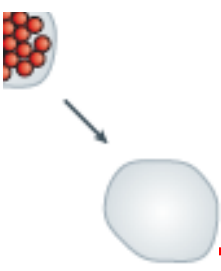


Killing

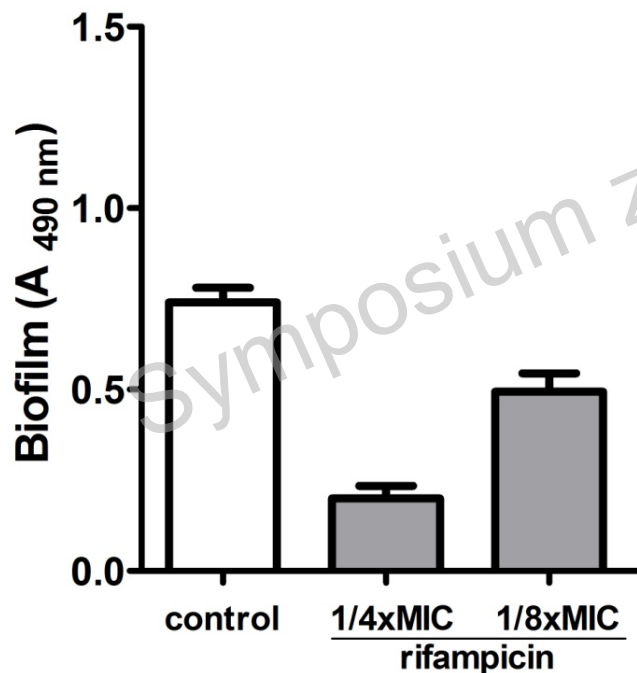
- PK/PD-guided antibiotic treatment
- Targeting subpopulations with different classes of antibiotics
- Targeting of the bacterial membrane



Welches AB wirkt?



- Killing**
- PK/PD-guided antibiotic treatment
 - Targeting subpopulations with different classes of antibiotics
 - Targeting of the bacterial membrane



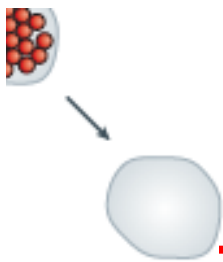
Aktivität im Biofilm und auf stationäre, nicht teilende Bakterien

Rifampin: Inhibiert bakterielle RNA Synthese, Wkg bei pH↓

AB mit Wirkung auf die Zellmembran :
Aktiv auch auf stationäre, nicht teilende Bakterien

Daptomycin:
Lipopeptid, Gram +

Colistin: Gram –
bei ↓O₂



Biofilm 'Toleranz'

Killing

- PK/PD-guided antibiotic treatment
- Targeting subpopulations with different classes of antibiotics
- Targeting of the bacterial membrane

Table 1 | **Biofilm tolerance of antibiotics and biocides**

Antibiotics and biocides	Physical (biofilm matrix-related) tolerance	Physiological tolerance	Adaptive tolerance*
β -lactam	Diffusion partially impaired ⁹¹	No activity on non-dividing cells ²³⁹	• Induction of β-lactamase, which can impair diffusion in the biofilm by hydrolysing the β-lactam molecules⁹⁸ • Subinhibitory concentrations induce alginate genes⁷³ • Upregulation of efflux pumps as stress response¹⁰¹
Quinolones	No impact ²⁴⁰	Impaired activity in anaerobic conditions [†]	• Upregulation of efflux pumps as stress response¹⁰¹ • Upregulation of PA1874-1877 multidrug efflux pump in combination with <i>ndv</i> (PA14)²⁴¹
Aminoglycosides	• Diffusion impaired by alginate²⁴² • Extracellular DNA chelates cations and thereby creates cation-limited conditions; this induces LPS modifications and impaired self-promoted uptake of antibiotics²⁴³	Impaired activity on non-dividing cells ³¹	• Upregulation of MexXY efflux pumps²⁴⁴ • Subinhibitory concentrations induce biofilm formation⁷⁴ • <i>ndv</i>-dependent glucan production in the periplasm and sequestration of aminoglycosides in this compartment²⁴⁶ • Upregulation of PA1874-1877 multidrug efflux pump in combination with <i>ndv</i>²⁴¹
Antimicrobial peptides and other membrane-active biocides	Extracellular DNA chelates cations and thereby creates cation-limited conditions; this induces LPS modifications and impaired self-promoted uptake of antibiotics ²⁴³	-	• Specific: induction of <i>arn</i> operon and LPS modification • Nonspecific: upregulation of efflux pumps^{17,100} • Upregulation of efflux pumps as stress response¹⁰¹
Azithromycin	-	-	Upregulation of MexCD-oprJ efflux pump ⁹⁹

LPS, lipopolysaccharide. *ndv*, glucosyltransferase involved in the formation of cyclic glucans. *Examples for *Pseudomonas aeruginosa*. [†]T.B., O.C., N.H., R. P. Brochmann, A. Toft, C. Haempel and P. Ø. Jensen, unpublished observations.

