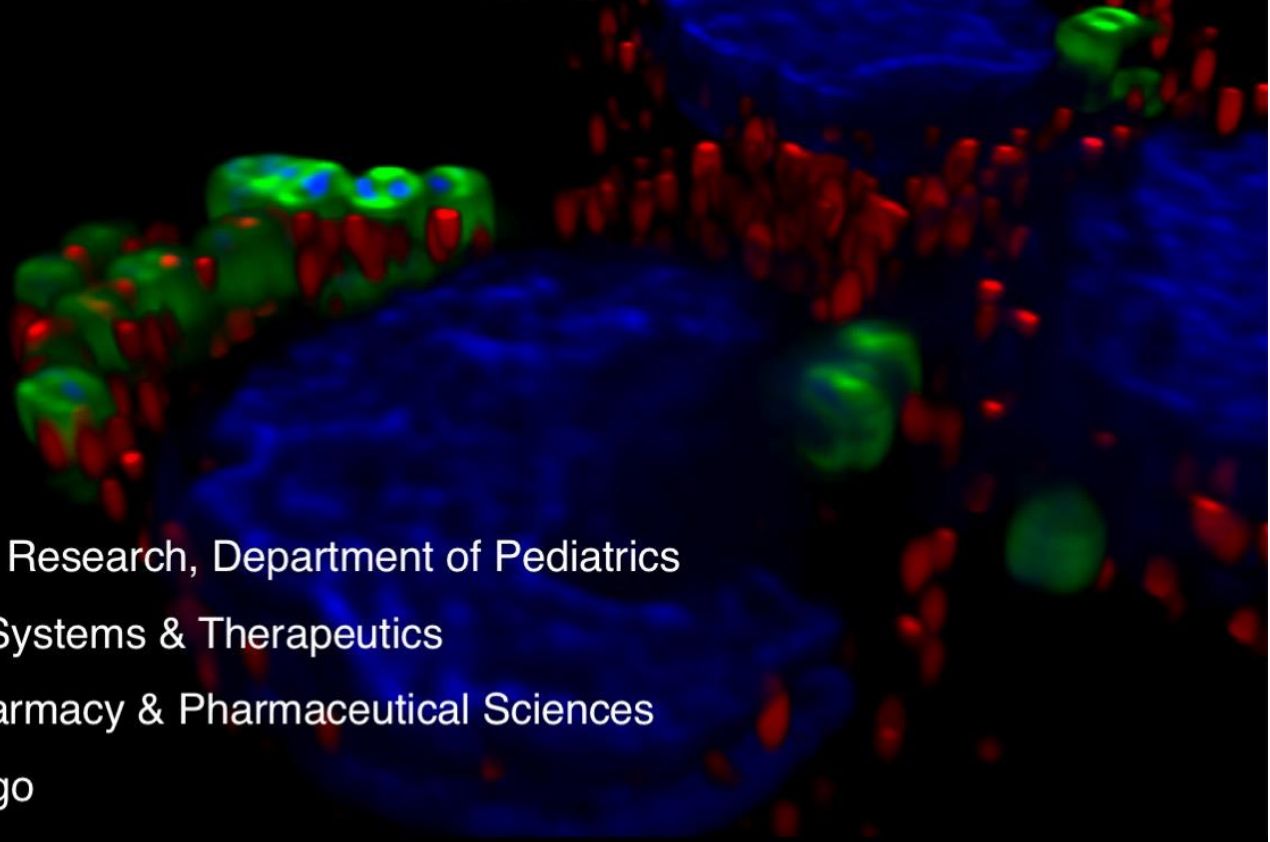
Is AST in standard bacteriologic media fully sufficient to guide management of certain highly MDR organisms or critically ill patients?



Victor Nizet, MD

Professor & Vice Chair for Basic Research, Department of Pediatrics

Chief, Division of Host-Microbe Systems & Therapeutics

Professor, Skaggs School of Pharmacy & Pharmaceutical Sciences

University of California, San Diego

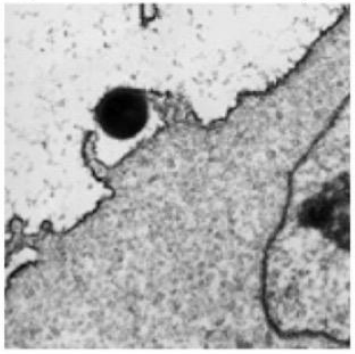
CLSI Antimicrobial Susceptibility Testing Meeting | San Diego, CA | June 4, 2018

Nizet Lab

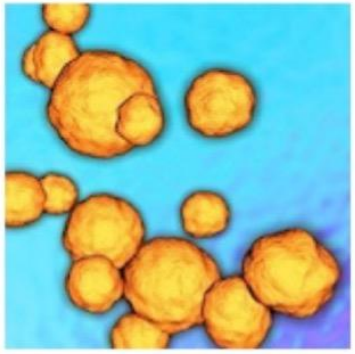
Research focus on common, invasive bacterial pathogens of humans …

S. pyogenes (Group A Strep)

S. agalactiae (Group B Strep)



S. aureus (Golden Staph)

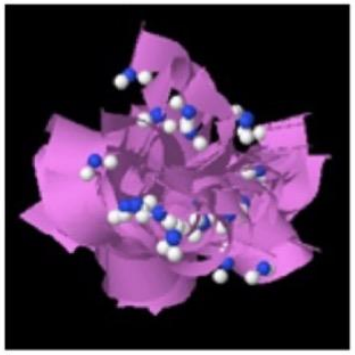


S. pneumoniae (Pneumococcus)

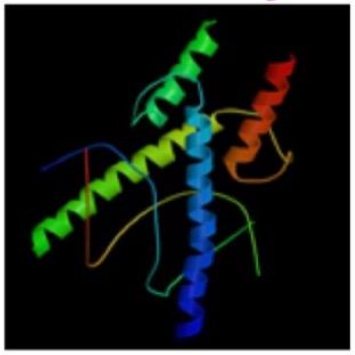
MDR Gram- (e.g. *Pseudomonas*)

… and their interaction with host innate immunity

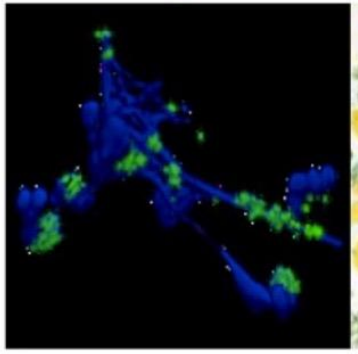
Cathelicidin AMPs



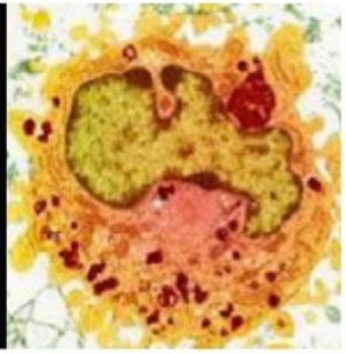
HIF and Immunity



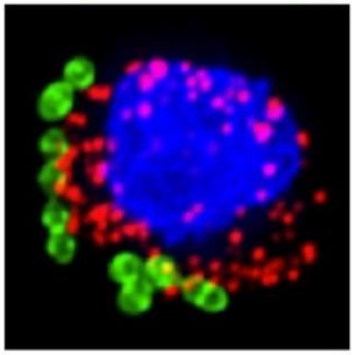
Neutrophil Traps



Macrophage Signaling



Host-Pathogen Glycobiology



Seeking alternatives to classical antibiotics, especially very broad-spectrum agents, that kill bacteria or block their growth

* **Drugs to block specific pathogen immune resistance factors**
  - Sensitize pathogens to clearance by normal host innate defenses
  - More targeted therapy, avoid "collateral damage" to microbiome

* **Modulation of innate immunity to treat bacterial infections**
  - Can we pharmacologically boost phagocyte function?

* **Explore "repurposing" existing drugs for the above properties**

* **Synergy between pharmaceutical and endogenous antibiotics**

Quiz: Can you name these Harvard researchers?

John Howard Mueller, PhD
(1891-1954)

Jane Hinton, DVM
DVM (1919-2003)

Proc Soc Exp Biol Med, 48: 330-333 (1941)

A Protein-Free Medium for Primary Isolation of the Gonococcus and Meningococcus.

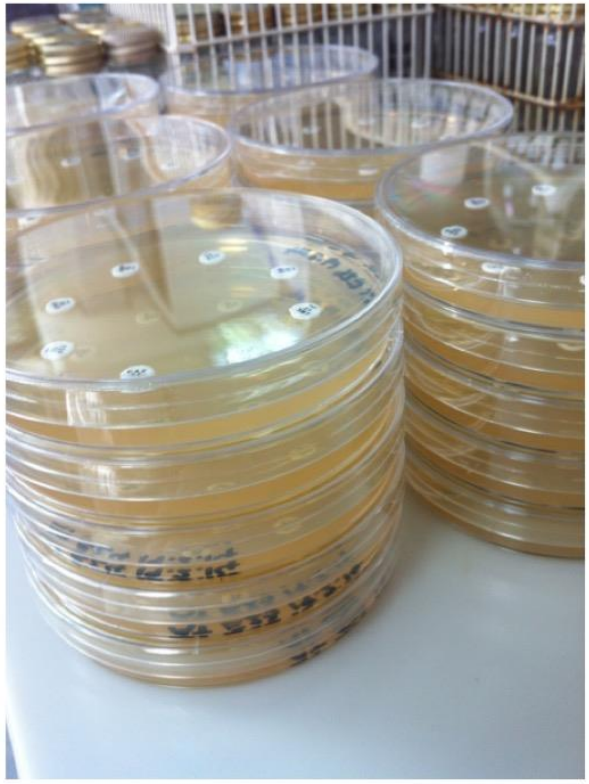
J. Howard Mueller and Jane Hinton.

From the Department of Bacteriology and Immunology, Harvard Medical School, and School of Public Health, and the Boston Dispensary, Boston, Mass.*

30.0%	Beef infusion
1.75%	Casein hydrosylate
0.15%	Starch
1.70%	Agar

pH to neutral at 25°C

Later – cation-adjusted
(for *Pseudomonas*)
Calcium 20-25 mg/L
Magnesium 10-12.5 mg/L

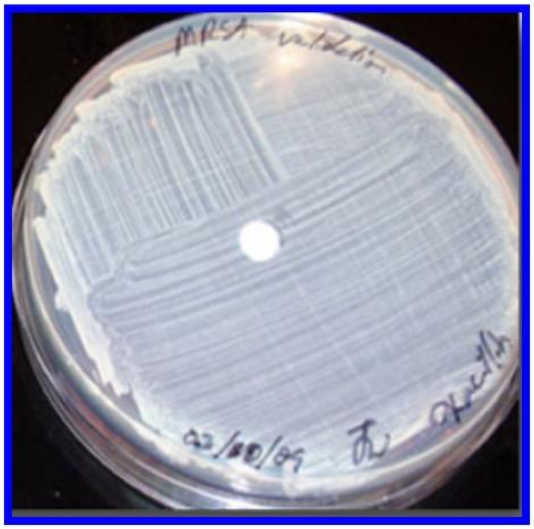


MIC/MBC TESTING IN BACTERIOLOGIC MEDIA (i.e. CA-MHB), *STRONGLY DELIMITS PHARMACOTHERAPY OF HUMAN BACTERIAL INFECTIONS*

ANTIBIOTIC DISCOVERY AND DEVELOPMENT

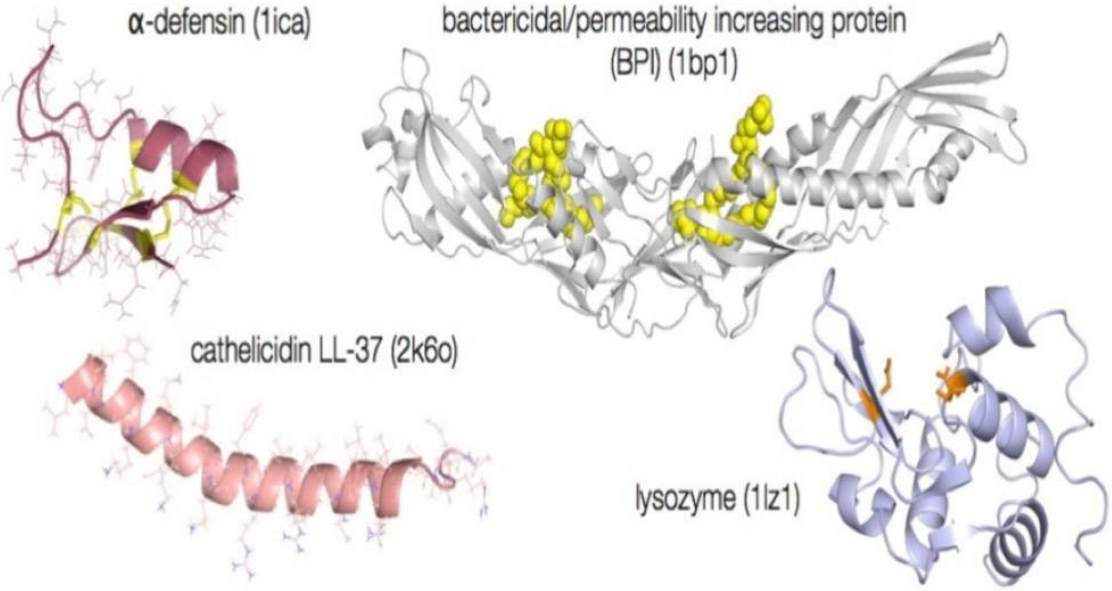
WHICH DRUGS CHOSEN FOR HOSPITAL FORMULARY

WHICH INFORMATION IS PROVIDED TO DOCTORS WHEN THE PATHOGEN IS CULTURED FROM THE PATIENT

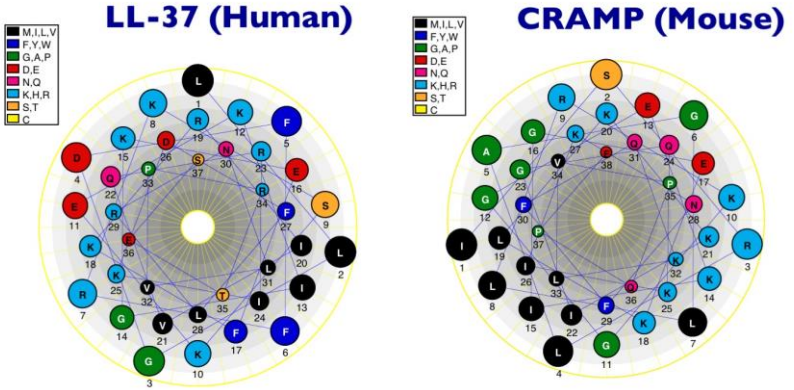


Before a patient has even seen the doctor ...

... their infection is already being treated by dozens of antibiotics



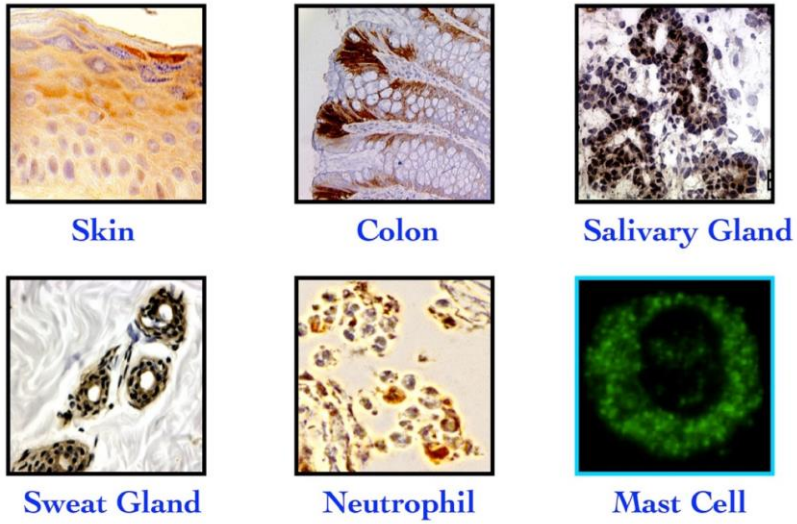
Cathelicidin Peptides: Natural Antibiotics of Innate Immunity



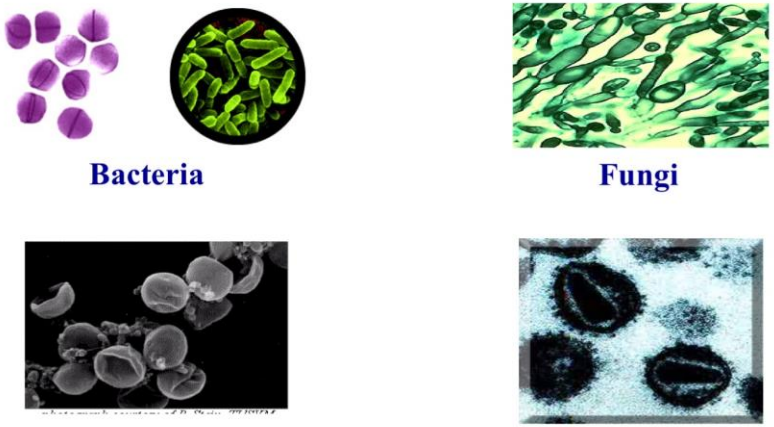
Similar:

- encoding genes
- alpha-helical structure
- tissue distribution
- spectrum of activity

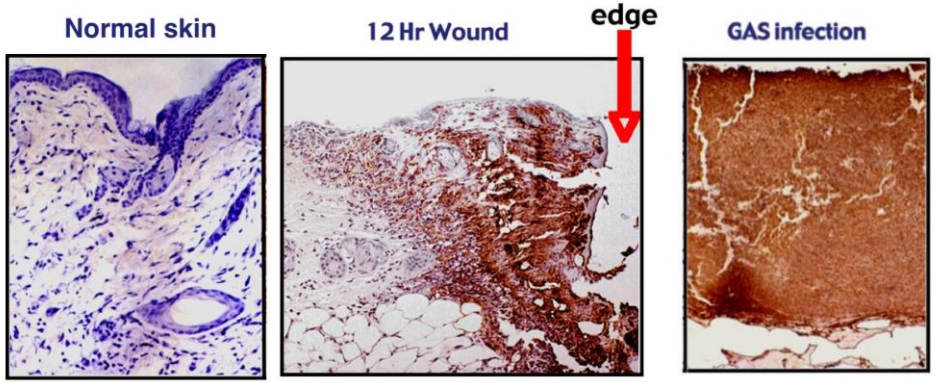
Produced on Epithelial Surfaces & By Granulocytes



