GCC AMR Conference 2024

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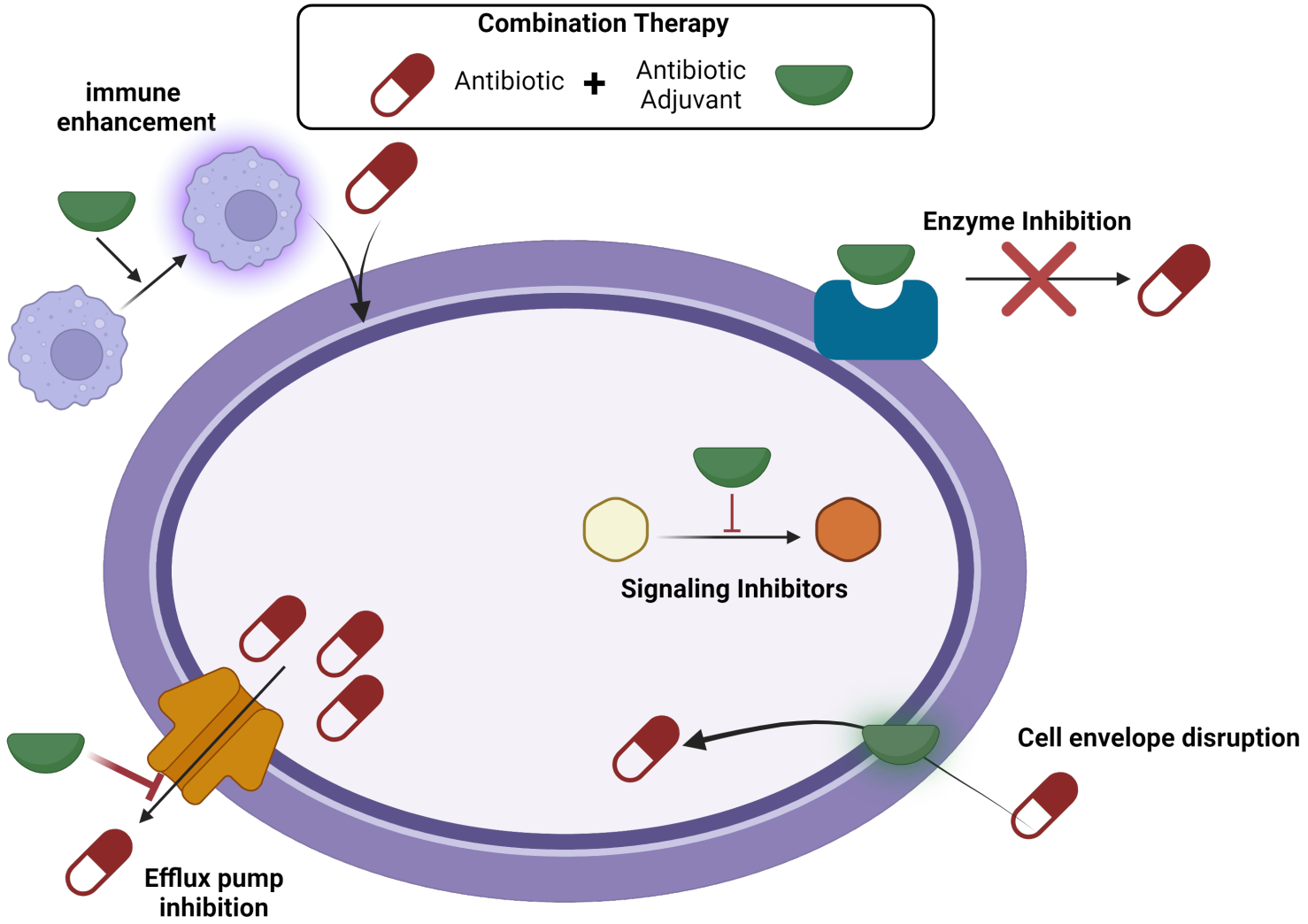
UNIVERSITY OF WISCONSIN–MADISON

Novel Drug Combinations *and Mechanisms* to Address Antimicrobial Resistance

Warren Rose, PharmD, MPH, FIDSA
Associate Professor

Combination Therapy Approaches for AMR

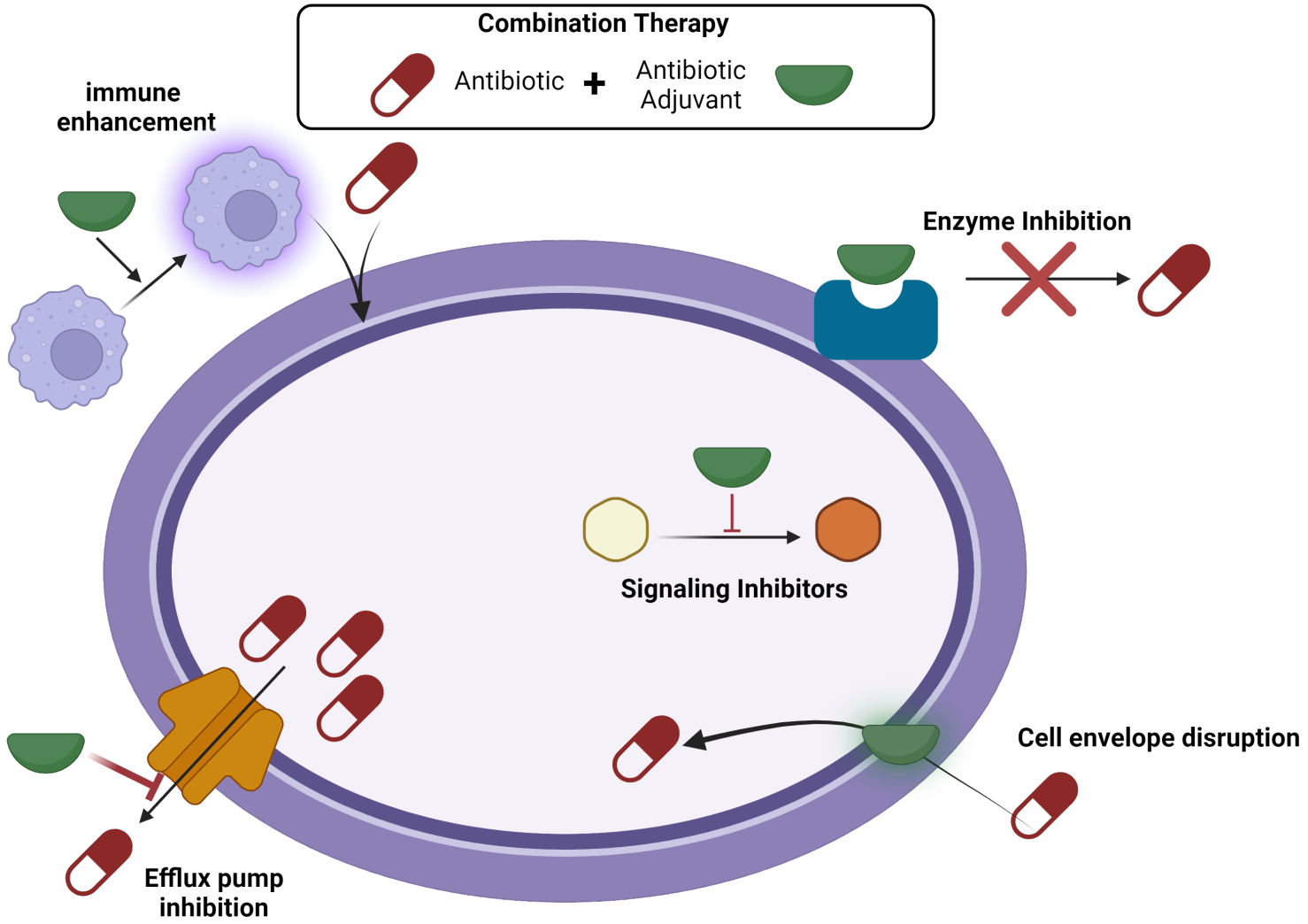


Drug Repurposing e.g.