It's all about location

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"I go south in the winter. I have a condo in Hades."

Bakterien

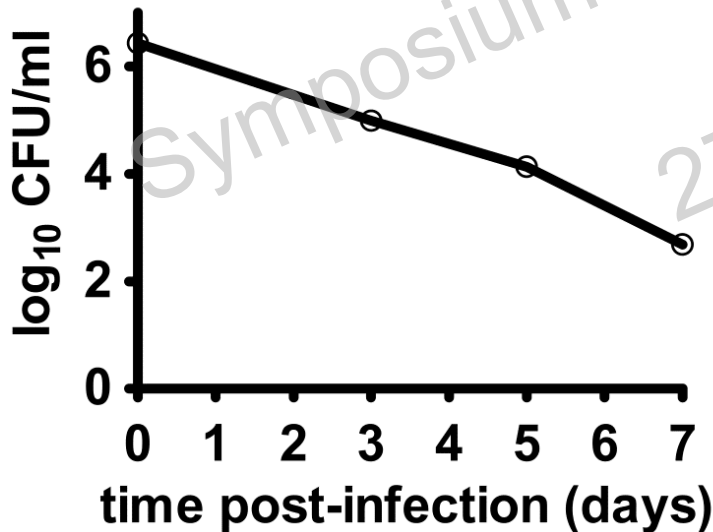
- Knochen
- Biofilm
- Intrazellulär

Antibiotika

Gewebepenetration

Biofilmaktivität

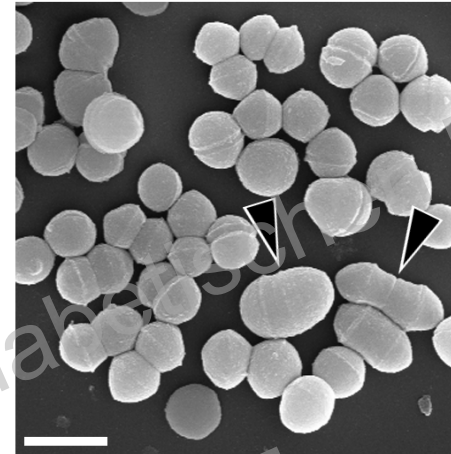