Broad-Spectrum Antimicrobial Activity



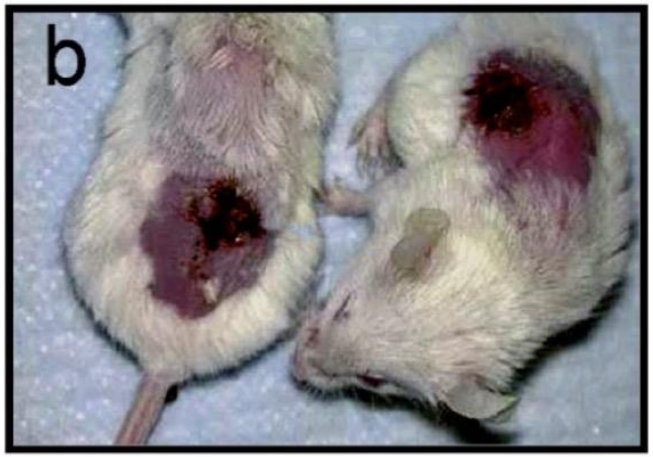
Immunostain for Cathelicidin



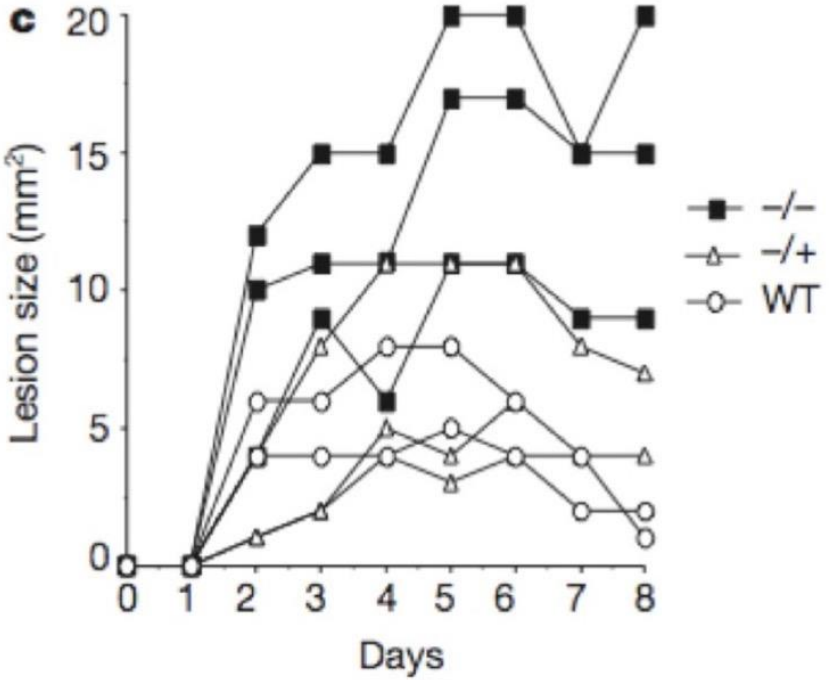
Dorschner et al. (2001) J Invest Derm

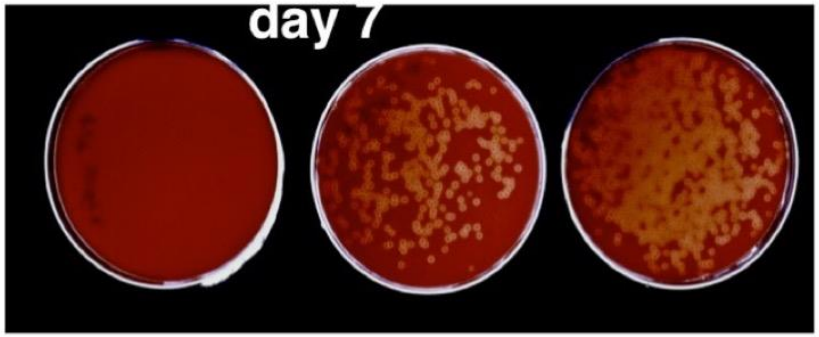
CRAMP-KO Mouse Has Immune Defect

Wild-type Mice



Knockout Mice





+/+ +/- -/-

with R. Gallo Lab

Nizet *et al.* Nature, 2001

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

80,461
SEVERE MRSA INFECTIONS PER YEAR

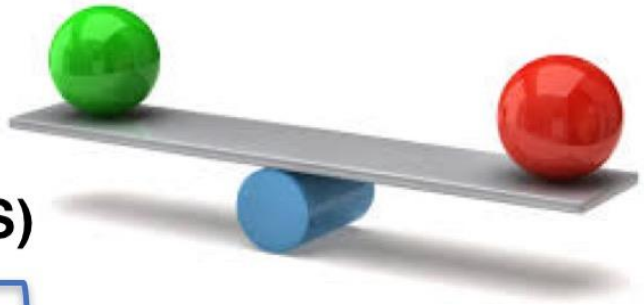
11,285
DEATHS FROM MRSA PER YEAR

THREAT LEVEL
SERIOUS

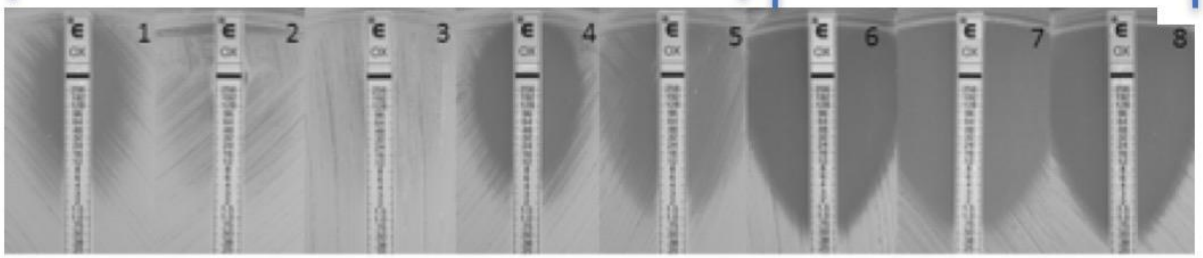
This bacteria is a serious concern and requires prompt and sustained action to ensure the problem does not grow.

STAPH BACTERIA ARE A LEADING CAUSE OF
HEALTHCARE-ASSOCIATED INFECTIONS

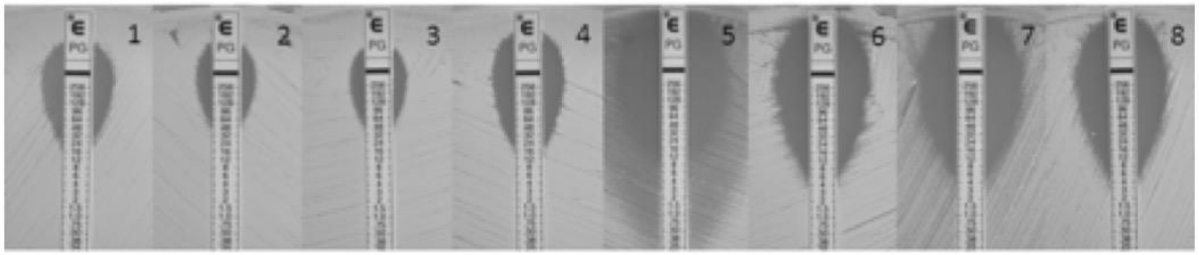
"Seesaw Effect" between Daptomycin Nonsusceptibility and β-Lactam Susceptibility in *Staph. haemolyticus*



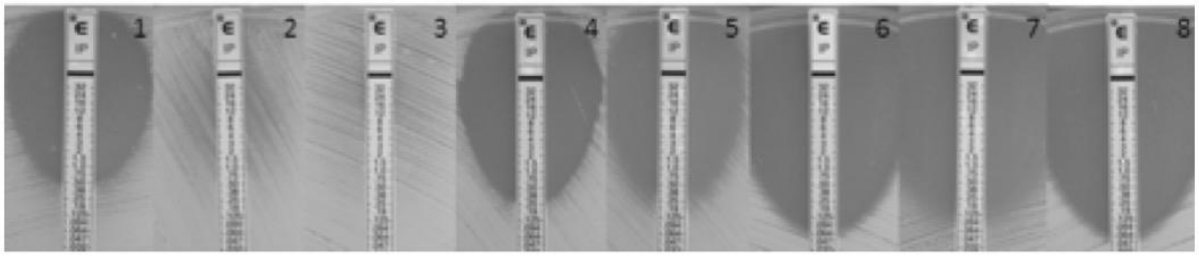
Daptomycin (S) | Daptomycin (NS)



Oxacillin



Penicillin

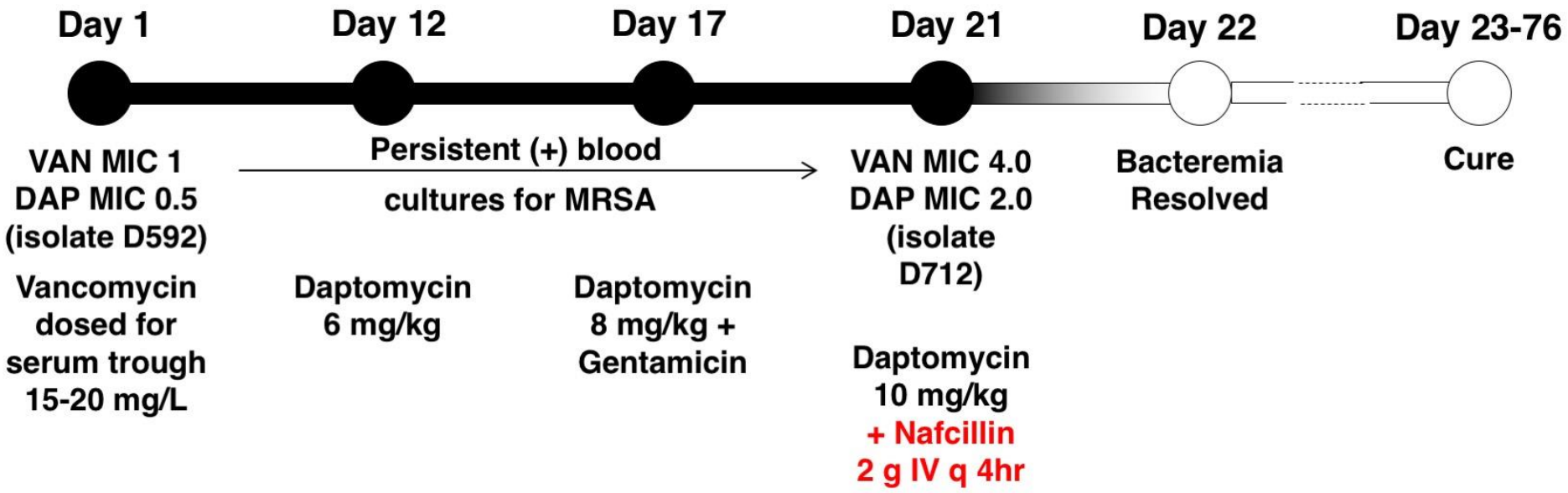


Imipenam

Continuation of patient therapy w/ Daptomycin

Vignaroli et al. Antimicrob Agents Chemother 2011

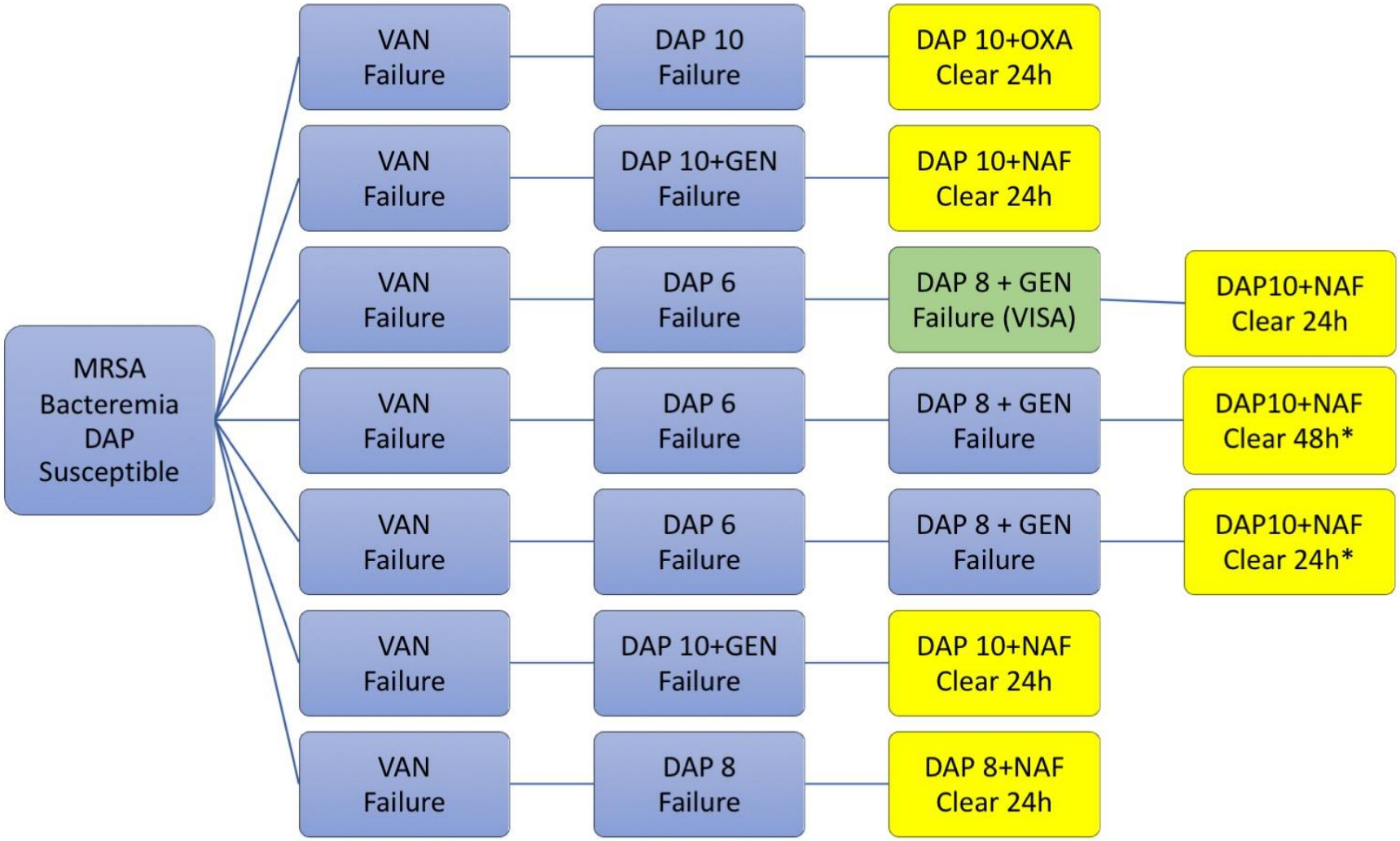
Reintroduction of β-Lactam Antibiotics in Refractory M.R.S.A. Bacteremia – With Surprising Results



George Sakoulas, MD

Sakoulas et al. J Mol Med 2014

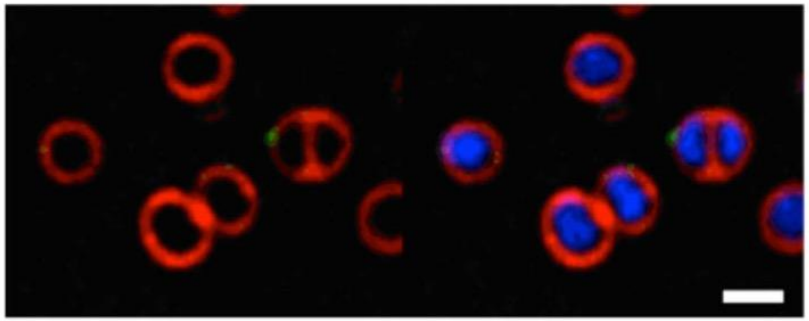
Rapid MRSA Bacteremia Clearance with High-Dose Daptomycin plus a β-lactam

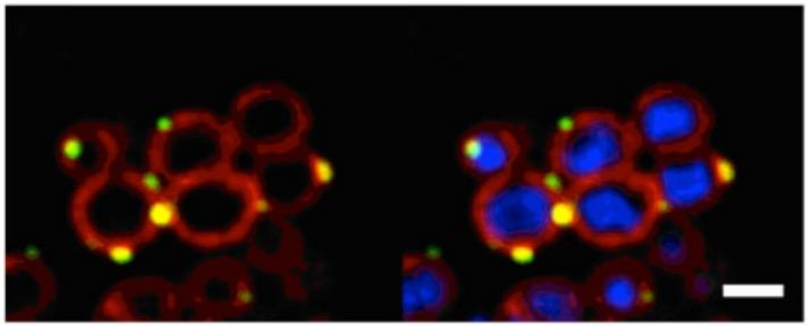


Dhand et al. Clin Infect Dis 2011

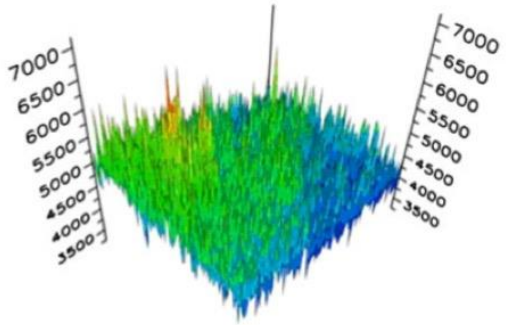
Exposure to Sub-MIC Nafcillin Increases Daptomycin Binding to *S. aureus* Cell Wall

Daptomycin-resistant VISA Clinical Isolate

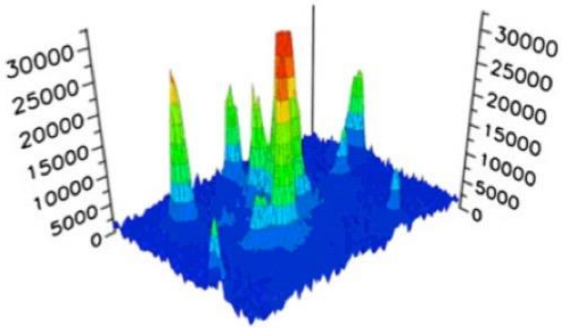




Bodipy-Dapto (Green/yellow)

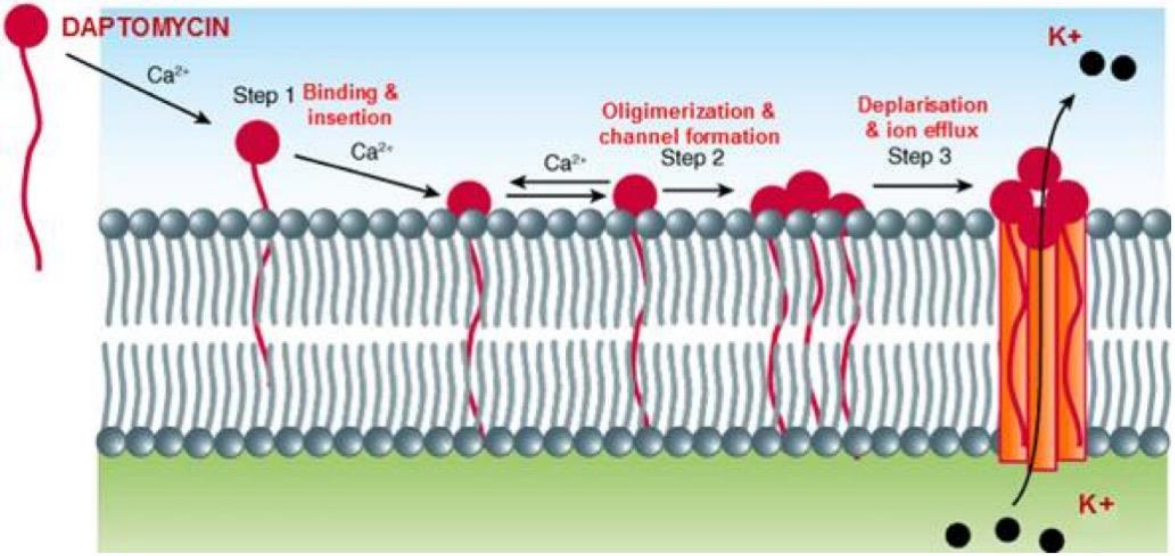


No nafcillin



Add nafcillin (40 μg/μl)