Fosfomycin
Minocycline
Daptomycin
Oritavancin
β-lactams
Ketoconazole
Zidovudine
Azithromycin

Adapted from Theranostics 2021; 11(10):4910-4928

Combination Therapy Approaches for AMR



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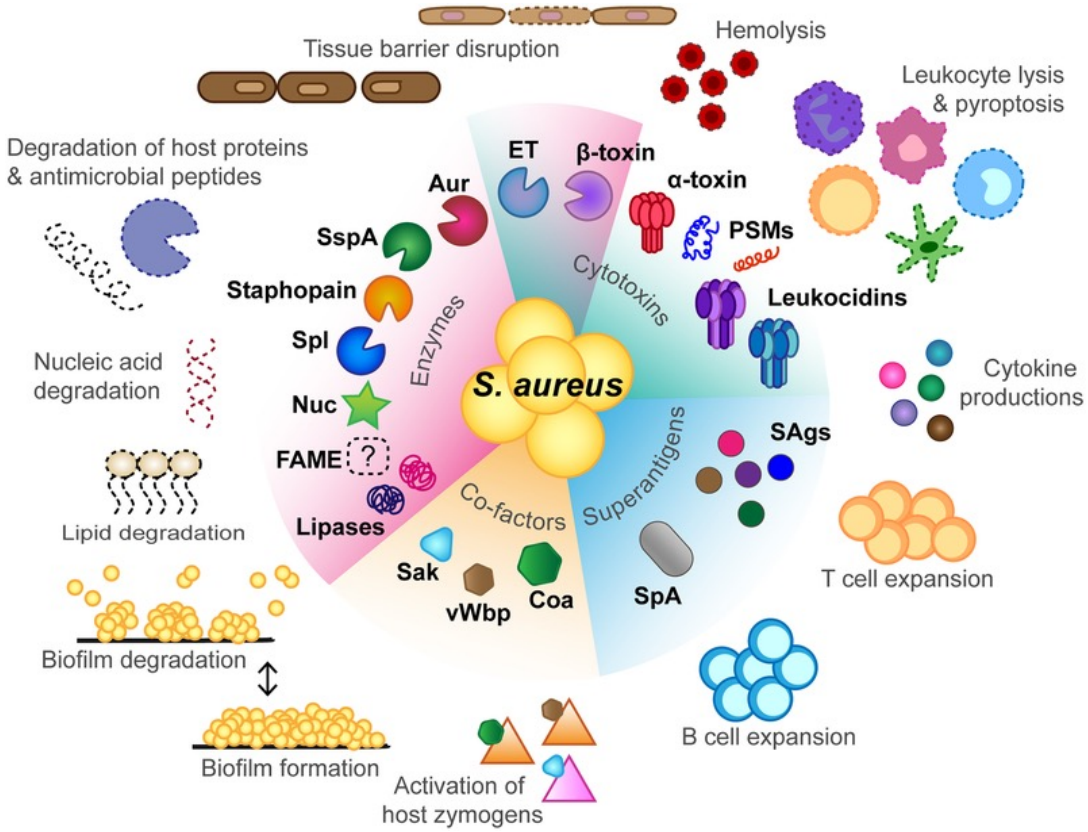
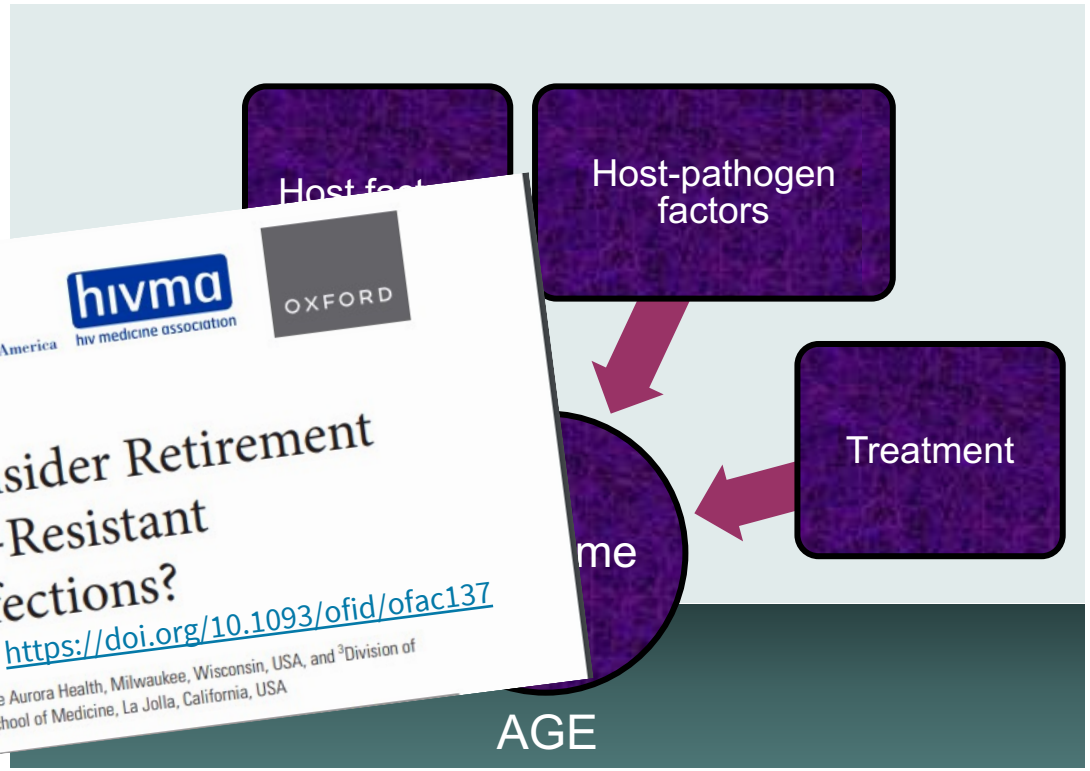
Staphylococcus aureus: the complicated pathogen

- Resistance documented to every class of antimicrobials
- Treatment failure persistently high for complicated infections
- Consistently changing epidemiology and patient risks
- Immune evasion….the non-commensal commensal



Tam and Torres. *Microbiol Spectr.* 2019 7(2)

MRSA Bacteremia
Can We Improve Upon the Standard of Care?