Intrazelluläre Persistenz trotz AB



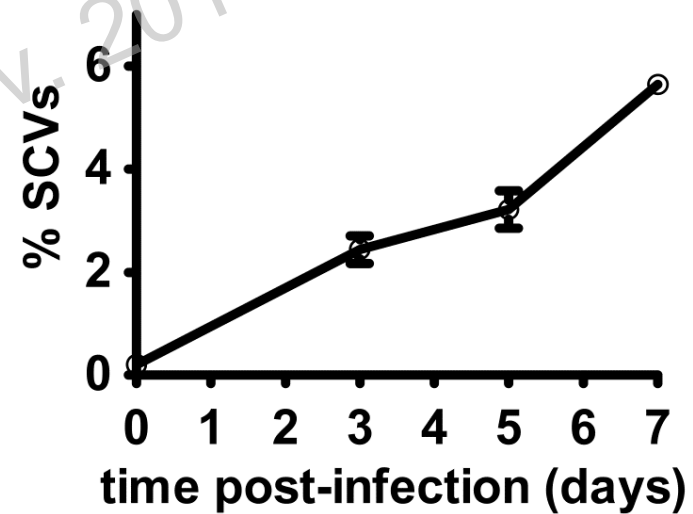
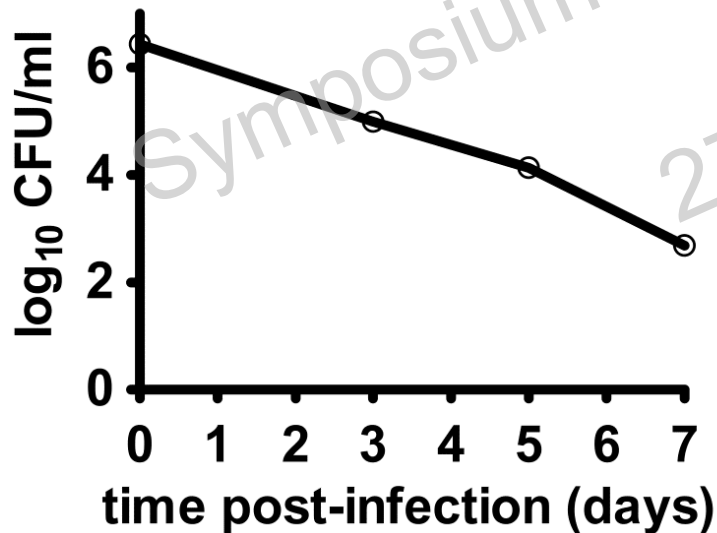
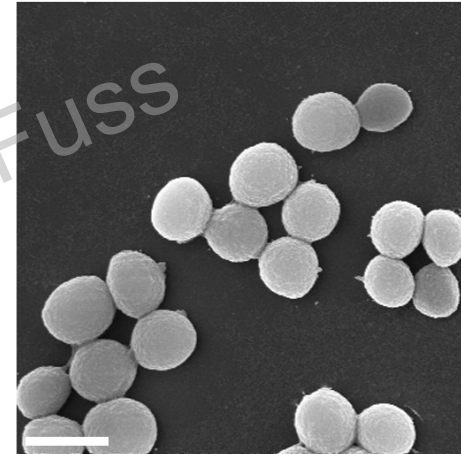
Intrazelluläre Persistenz trotz AB



SCV

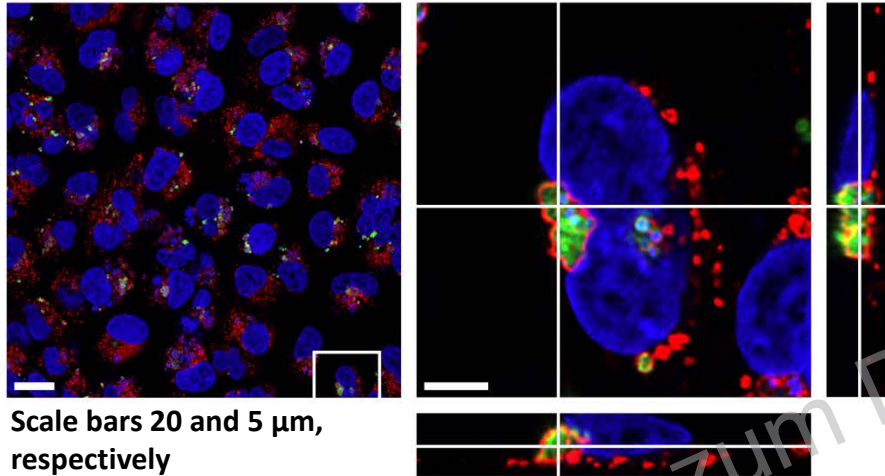


LCV



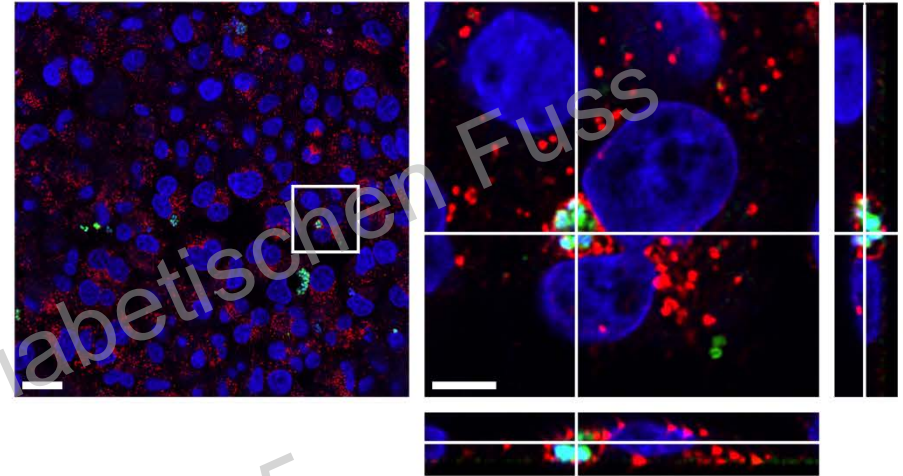
Intrazelluläre *S.aureus* sind geschützt vor AB

