Combination Therapy for Serious Infections: *Staphylococcus aureus*

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(Clinical)

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Limited

CLINICAL TRIALS

Once a disease target is identified, drugs are designed and tested. Both public and privately funded research are involved.

REGULATORY APPROVAL

Human trials are completed. FDA approval. Industry is responsible for bringing a drug to market. Safety and evaluation continue after approvals.

IDEA

PHASE I **PHASE II** **PHASE III**

17 years Process

BASIC RESEARCH

The majority of the research at this stage is publicly funded at universities, colleges and independent research institutions in every state.

PATIENT CARE

Questions to Consider in Combination Therapy…

- Why Talk About Combination Antibiotic Therapy to Treat Bacterial Infections?
- Do we use combination therapy to treat some infections currently?
- If so, why is it such a big deal when the concept is introduced to clinicians?
- Why are serious S. aureus infections a good example where combination antibiotic therapy should be considered?

The Beginning of the Modern (Human) Antibiotic Era

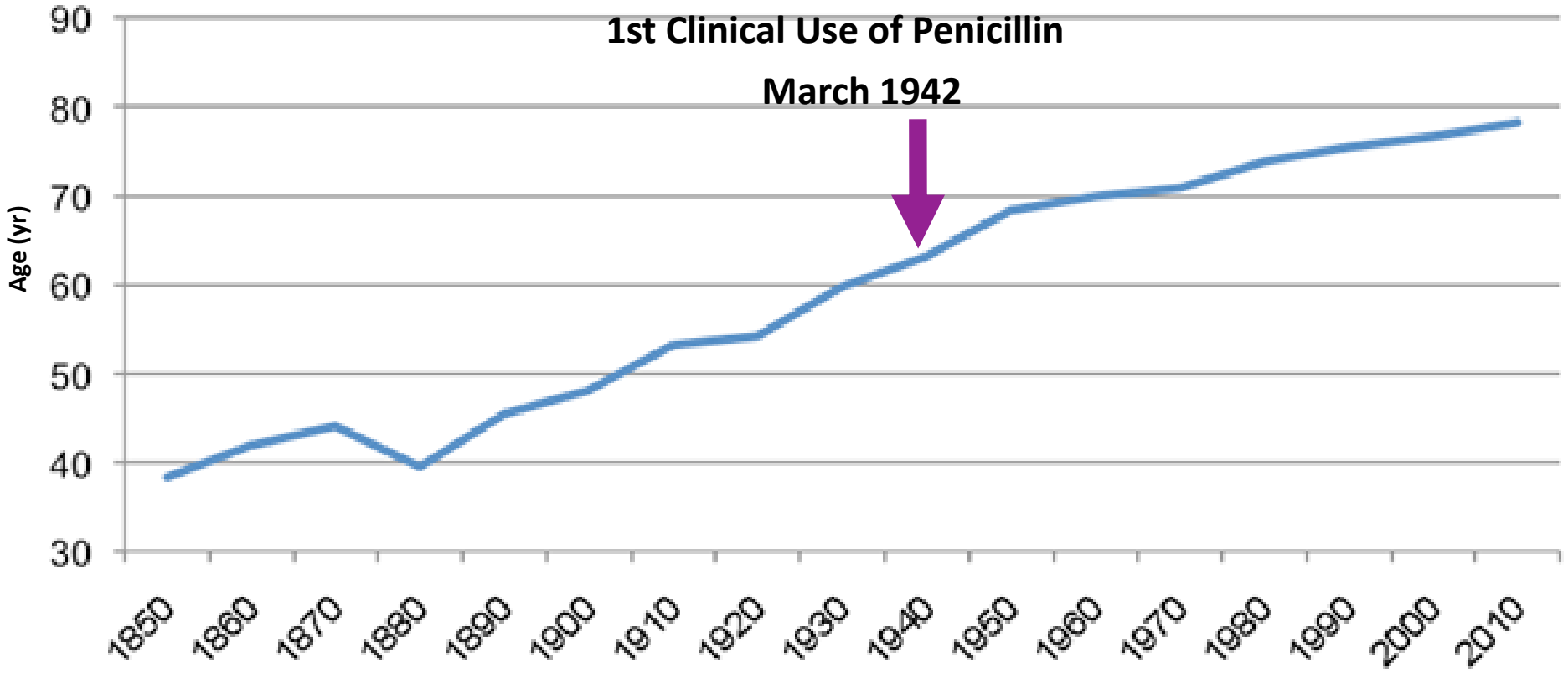
1943 - 4 year old.

14 days penicillin

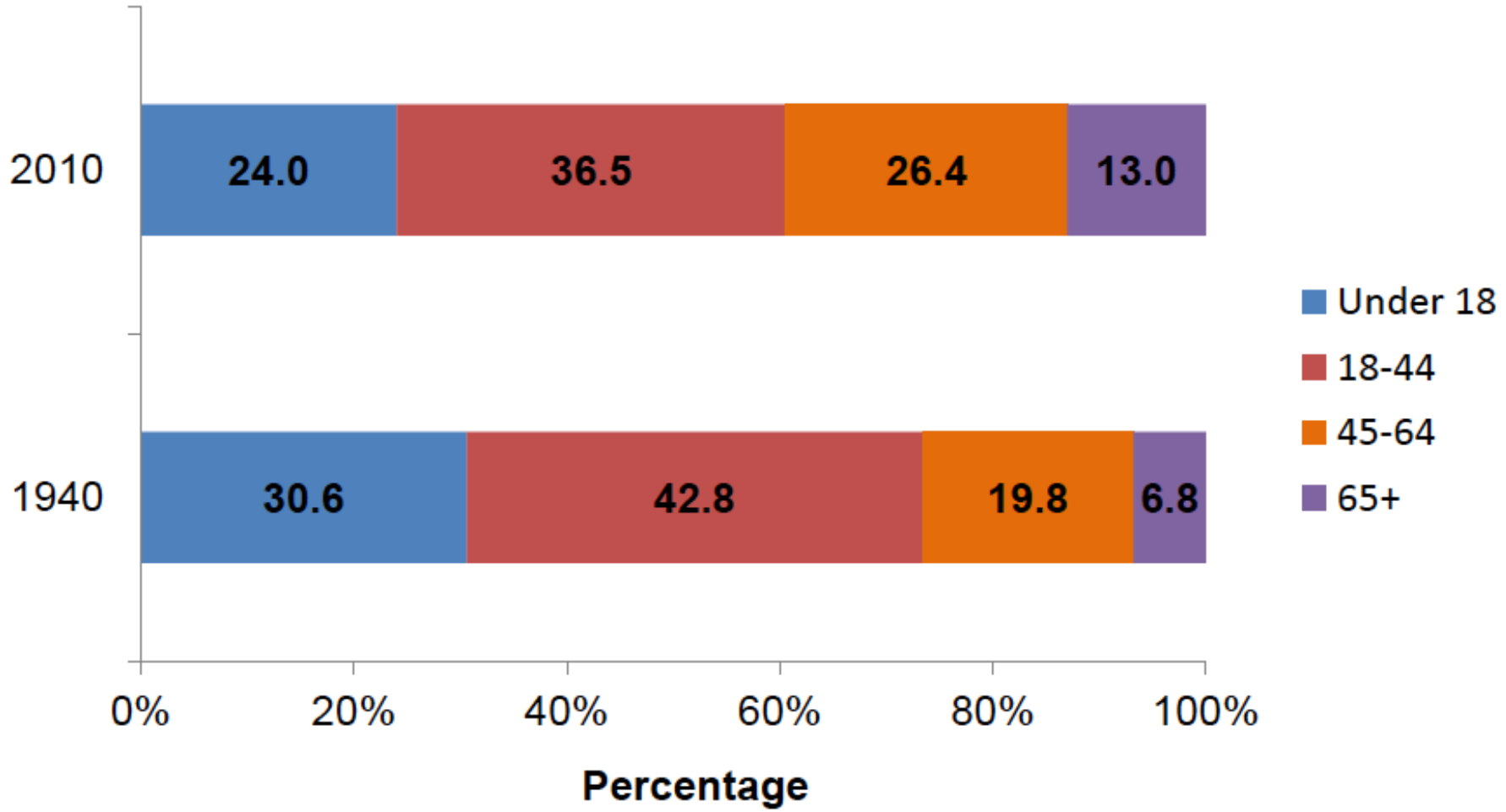
There was was never a comparator—does that matter?
Why 14 days?
How different were patient hosts in 1943 vs the 21st century?
How many beta-lactam resistant bacterial pathogens were clinically relevant in the 1940's?

Herrell '43 Proc Staff Meetings Mayo Clinic 18:65-76

Life Expectancy at Birth



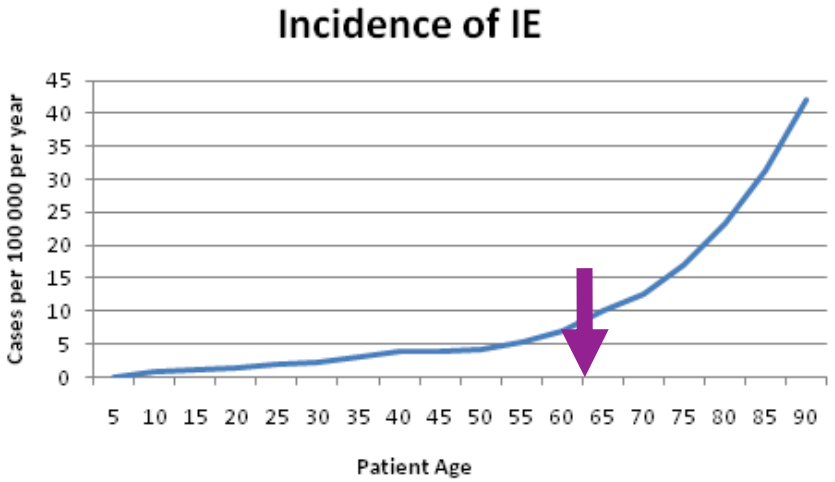
Age Distribution: 1940 and 2010



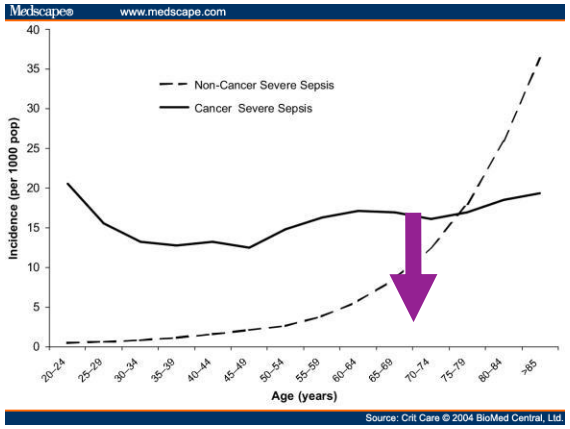
Sources: U.S. Census Bureau, *1940 Census* and *2010 Census*.

United States Census Bureau

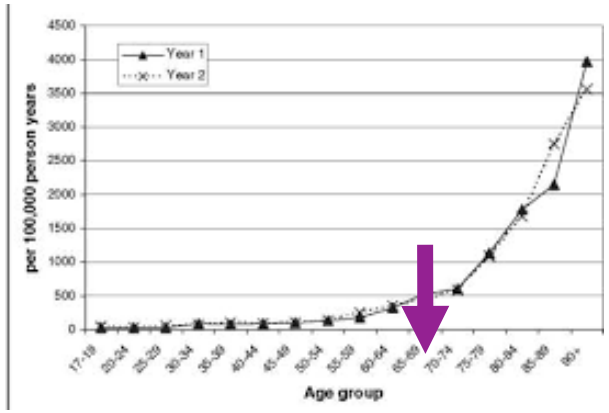
Consequence of Medical Progress: Age Senescence of Innate Immunity



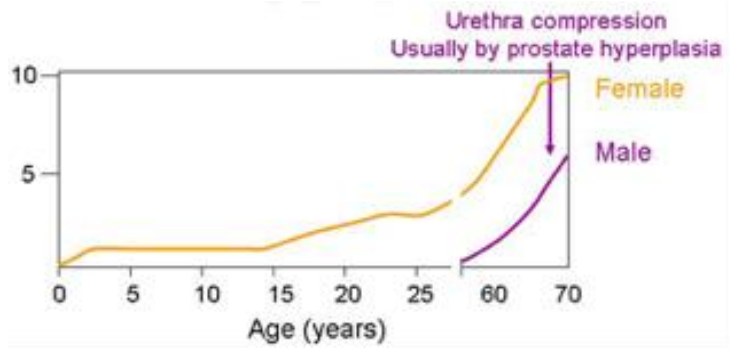
Does the Human Innate Immune System Have an Approximate 6 Decade Shelf Life??