Open Forum Infectious Diseases

PERSPECTIVES

IDSA Infectious Diseases Society of America | hivma hiv medicine association | OXFORD

Approaching 65 Years: Is It Time to Consider Retirement of Vancomycin for Treating Methicillin-Resistant *Staphylococcus aureus* Endovascular Infections?

https://doi.org/10.1093/ofid/ofac137

Warren Rose,[1] Cecilia Volk,[1] Thomas J. Dilworth,[2] and George Sakoulas[3]

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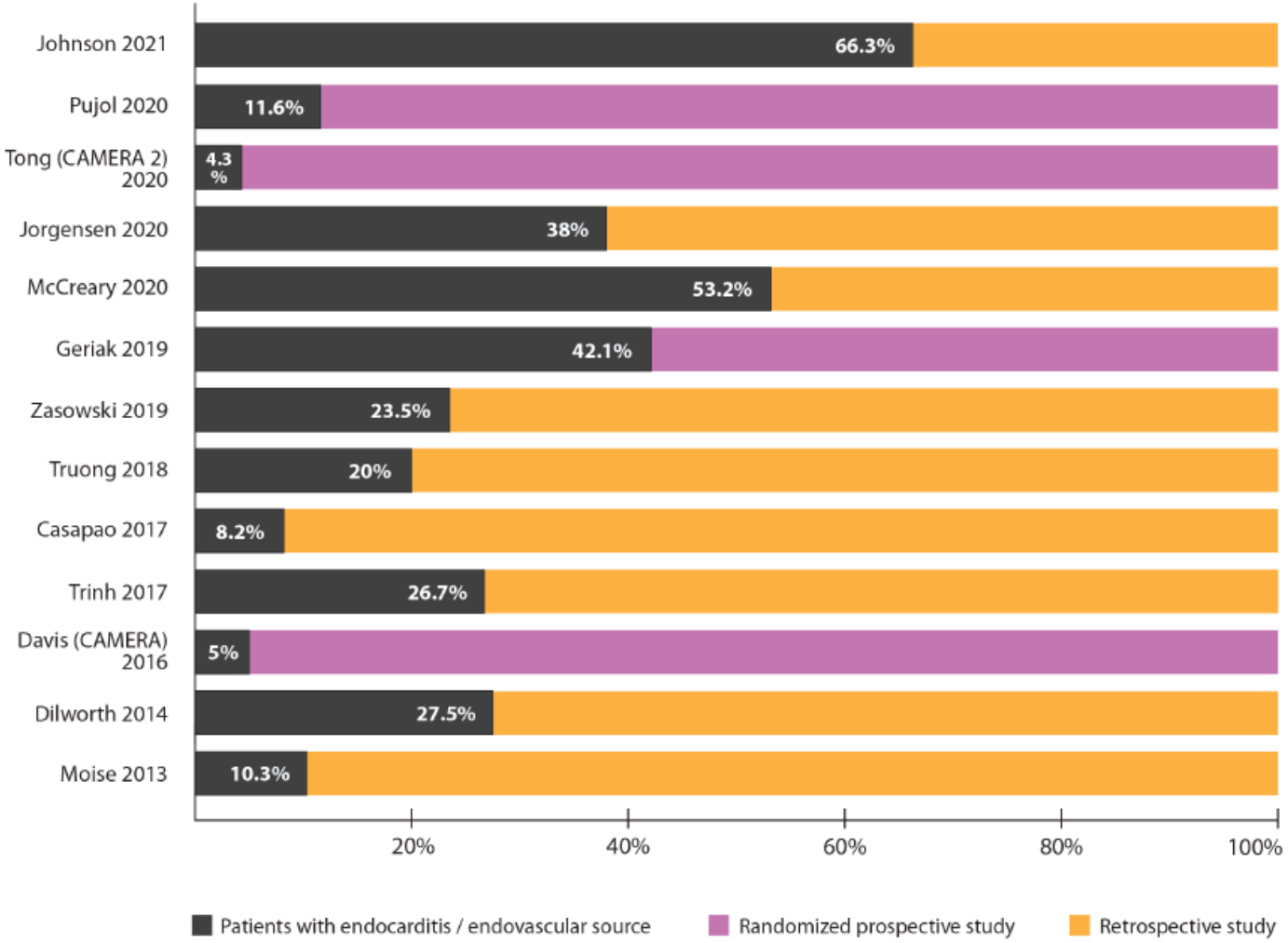
- Assessing
  - Mortality
  - Treatme
  - Bundled care and ID consult

Difficulty in applying combination clinical study results to your patients

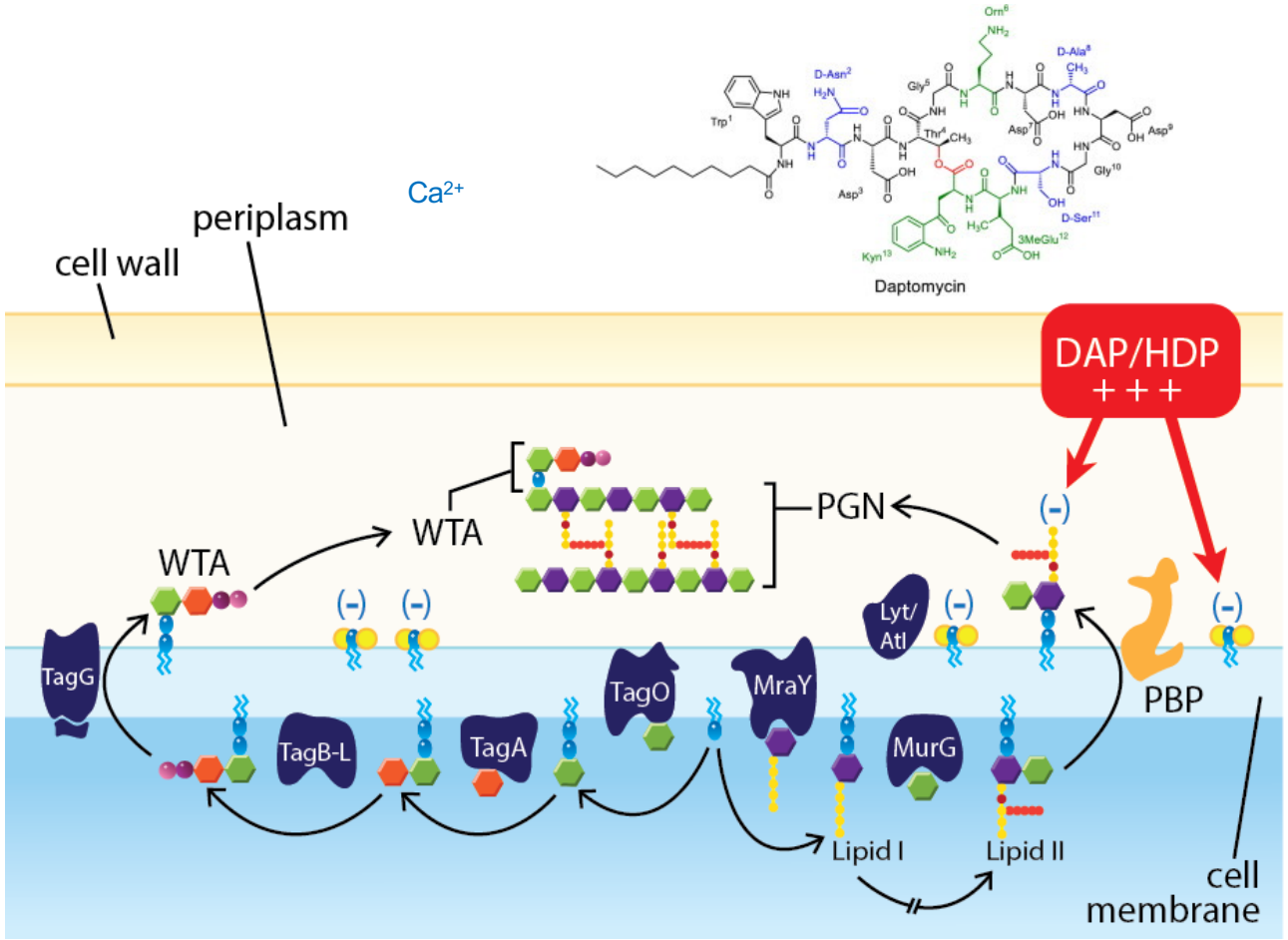


Definitive IE among clinical studies of MRSA combination therapy:	
Prospective clinical trials	Mean 15.8% Median 11.6%
Retrospective cohort studies	Mean 30.4% Median 26.7%