Day 0

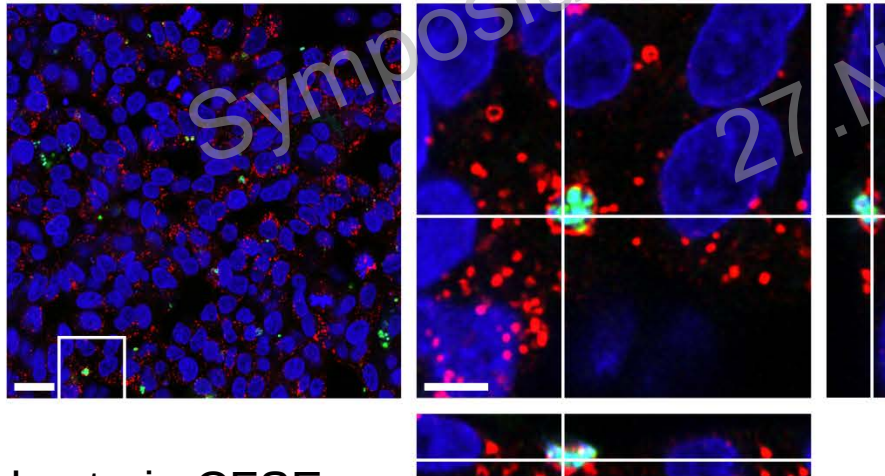


Scale bars 20 and 5 μm , respectively

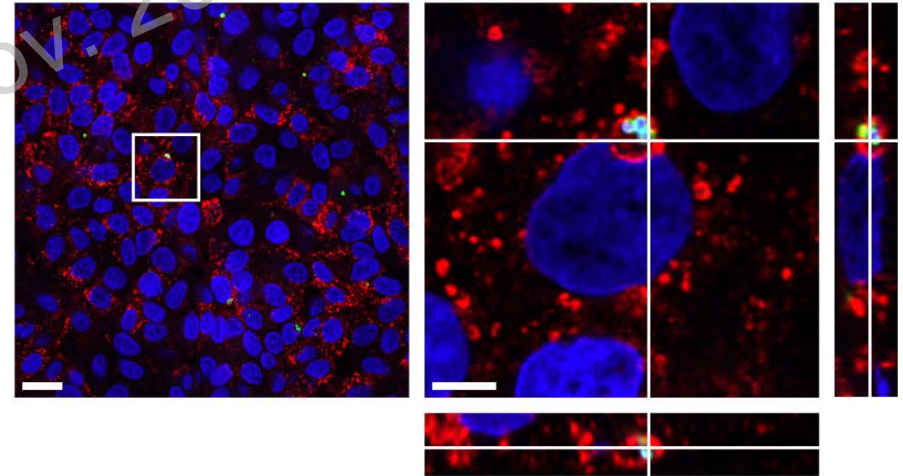
Day 3



Day 5



Day 7



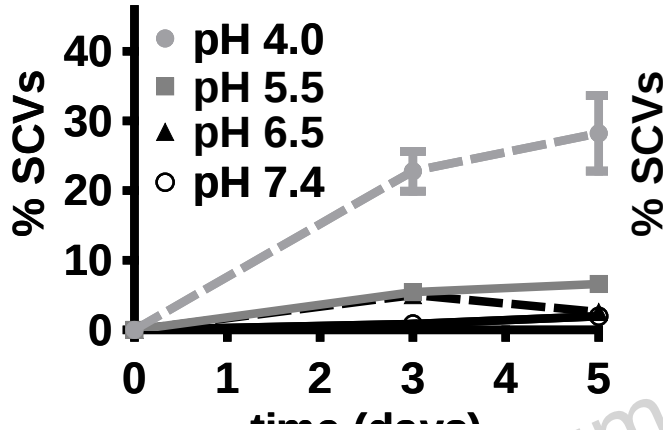
bacteria CFSE, green
lysosomes LAMP-2 antibody, Alexa Fluor 594, red
nuclei DAPI, blue