Sepsis

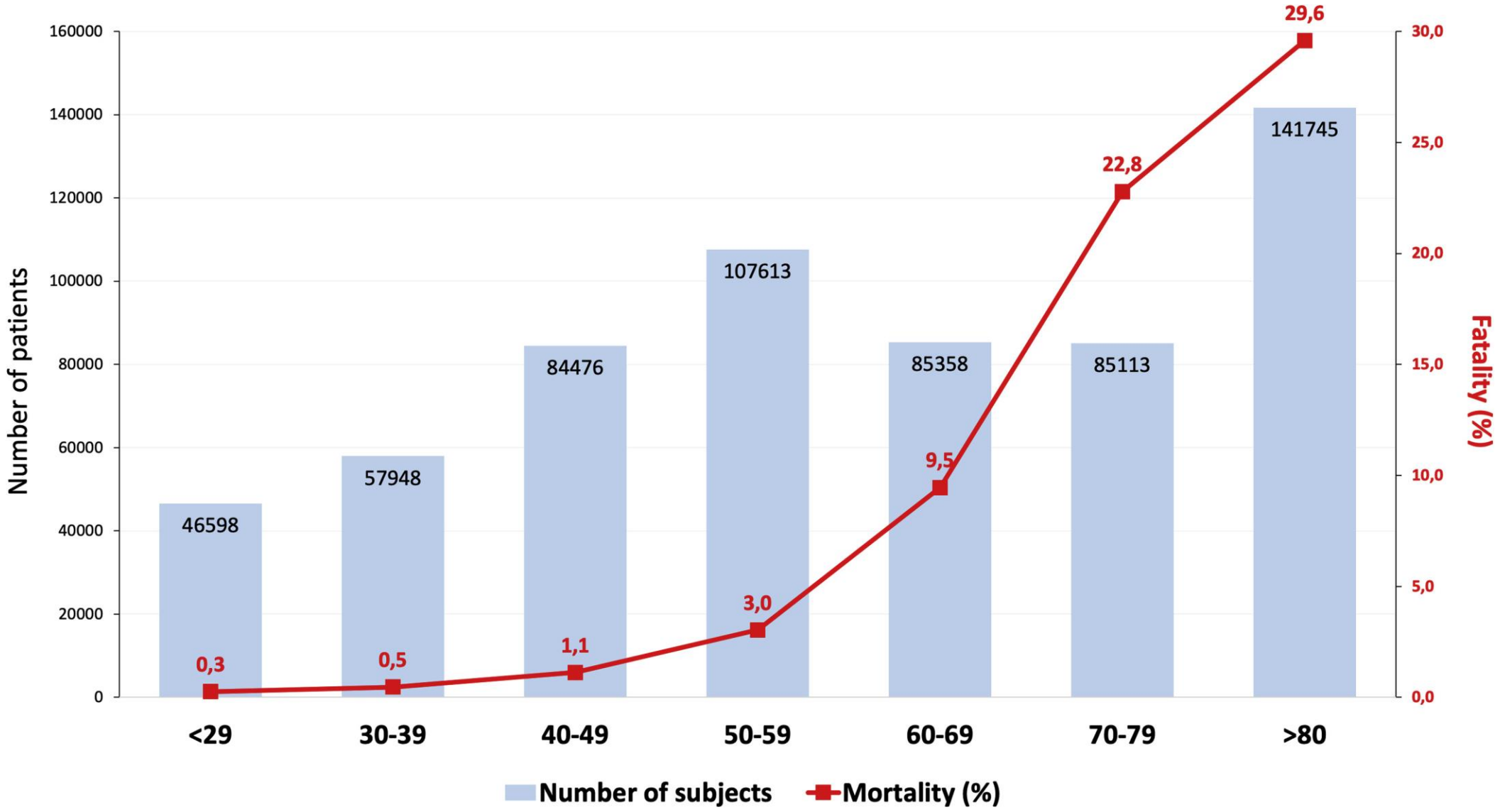


CAP

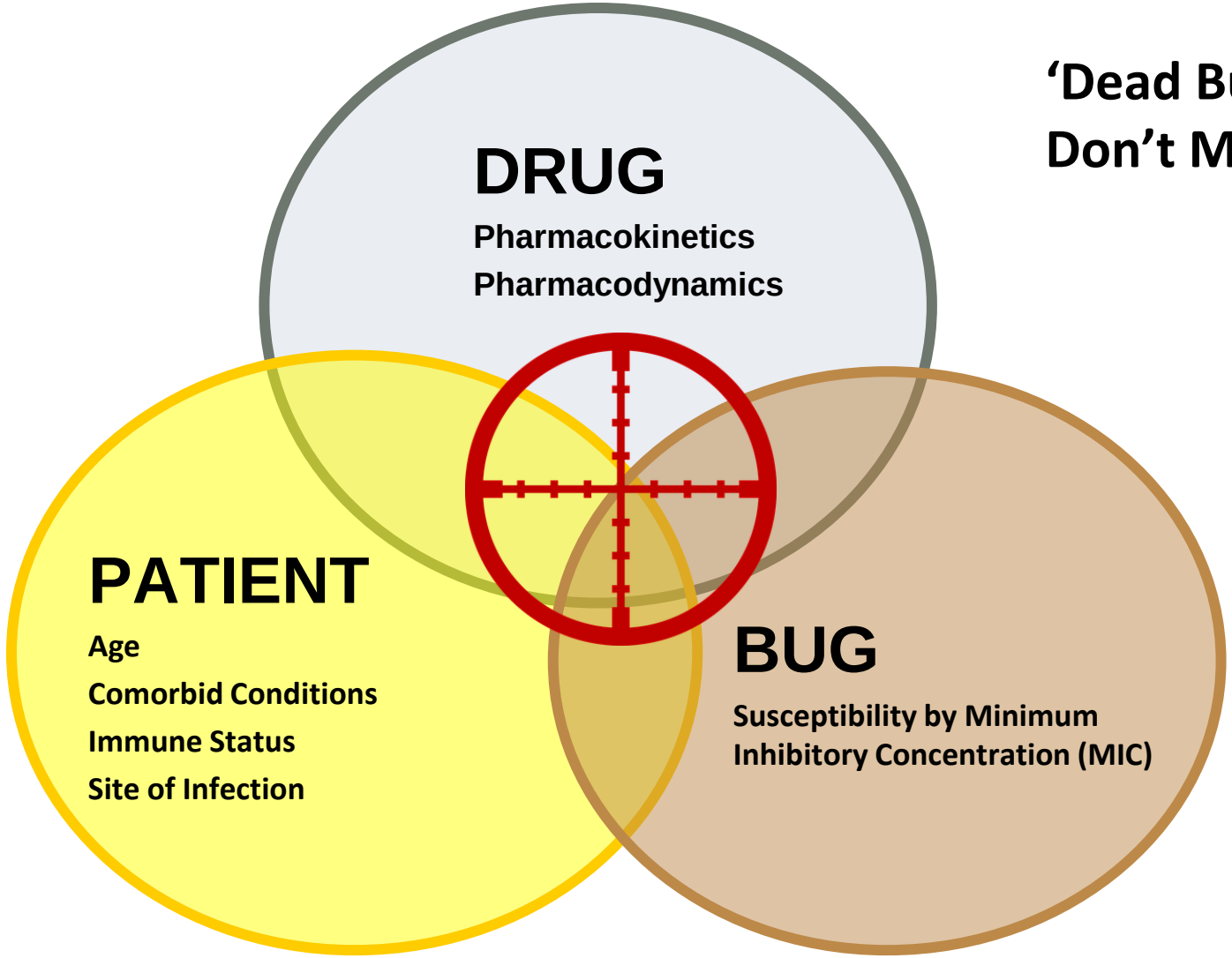


UTI

COVID-19 Mortality by Age



Combating Antimicrobial Resistance Means We Have Set Our Targets and Hit the Mark



‘Dead Bugs Don’t Mutate’

Rationale for Combination Antibiotic Therapy

- **Synergy: More potent and efficient killing of bacteria (1+1>2)**
  - **Immunocompromised hosts**
  - **High inoculum**
  - **Attack bacteria in different ‘compartments’ (eg. intracellular vs extracellular; biofilms)**
  - **Target different aspects of pathogenesis (eg. killing; virulence attenuation)**
- **Broaden spectrum of activity**
  - **Empiric regimen**
  - **Beta-lactamase inhibitors**
- **Reduce the likelihood of antimicrobial resistance**
  - **High inoculum**
  - **Organism-specific concerns**
- **Reduce the incidence of side-effects**
  - **Reduce dose and boost pharmacodynamics**
- **Reduce costs**
  - **Pharmacodynamic boosting of a costly single agent by a second cheaper agent**
  - **Shorten duration of hospitalization**

Factors Determining A Successful Outcome in the Treatment of Infection

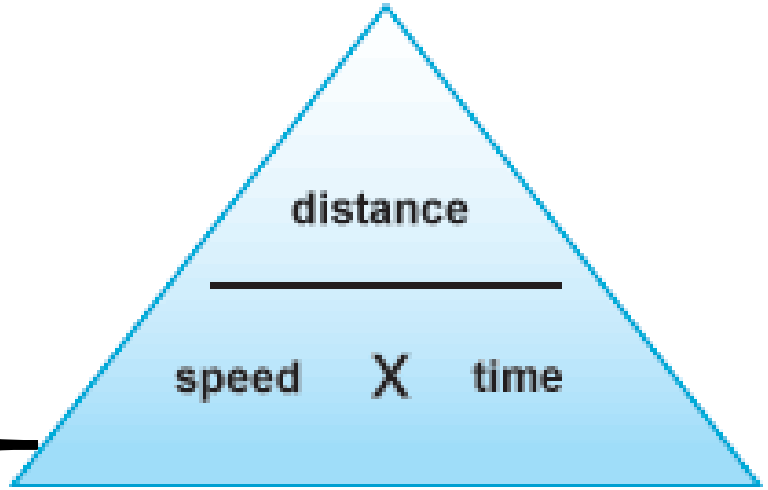
Host Immunity
- Age
- Immunosuppression

Pathogen
- Virulence (invasion/evasion)
- Antimicrobial Resistance
- Infecting Site

Surgical Source Control

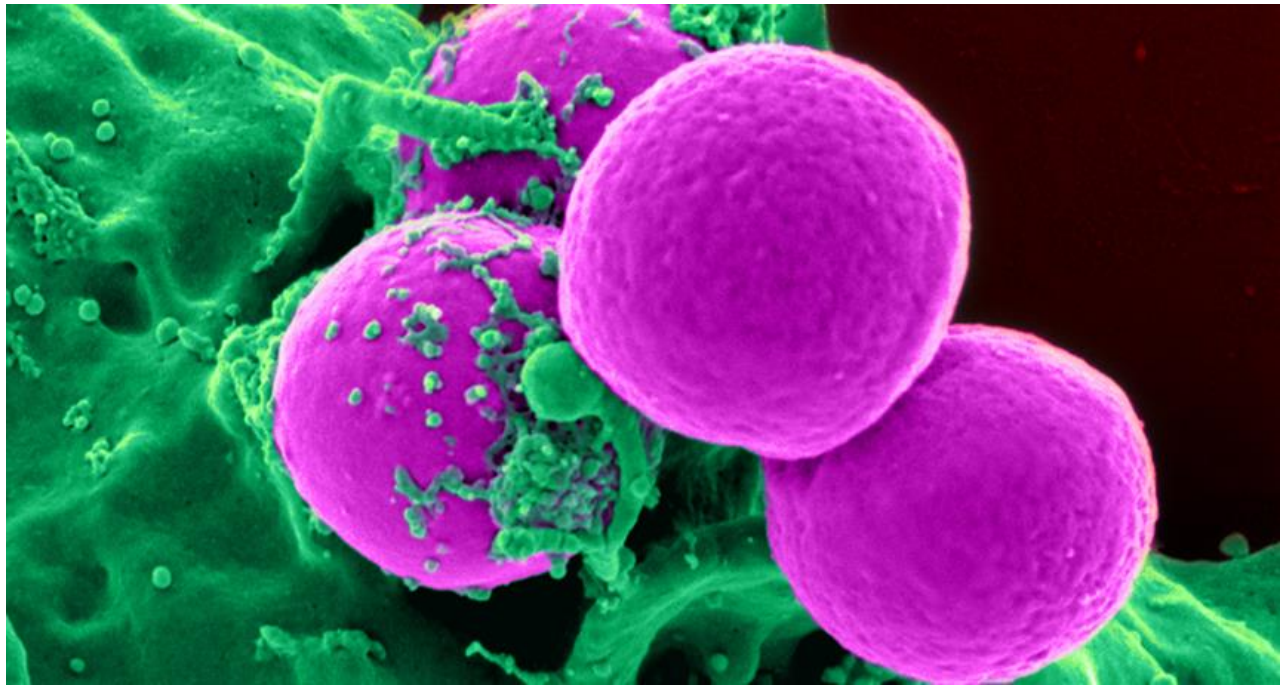
Antibiotic Properties: MIC (S vs R)
- Bactericidal vs Bacteriostatic
- PK/PD
- Penetration into biofilms
- Activity against ‘inert’ forms
- **Synergy with Innate Immunity**

Burden of Infection (inoculum)

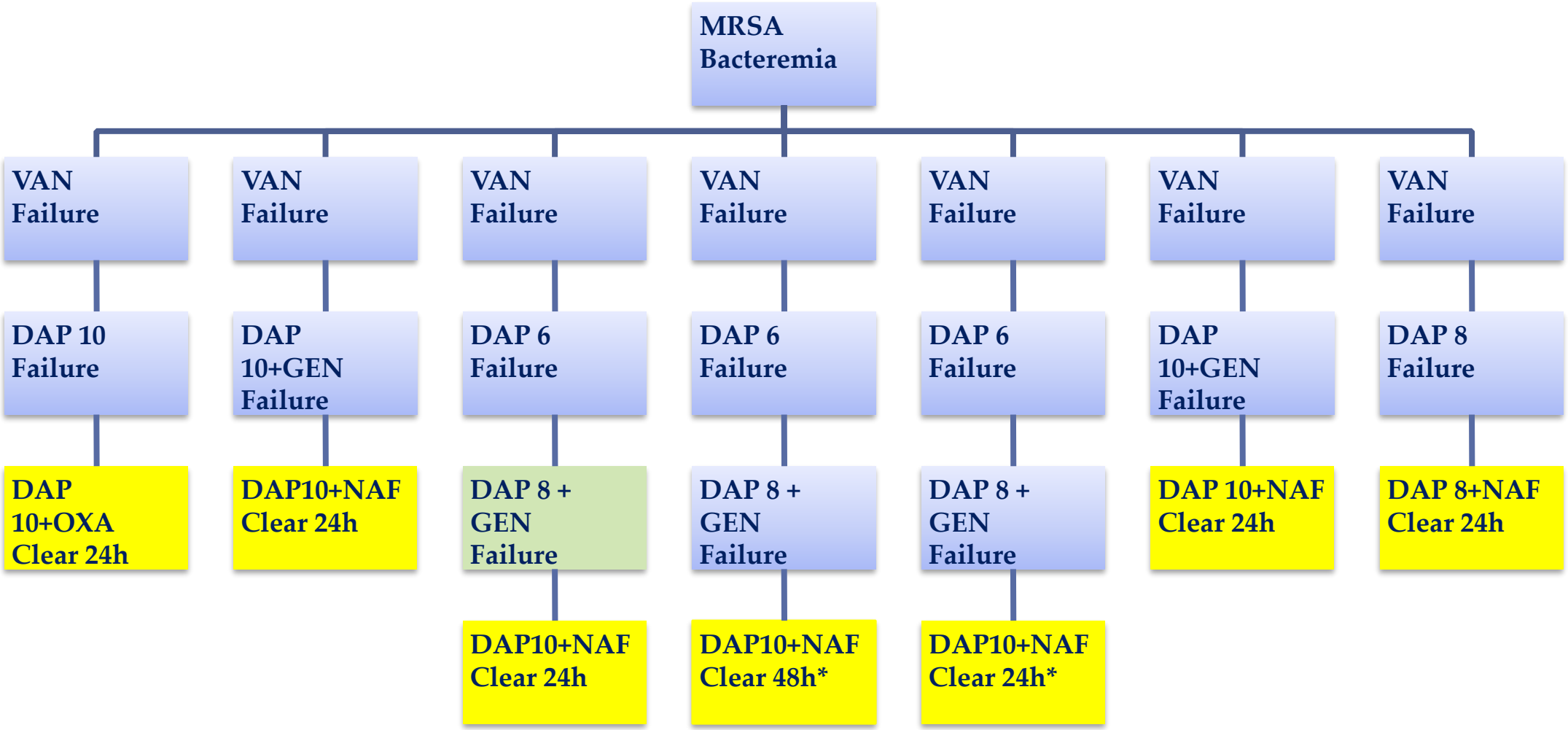


Duration of Therapy

REFRACTORY MRSA BACTEREMIA

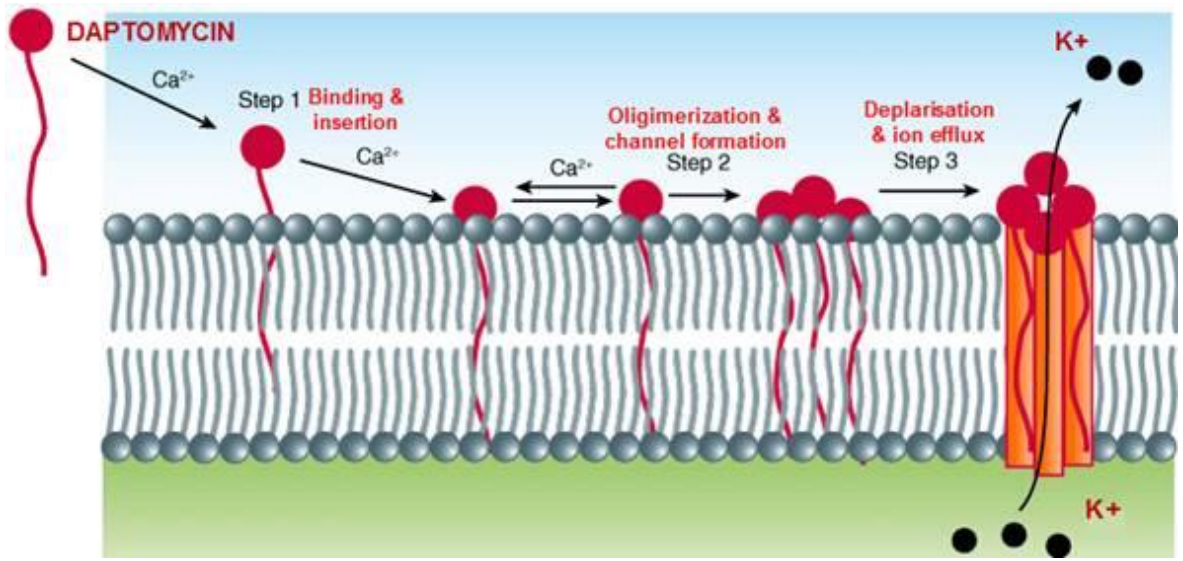


Rapid MRSA Bacteremia Clearance with High-Dose Daptomycin plus a β-lactam



*Relapsed – 12 wks & 8 wks post-therapy – 1 cleared w/another course,;1 died w/VISA PV IE VAN MIC 3; DAP MIC 1.5
Red VISA; DAP MIC 2-4 - Additional studies performed on the isolates from this case