Rose *et al*. *Clin Infect Dis* 2021

Daptomycin (DAP) Mechanism and Resistance

lipopeptide antibiotic that is a functional cation

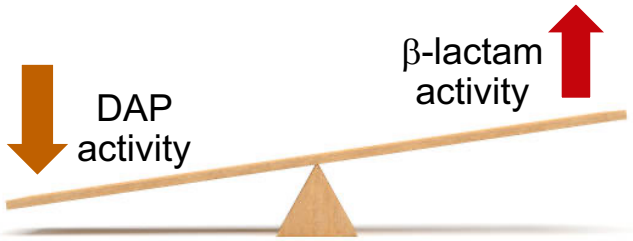


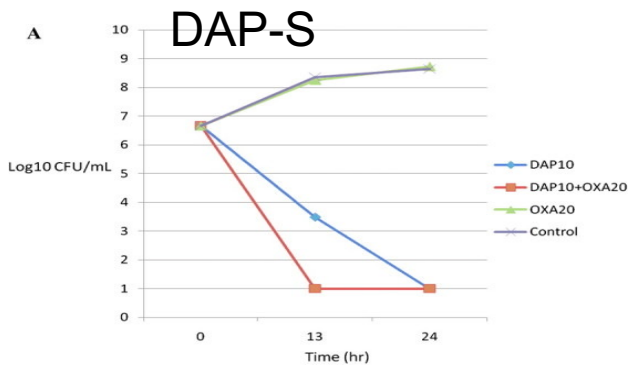
Host Defense Peptides (HDP): LL-37, tPMP (Thrombin-induced platelet microbial protein), HNP1 (human neutrophil peptide)

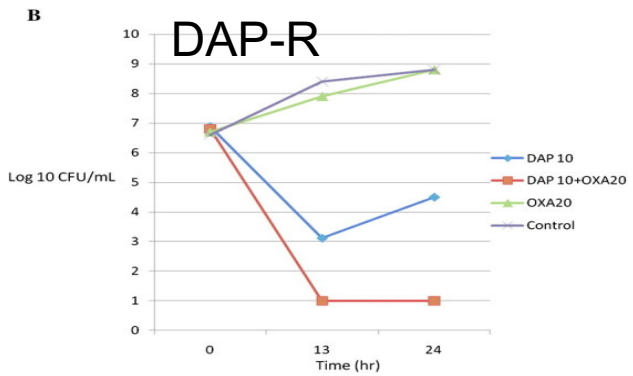
B-lactam effects on DAP in MRSA

Use of antistaphylococcal β-lactams to increase daptomycin activity in eradicating persistent MRSA

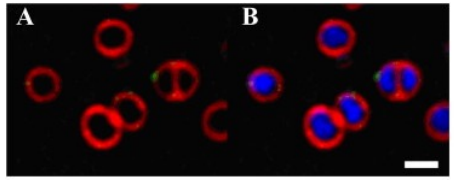
Seesaw effect

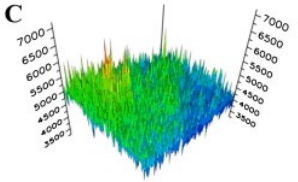




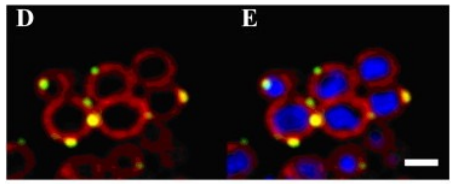


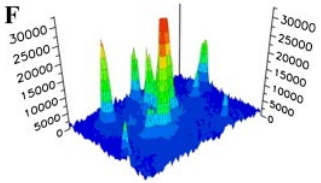
Incorporation of fluorescently-labelled DAP grown in the presence or absence of nafcillin





DAP only





DAP+NAF

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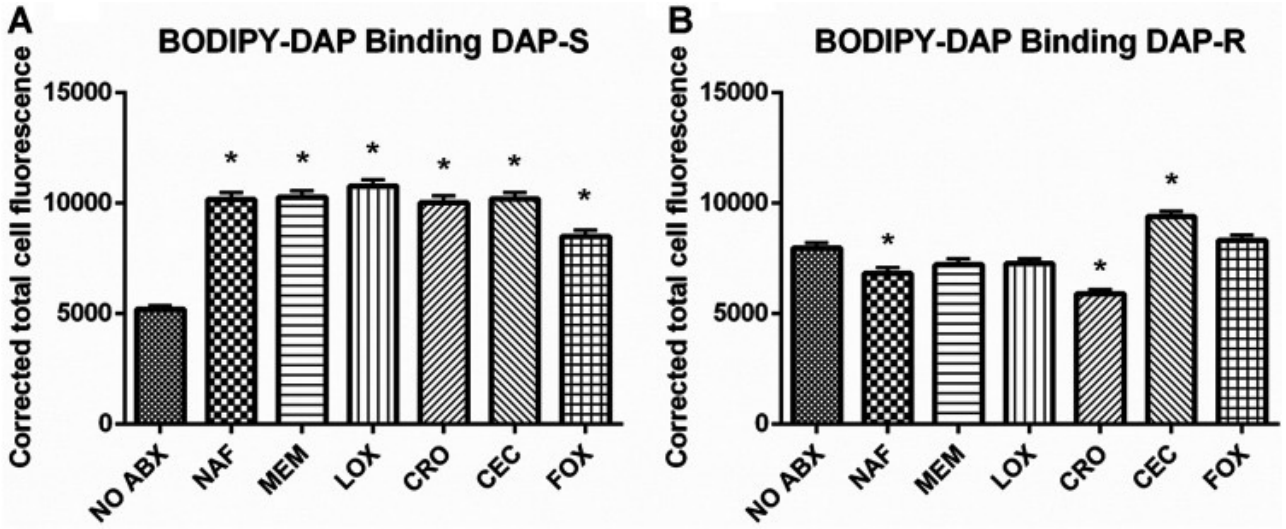
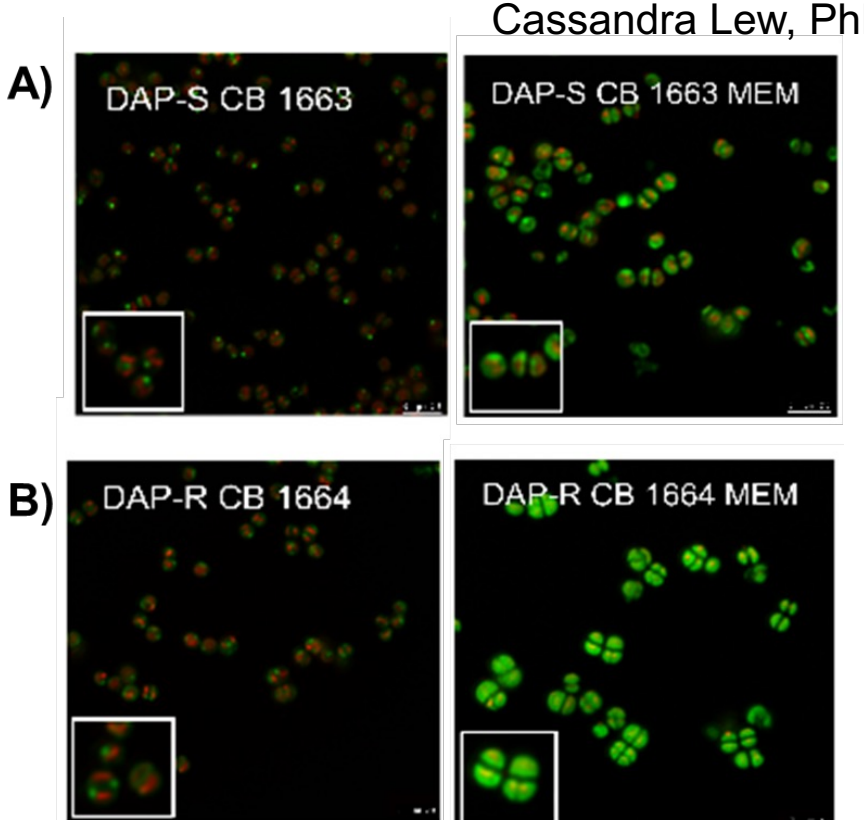
Dhand et al. Clin Infect Dis. 2011; 53(2): 158–163.