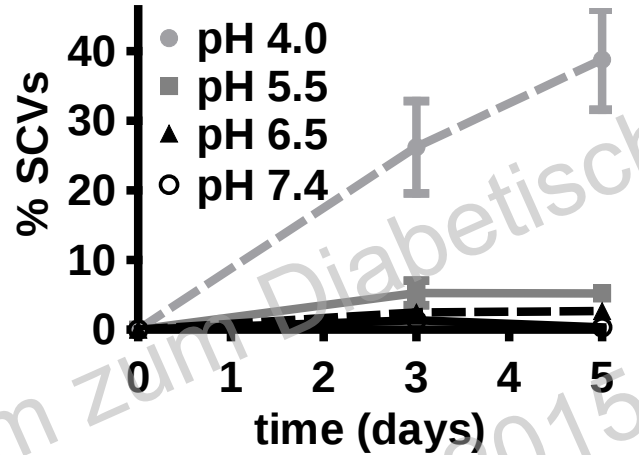
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Niedriger pH induziert persistierender Bakterien

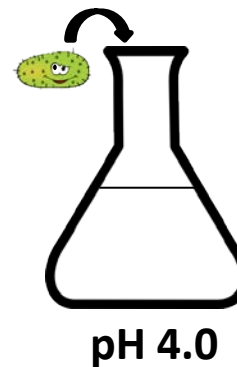
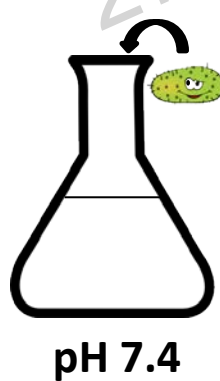
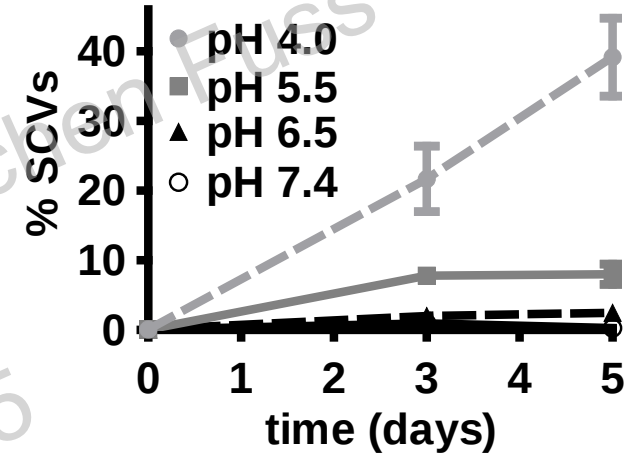
MSSA Cowan



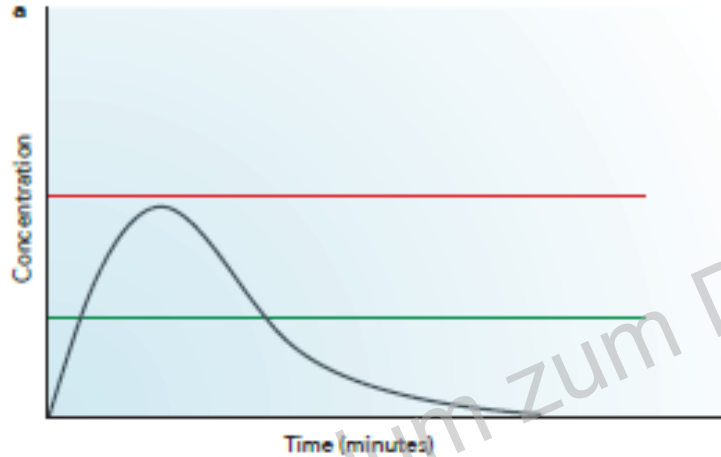
MSSA 6850



MRSA JE2



Wieviel?