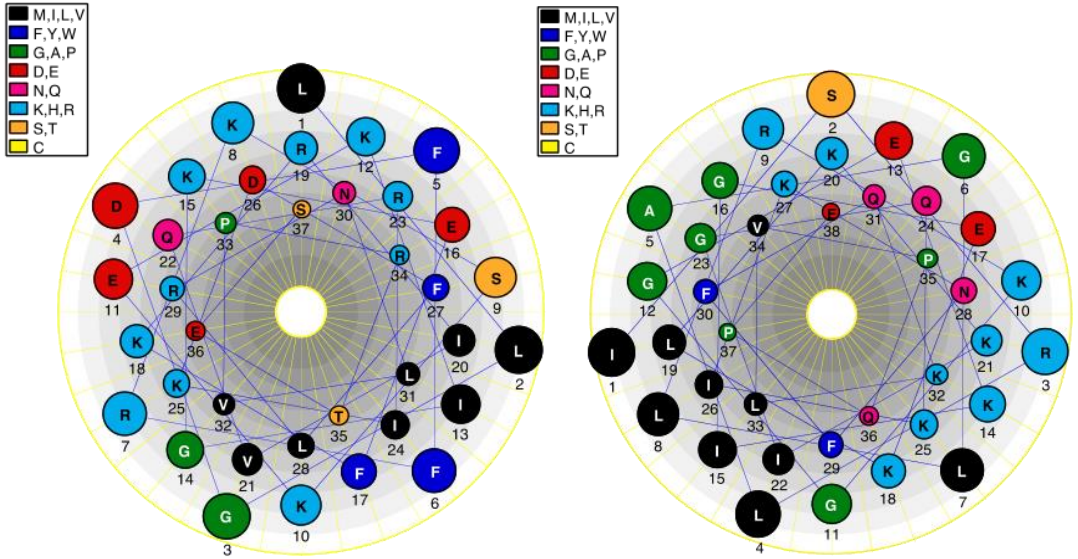
Dhand A, Bayer AS, Pogliano J et al. Use of antistaphylococcal β-lactams to increase daptomycin activity in eradicating persistent bacteremia due to methicillin-resistant *Staphylococcus aureus*: role of enhanced daptomycin binding. CID 2011:53:158-63.



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 are a critical component of mammalian innate immunity

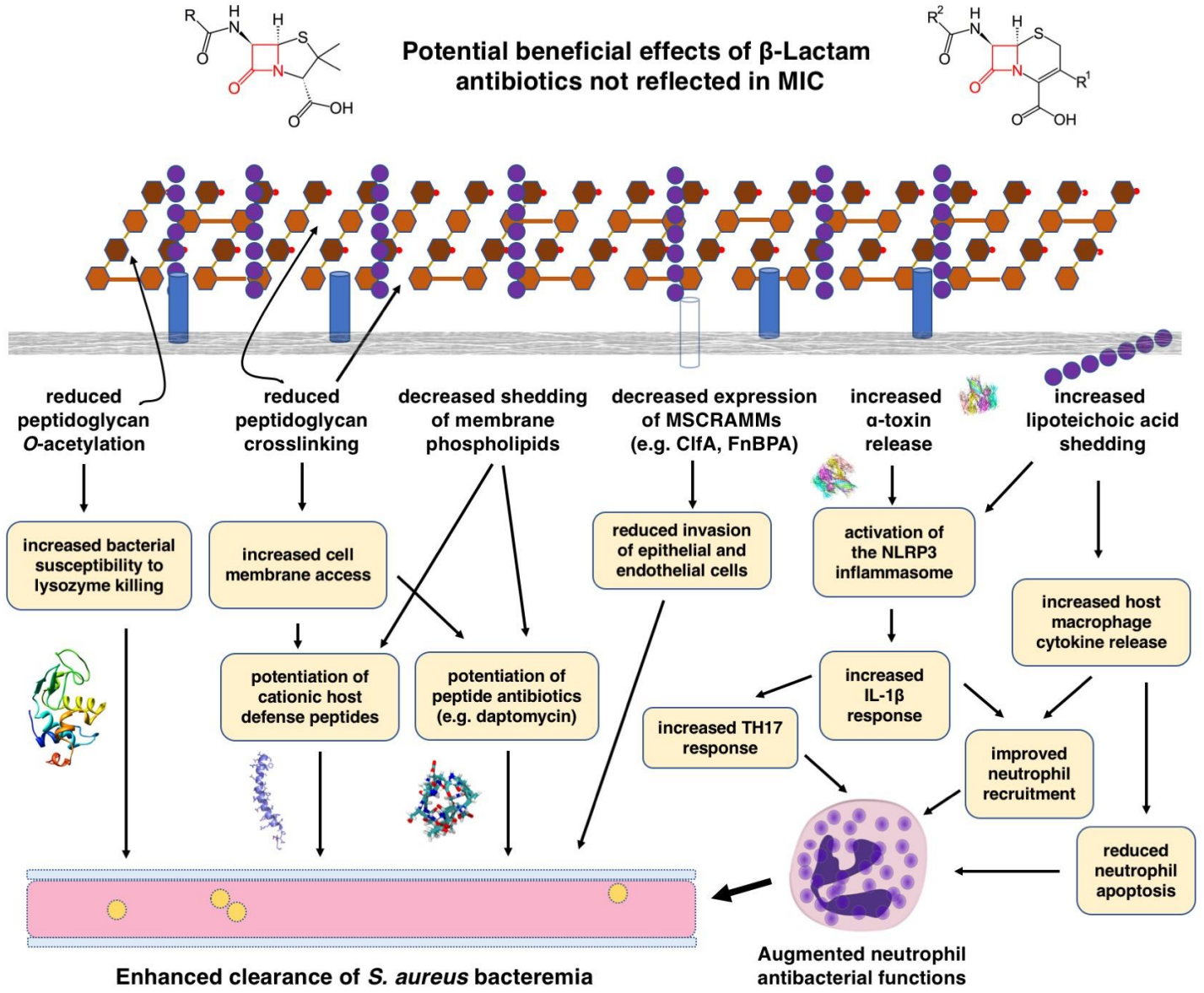
CATHELICIDIN



LL-37 (Human)

CRAMP **(Mouse)**

Nizet V. Curr Issues Mol Biol 8:11-26



Sakoulas G, Geriak M, Nizet V. Clin Infect Dis 2018 Jul 9. doi: 10.1093/cid/ciy557. [Epub ahead of print]

CAMERA-2 Study: A Rigorous Look At Combination Therapy for MRSA

- **MRSA Bacteremia**
- **August 2015 - July 2018, 352 adults, 27 sites ,4 countries (Australia, New Zealand, Singapore, and Israel)**
- **Monotherapy Vancomycin or Daptomycin VS plus an Combination with anti-staphylococcal β-lactam (flucloxacillin, cloxacillin, or cefazolin)**
- **Mention of daptomycin is highly deceiving**
- **Vast Majority of combination received vancomycin +flucloxacillin**
  - **171/174 (98%) combination group**
  - **178/178 (100%) monotherapy group**
- **Stopped Early Due to Safety Concerns**

Tong et al. JAMA Feb 2020

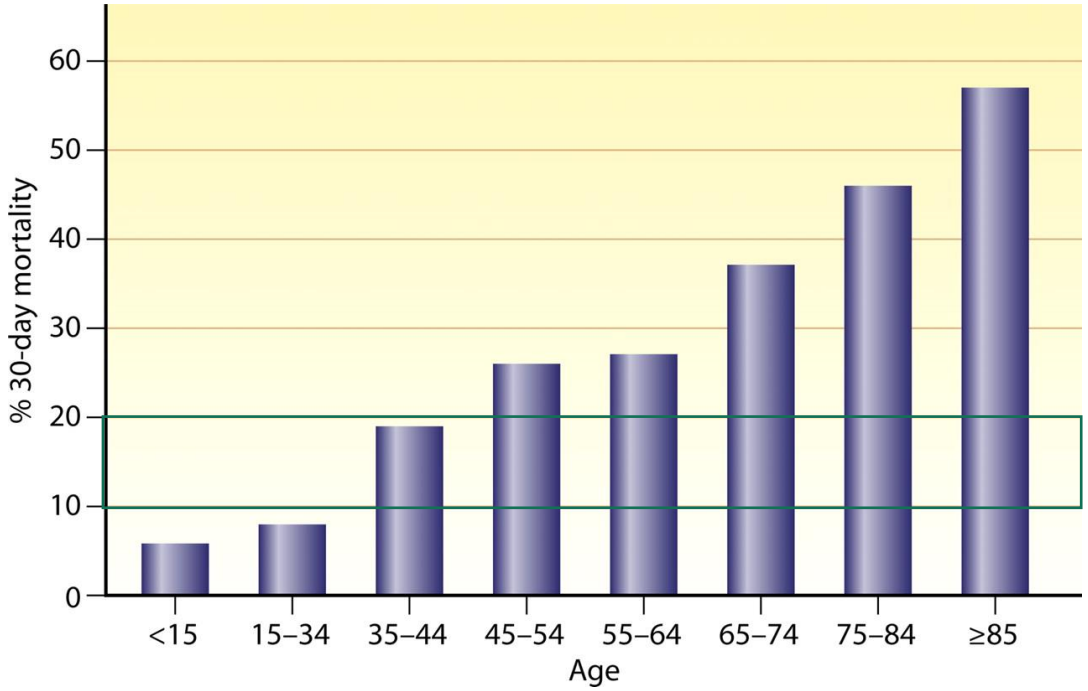
CAMERA-2 Study

- **30% developed acute kidney injury vs 9% in the standard therapy arm**
- **Increased 90-day mortality in the combination arm vs the standard therapy arm (21% vs 16%)**
- **Combination arm had less bacteremia persistence, consistent with CAMERA-1**
- **NOT REALLY THE END OF THE COMBINATION THERAPY DISCUSSION**

Tong et al. JAMA Feb 2020

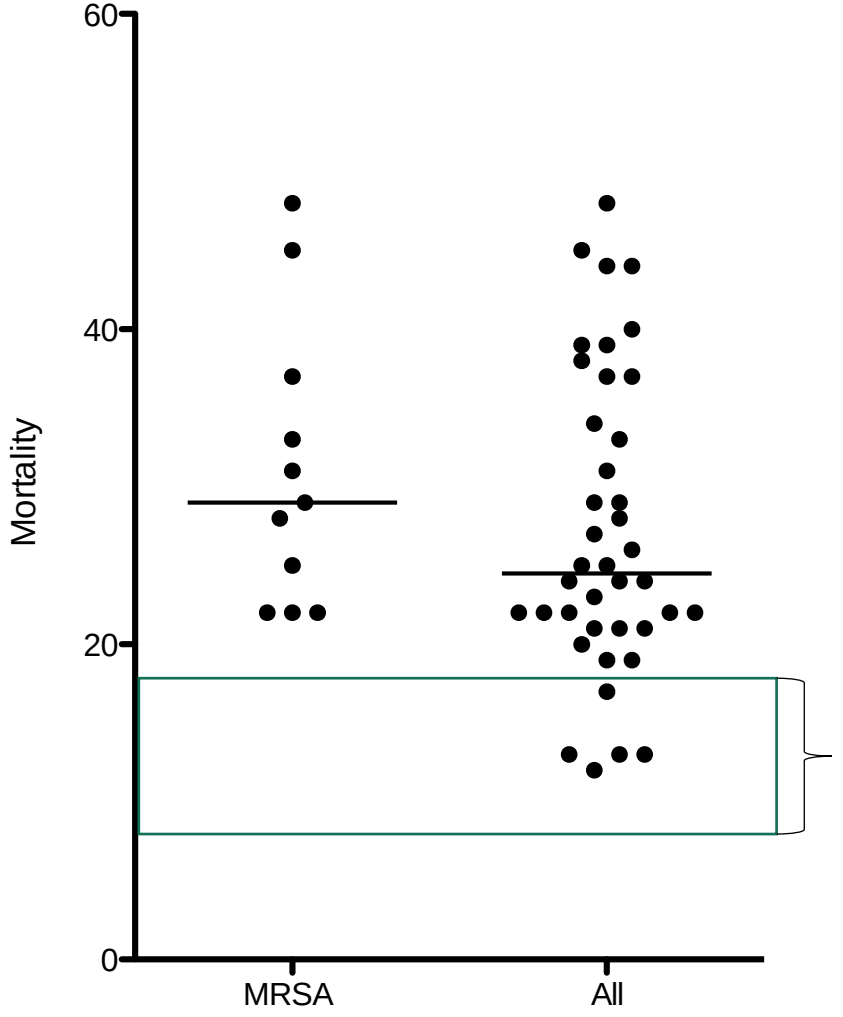
Putting the CAMERA Under the Microscope

Mortality in MRSA Bacteremia: Where Did CAMERA-2 Fall?