But its more than just DAP binding

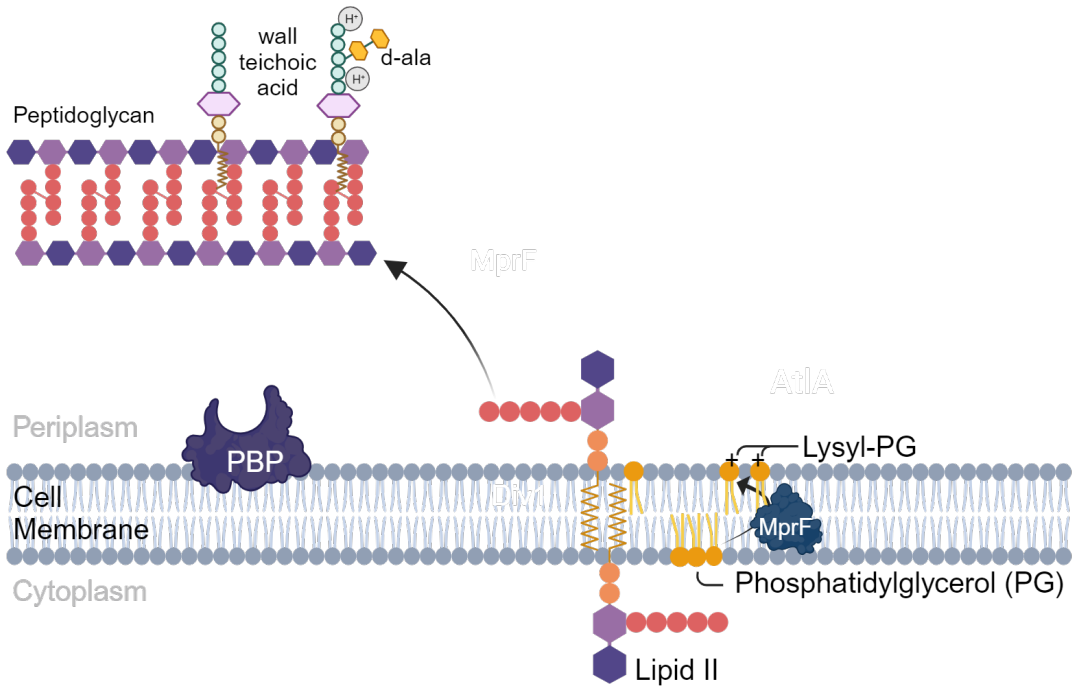
Cassandra Lew, PhD



DAP binding NOT universally increased with b-lactams



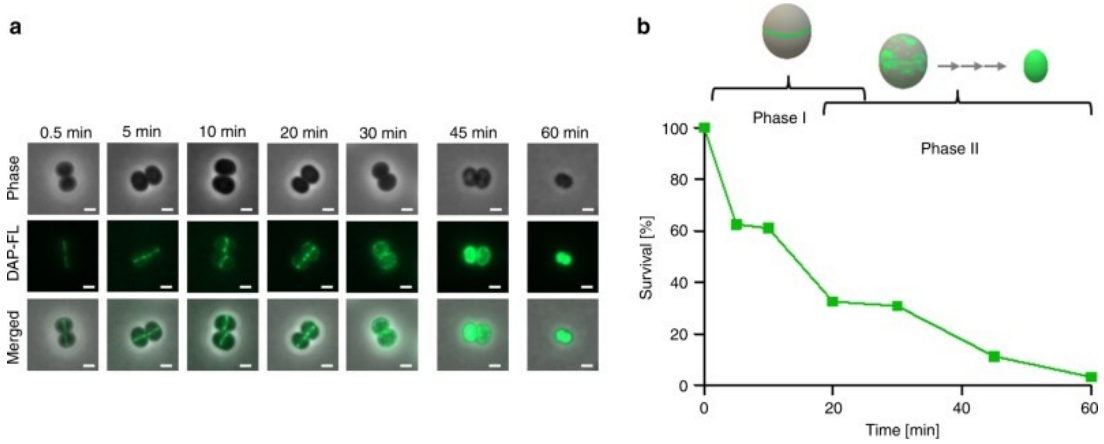
β-lactam conditioning increases cardiolipin distribution (NAO in green)


wall
teichoic
acid
d-ala
H+
H+
Peptidoglycan
MprF
AtlA
Periplasm
PBP
Cell
Membrane
Cytoplasm
Lysyl-PG
MprF
Phosphatidylglycerol (PG)
Lipid II

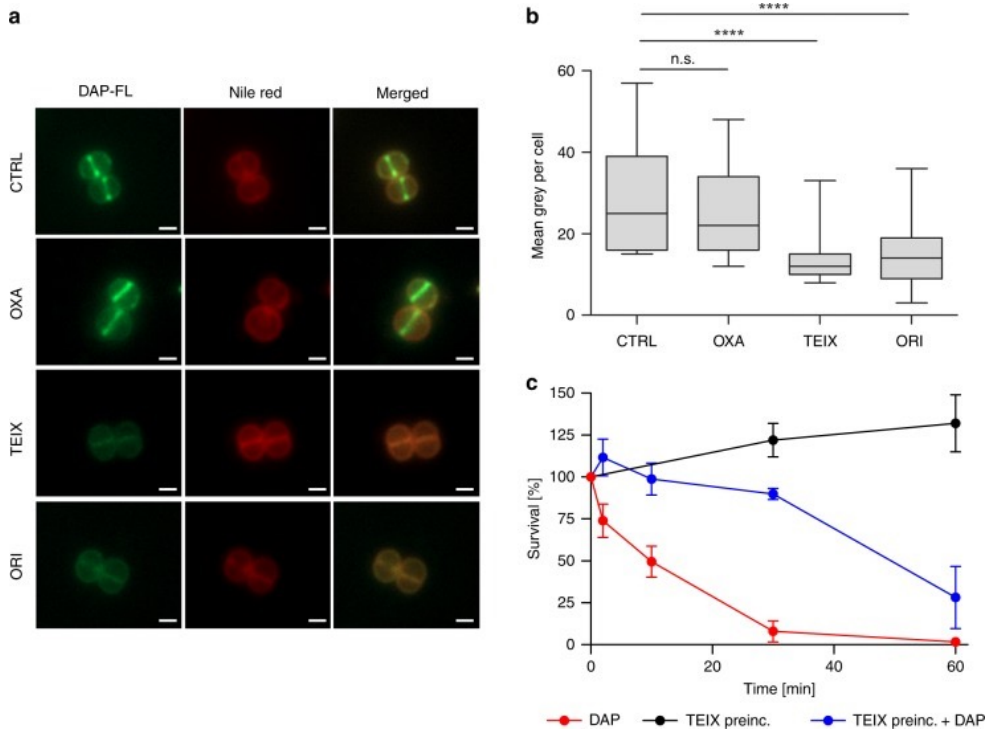
DAP forms a tripartite complex with lipid II and PG and inhibits cell wall biosynthesis *in vitro*

Tanja Schneider, PhD

DAP binds to S. aureus in a biphasic manner.