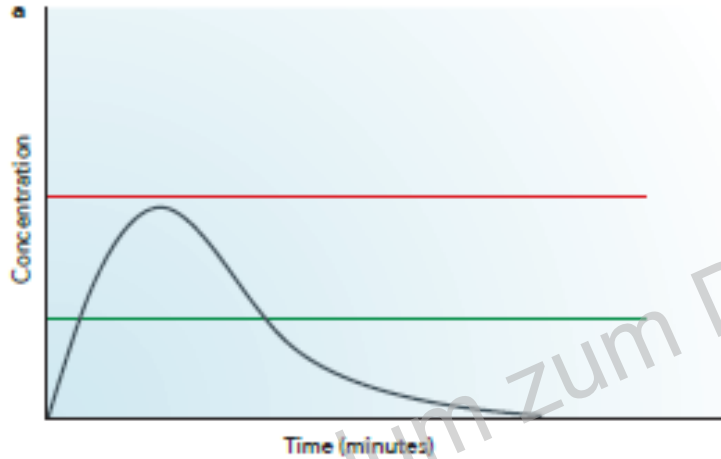


Grün: AB conc notwendig um planktonische Bakterien zu töten

Rot: Biofilm assoz. Bakterien

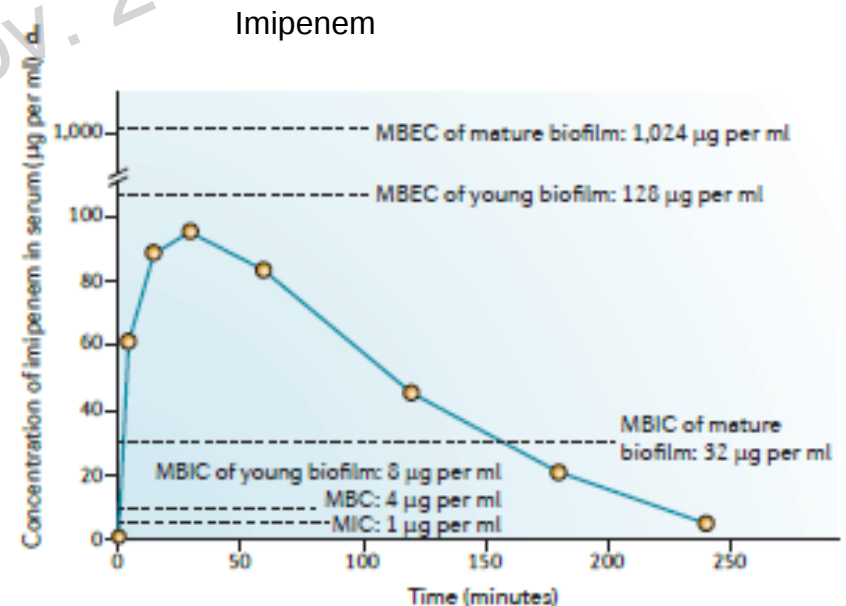
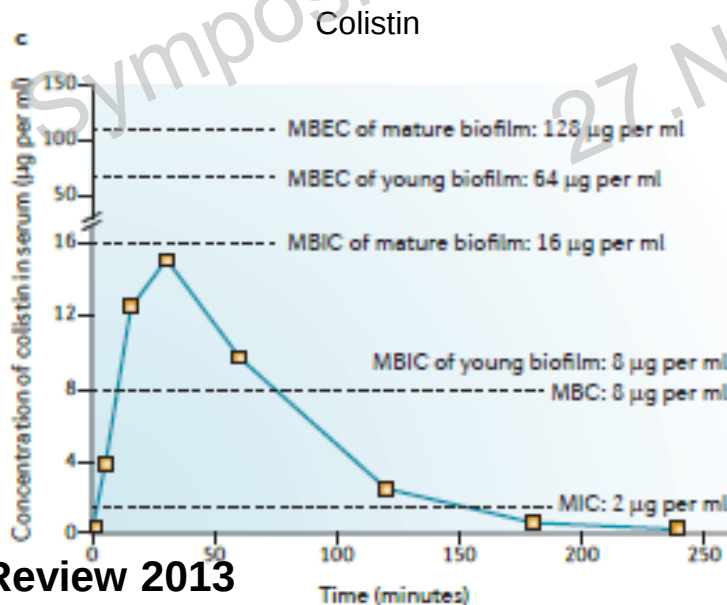
Biofilm assoz. Bakterien bis 10'000 x resistenter

Wieviel - Was?



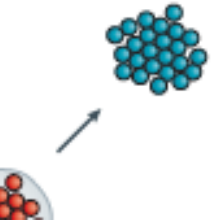
Grün: AB conc notwendig um planktonische Bakterien zu töten

Rot: Biofilm assoz. Bakterien



Schwächen der Kommunikation

- Weakening**
- Inhibition of efflux pumps
 - Degradation of extracellular matrix
 - Targeting of extracellular and intracellular signalling molecules



Rote Alge *Delisea pulchra*
Furanone: inhibieren Bakterienadherenz
auf Algenoberfläche