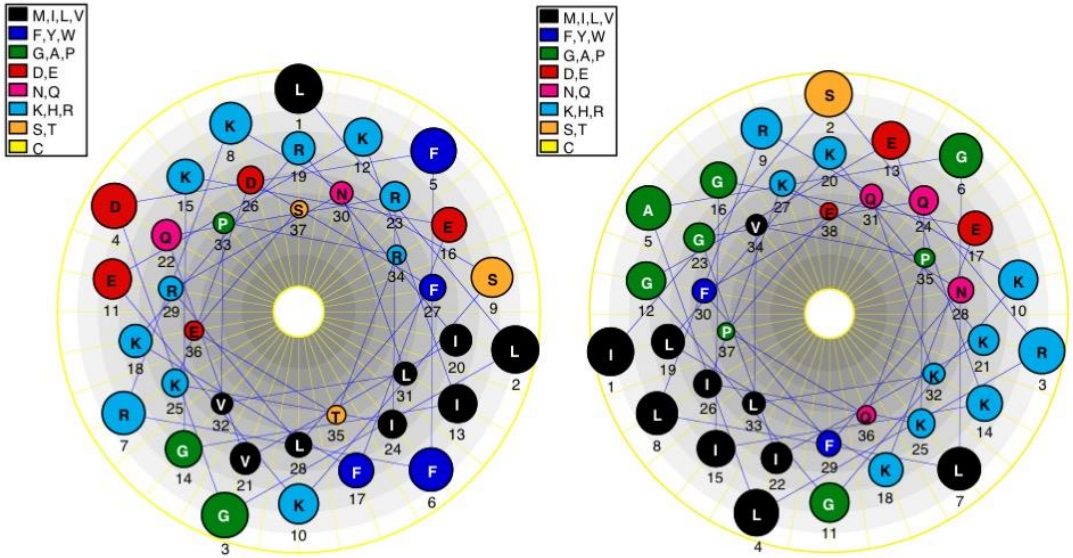
Dhand et al. Clin Infect Dis 2011



Daptomycin binds Ca^{2+} *in vivo* as an integral part of its mechanism of action – i.e. it becomes a *de facto* cationic peptide

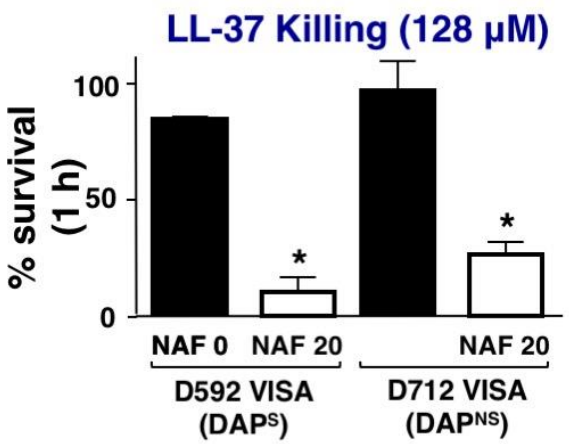
Cationic antimicrobial peptides such as cathelicidin are a critical component of mammalian innate immunity ……

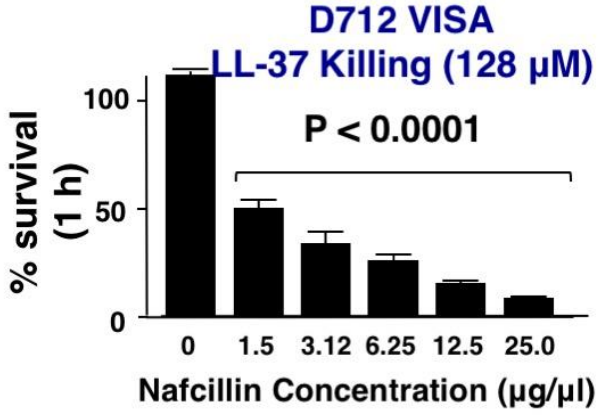
CATHELICIDIN

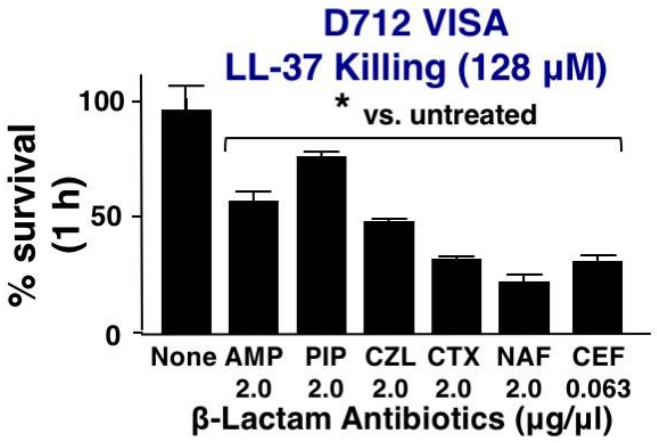


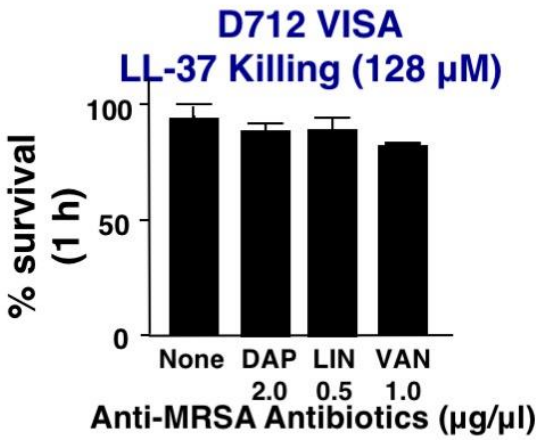
LL-37 (Human) **CRAMP (Mouse)**

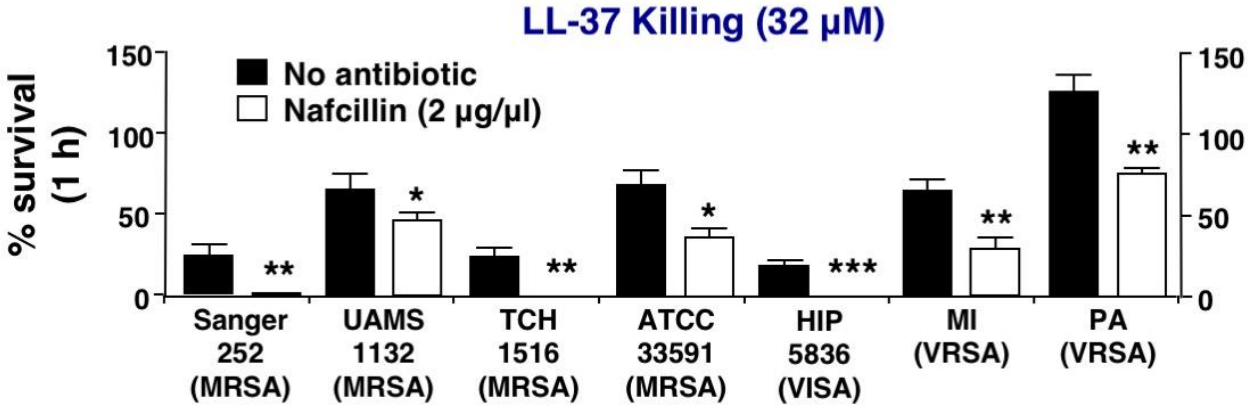
Sublethal Nafcillin Dramatically Sensitizes MRSA/VISA Strains to Human Cathelicidin AMP LL-37 Killing







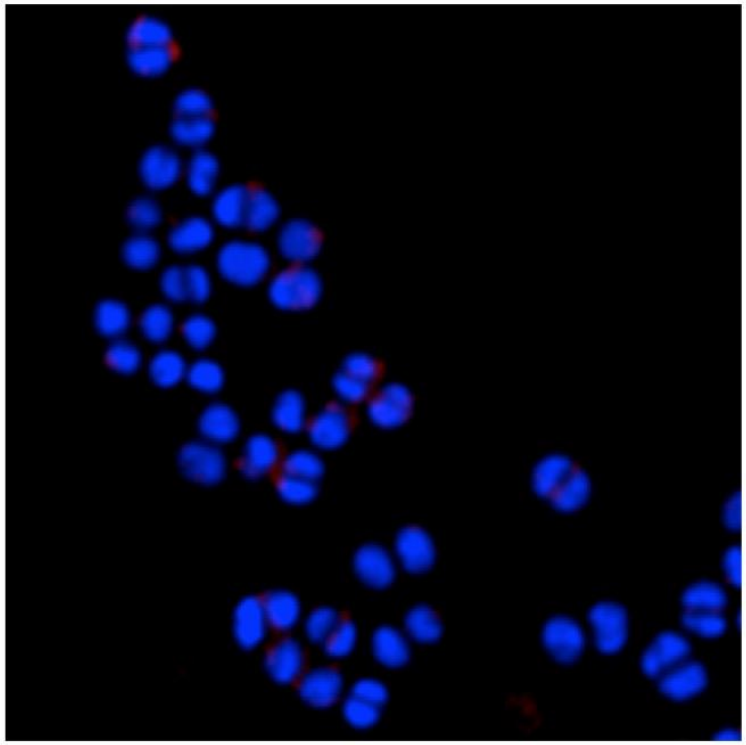




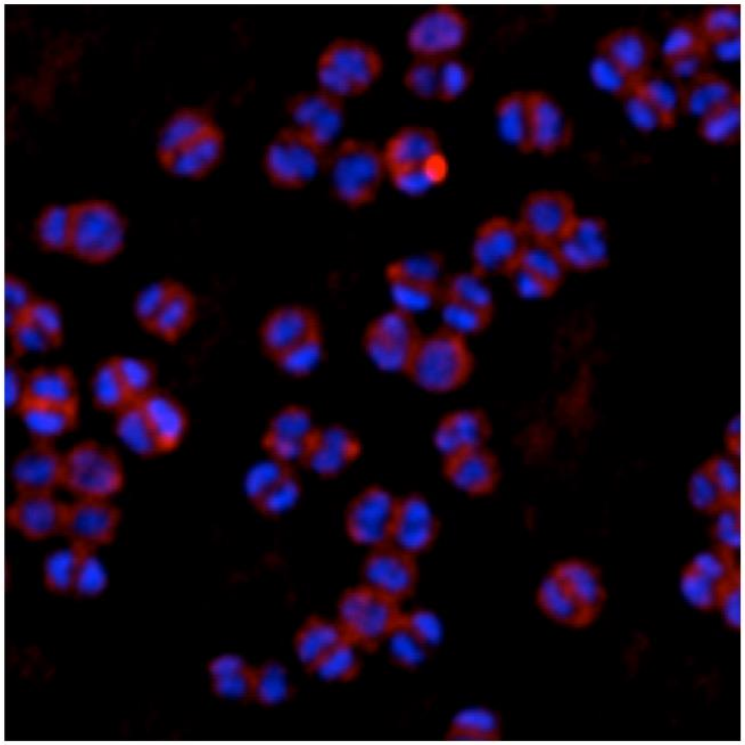
Similar results with human alpha-defensin, platelet-derived AMP, and murine cathelicidin

Sakoulas et al. J Mol Med 2014

Nafcillin Increases Binding to MRSA by Rhodamine-Labeled Cathelicidin LL-37



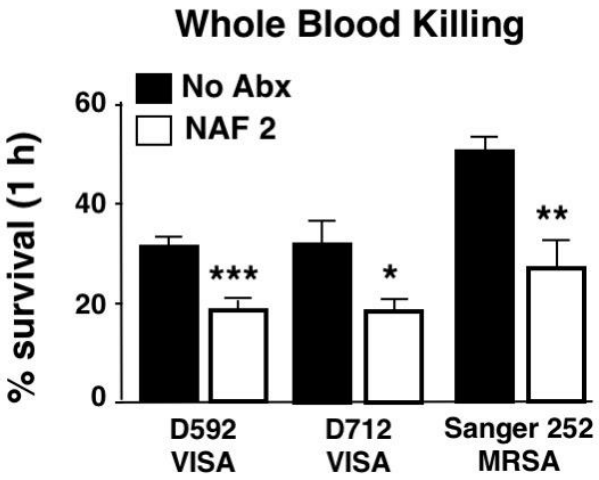
MRSA + LL-37

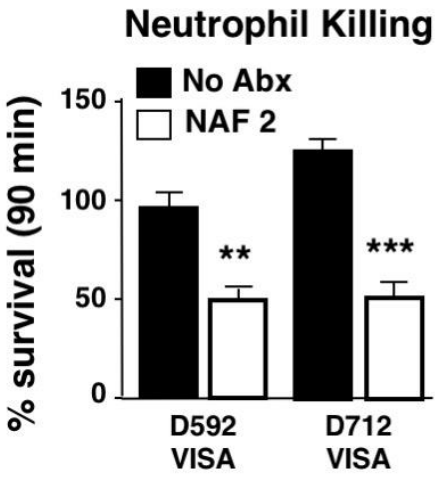


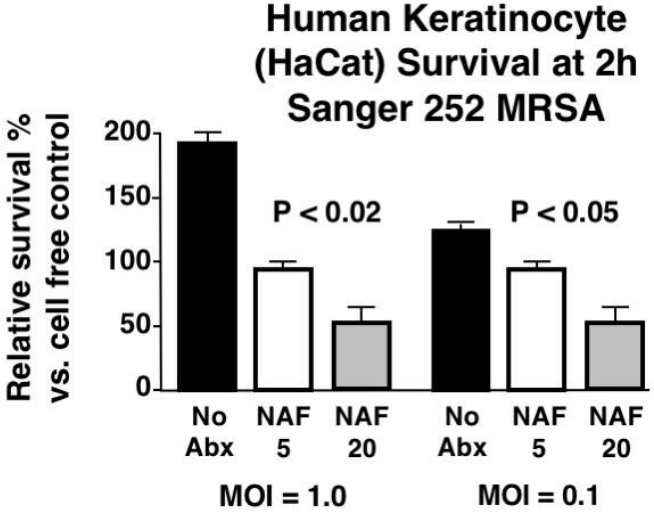
MRSA + LL-37
+ Naf 10

Sakoulas et al. J Mol Med 2014

Sublethal Nafcillin Sensitizes MRSA/VISA Strains to Whole Blood, Neutrophil & Keratinocyte Killing



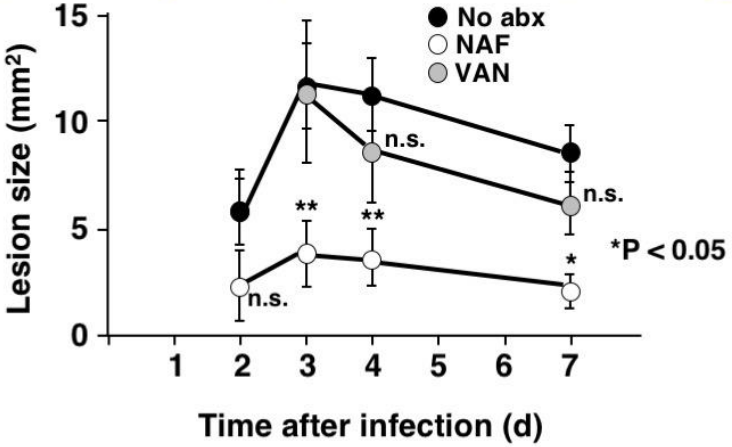




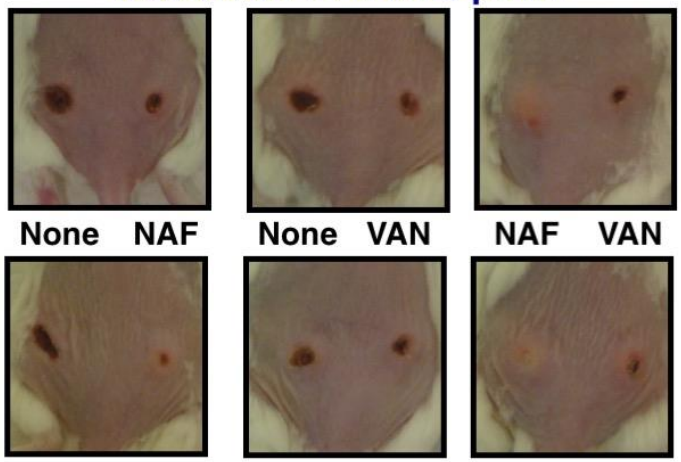
Sakoulas et al. J Mol Med 2014

Sublethal Nafcillin (Monotherapy) Influences MRSA Lesion Development in Mouse Skin Infection Models

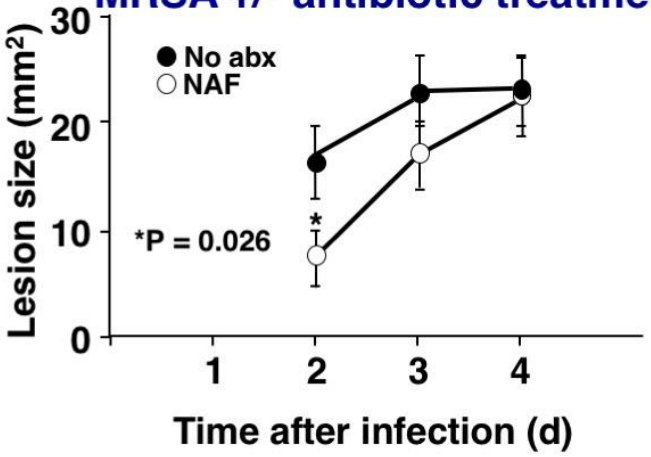
Antibiotic pretreatment of Sanger 252 MRSA followed by mouse subcutaneous challenge



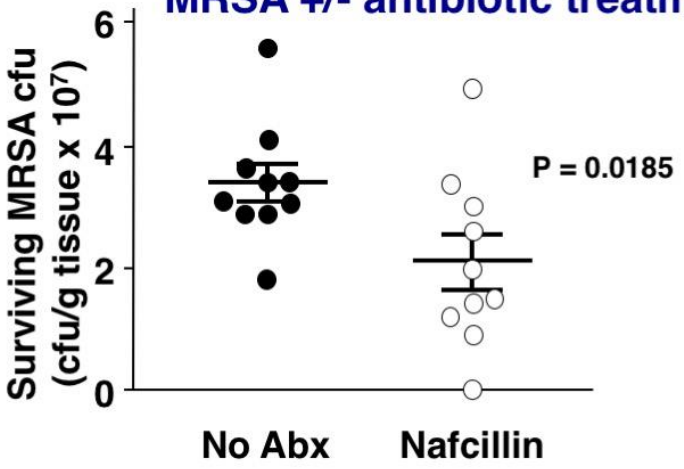
Representative gross appearance of skin lesions at 48 h time point



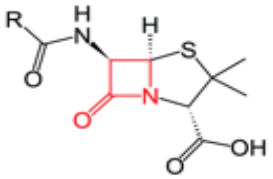
Mouse s.c. challenge with Sanger 252 MRSA +/- antibiotic treatment