Cell wall precursor lipid II DAP binding.



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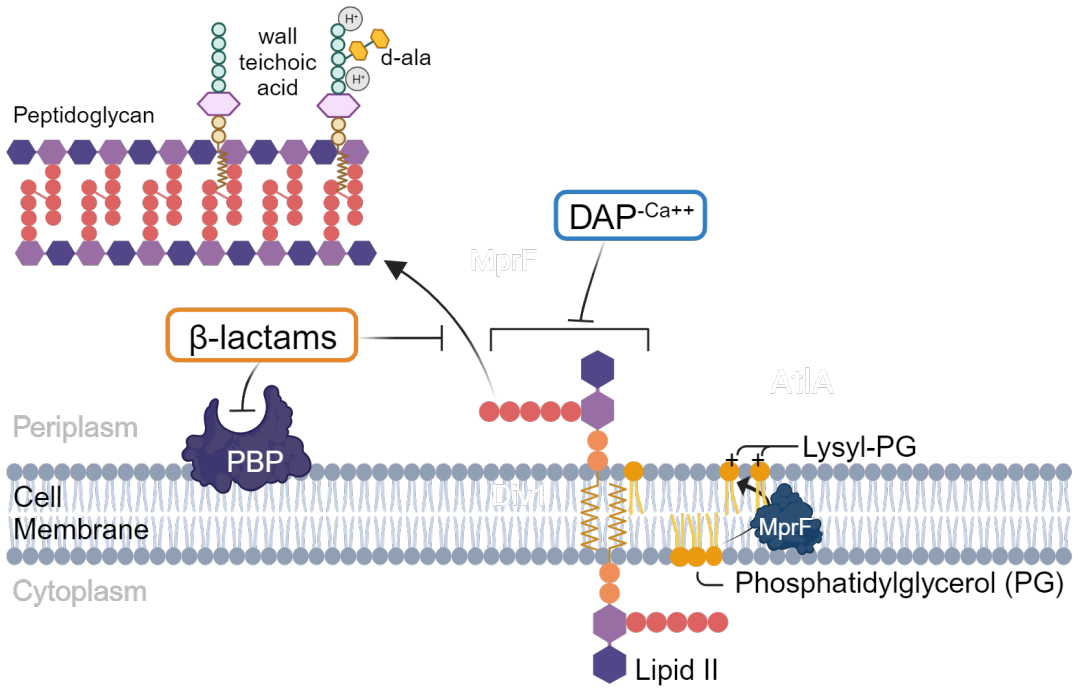
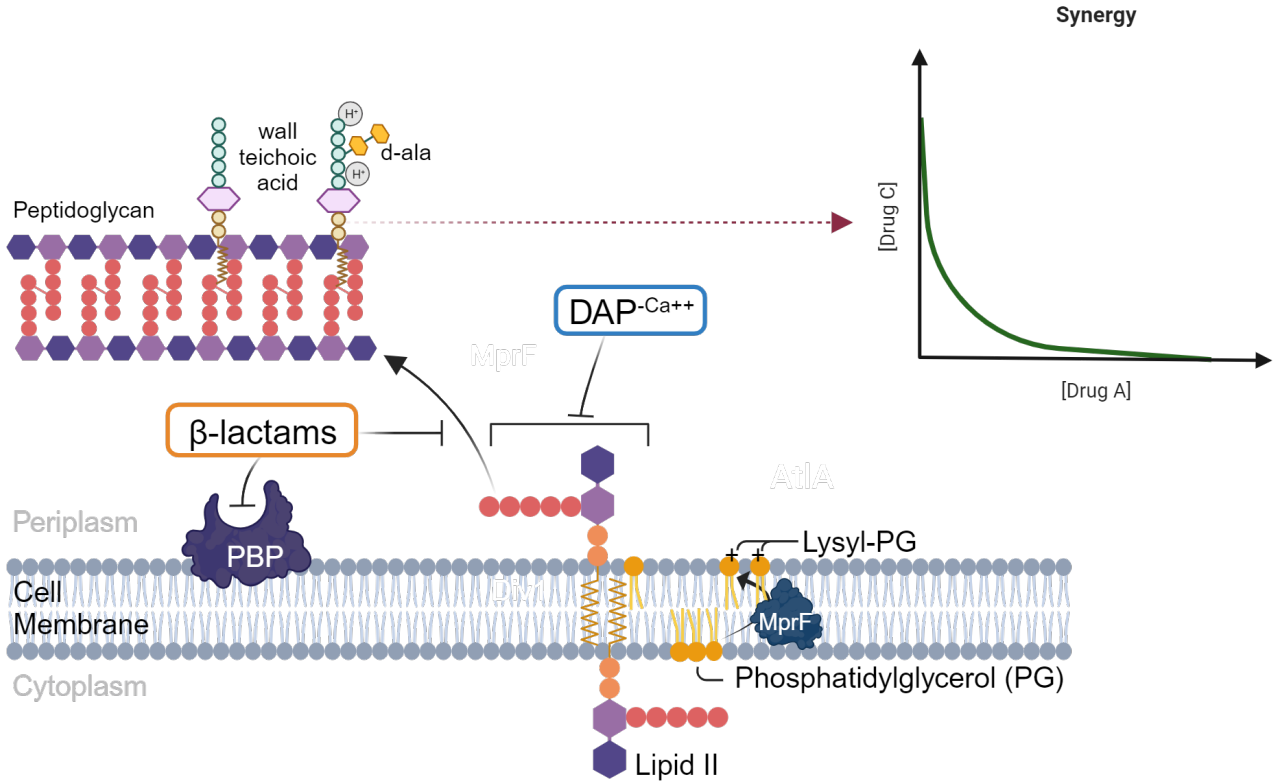
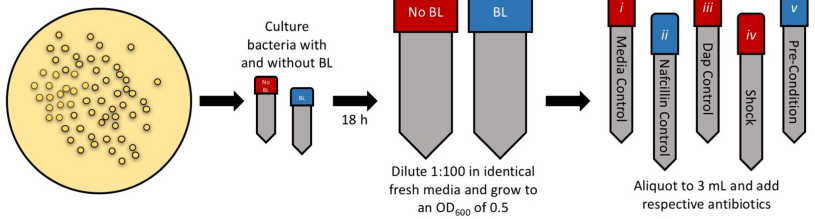
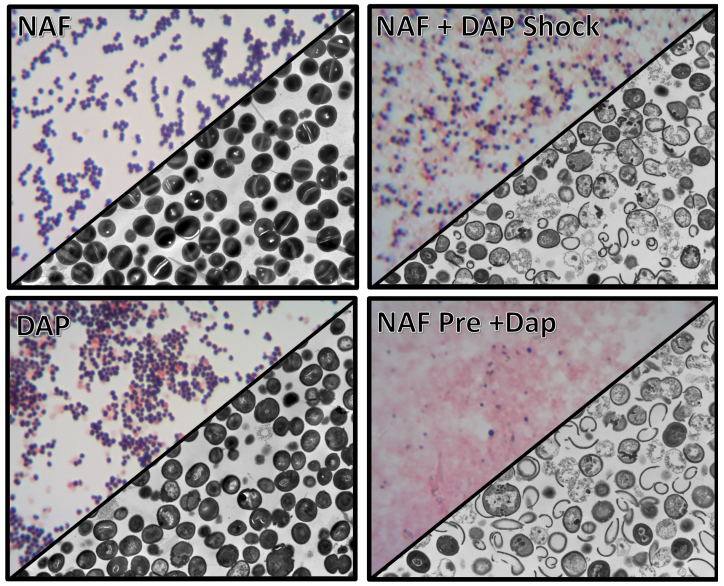
Nature Communications volume 11, Article number: 1455 (2020)


wall
teichoic
acid
d-ala
H+
H+
Peptidoglycan
DAP-Ca++
MprF
β-lactams
AtlA
Periplasm
PBP
Lysyl-PG
Cell
Membrane
MprF
Phosphatidylglycerol (PG)
Cytoplasm
Lipid II