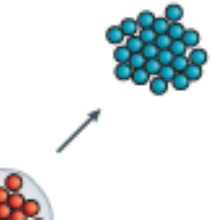


Furanone
interferieren mit dem
Quorum sensing von Gram –
kein Biofilm

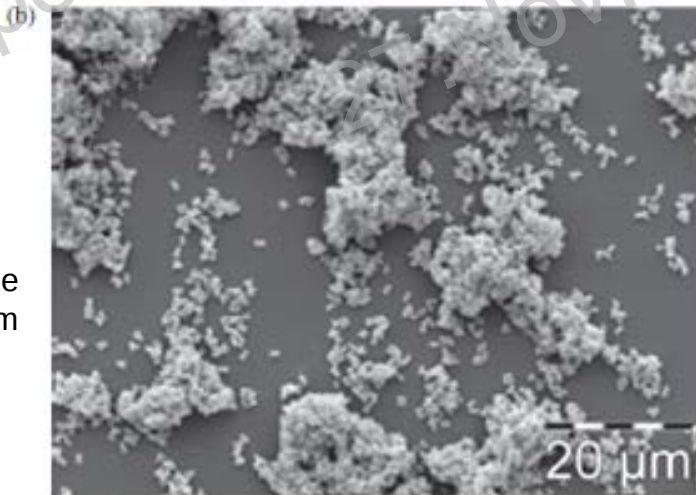
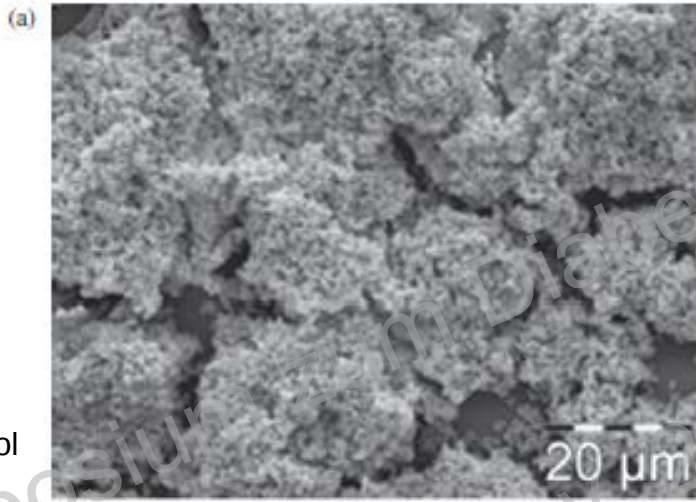


Schwächen der Kommunikation

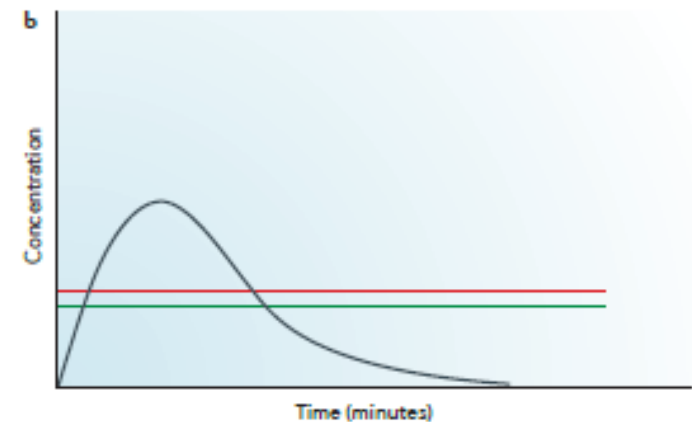
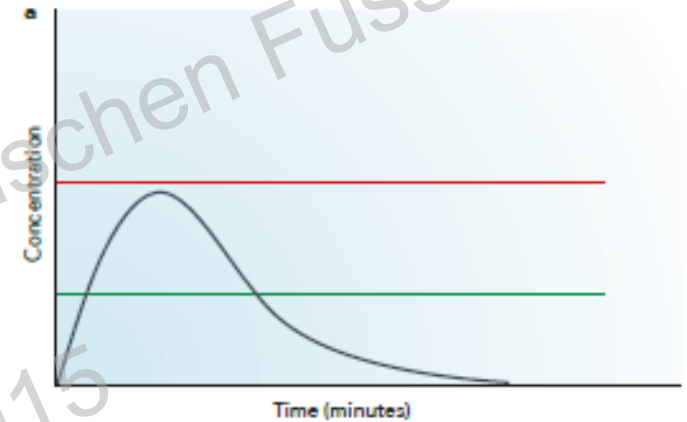
- Weakening**
- Inhibition of efflux pumps
 - Degradation of extracellular matrix
 - Targeting of extracellular and intracellular signalling molecules



control



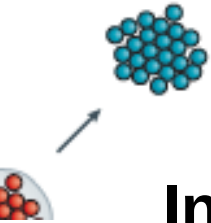
Synthetische Furanone
Weniger *S.epidermidis* Biofilm