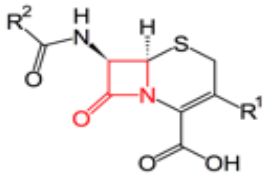
Mouse s.c. challenge with Sanger 252 MRSA +/- antibiotic treatment

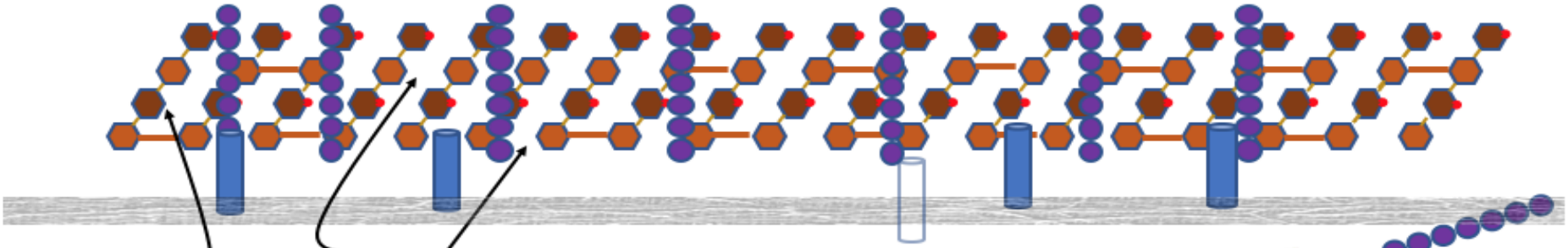


Sakoulas et al. J Mol Med 2014



Potential beneficial effects of β-Lactam antibiotics not reflected in MIC





reduced peptidoglycan *O*-acetylation

reduced peptidoglycan crosslinking

decreased shedding of membrane phospholipids

decreased expression of MSCRAMMs (e.g. ClfA, FnBPA)

increased α-toxin release

increased lipoteichoic acid shedding

increased bacterial susceptibility to lysozyme killing

increased cell membrane access

reduced invasion of epithelial and endothelial cells

activation of the NLRP3 inflammasome

increased host macrophage cytokine release

potentiation of cationic host defense peptides

potentiation of peptide antibiotics (e.g. daptomycin)

increased IL-1β response

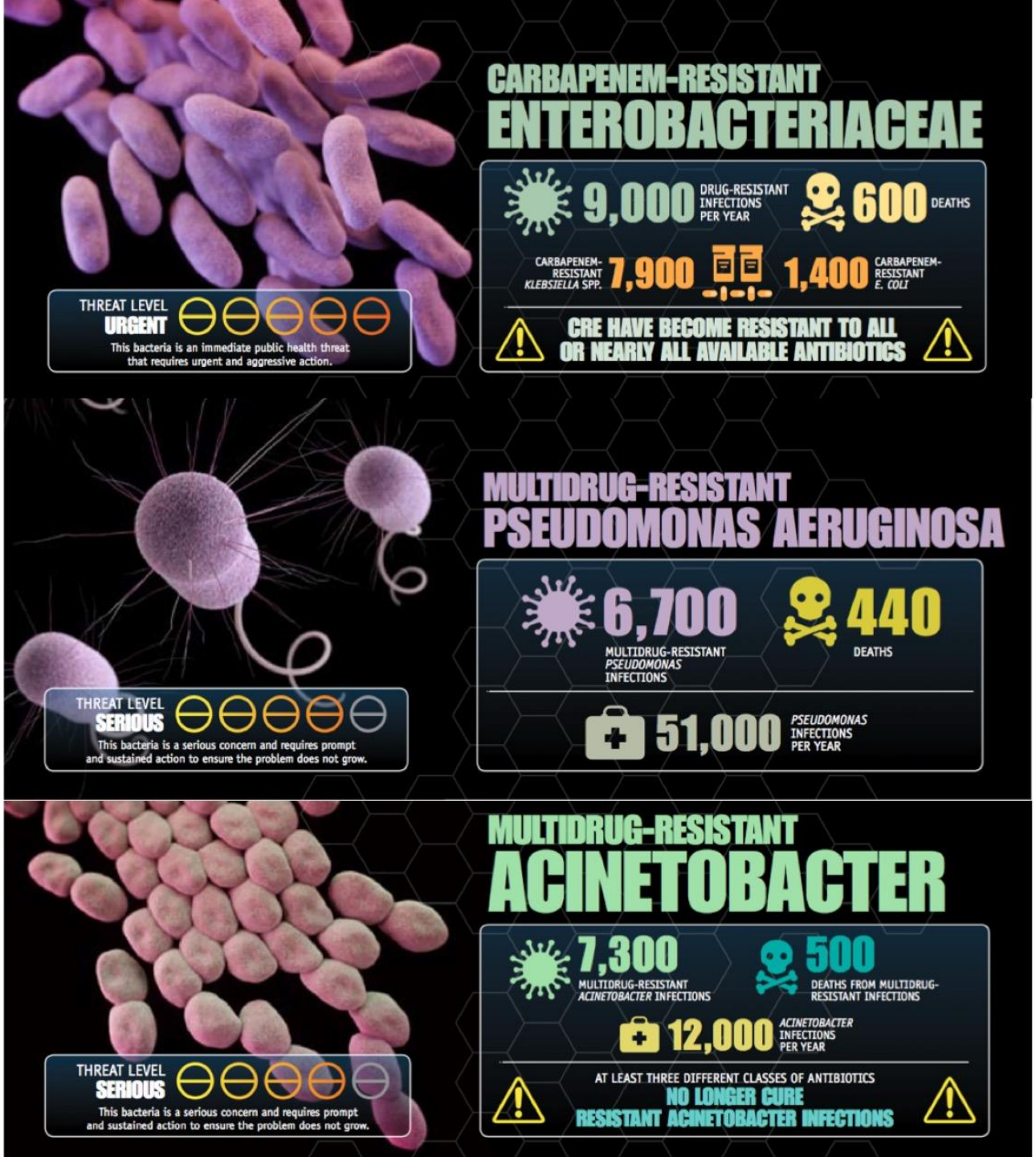
increased TH17 response

improved neutrophil recruitment

reduced neutrophil apoptosis

Enhanced clearance of *S. aureus* bacteremia

Augmented neutrophil antibacterial functions

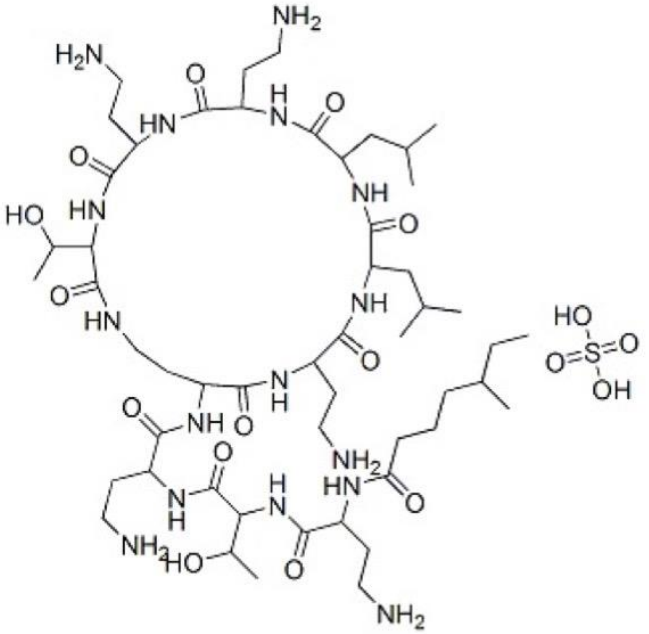

CARBAPENEM-RESISTANT
ENTEROBACTERIACEAE
9,000 DRUG-RESISTANT INFECTIONS PER YEAR
600 DEATHS
CARBAPENEM-RESISTANT *KLEBSIELLA* SPP. 7,900
1,400 CARBAPENEM-RESISTANT *E. COLI*
CRE HAVE BECOME RESISTANT TO ALL OR NEARLY ALL AVAILABLE ANTIBIOTICS
THREAT LEVEL
URGENT
This bacteria is an immediate public health threat that requires urgent and aggressive action.
MULTIDRUG-RESISTANT
PSEUDOMONAS AERUGINOSA
6,700 MULTIDRUG-RESISTANT *PSEUDOMONAS* INFECTIONS
440 DEATHS
51,000 *PSEUDOMONAS* INFECTIONS PER YEAR
THREAT LEVEL
SERIOUS
This bacteria is a serious concern and requires prompt and sustained action to ensure the problem does not grow.
MULTIDRUG-RESISTANT
ACINETOBACTER
7,300 MULTIDRUG-RESISTANT *ACINETOBACTER* INFECTIONS
500 DEATHS FROM MULTIDRUG-RESISTANT INFECTIONS
12,000 *ACINETOBACTER* INFECTIONS PER YEAR
AT LEAST THREE DIFFERENT CLASSES OF ANTIBIOTICS
NO LONGER CURE
RESISTANT ACINETOBACTER INFECTIONS
THREAT LEVEL
SERIOUS
This bacteria is a serious concern and requires prompt and sustained action to ensure the problem does not grow.

ANTIBIOTIC	*Pseudomonas aeruginosa*, P4 (MDR)		*Klebsiella pneumoniae*, K1100 (MDR, KPC)		*Acinetobacter baumannii*, AB5075 (MDR)	
	MIC	Interpretation	MIC	Interpretation	MIC	Interpretation
Ampicillin	≥ 32	R	≥ 32	R	≥ 32	R
Amoxicillin/Clavulanate	≥ 32	R	≥ 32	R	≥ 32	R
Ampicillin/Sulbactam	≥ 32	R	≥ 32	R	≥ 32	R
Ticarcillin			≥ 128	R	≥ 128	R
Ticarcillin/Clavulanate	≥ 128	R				
Pipericillin	≥ 128	R	≥ 128	R	≥ 128	R
Pipericillin/Tazobactam	≥ 128	R	≥ 128	R	≥ 128	R
Cefalotin	> 64	R	> 64	R	> 64	R
Cefazolin	≥ 64	R	≥ 64	R	≥ 64	R
Cefuroxime	> 64	R	> 64	R	> 64	R
Cefuroxime Axetil	≥ 64	R	≥ 64	R	≥ 64	R
Cefotetan	> 64	R	8	*R	> 64	R
Cefoxitin	≥ 64	R	32	R	≥ 64	R
Cefpodoxime	≥ 8	R	≥ 8	R	≥ 8	R
Cefotaxime	≥ 64	R	8	R	≥ 64	R
Ceftazidime	≥ 64	R	≥ 64	R	≥ 64	R
Ceftizoxime	≥ 64	R	4	*R	≥ 64	R
Ceftriaxone	≥ 64	R	≥ 64	R	≥ 64	R
Cefepime	≥ 64	R	4	*R	≥ 64	R
Aztreonam	≥ 64	R	≥ 64	R	≥ 64	R
Doripenem	≥ 8	R	≥ 8	R	≥ 8	
Ertapenem			≥ 8	R		
Imipenem	≥ 16	R	8	R	≥ 16	R
Meropenem	≥ 16	R	≥ 16	R	≥ 16	R
Amikacin	32	I	> 64	R	> 64	R
Gentamicin	8	I	≥ 16	R	≥ 16	R
Tobramycin	≤ 1	S	≥ 16	R	8	I
Nalidixic Acid	≥ 32	R	≥ 32	R	≥ 32	R
Ciprofloxacin	≥ 4	R	≥ 4	R	≥ 4	R
Levofloxacin	≥ 8	R	≥ 8	R	4	I
Moxifloxacin	≥ 8	R	≥ 8	R	≥ 8	R
Norfloxacin	8	I	≥ 16	R	≥ 16	R
Tetracycline	≥ 16	R	4	S	≤ 1	S
Tigecycline	≥ 8	R	4	I	≤ 0.5	S
Nitrofurantoin	≥ 512	R	128	R	≥ 512	R
TMP/SFX	≥ 320	R	40	S	≥ 320	R

Colistin (Polymyxin E2) from *Paenibacillus polymyxa*
"Drug of Last Resort" for MDR Gram- Pathogens

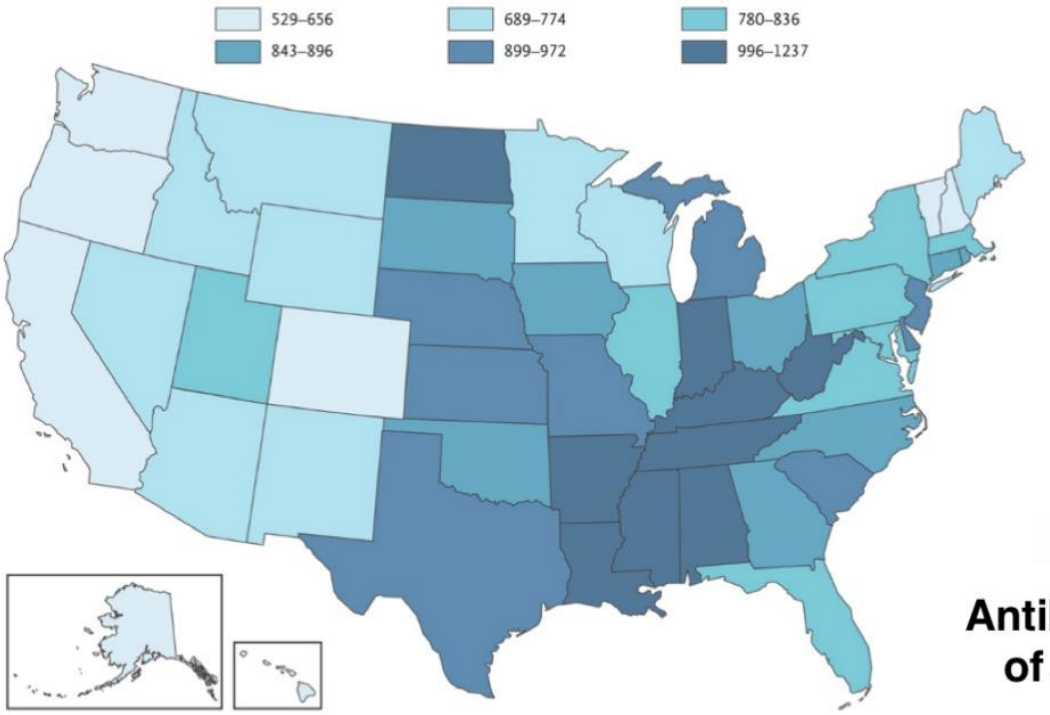
Pentacationic polypeptide consisting of a cyclic heptapeptide, a linear tripeptide and a fatty acid tail linked to the N-terminal of the tripeptide.

The five L-diaminobutyric acid (L-Dab) molecules are positively charged.



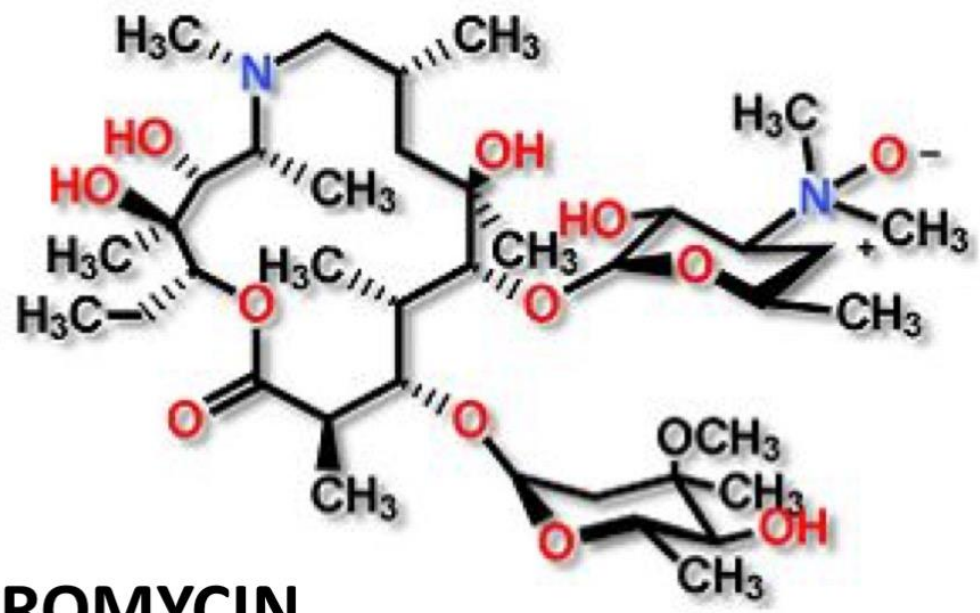
L-Thr ← (α)L-Dab ← (α)L-Dab ← L-Leu

Fatty acid → (α)L-Dab → L-Thr → (α)L-Dab → (α,γ)L-Dab → (α)L-Dab → D-Leu

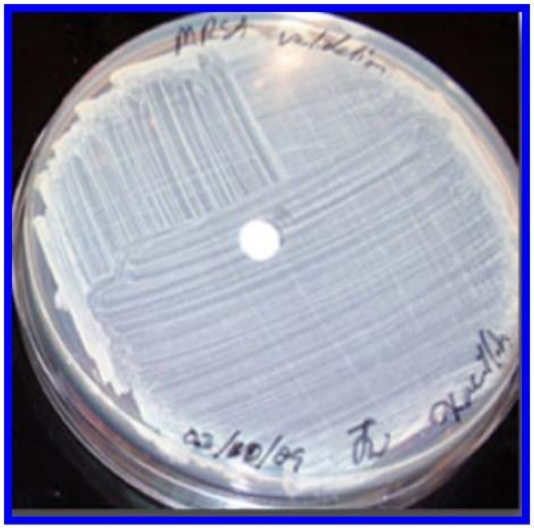


Antibiotic Prescriptions per 1000 Persons of All Ages , According to State (2010)
Hicks et al. New Engl J Med 2013

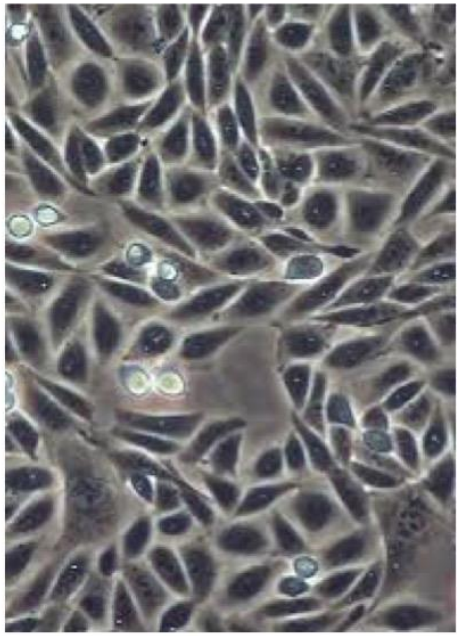
What is the most commonly prescribed antibiotic in the United States? (> 60 million/year)