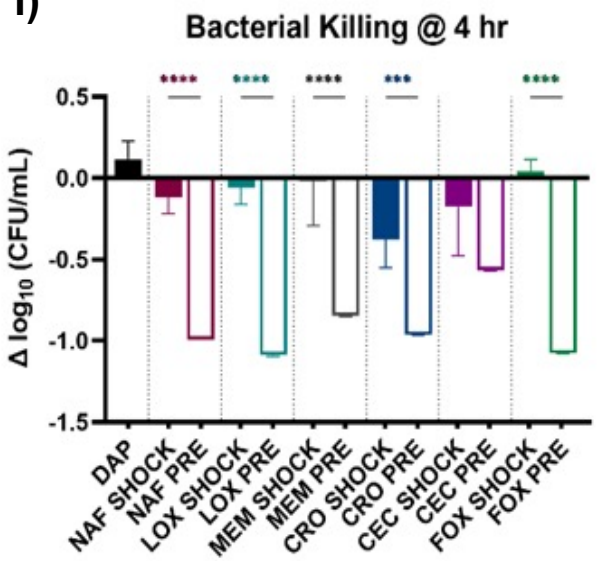

Synergy
[Drug C]
[Drug A]
wall
teichoic
acid
d-ala
H+
Peptidoglycan
DAP-Ca++
MprF
β-lactams
AtlA
Periplasm
PBP
Lysyl-PG
Cell
Membrane
MprF
Phosphatidylglycerol (PG)
Cytoplasm
Lipid II

B-lactam induced cell lysis with DAP vs MRSA



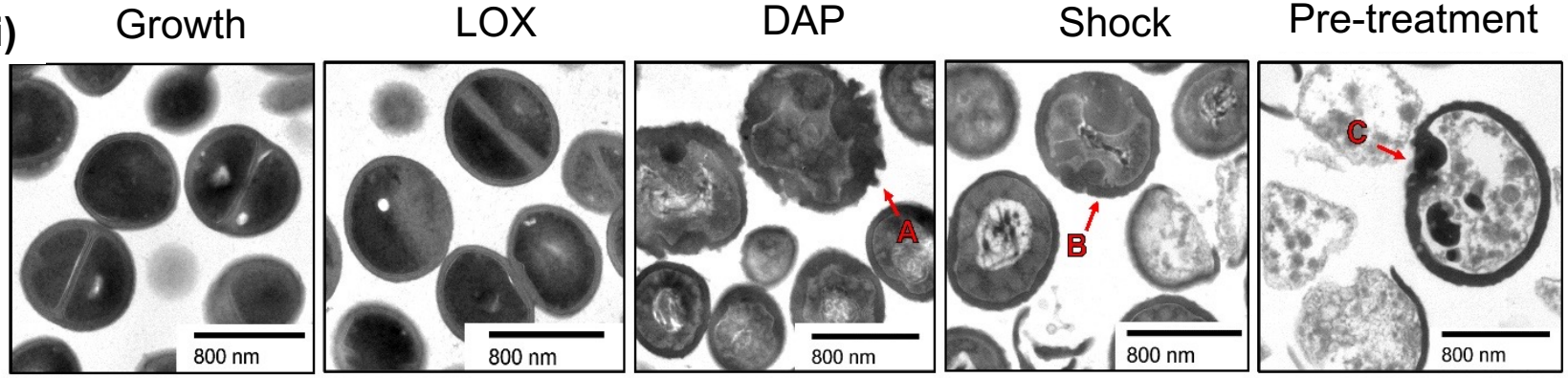


i)

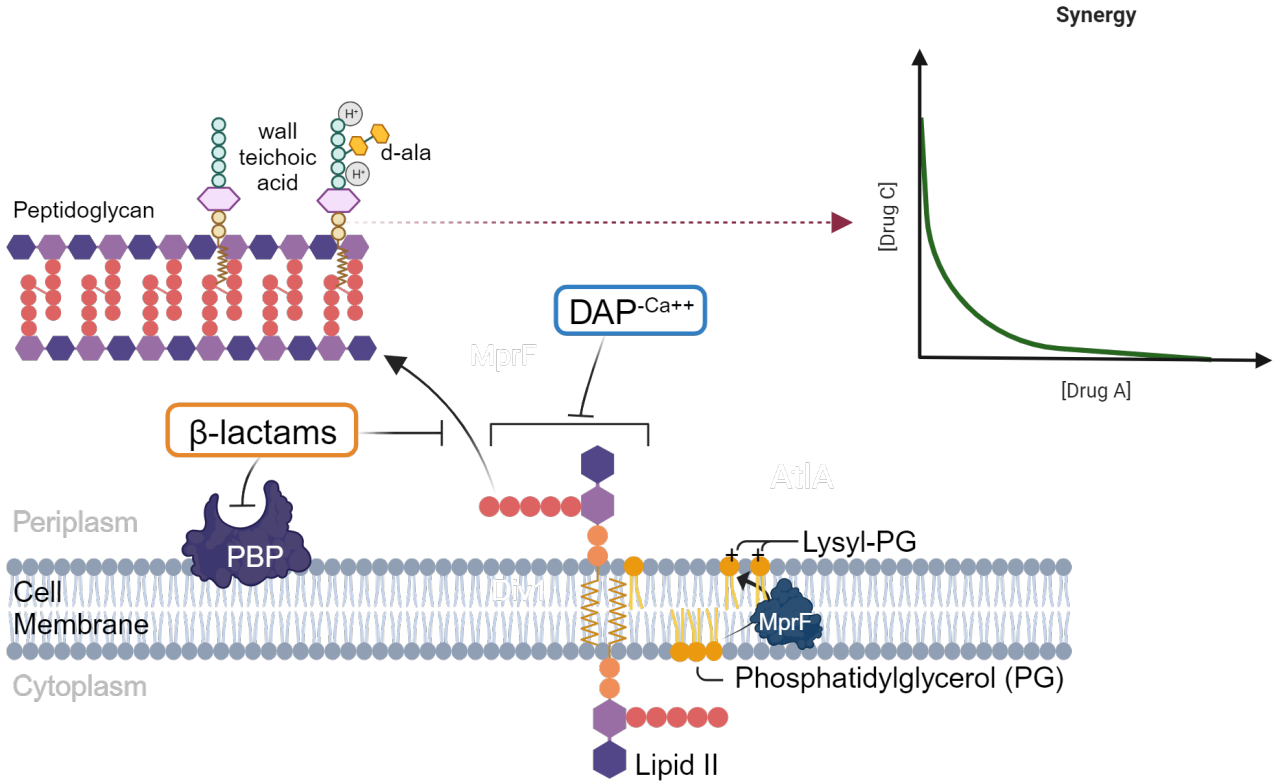


Aaron Rottier C. Lew

ii)



Lew C, Antimicrob Agents Chemother. 2022 Mar; 66(3): e02017-21.