All-cause mortality[d]

Day 14	13/174 (7%)	13/170 (8%)	0.2 (-5.4,5.8)	0.95
Day 42	19/174 (11%)	25/170 (15%)	3.8 (-3.3,10.8)	0.29
Day 90	28/174 (16%)	35/170 (21%)	4.5 (-3.7,12.7)	0.28

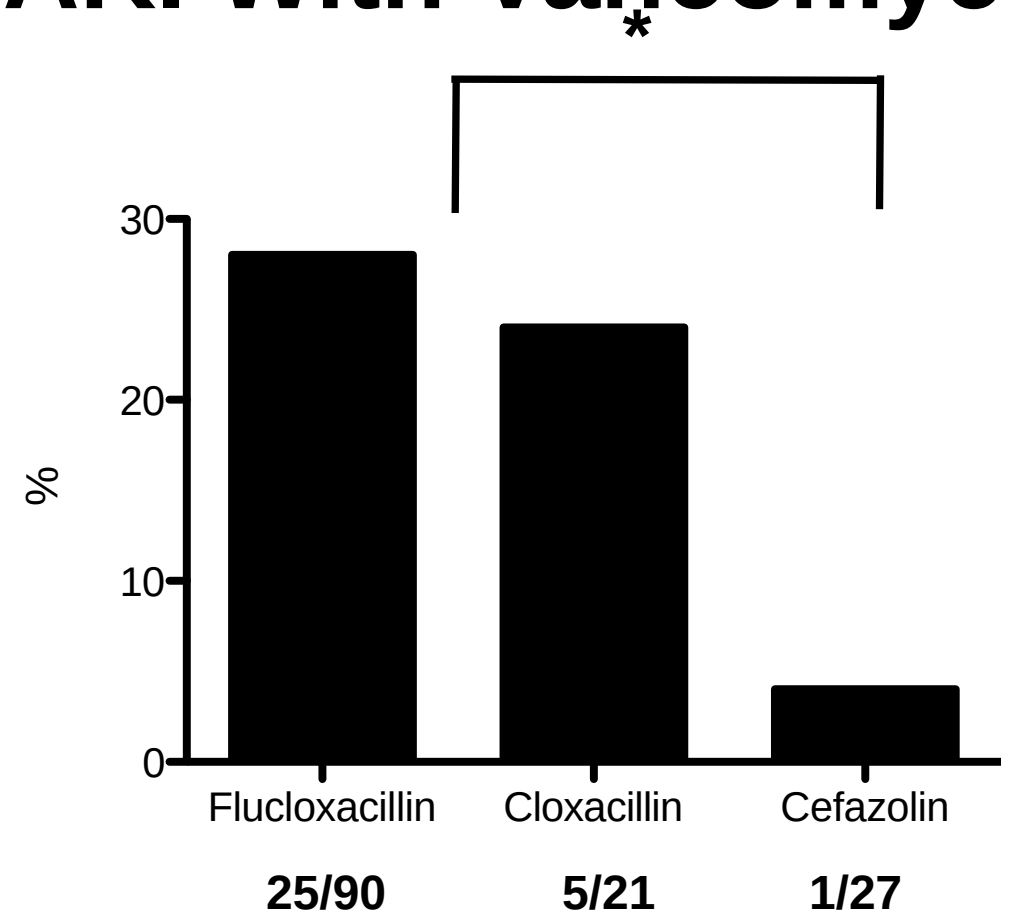


Van Hal S etal. Clin Microbiol Rev 2012. https://doi.org/10.1128/CMR.05022-11
Tong et al. 2020 Feb 11;323(6):527-537

CAMERA 2 Did Not Have the Study Population to Answer the Combination Therapy Question

Gandhi TN et al. 2020 Feb 11;323(6):515-516.doi: 10.1001/jama.2019.21472
Tong et al. 2020 Feb 11;323(6):527-537

The Real Take Home Message of CAMERA-2 AKI With Vancomycin and Beta-Lactams



P=0.008 Cefazolin vs Flucloxacillin+ Cloxacillin

P=0.007 Cefazolin vs Flucloxacillin

P= 0.07 Cefazolin vs Cloxacillin

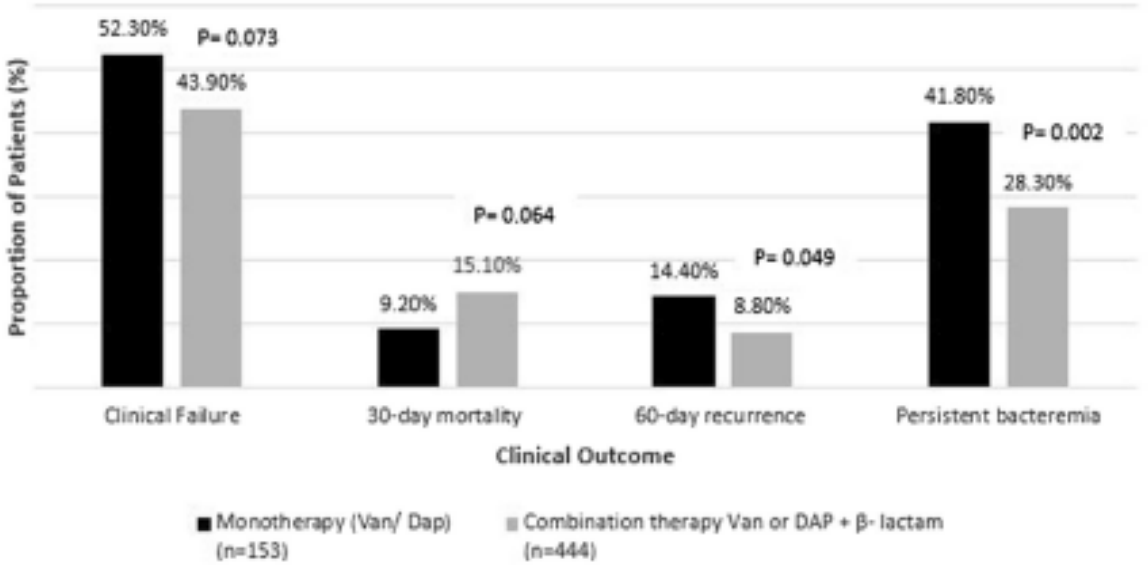
Tong et al. 2020 Feb 11;323(6):527-537

Infect Dis Ther
https://doi.org/10.1007/s40121-020-00292-8

ORIGINAL RESEARCH

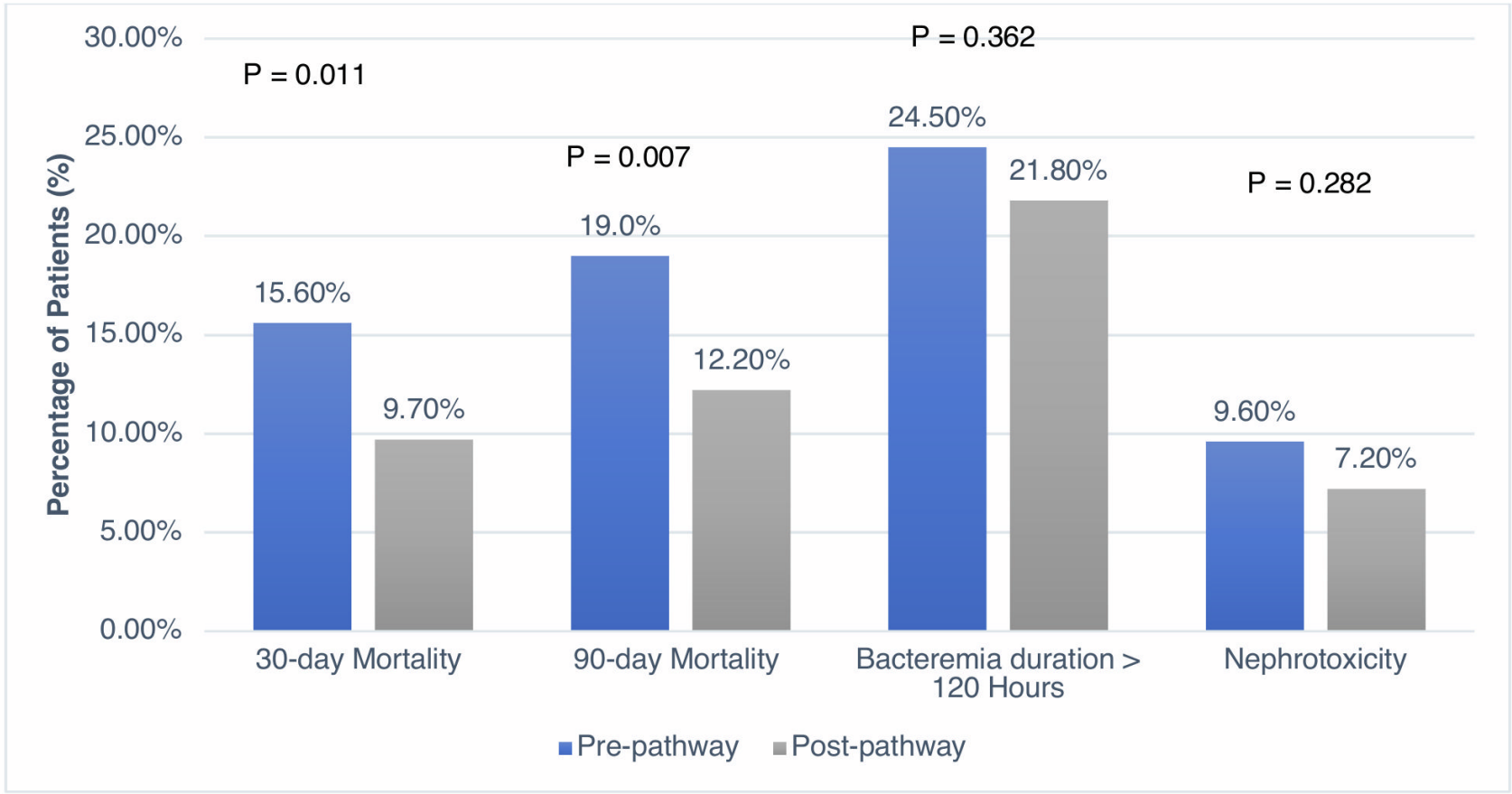
Monotherapy with Vancomycin or Daptomycin versus Combination Therapy with β-Lactams in the Treatment of Methicillin-Resistant *Staphylococcus Aureus* Bloodstream Infections: A Retrospective Cohort Analysis

Sara Alosaimy · Noor L. Sabagha · Abdalhamid M. Lagnf · Evan J. Zasowski · Taylor Morrisette · Sarah C. J. Jorgensen · Trang D. Trinh · Ryan P. Mynatt · Michael J. Rybak



- 153 Monotherapy, 444 Combination Therapy (Mostly with cephalosporins)
- 14% Nephrotoxicity In Both Groups (15% Pip/tazo, 3% Amp/Sulbactam)
- Multivariate logistic regression model predictive of clinical failure
  - Endocarditis 3.294 (2.115-5.132)
  - APACHE II Score 1.045 (1.029-1.072)
  - Combination Therapy 0.545 (0.364-0.817)
- Multivariate logistic regression model predictive of persistent bacteremia
  - Endocarditis 3.331 (2.132-5.205)
  - Source Control 1.236 (0.828-1.844)
  - Combination Therapy 0.597 (0.393-0.907)

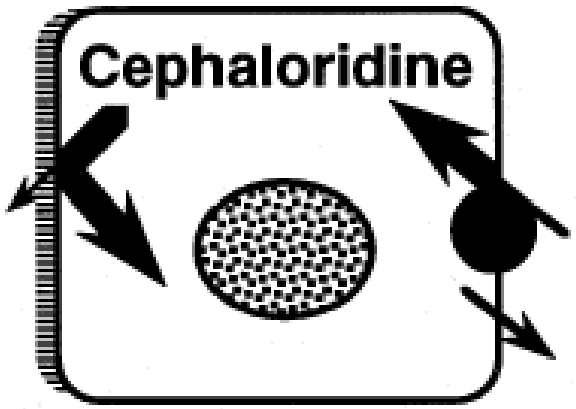
Comparison of Clinical Outcomes Between Patients in Pre-pathway or Post-pathway



Alosaimy S, Lagnf AM, Morrisette T, et al.Standardized Treatment and Assessment Pathway Improves Mortality in Adults with Methicillin-resistant *Staphylococcus aureus* Bacteremia: STAPH-Study. Open Forum Infect Dis. Accepted pending publication. April 2021.

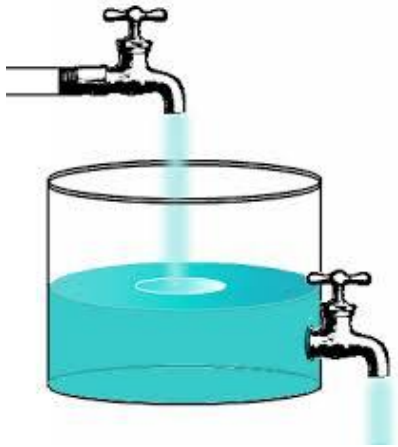
Proximal Tubular Cells

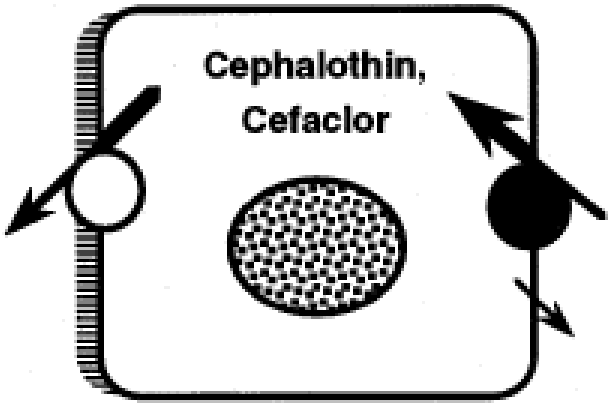
Nephrotoxic



TUBULAR LUMEN

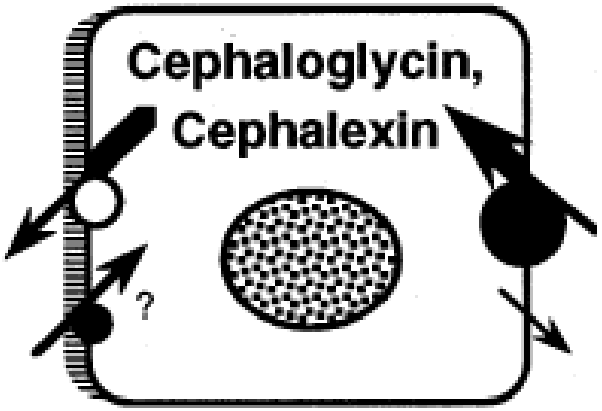
BLOOD





TUBULAR LUMEN

BLOOD



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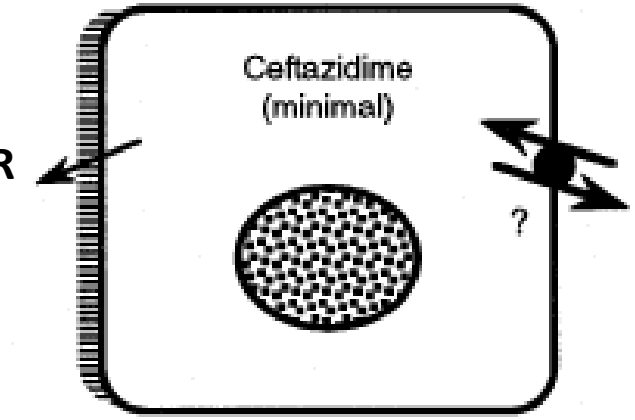

TUBULAR LUMEN

BLOOD

Pediatr Nephrol (1997) 11: 768–772

1521-009X/41/4/791–800$25.00
DRUG METABOLISM AND DISPOSITION
http://dx.doi.org/10.1124/dmd.112.049569
Drug Metab Dispos 41:791–800, April 2013
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Organic Anion Transporter 3 Interacts Selectively with Lipophilic β-Lactam Antibiotics

Aaron T. Wolman, Michael R. Gionfriddo, Gregory A. Heindel, Paran Mukhija, Sarah Witkowski, Ajay Bommareddy, and Adam L. VanWert

Department of Pharmaceutical Sciences, Nesbitt College of Pharmacy and Nursing, Wilkes University, Wilkes-Barre, Pennsylvania (A.T.W., G.A.H., P.M., S.W., A.B., A.L.V.); and Knowledge and Evaluation Research Unit, Clinical and Translational Sciences, Mayo Graduate School, Mayo Clinic, Rochester, Minnesota (M.R.G.)