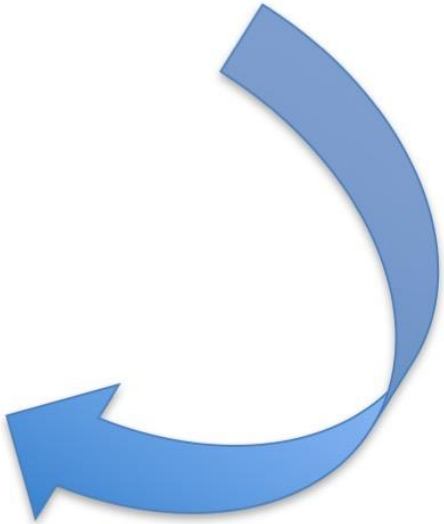
AZITHROMYCIN




dreamstime.com




RPMI-1640
0.2 um Filtered
Contains: L-Glutamine
Catalog #: 30041
Lot #: E067-6



Dramatic Differences in Azithromycin Activity vs. Multidrug-Resistant Gram-Negative Rods in Tissue Culture Media vs. Bacteriologic Media

Bacterial Strain	Azithromycin MIC in Ca-MHB (ug/ml)	Azithromycin MIC in 5% LB-RPMI (ug/ml)
MDR Pseudomonas aeruginosa – P4	>64	4
Pseudomonas aeruginosa – PA01	>64	2
Carbapenemase-Producing Klebsiella pneumoniae (KPC) – K1100	32	1
Klebsiella pneumoniae – K700603	64	2
MDR Acinetobacter baumanii – AB5075	32	0.5
Acinetobacter baumanii – AB19606	64	0.25

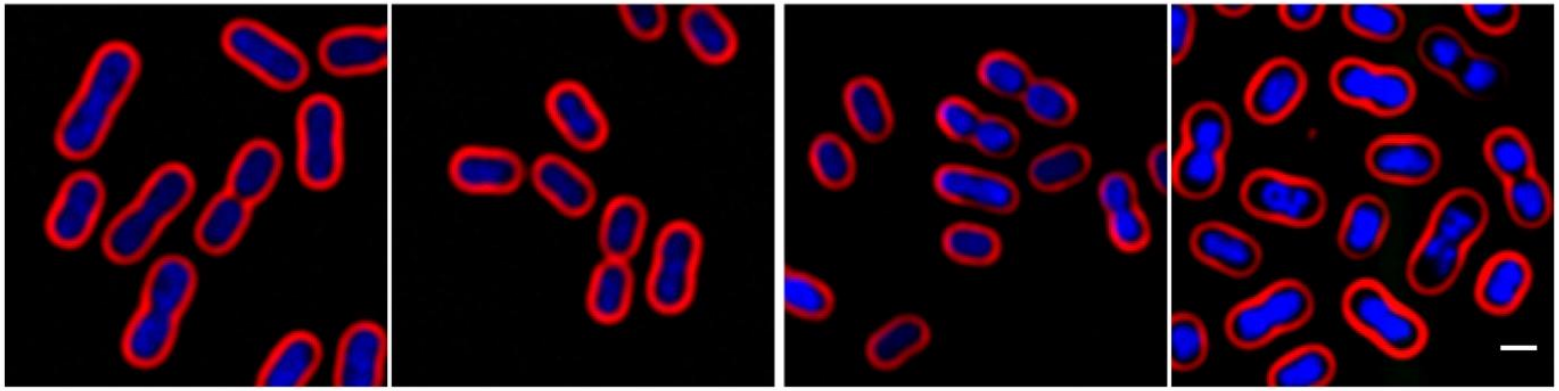
Leo Lin
(UCSD MSTP)

Lin et al. eBiomedicine 2015

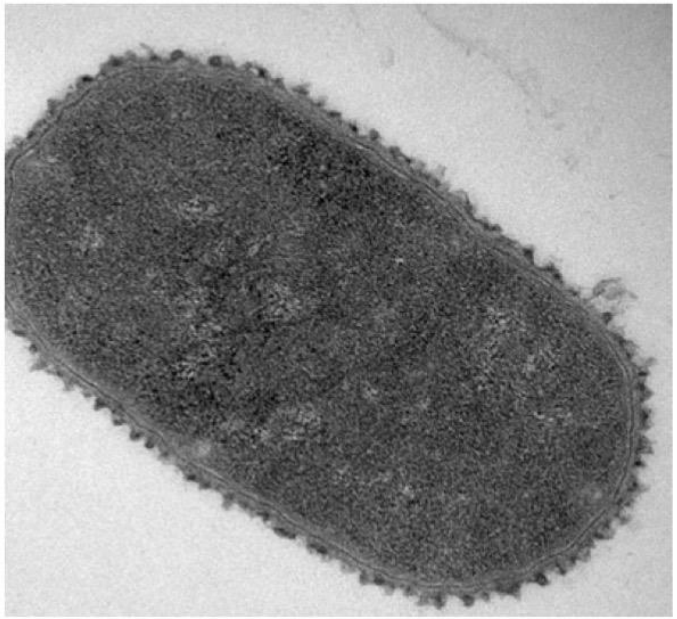
MDR *A. baumannii*

Ca-MHB

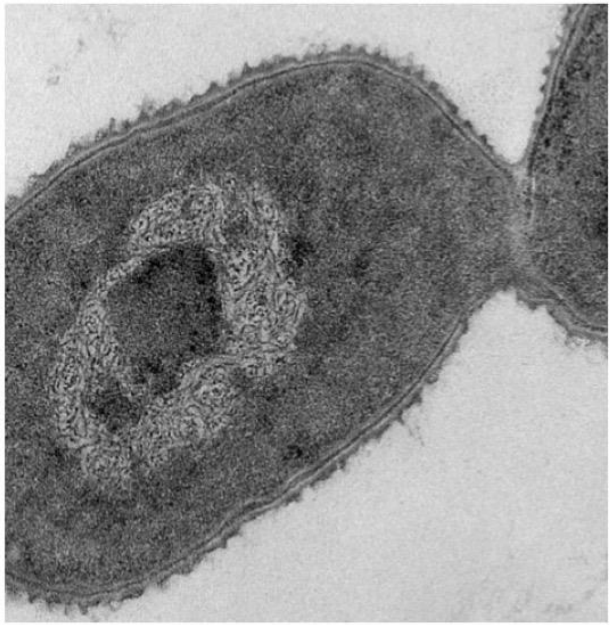
RPMI (5% LB)



No abx AZM 2 No abx AZM 2



RPMI (5% LB), no Abx



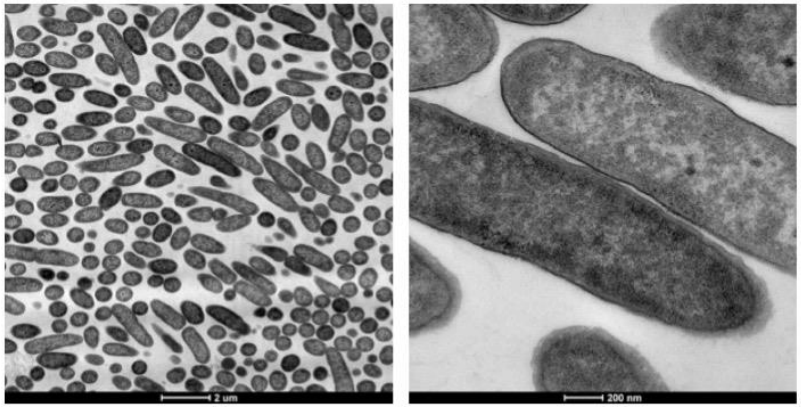
RPMI (5% LB) + AZM 2

Lin et al. submitted

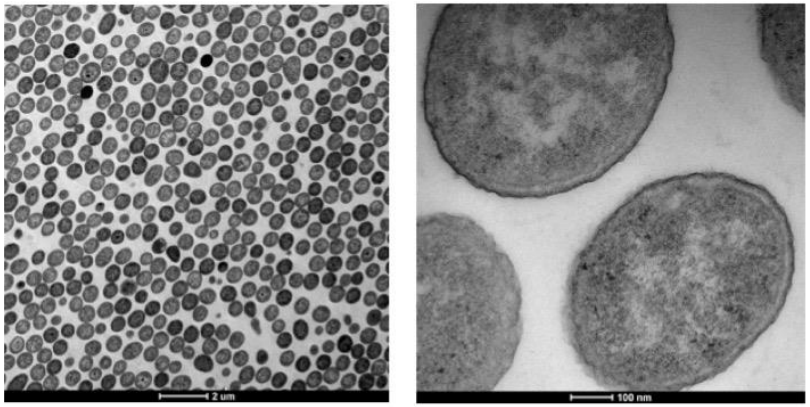
Subinhibitory Azithromycin Induces Marked Ultrastructural Changes in Pseudomonas

MDR *P. aeruginosa* in RPMI (5% LB)

Control

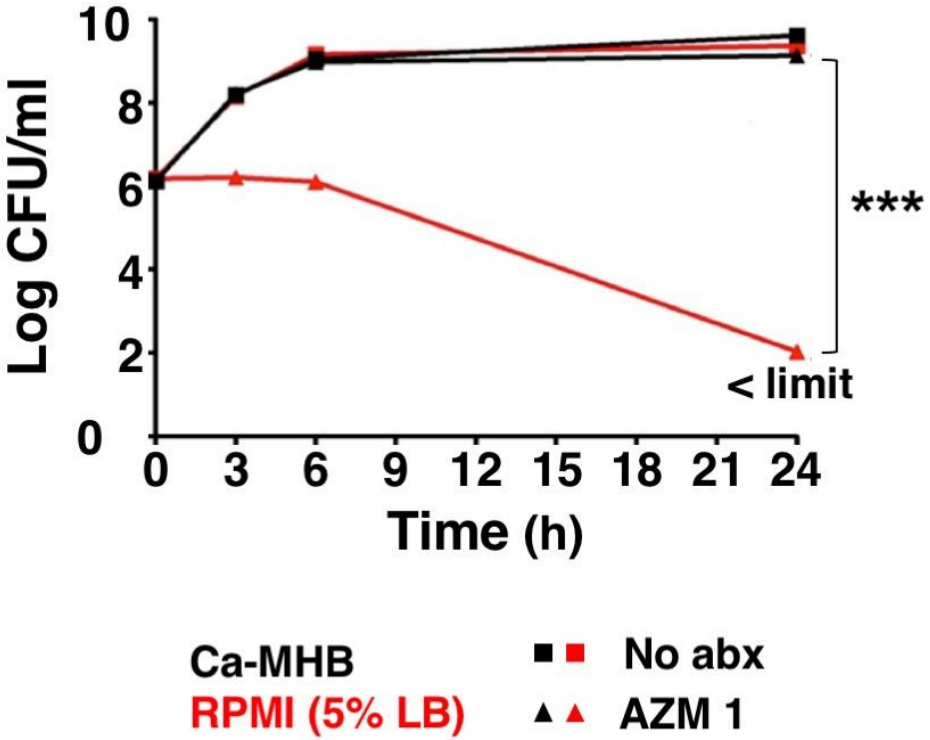


Azithromycin 1/8th MIC

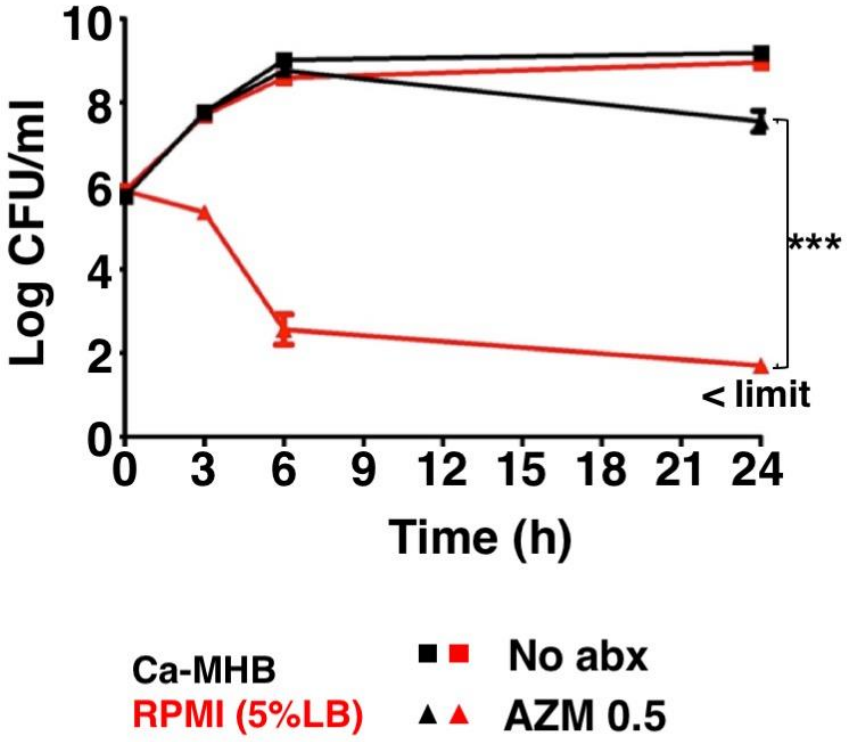


Azithromycin is Cidal for MDR Gram-Negative Rods at low Concentrations in RPMI + 5% LB

MDR *K. pneumoniae*

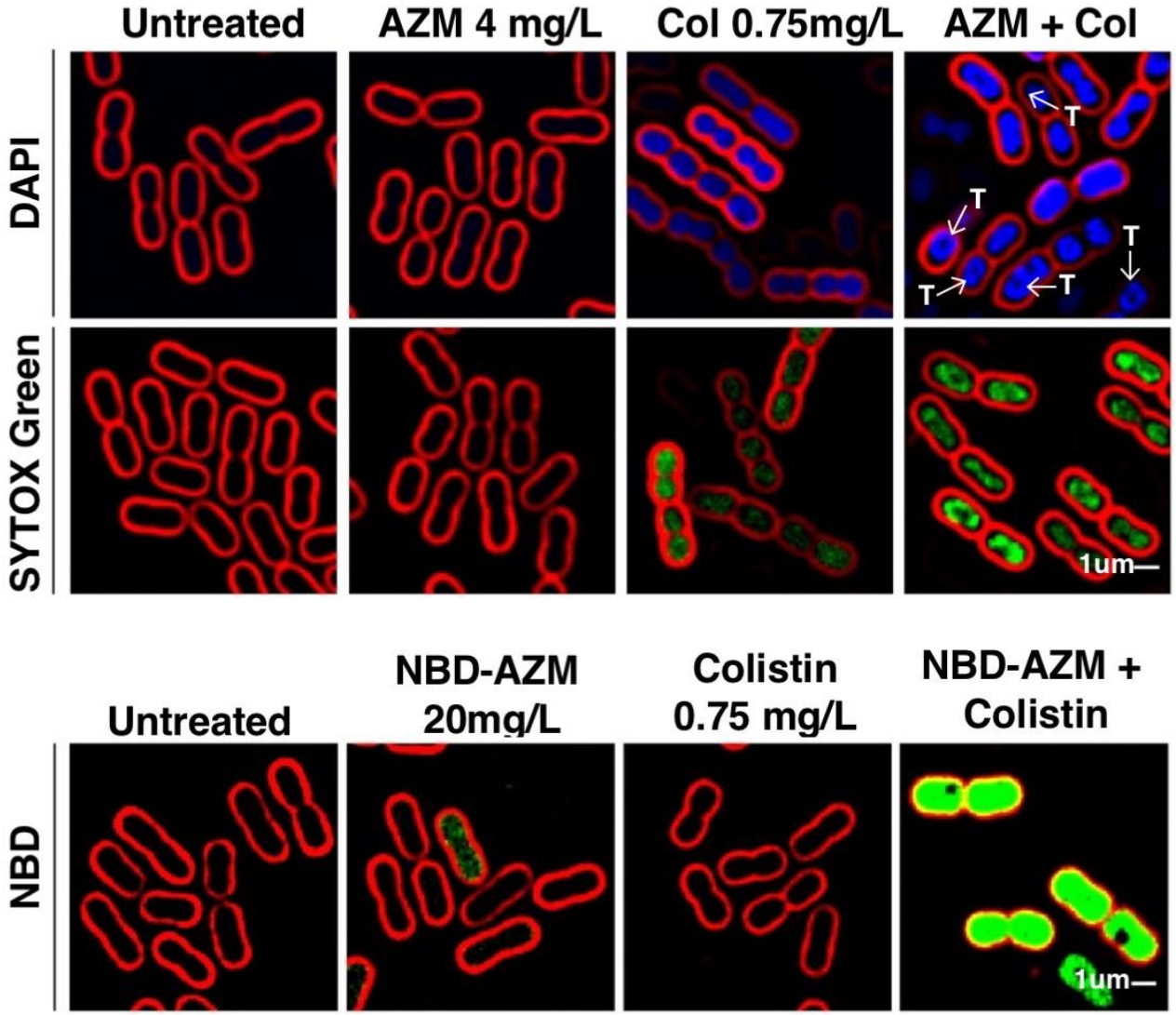


MDR *A. baumannii*



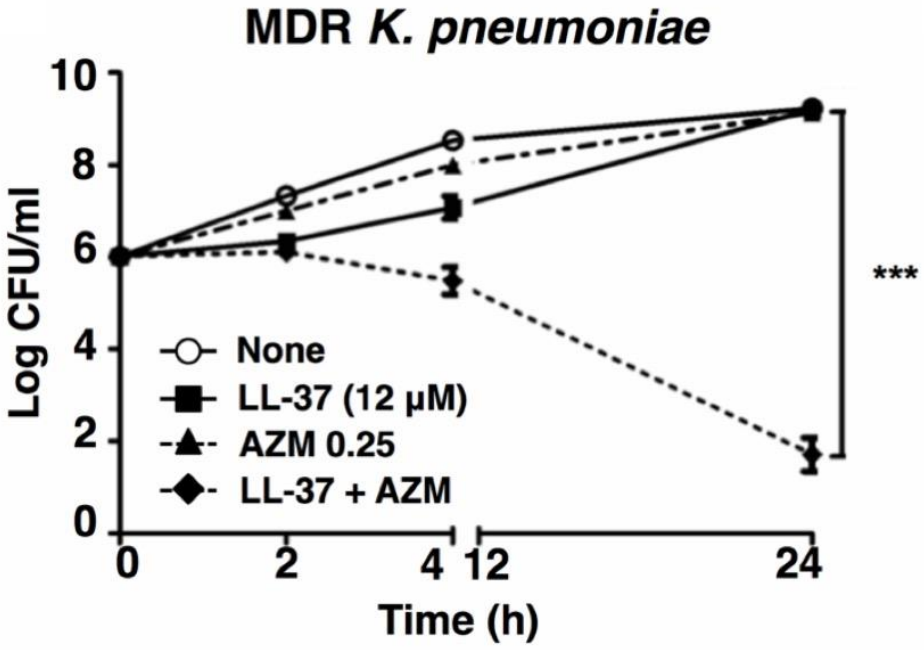
Lin et al. eBiomedicine 2015

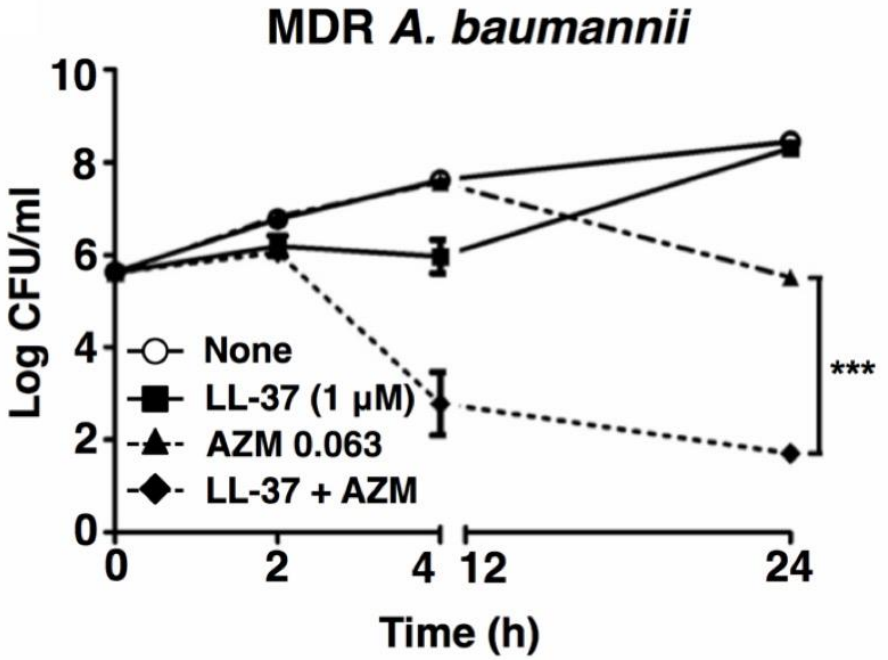
Synergy Between Azithromycin and Colistin in Killing MDR *Acinetobacter baumannii* (done in MHB)