Schwächen der Kommunikation

Weakening

- Inhibition of efflux pumps
- Degradation of extracellular matrix
- Targeting of extracellular and intracellular signalling molecules



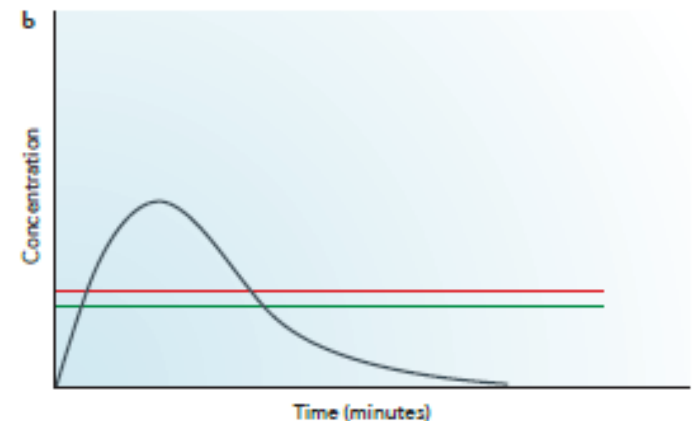
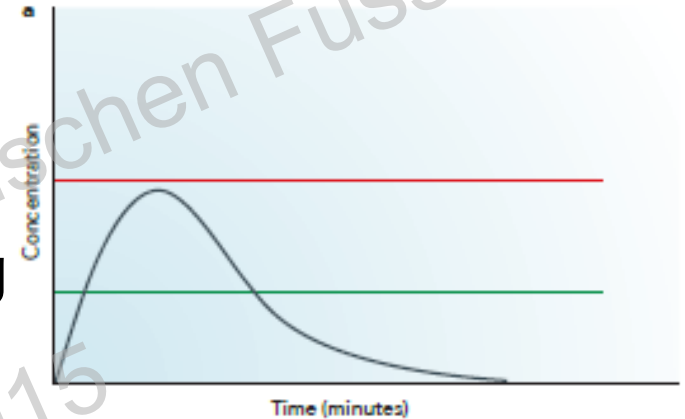
Interference mit Quorum Sensing

Knoblauch

Ginseng

AB

- Makrolide
- Ceftazidime
- Ciprofloxacin



Gewebepenetration und Biofilm-Aktivität

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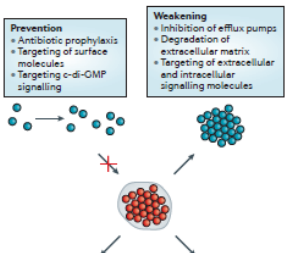
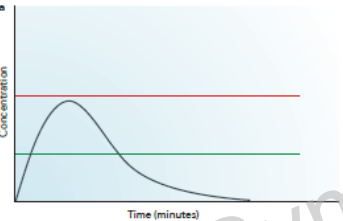
"I go south in the winter. I have a condo in Hades."

- **Lokalisation - Bakterien sind geschützt**
(Wirtabwehr + AB)

- Knochen (po)
- intrazellulär – Persister
- Biofilm

- **Therapie: Prävention**

- Gewebepenetration
- Aktivität im Biofilm (mechan.- Kommunikation)
- Intrazellulär aktiv
- Persister -stationäre, nicht teilende Bakterien
 - Zellmembran: Colistin, Daptomycin
 - Modulation: Makrolide, Cipro, Ceftazidim
 - Penetration: Rifampicin



Diabetischer Fuss - Infektionen



- Hausarzt
- Wundexperten
- Orthopäde
- Endokrinologe
- Angiologe
- Mikrobiologe
- Infektiologe

Table 1. Partial list of human infections involving biofilms.

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>

Gewebepenetration - Diabetes



- Durchblutungsproblematik
- Hautdefekte

Symposium zum Diabetischen Fuss
27. Nov. 2015

Infektanfälligkeit - Diabetes



- Durchblutungsproblematik
- Hautdefekte
- Infektanfälligkeit:
 - Makrophagendysfunktion
 - Optimaler Nährboden
 - Bakterien -> Antibiotika

DIABETES

KNOW THE SYMPTOMS