- OAT-3 is important in renal and CSF beta-lactam transport
- Used cells expressing mouse and human OAT-3 to assess interactions with 26 beta-lactams
  - 12 inhibitors
  - 14 poorly interactive
- The beta—lactam that were inhibitors were also substrates
- OAT-3 interactive antibiotics were lipophilic (log partition coefficient +1.41 inhibitors vs -1.54 for non-inhibitors)
- Beta-lactams that interact with OAT-3 correlate with nephrotoxicity
  - Piperacillin 0.737
  - Ampicillin -0.873

AAC
Journals.ASM.org

Overton's Rule Helps To Estimate the Penetration of Anti-Infectives into Patients' Cerebrospinal Fluid

TABLE 1 CSF penetration versus physicochemical properties of anti-infectives in patients with uninflamed meninges

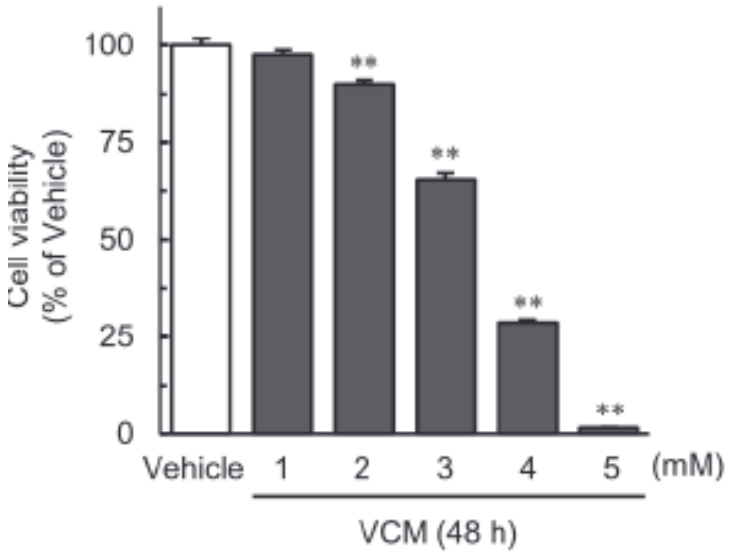
Drug	Molecular mass (g/mol)	Partition coefficient[a]		Protein binding (%)	Protein binding reference	AUC_{CSF}/AUC_{S}	$AUC_{CSF}/AUC_{S,free}$	Pharmacokinetic reference(s)
		Log $P_{O/W}$	Log $D_{O/W}$					
Penicillins								
Cloxacillin	436	2.53 ± 0.34[b]	−0.87[c]	93	68	0.0087	0.12	114
Piperacillin	518	1.88 ± 0.37[b]	−2.69[c]	25	118	0.034	0.045	87
β-Lactamase inhibitors								
Clavulanate	199	−1.98 ± 0.78[b]	−3.47[c]	26	67	0.037	0.05	F. Sörgel et al., unpublished data
Tazobactam	300	−1.70 ± 0.84[b]	−3.10[c]	21.5	118	0.106	0.135	87
Cephalosporins								
Cefotaxime	455	1.20 ± 0.89[b]	−2.47[b]	25.5	71	0.12	0.16	93
Ceftriaxone	555	−0.25 ± 1.12[b]	−4.43[c]	94.5	73	0.007	0.13	93
Ceftazidime	547	−1.21[d]	−2.07 (135)	14.5	72	0.057	0.067	90
Carbapenem meropenem	383	−3.13 ± 0.75[b]	−3.74[c]	2	7	0.047, 0.21, 0.25	0.048, 0.21, 0.26	61, 89

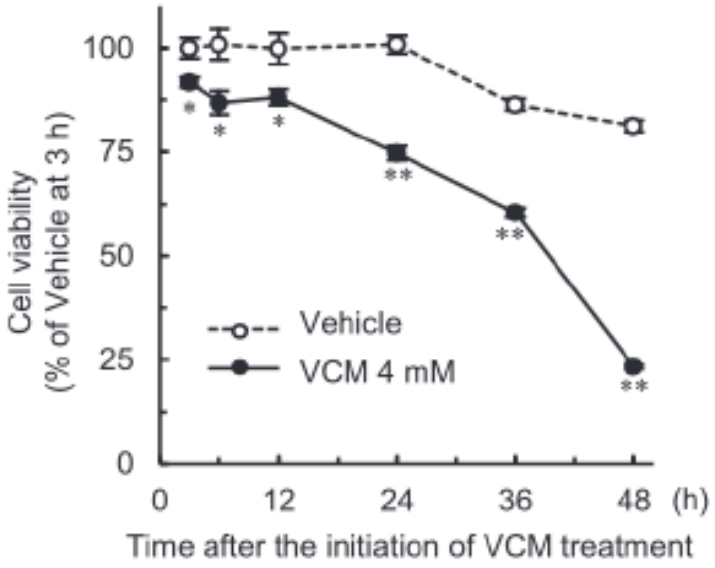
In 1900 Ernst Overton found entry of dyes through cell membranes of living cells predicted lipophilicity
This predicts beta-lactam CSF penetration (and likely proximal tubule accumulation and vanco-associated nephrotoxicity

Vancomycin induces reactive oxygen species-dependent apoptosis via mitochondrial cardiolipin peroxidation in renal tubular epithelial cells

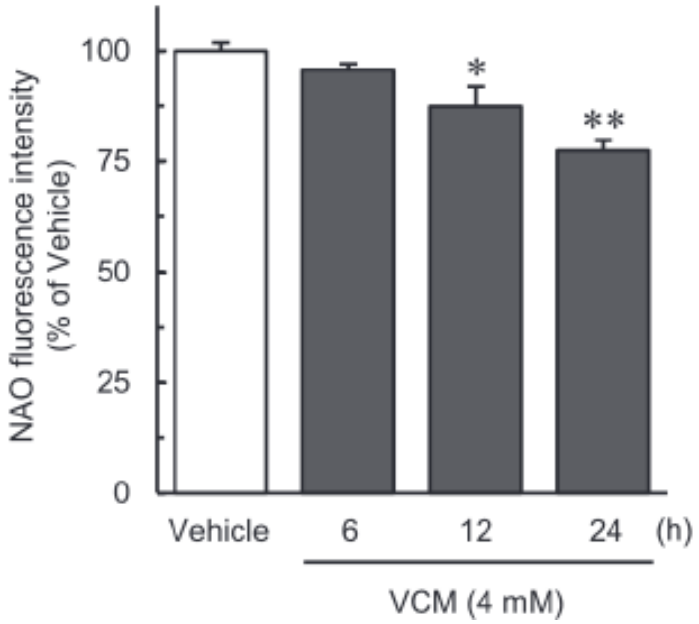
Yuya Sakamoto[a,b,1], Takahisa Yano[c,,1], Yuki Hanada[a], Aki Takeshita[a], Fumika Inagaki[a], Satohiro Masuda[b,c], Naoya Matsunaga[d], Satoru Koyanagi[d], Shigehiro Ohdo[a]

Concentration and Time-Dependent Vancomycin Cytotoxicity





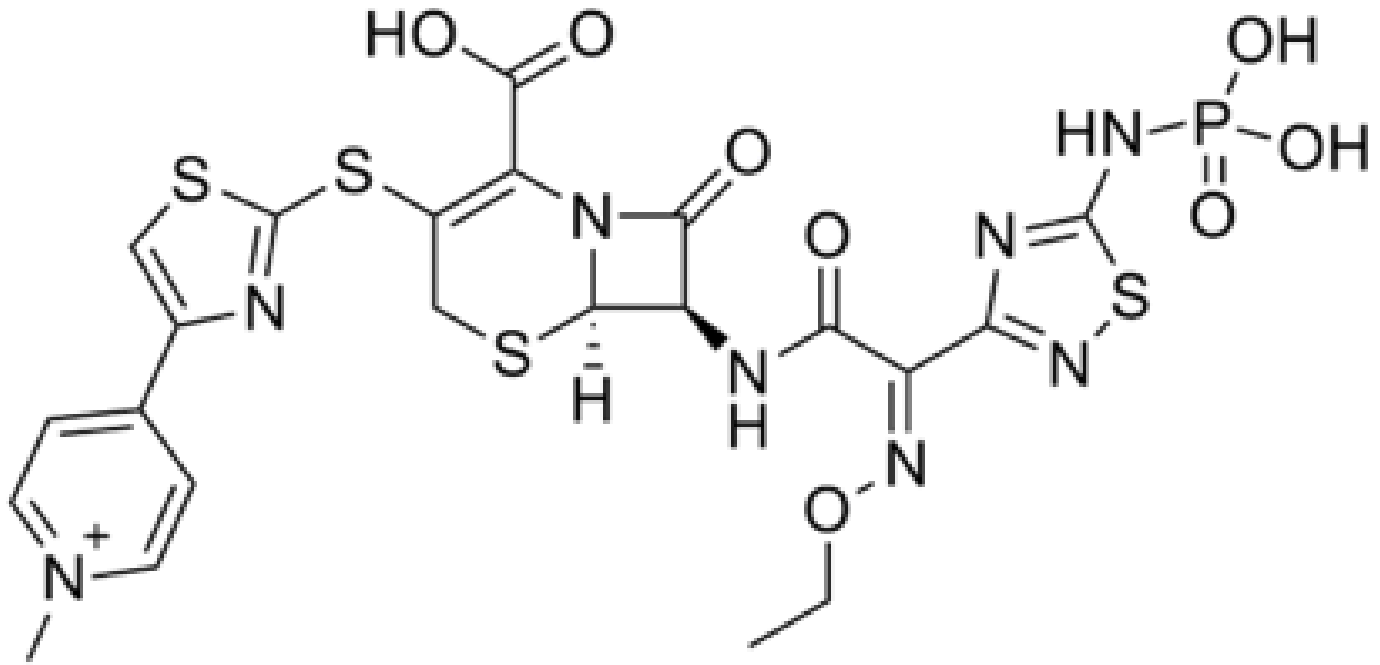
Vancomycin Mitochondrial Cardiolipin Peroxidation



Porcine Proximal Tubular Epithelial Cell line LLC-PK

http://dx.doi.org/10.1016/j.ejphar.2017.02.025

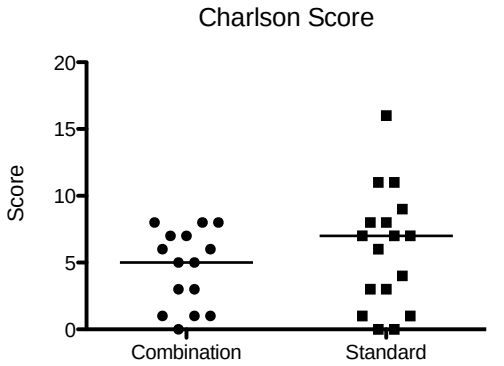
Using Ceftaroline as the Beta-Lactam in Daptomycin Combination Therapy in MRSA Bacteremia

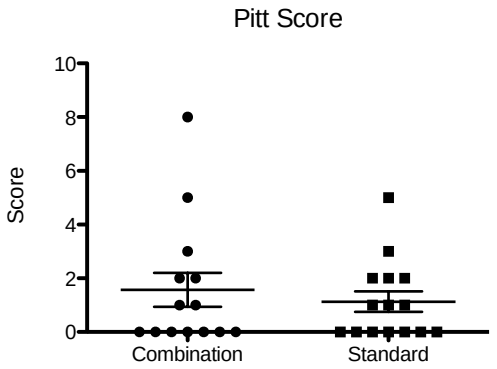


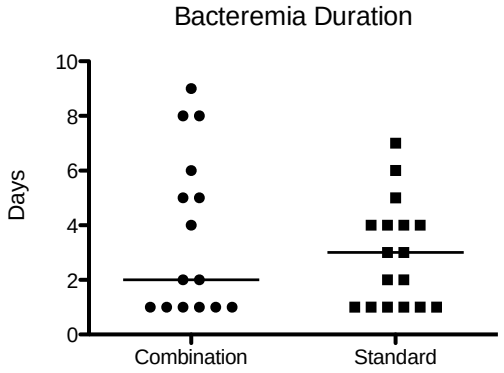
Case Series Salvage Regimen
Sakoulas G et al. Clin Ther 2014; 36 (10): 1317-1333

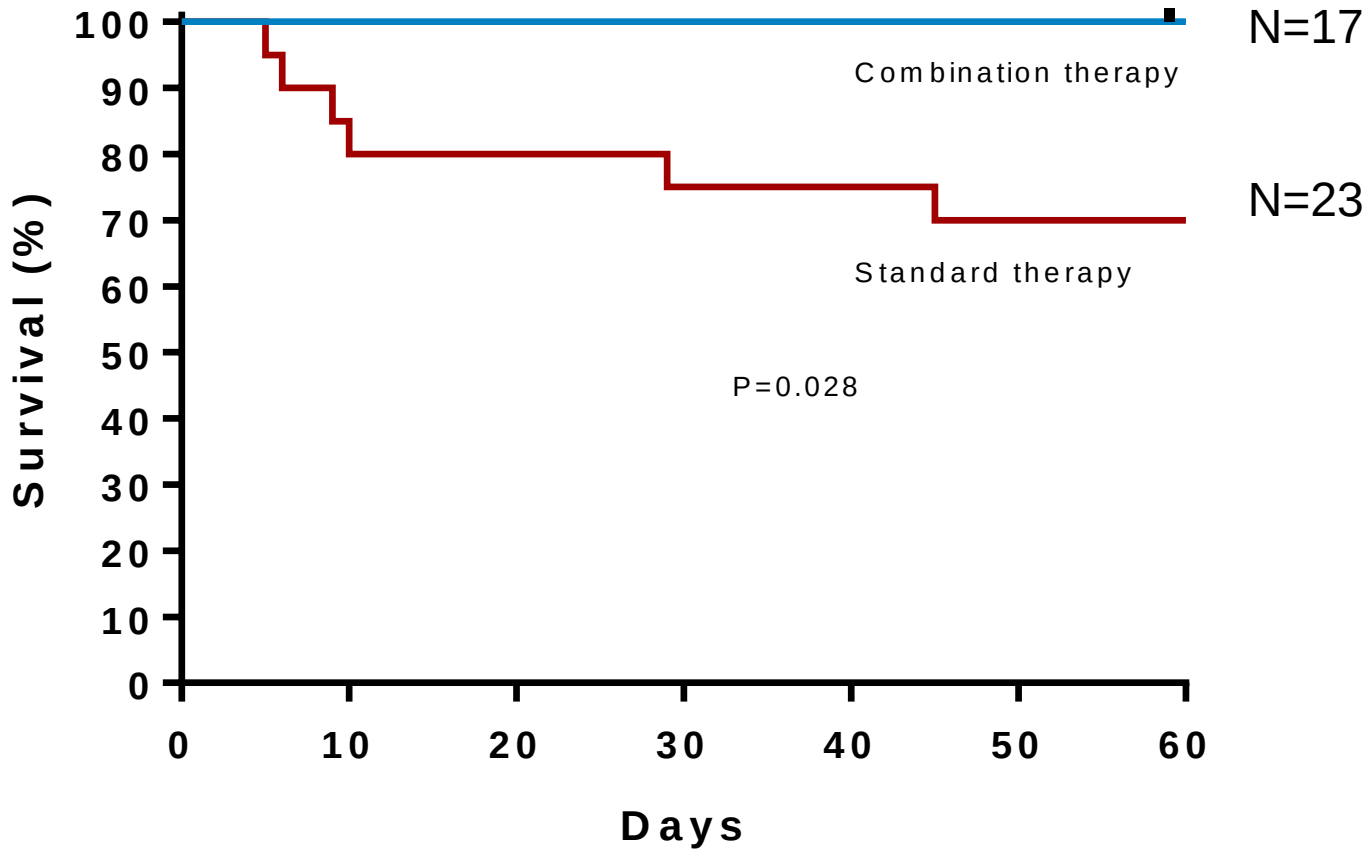
Antimicrobial Stewardship Pharmacist Emoji

Prospective Randomized Study of Vancomycin vs DAP+CPT in MRSA Bacteremia









Geriak M et al. Antimicrob Agents Chemother 2019 Apr 25;63(5):e02483-18.