Lin et al. submitted

Synergy Between Azithromycin and LL-37 in Killing MDR Gram-Negative Rods

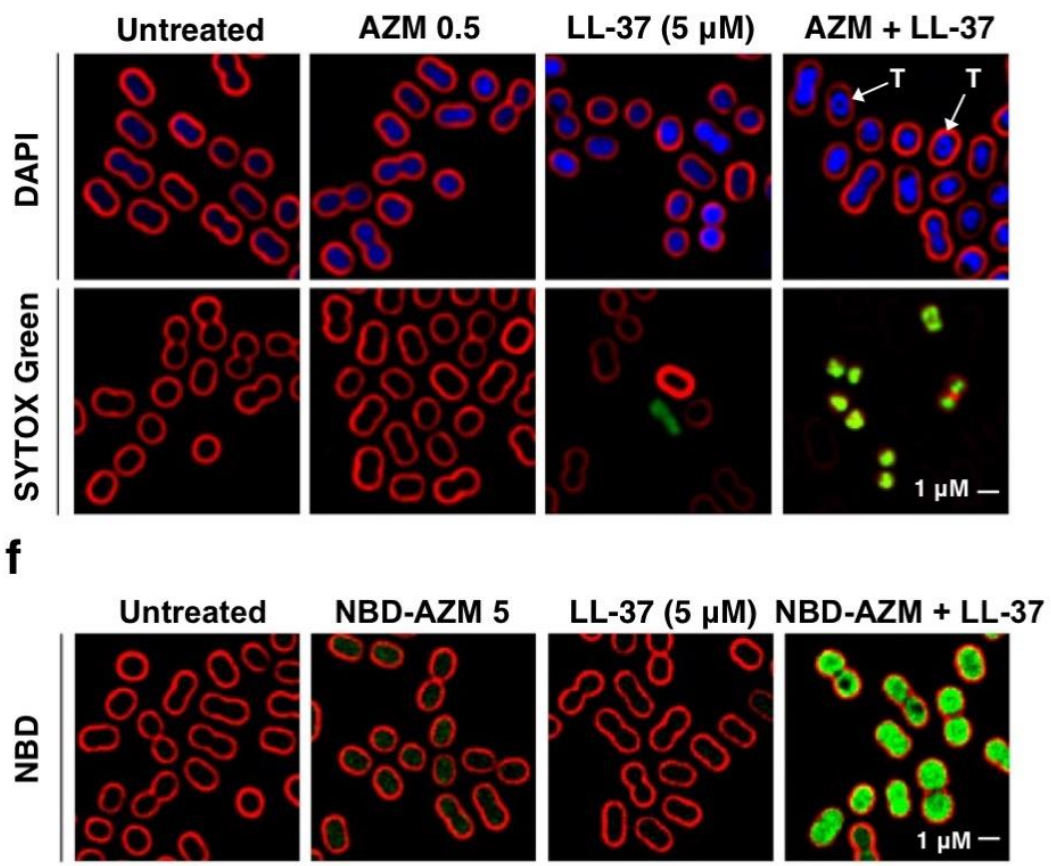


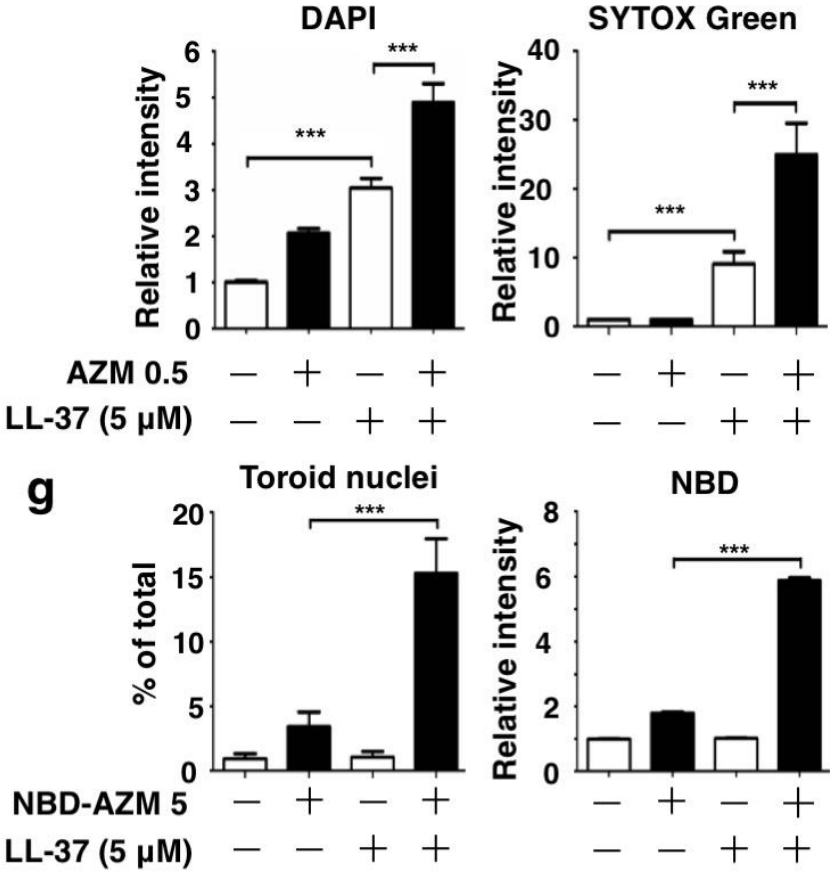


Lin et al. eBiomedicine 2015

Azithromycin Synergy with LL-37: Increased Cell Wall Permeability and Azithromycin Entry

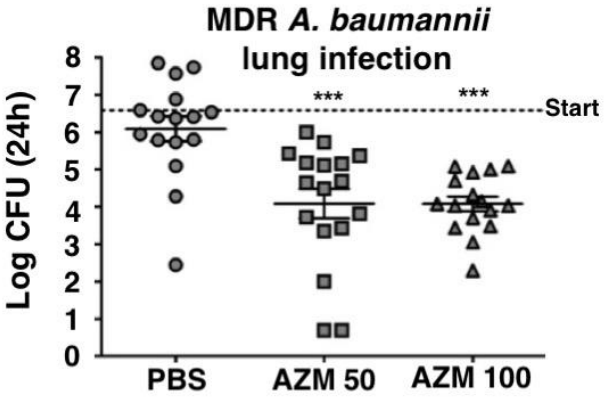
MDR *Acinetobacter baumannii*

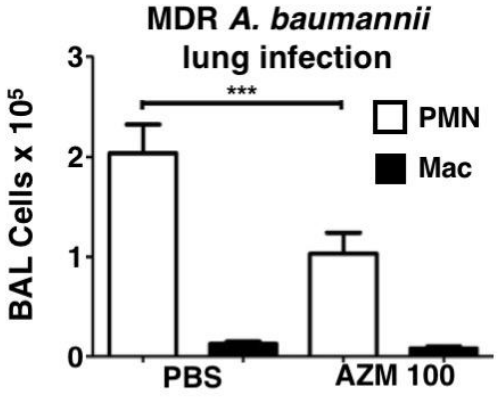


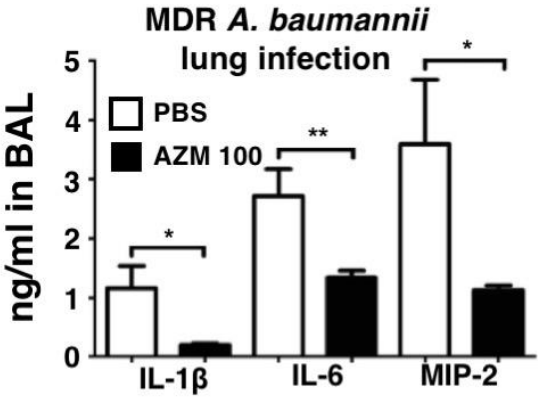


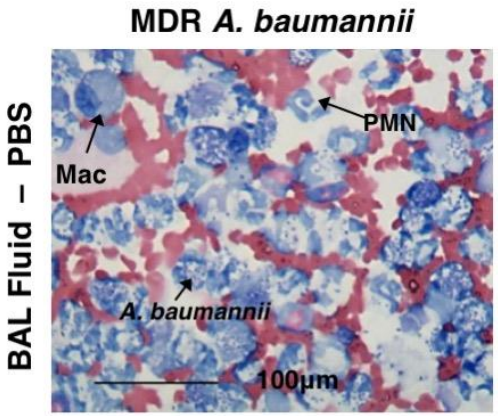
Lin et al. eBiomedicine 2015

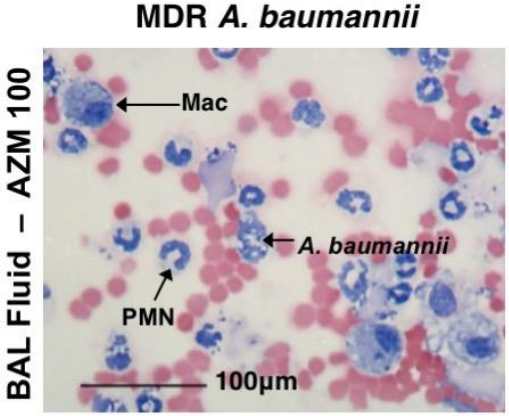
Azithromycin Monotherapy Reduces CFU, Lung Inflammation and Mortality in Mouse Model of *A. baumannii* Pneumonia

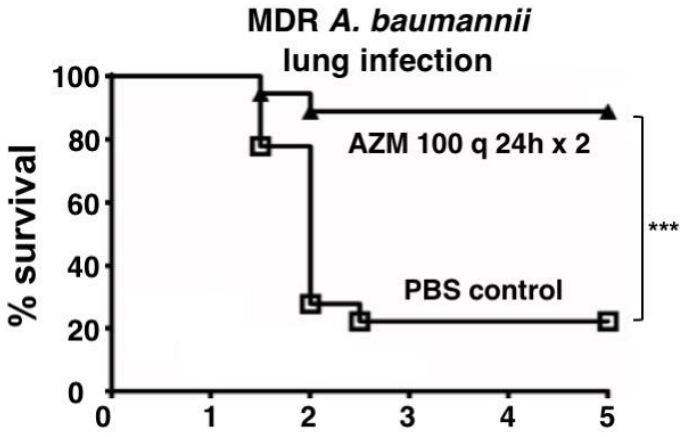






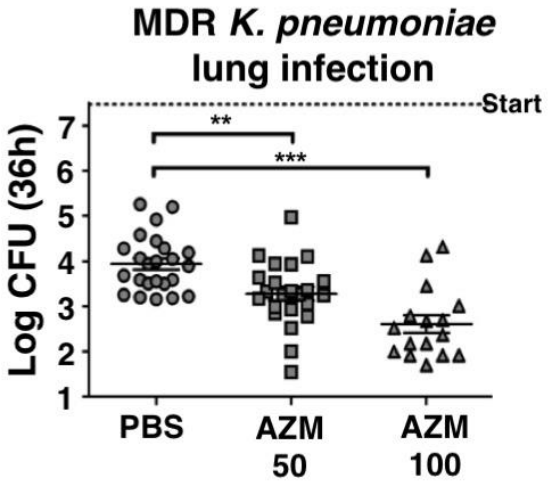


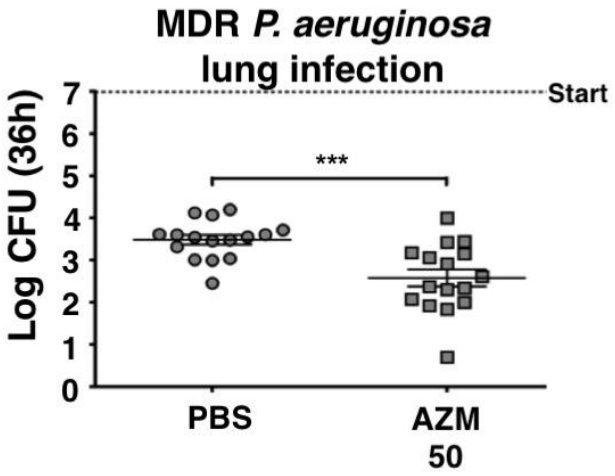




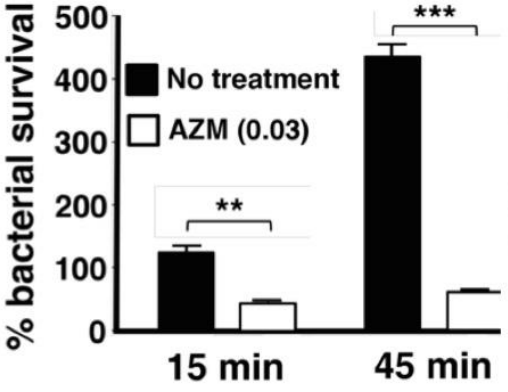
Lin et al. eBiomedicine 2015

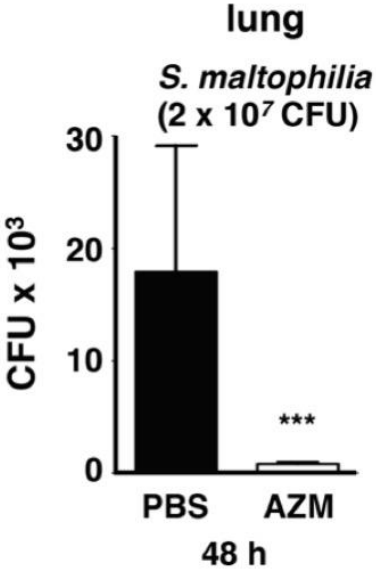
Azithromycin Activity vs. Carbapenem-Resistant *P. aeruginosa & K. pneumoniae*
(Lin et al. eBiomedicine 2015)





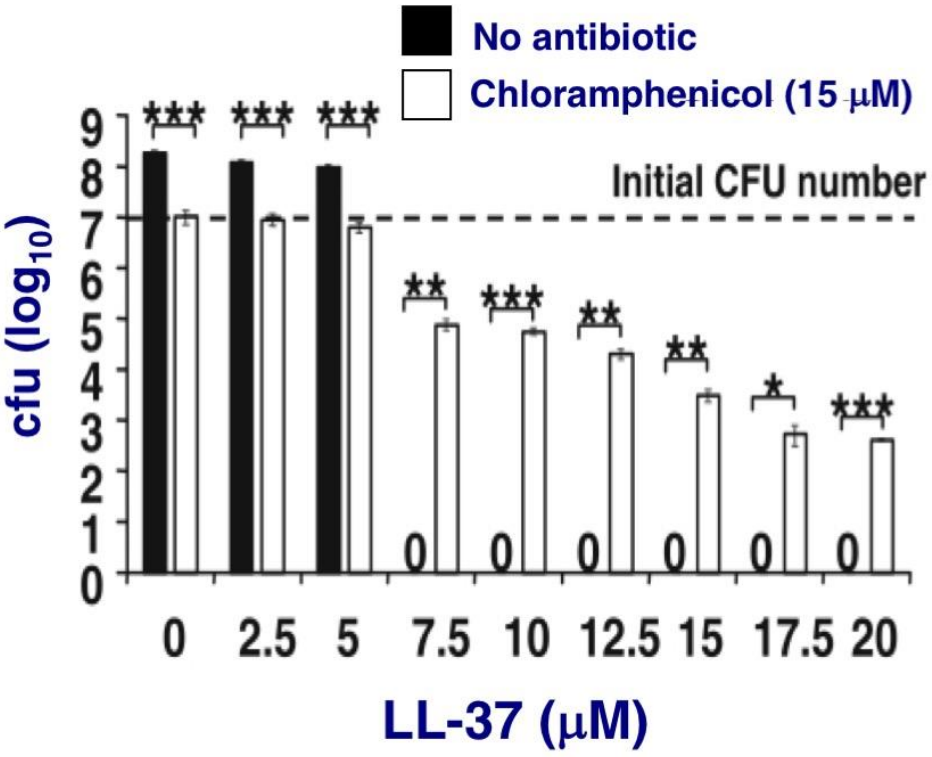
Unrecognized Azithromycin Activity vs. MDR *Stenotrophomonas maltophilia*
(Kumaraswamy et al. J Antimicrob Chemother 2016)





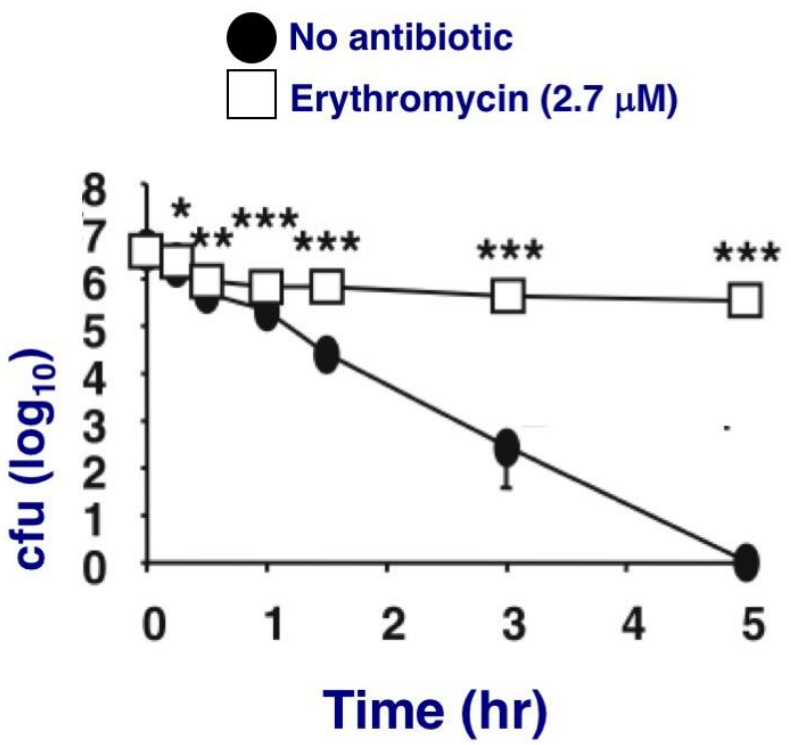
Bacteriostatic Antibiotics INHIBIT Cathelicidin Function

E. coli



S. aureus

(CRAMP 32 μM)



LL-37 binds to plane of cell division

Kristian et al., FASEB J 2007

Research Paper

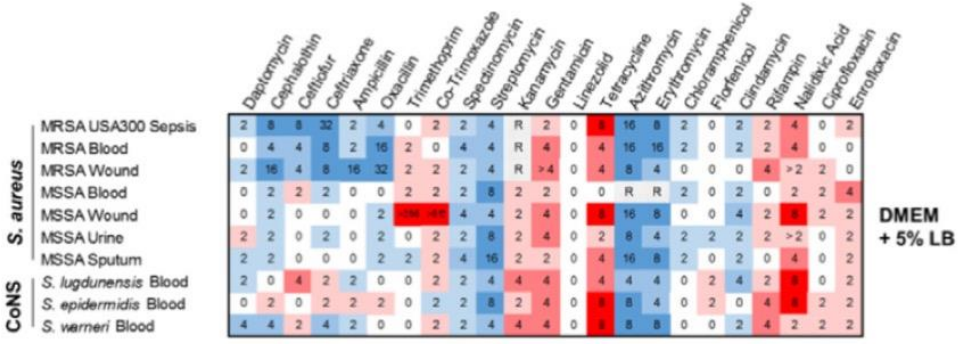
Correcting a Fundamental Flaw in the Paradigm for Antimicrobial Susceptibility Testing

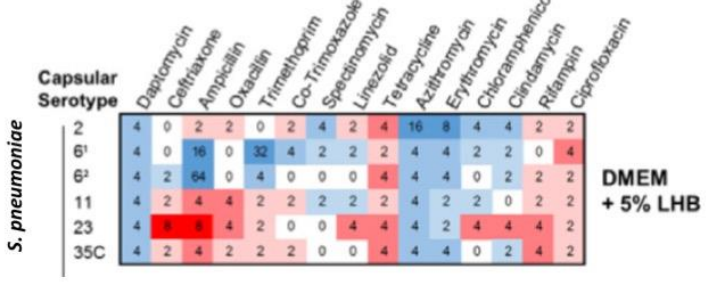
Selvi C. Ersoy [a,1], Douglas M. Heithoff [a,b,1], Lucien Barnes V [a], Geneva K. Tripp [a], John K. House [c], Jamey D. Marth [a,b,d], Jeffrey W. Smith [d], Michael J. Mahan [a,b,*]

[a] *Dept. of Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, CA 93106, USA*

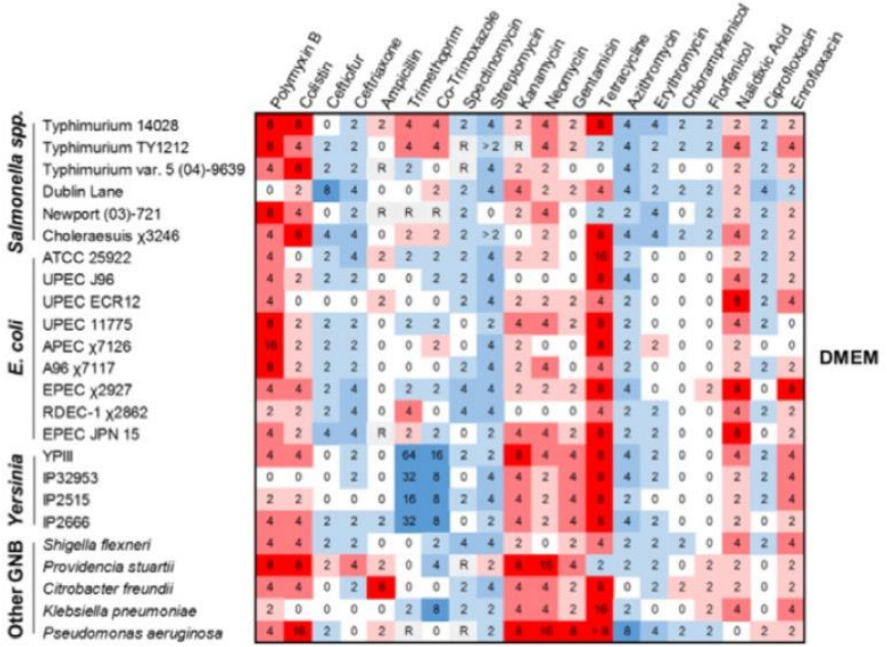
Fold change in MIC in "physiological" mammalian tissue culture media vs. Mueller-Hinton Broth
(blue = MIC reduced; red = MIC increased)

Gram-positive bacteria

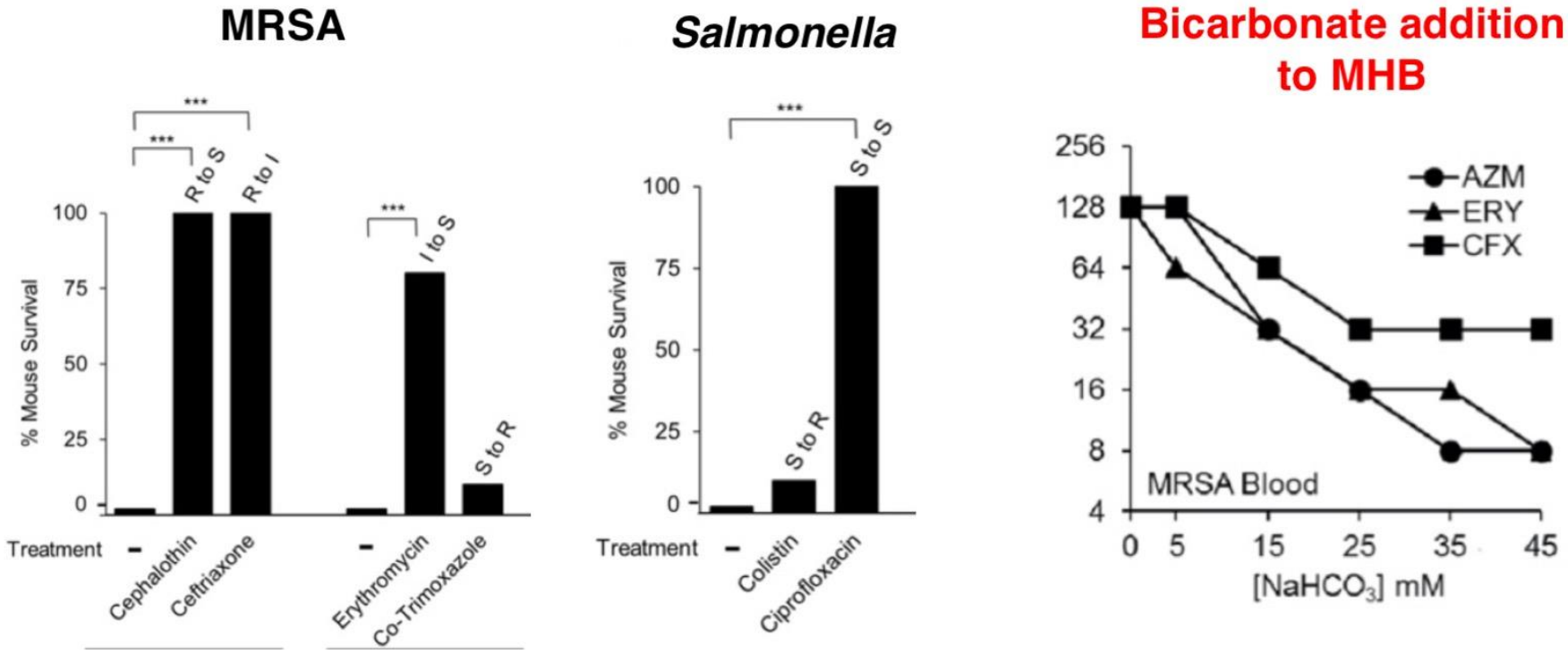




Gram-negative bacteria



Prediction of *in vivo* efficacy comparing MIC determine in MHB vs. that determined in "physiological" tissue culture media



Bicarbonate is absent in MHB yet an important physiological buffer required for endogenous cathelicidin antimicrobial peptide activity (Dorschner et al. 2006) Simply adding bicarbonate to MHB helped adjust MICs toward tissue culture media results and was better predictive of in vivo efficacy.

Ersoy et al. eBiomedicine 2017

ACS Infectious Diseases

Article

Cite This: ACS Infect. Dis. XXXX, XXX, XXX−XXX

pubs.acs.org/journal/aidcbc

Bicarbonate Alters Bacterial Susceptibility to Antibiotics by Targeting the Proton Motive Force

Maya A. Farha, Shawn French, Jonathan M. Stokes, and Eric D. Brown*

Michael G. DeGroote Institute for Infectious Disease Research, Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada

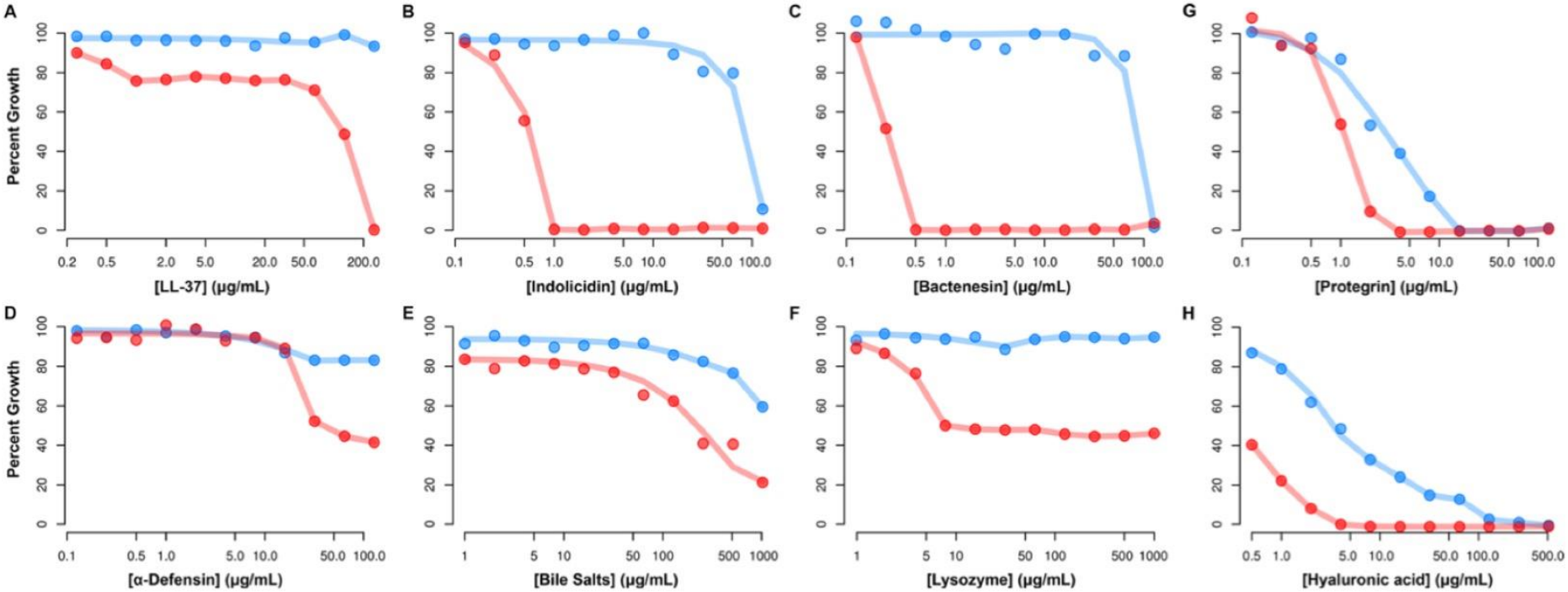


Figure 4. Physiological concentrations of bicarbonate enhance the antibacterial activity of various chemical factors involved in innate immunity.

ACS Infectious Diseases

Article

Cite This: ACS Infect. Dis. XXXX, XXX, XXX−XXX

pubs.acs.org/journal/aidcbc

Bicarbonate Alters Bacterial Susceptibility to Antibiotics by Targeting the Proton Motive Force

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Michael G. DeGroote Institute for Infectious Disease Research, Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, Ontario L8N 3Z5, Canada

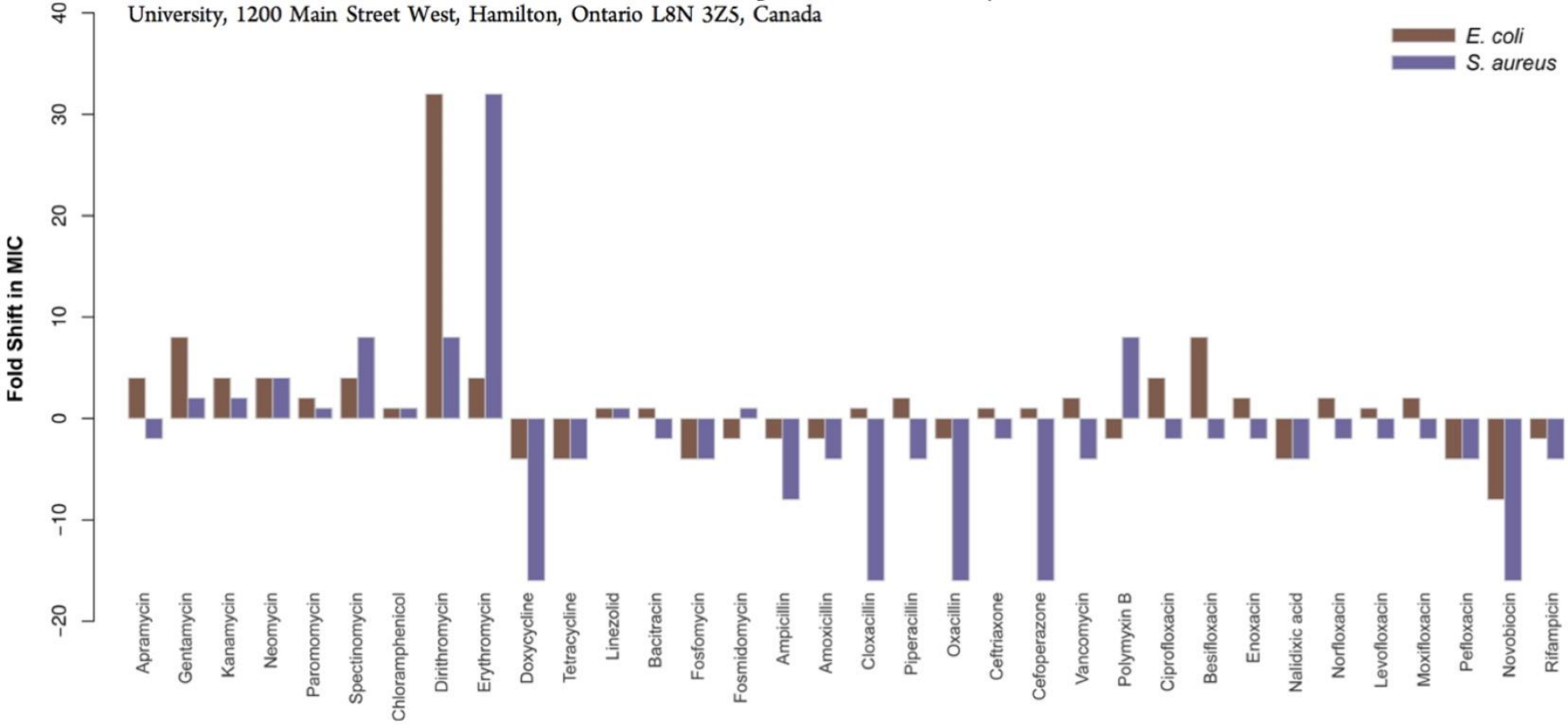
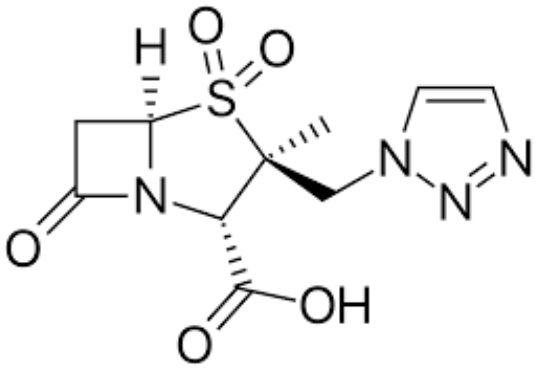


Figure 1. Bicarbonate affects the activity of various classes of antibiotics.