Expensive

- **Limitations on Disposition**
- **Third Party Payors**
- **Subacute Nursing Facilities**

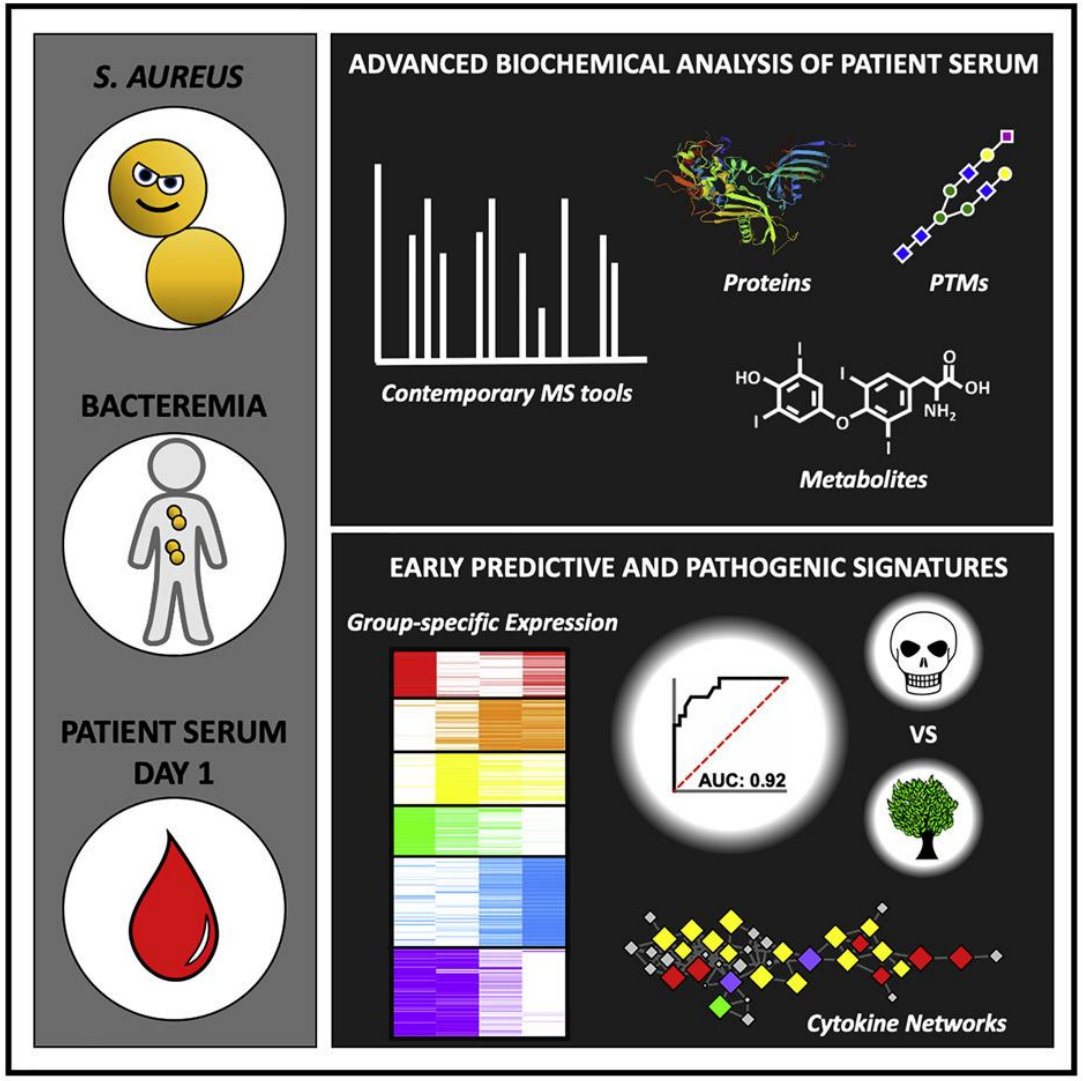
Cumbersome

- **Many Doses**

More drugs=more risk of medical errors and AE's

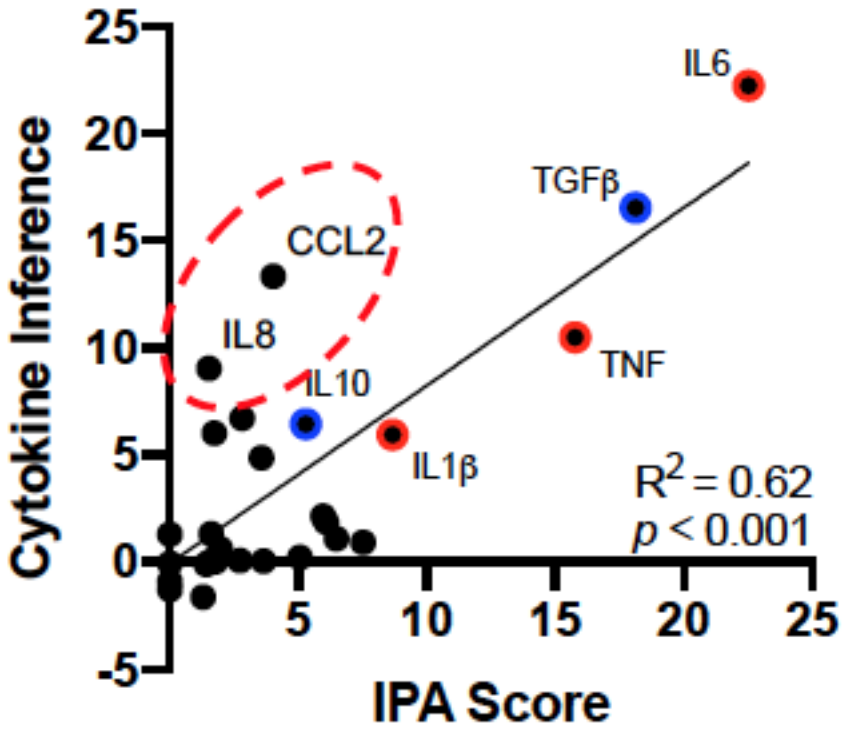
CAN DAP+CPT BE TARGETTED TO HIGH-RISK PATIENTS?

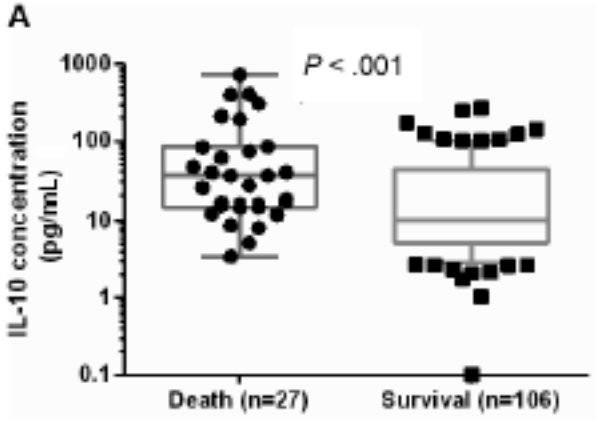
HOW TO IDENTIFY HIGH RISK PATIENTS UP FRONT?

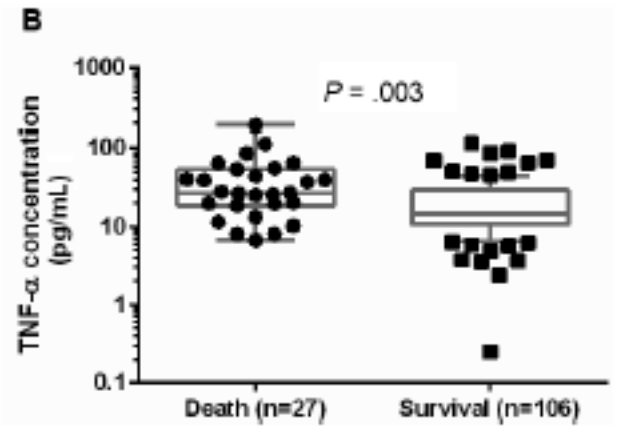


Wozniak JM, Mills RH, Olson J, Caldera JR, Sepich-Poore GD, Carrillo-Terrazas M, Tsai CM, Vargas F, Knight R, Dorrestein PC, Liu GY, Nizet V, Sakoulas G, Rose W, Gonzalez DJ. Mortality Risk Profiling of Staphylococcus aureus Bacteremia by Multi-omic Serum Analysis Reveals Early Predictive and Pathogenic Signatures. Cell. 2020 Sep 3;182(5):1311-1327.e14.

Cytokines Predicting Mortality in SaB







	ROC AUC	P Value
IL–8	0.832	<0.001
CCL2	0.814	<0.001
IL–6	0.785	<0.001

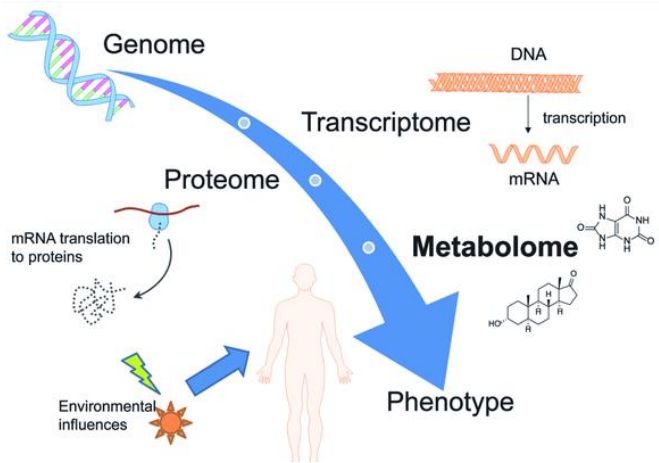
Wozniak JM, Mills RH, Olson J, Caldera JR, Sepich-Poore GD, Carrillo-Terrazas M, Tsai CM, Vargas F, Knight R, Dorrestein PC, Liu GY, Nizet V, Sakoulas G, Rose W, Gonzalez DJ. Mortality Risk Profiling of Staphylococcus aureus Bacteremia by Multi-omic Serum Analysis Reveals Early Predictive and Pathogenic Signatures. Cell. 2020 Sep 3;182(5):1311-1327.e14.

- Multi-omic analysis of *S. aureus* bacteremia captured strong mortality biomarkers

- Clustering of proteins and metabolites highlights host metabolic dysfunction

IGF System

Acyl-carnitines ↑ Thyroxine ↓ Ternary Complex → Binary Complex



- Therapeutic interventions of host metabolism can influence survival

Wozniak JM, Mills RH, Olson J, Caldera JR, Sepich-Poore GD, Carrillo-Terrazas M, Tsai CM, Vargas F, Knight R, Dorrestein PC, Liu GY, Nizet V, Sakoulas G, Rose W, Gonzalez DJ. Mortality Risk Profiling of Staphylococcus aureus Bacteremia by Multi-omic Serum Analysis Reveals Early Predictive and Pathogenic Signatures. Cell. 2020 Sep 3;182(5):1311-1327.e14.