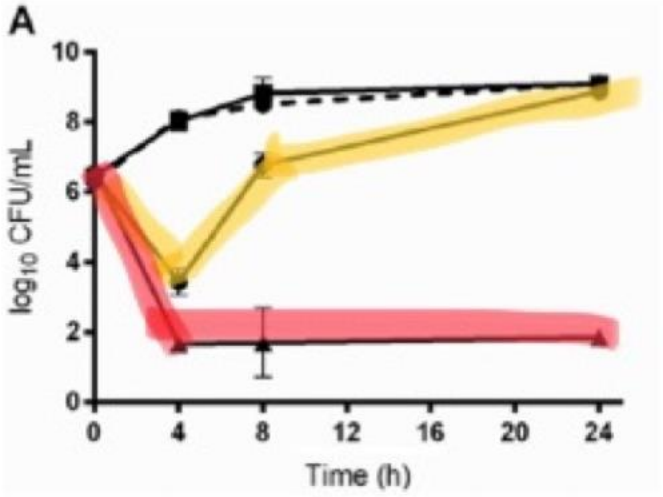
Classical β-Lactamase Inhibitors Potentiate the Activity of Daptomycin against Methicillin-Resistant *Staphylococcus aureus* and Colistin against *Acinetobacter baumannii*

George Sakoulas,[a,b] Warren Rose,[c] Andrew Berti,[c] Joshua Olson,[a] Jason Munguia,[a] Poochit Nonejuie,[d] Eleanna Sakoulas,[a] Michael J. Rybak,[e] Joseph Pogliano,[d] Victor Nizet[a,f]



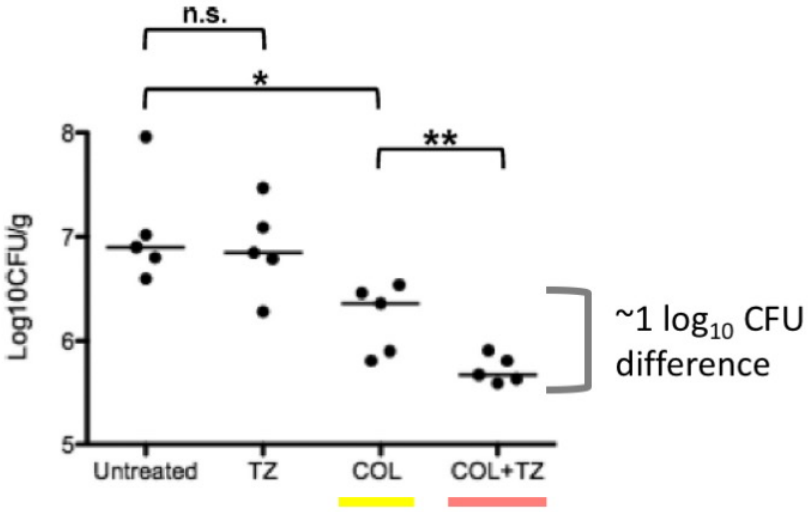
TAZOBACTAM

MDR *Acinetobacter baumannii*

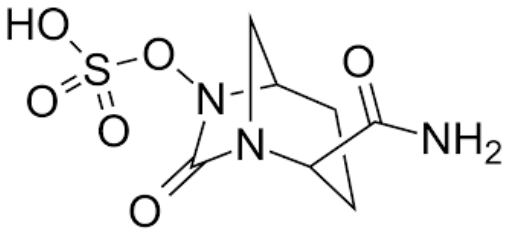


Colistin

Colistin + TAZ

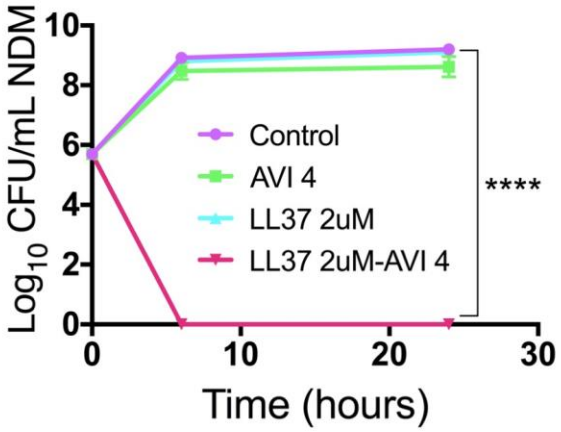


New Delhi metallo-beta-lactamase 1 (NDM-1) *Klebsiella pneumoniae*

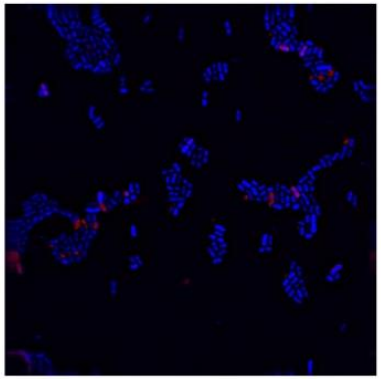


AVIBACTAM

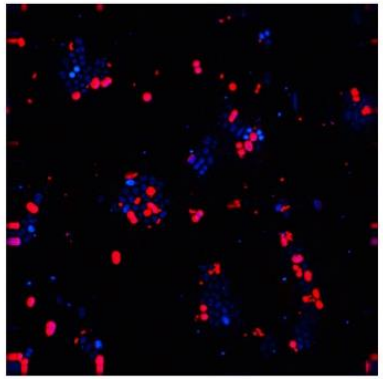
AVI Enhances Killing by Antimicrobial Peptides (LL37)



NDM *K. pneumoniae* + TAMRA-LL37 (2μM)



LL37 alone

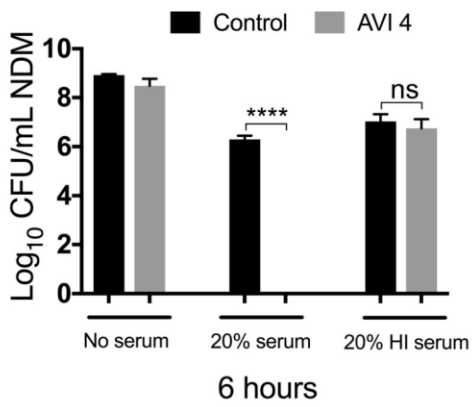


AVI 4μg/mL + LL37

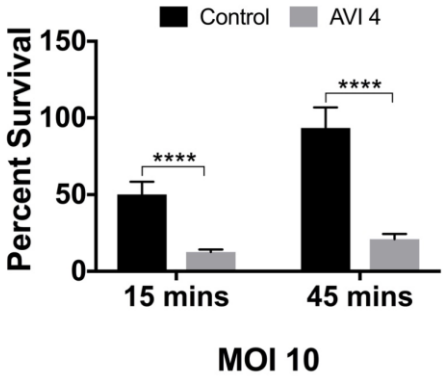
Chulie Ulloa, MD (in prep)

Avibactam Enhances Innate Immune Clearance of (NDM-1) *Klebsiella pneumoniae*

AVI Enhances Human Serum Killing

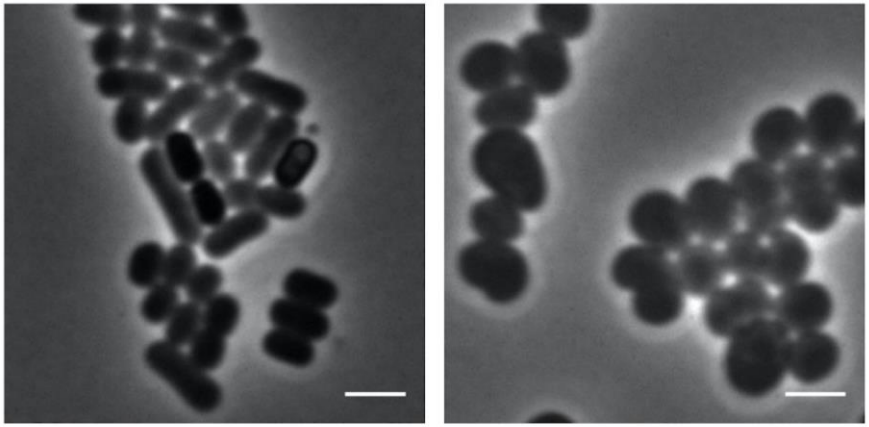


AVI Enhances Human Neutrophil Killing



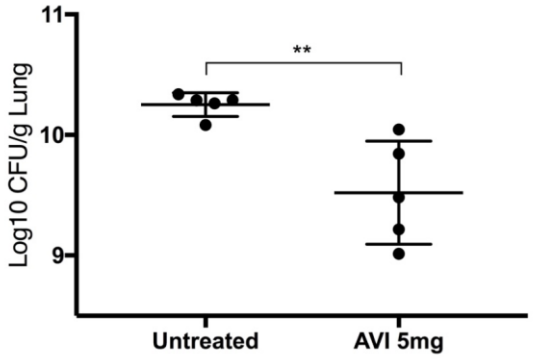
Untreated or LL37 alone

AVI 4 or AVI 4+LL37



AVI Enhances Killing of NDM-KP

Murine Pneumonia Model



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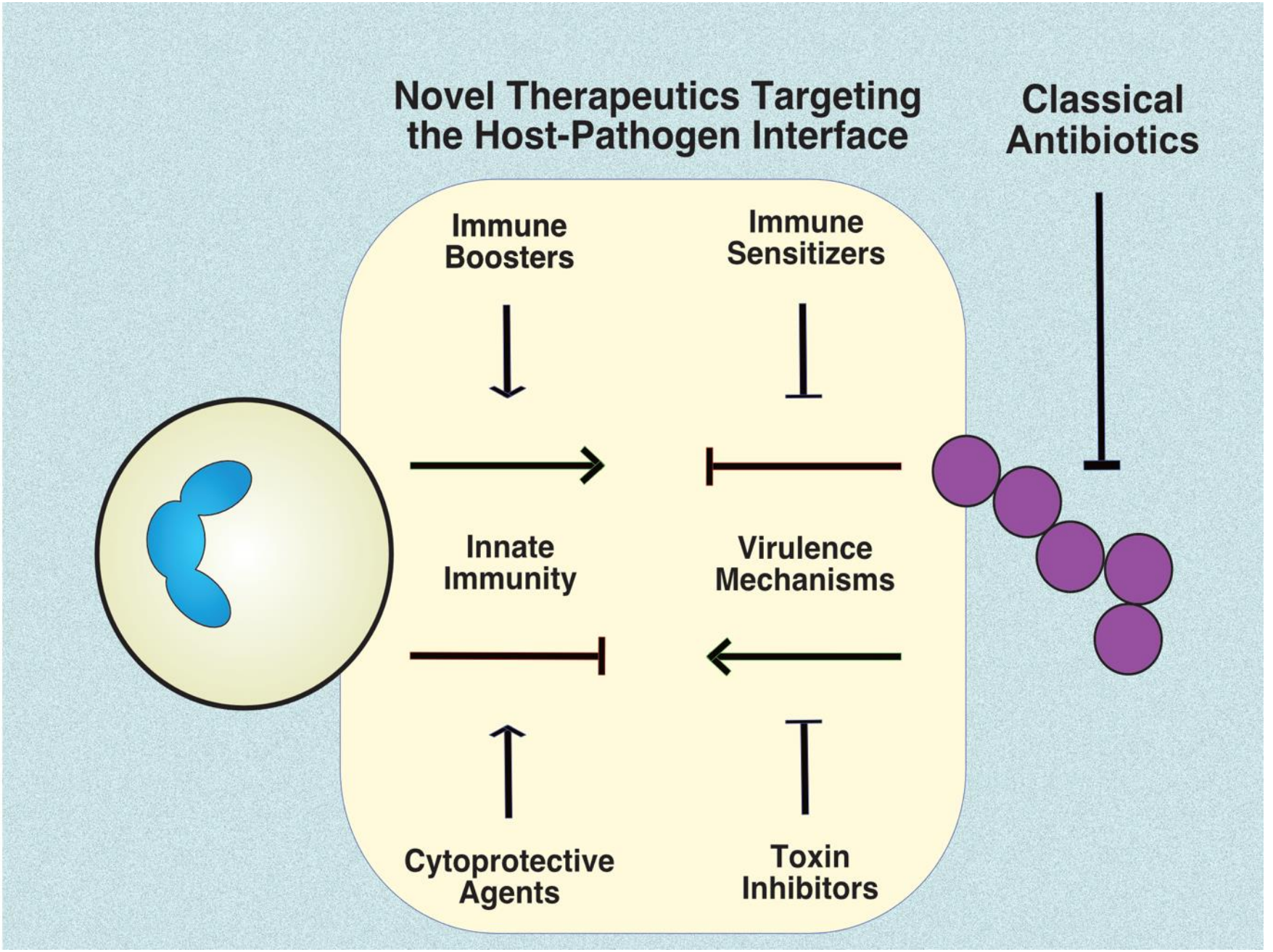

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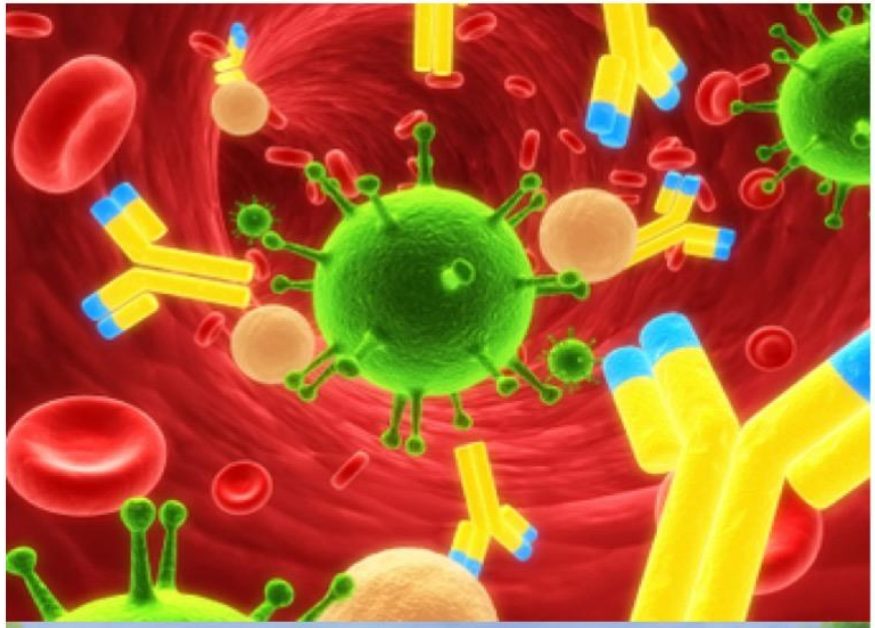
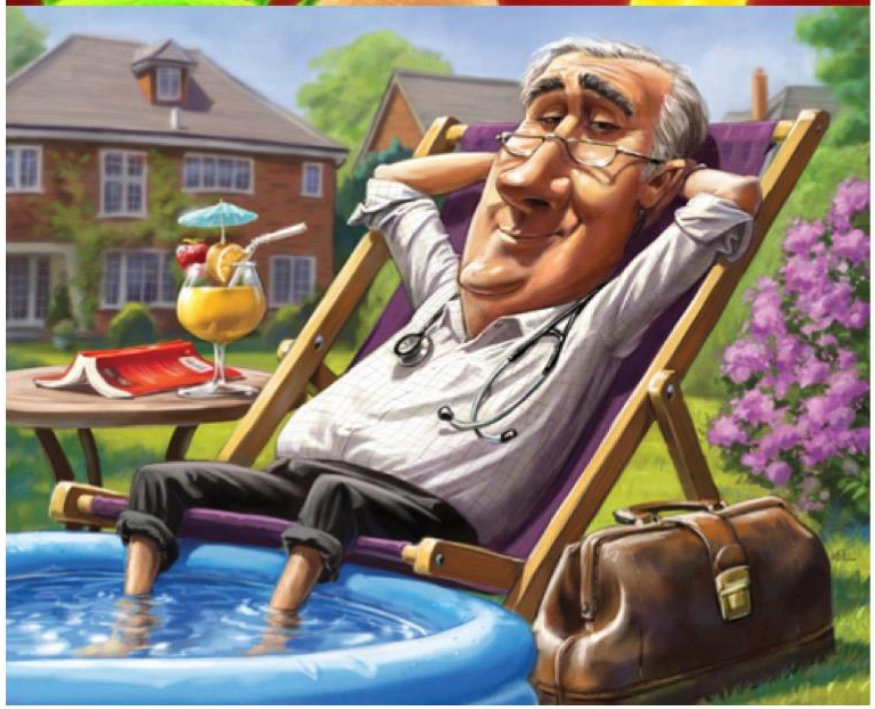
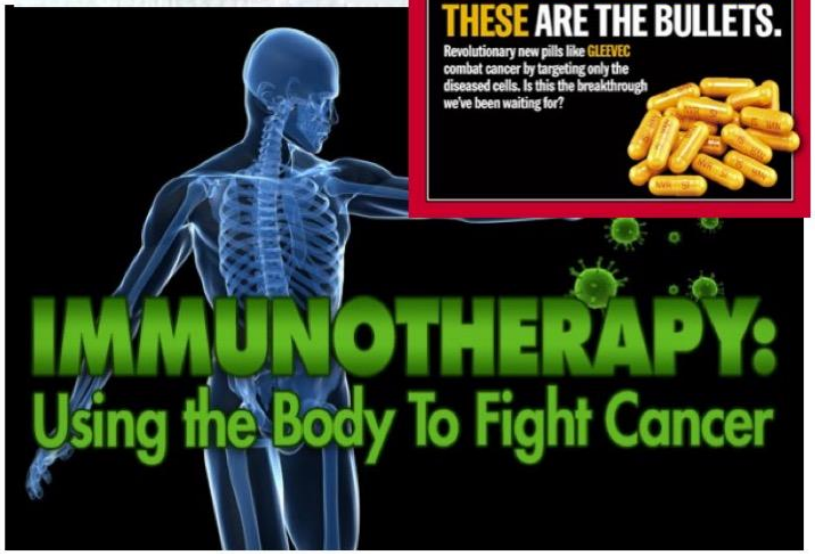




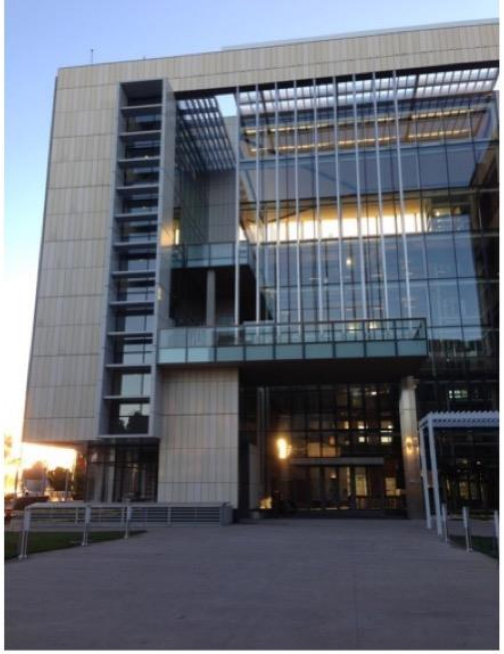
TIME

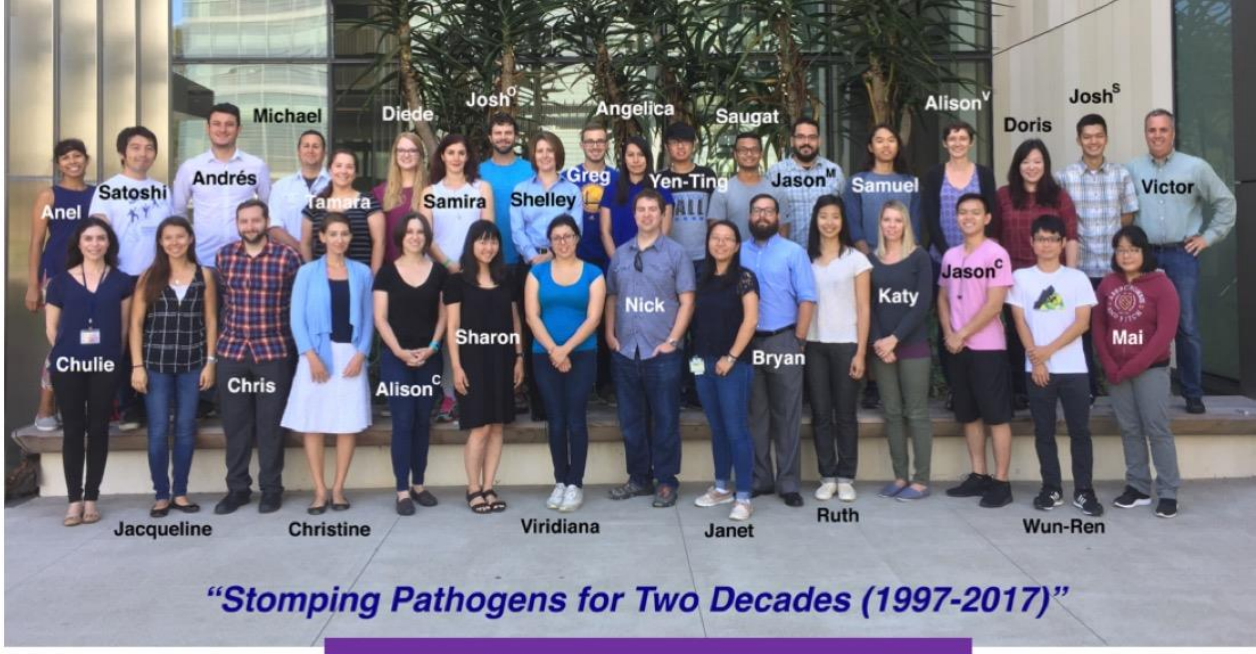
THERE IS NEW AMMUNITION IN THE WAR AGAINST CANCER. THESE ARE THE BULLETS.

Revolutionary new pills like GLEEVEC combat cancer by targeting only the diseased cells. Is this the breakthrough we've been waiting for?



IMMUNOTHERAPY:
Using the Body To Fight Cancer





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