Induction->Consolidation Paradigm

- MRSA Bacteremia is a dynamic condition, so therapy must be dynamic
- Combination therapy at the beginning to de-escalation to monotherapy
- One small study of 30 patients rx with combo: 15 de-escalated to monotherapy, 15 remained on combination
  - Median bacteremia duration preceding CPT 6 days
  - 1 recurrence in monotherapy; 2 30-day readmission monotherapy; 1 death monotherapy, 3 deaths combo
  - Ahmad O et al. Infect Dis Ther. 2020 Mar;9(1):77-87
- Factors to consider:
  - Bridge to definitive source control (eg. left-sided IE with CNS emboli)
  - Bacteremia cleared 5-7 days
  - Time to discharge and disposition (home vs SNF vs LTAC)
  - CRP reduced >50%

Beta-Lactams in MRSA

- Important adjuncts to daptomycin backbone
- Some are using vancomycin backbone with improved outcomes
- Vancomycin + cephalosporin > Vancomycin + Penicillin due to nephrotoxicity
- How do anti-staphylococcal beta-lactams compare to ceftaroline when added to daptomycin ?
- **COMBINATION THERAPY IS NOT REQUIRED FOR THE WHOLE DURATION**
  - **De-escalate to vancomycin (eg LTAC/SNF)**
  - **Single therapy**
  - **CRP reduction >50%**

Daptomycin+Fosfomycin

- Randomized 1:1 open label 18 medical centers in Spain
- Daptomycin 10 mg/kg IV QD+ Fosfomycin 2g IV Qhr vs Daptomycin 10 mg/kg alone
- Treatment success day 7, day 42
- 6 weeks: Combo 40/74 (54%) vs Mono 34/81 (42%)
- 7 days: Combo 69/74 (93%) vs Mono 62/81 (77%)
- Combo lower rate of microbiological failure at 6 weeks: 0 vs 9 patients (p=0.009)

Open Forum Infectious Diseases, Volume 5, Issue suppl_1, November 2018, Page S760,

J Antimicrob Chemother
doi:10.1093/jac/dkaa144

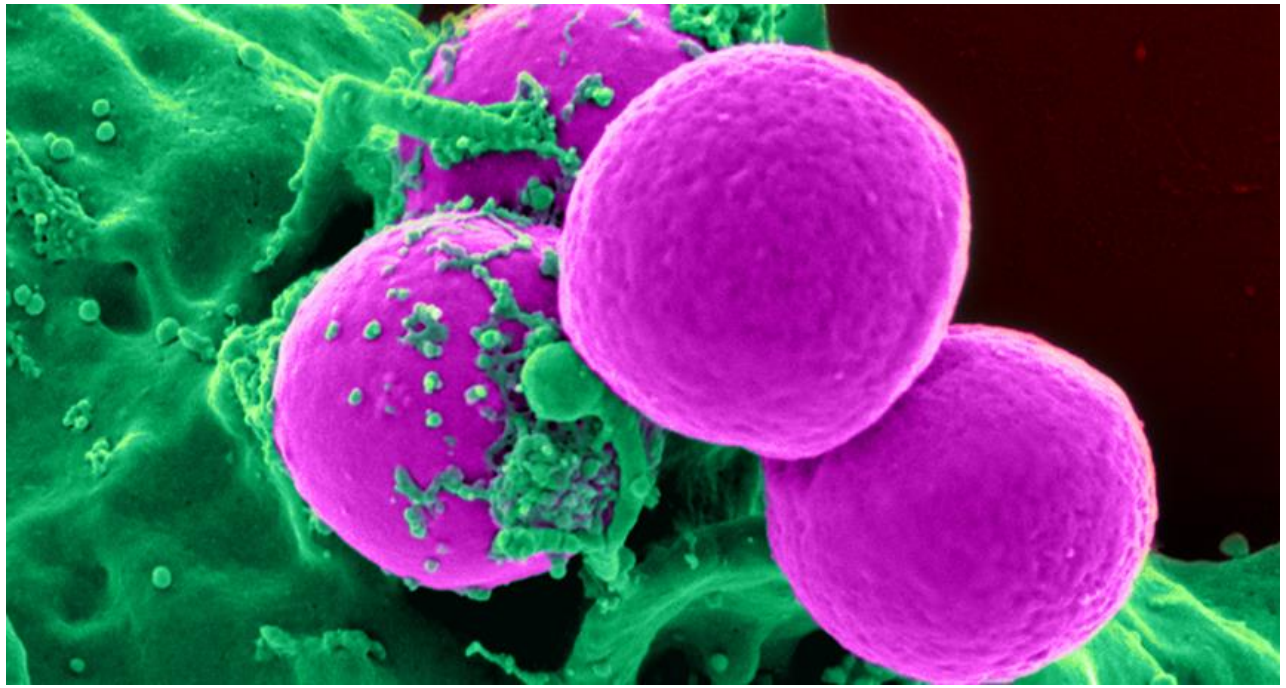
Journal of Antimicrobial Chemotherapy

Combination therapy with rifampicin or fosfomycin in patients with *Staphylococcus aureus* bloodstream infection at high risk for complications or relapse: results of a large prospective observational cohort

Siegbert Rieg [1*], Angela Ernst [2], Gabriele Peyerl-Hoffmann[1], Insa Joost[3], Johannes Camp[1], Martin Hellmich[2], Winfried V. Kern[1], Achim J. Kaasch[3]† and Harald Seifert[4,5]†

- 578 patients with high-risk SaB, 313 (54%) received combination therapy
- Evaluated composite outcome of 90-day all-cause mortality plus rates of late complications at 180 days
- 242 received combination with rifamycin; 58 with IV fosfomycin.
- Combination therapy better composite outcome (hazard ratio, 065; 95% confidence interval, 0.46–0.92).
- Primary benefit was seen in patients with biomedical devices (HR, 0.53; 95% CI, 0.35–0.79).
- Fosfomycin and rifampicin had similar outcome benefits

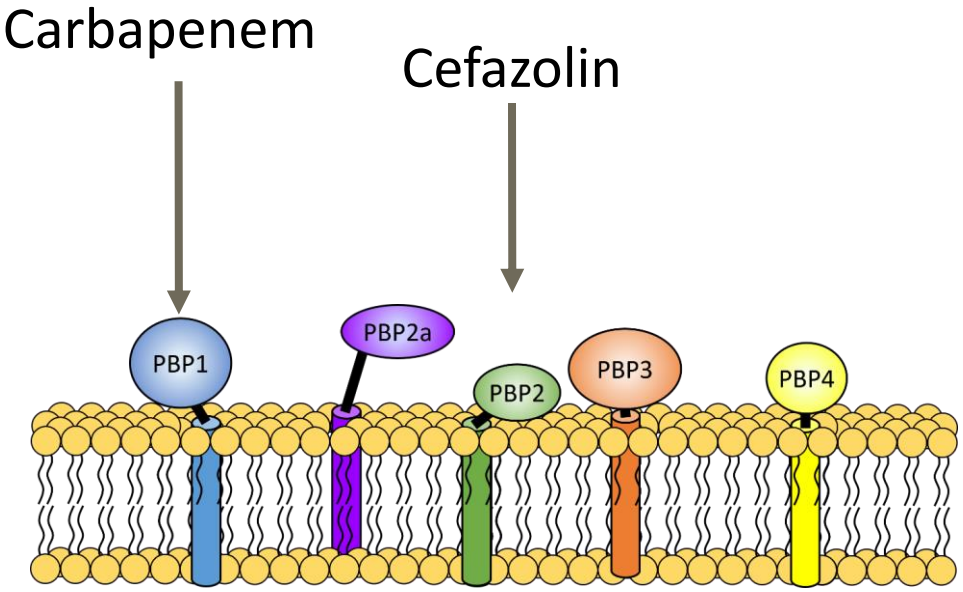
REFRACTORY MSSA BACTEREMIA



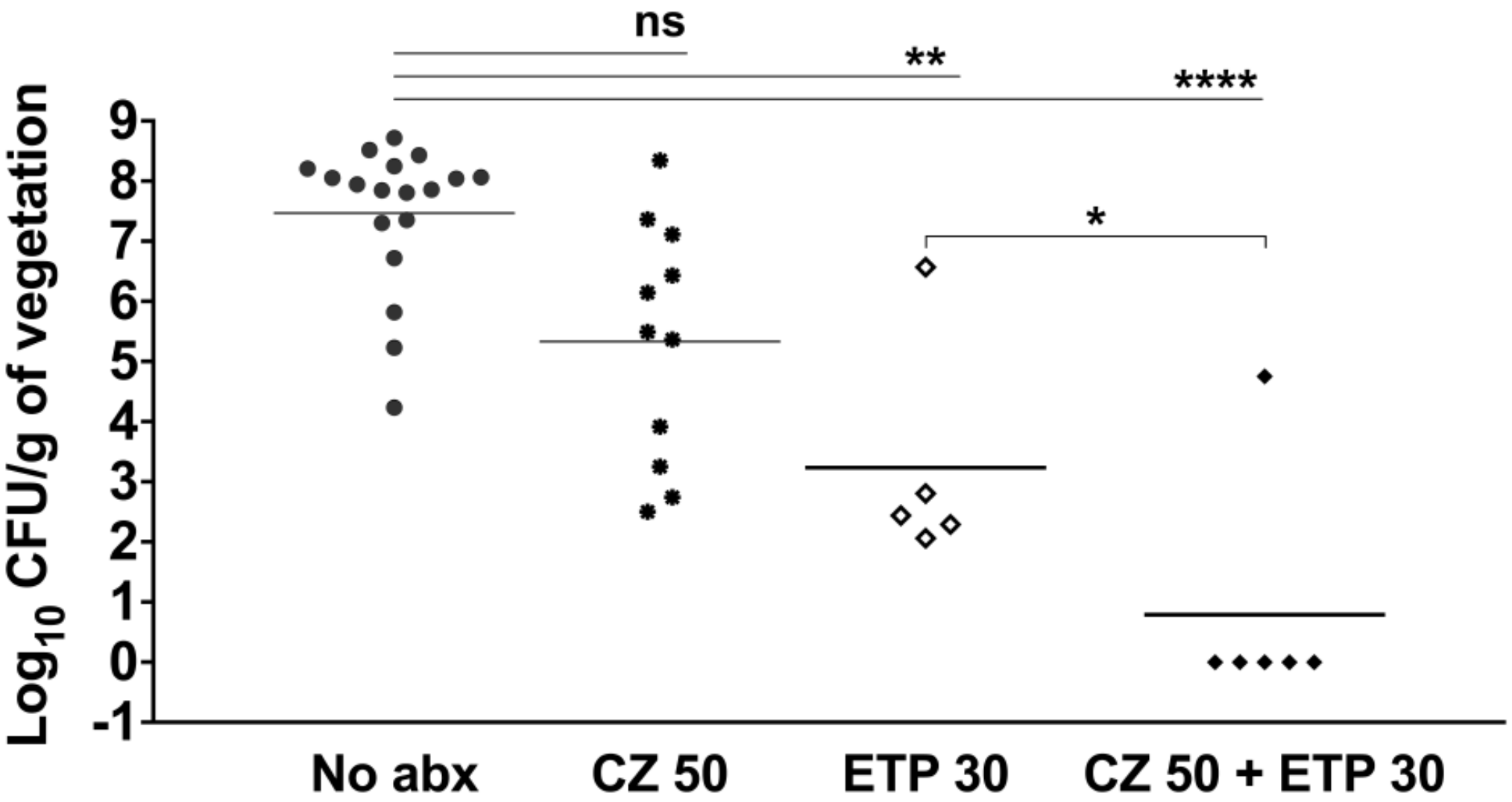
- **32 yo IVDA but otherwise healthy admitted with worsening fevers x 1 week**
- **Accompanied by SOB, lethargy**
- **Found to have leukocytosis, tachycardia, hypotension, admitted to ICU**
- **Vancomycin+ceftriaxone for 1st 24 hrs**
- **Blood Cx GPC->ID called->Ceftaroline 600 mg iv q8 hr+ Dapto 8 mg/kg/24 hr x 24 hr**
- **Verigene Show MSSA in 24 hrs->Nafcillin 2g iv 4 hr**
- **Imaging Chest, Abdomen, Pelvis: Multifocal pneumonia suggestive of septic pulmonary emboli, some early cavitation**
- **TEE shows 3.2 cm tricuspid valve vegetation**
- **Blood Cultures Remain + Despite 5 days nafcillin**
- **Ertapenem 1g iv q24 hr + cefazolin 2g iv q8hr →Blood cultures clear in 24 hrs!!**
- **Angiovac was performed→Partial success in debulking the Tricuspid Valve**
- **Signed out AMA after 4 weeks in the hospital**

MEDICAL THERAPY: OPTIONS

Consider DAP +Beta-lactam But May Be Antagonistic in MSSA



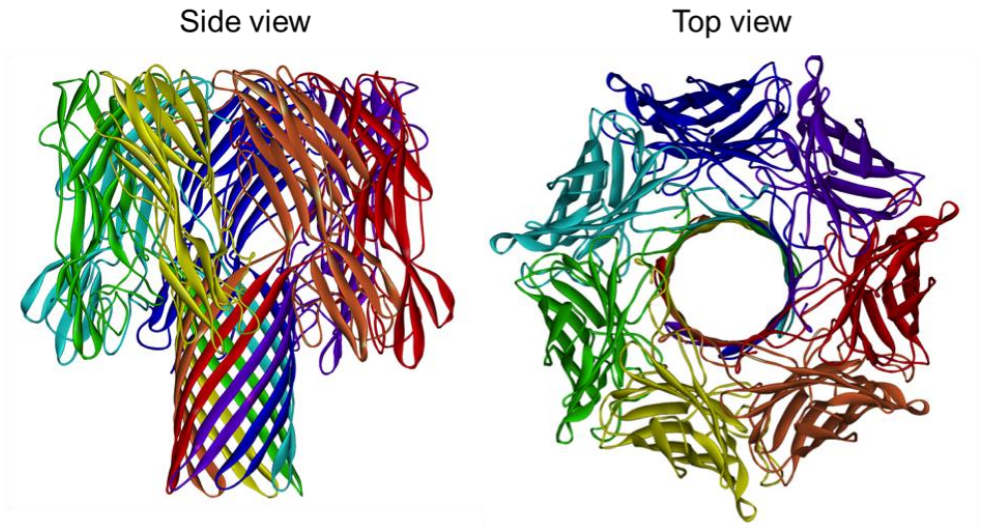
MSSA RAT ENDOCARDITIS MODEL



Ulloa E et al. Clin Infect Dis 2020 Sep 12;71(6):1413-1418

FUTURE THERAPY COMBINATIONS

- Not necessarily with two antibiotics
- Host response therapies
- Virulence Neutralization
- Repurposing Drugs Used by Other Specialists



MAD-ID
MAKING A DIFFERENCE
IN INFECTIOUS DISEASES®

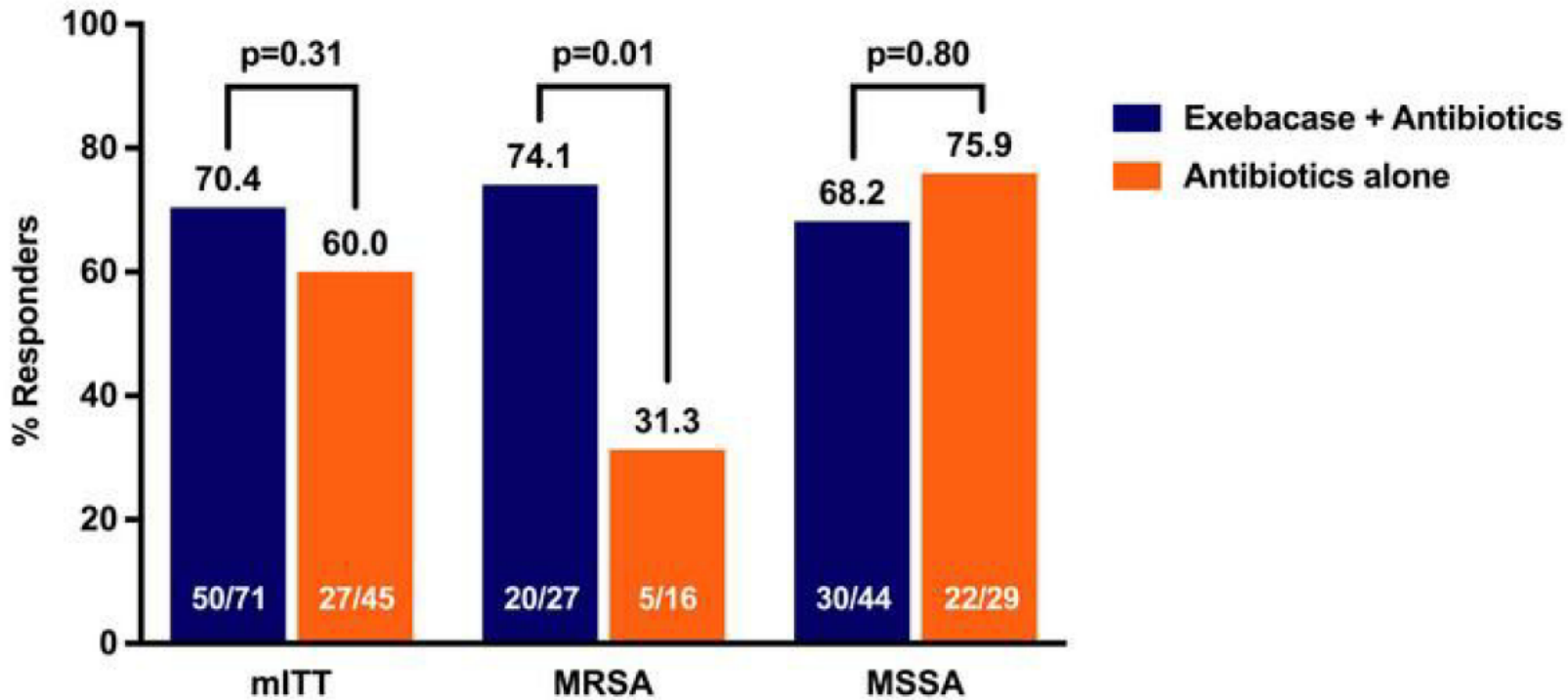
Exebacase for patients with *Staphylococcus aureus* bloodstream infection and endocarditis

Vance G. Fowler Jr., … , Pamela S. Douglas, Cara Cassino

J Clin Invest. 2020;130(7):3750-3760. https://doi.org/10.1172/JCI136577.

Clinical Medicine | Clinical trials | Infectious disease

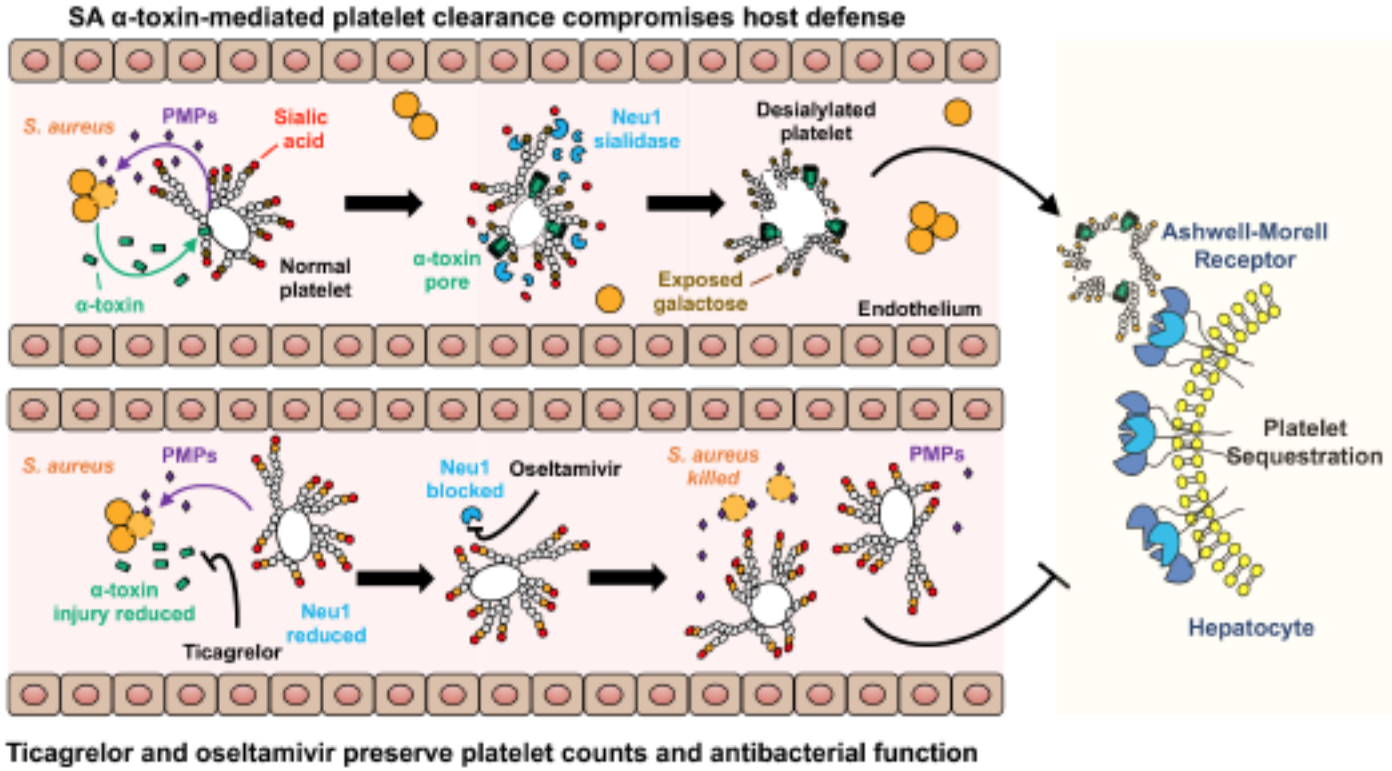
Graphical abstract



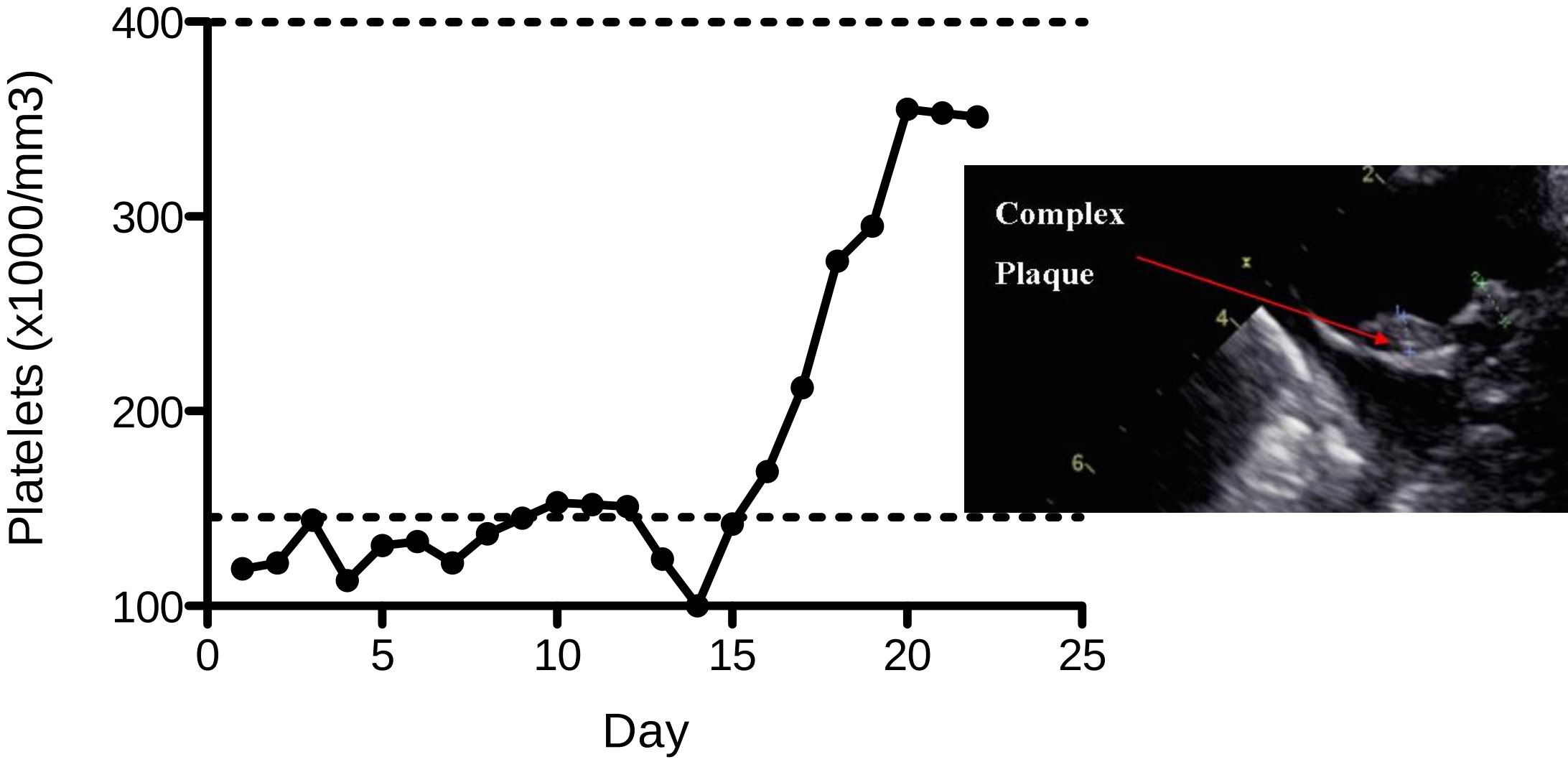
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

Repurposed Drugs Block Toxin-Driven Platelet Clearance by the Hepatic Ashwell-Morell Receptor to Clear *Staphylococcus aureus* Bacteremia

Josh Sun[1,2,3*], Satoshi Uchiyama[1*], Joshua Olson[1], Yosuke Morodomi[4], Ingrid Cornax[1], Nao Ando[1], Yohei Kohno[1], May M. T. Kyaw[1], Bernice Aguilar[1], Nina M. Haste[1,2,3], Sachiko Kanaji[4], Taisuke Kanaji[4], Warren E. Rose[5], George Sakoulas[2], Jamey D. Marth[6,7], Victor Nizet[1,2,3 †]



A CASE OF MSSA BACTEREMIA AND PLATELELTS

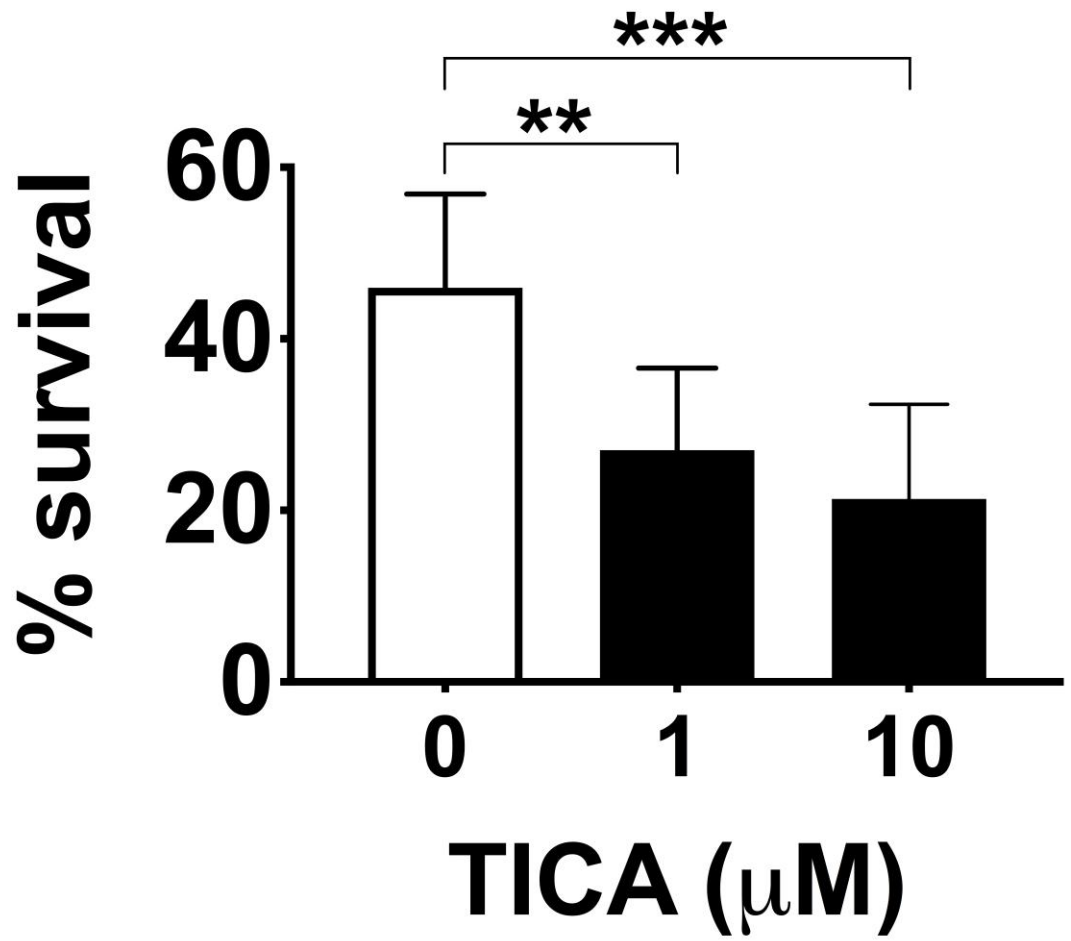


Ticagrelor Therapy **++++++++++++** **++++++++++++++++>> 90 day**

MSSA Bacteremia **++++++++++**

Ulloa E et al. J Infect Dis. 2021 May 9:jiab146. doi: 10.1093/infdis/jiab146.

Platelet Killing of *S. aureus*



Ulloa E et al. J Infect Dis. 2021
May 9:jiab146. doi: 10.1093/infdis/jiab146

Figure 1. Ticagrelor (TICA) boosts the bactericidal activity of platelets, frontline components of innate immunity, to kill methicillin-susceptible *S. auerus in vitro* at physiological attainable concentrations (1μM).

Summary and Conclusions

- Clearly in some more difficult cases of S. aureus bacteremia, combination therapy is better than monotherapy (membrane+cell wall agent; complementary beta-lactams)
- As in most cases of optimal therapy, benefit declines with delays in use
- Unfortunately, days go by until difficult cases define themselves during which the patient's clinical status and prognosis may decline
- An important goal of future studies will be the a priori identification of challenging cases before they become challenging
- Concept of 'Induction' and 'Consolidation' Phases of Treatment for SaB
  - Beginning/Middle/'Mop up' Regimens
- Future therapies will need to consider host-virulence factor attenuation
- Lysin Therapy
- Phage Therapy

Questions to Consider…Answers

- Why Talk About Combination Antibiotic Therapy to Treat Bacterial Infections?
  - Some infections are treated more successfully when using multiple antibiotics (high inoculum size, host level of immunosuppression)
  - Monotherapy Infection Treatment Paradigms Were Established At A Time With More Immunologically Robust Hosts
- Do we use combination therapy to treat some infections currently?
  - Routine in malaria, HIV, Tuberculosis, Early Fungal Meningitis
- If so, why is it such a big deal when the concept is introduced to clinicians?
  - Treatment and diagnostic paradigms that are decades old are very difficult to change
  - Identifying the subset of infections that benefit with 2 vs 1 antibiotics are difficult in broad clinical trials (granularity issue)
  - Antibiotic drug acquisition costs
  - Concern for resistance when using antibiotics
- Why are serious *S. aureus* infections a good example where combination antibiotic therapy should be considered?
  - High inoculum bloodstream infections do not respond well to single therapy
  - Adjunctive antibiotics may potentiate immune clearance
  - Small low-powered study was able to show a mortality benefit

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