Synergy
[Drug C]
[Drug A]
wall
teichoic
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Periplasm
PBP
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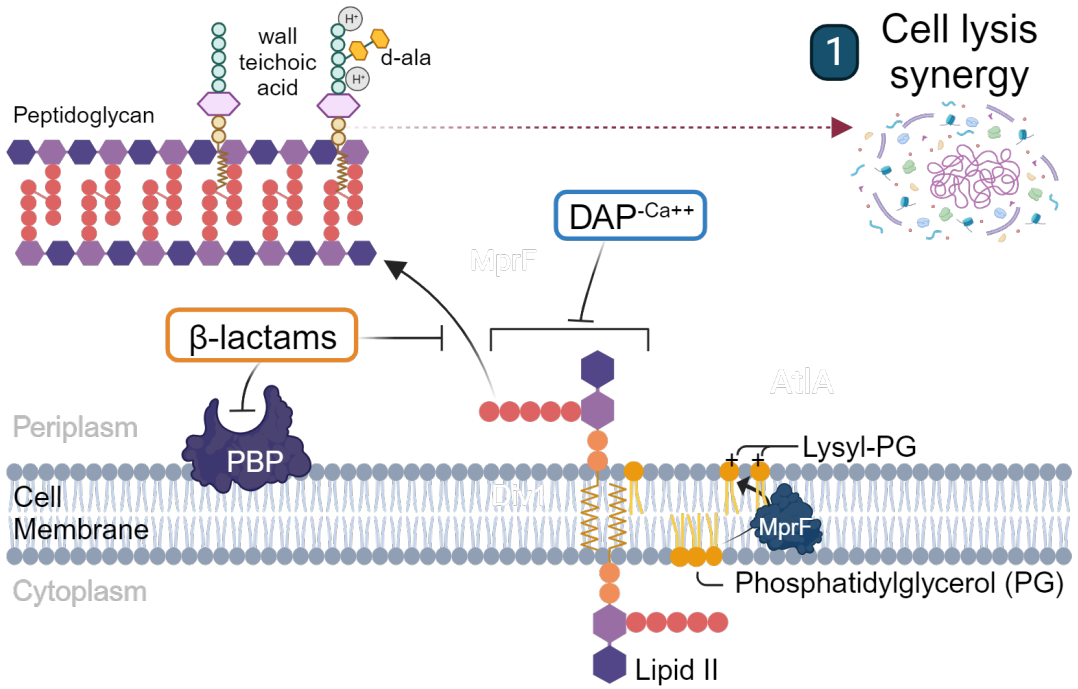
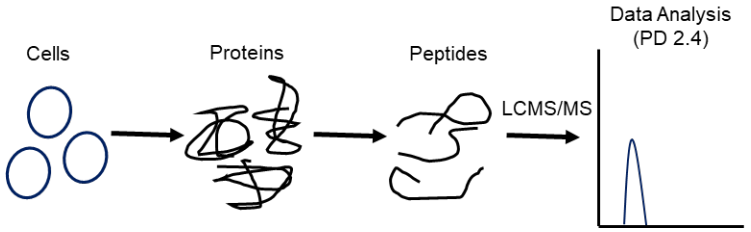
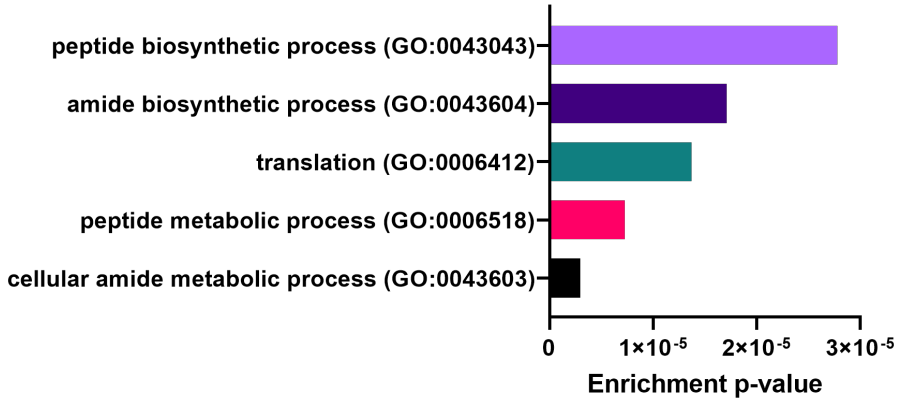
Cell
Membrane
MprF
Phosphatidylglycerol (PG)
Cytoplasm
Lipid II

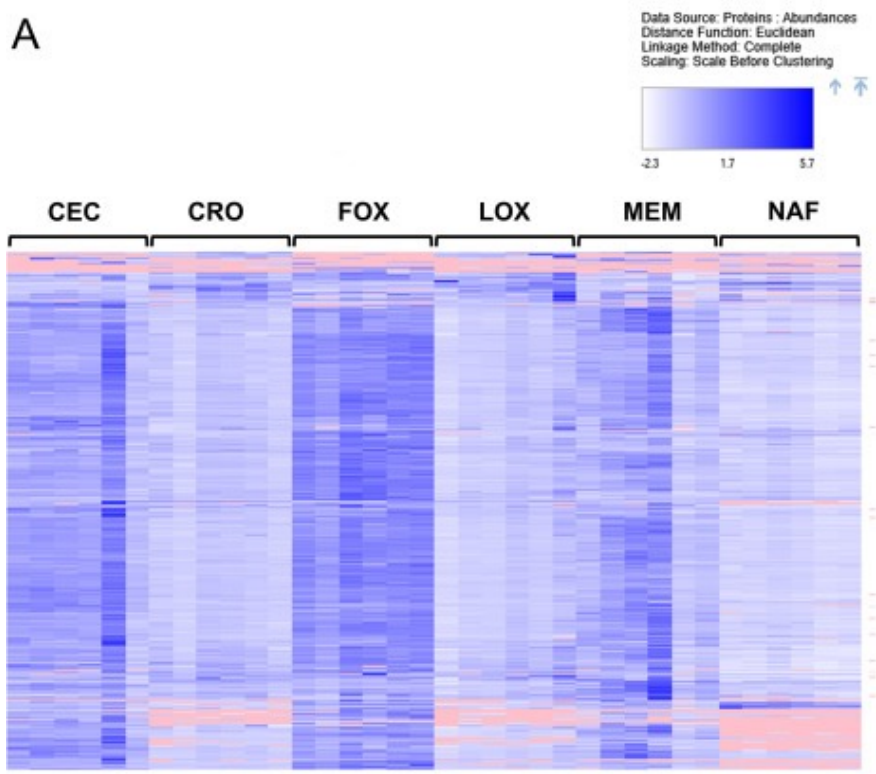

wall teichoic acid
d-ala
Peptidoglycan
1
Cell lysis synergy
DAP-Ca++
MprF
β-lactams
AtlA
Periplasm
PBP
Lysyl-PG
Cell Membrane
MprF
Phosphatidylglycerol (PG)
Cytoplasm
Lipid II

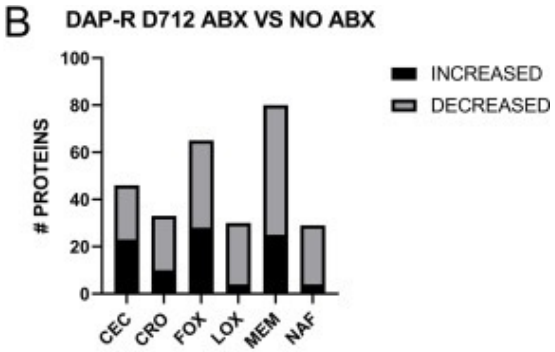
Proteomic correlates of DAP+β-lactam synergy

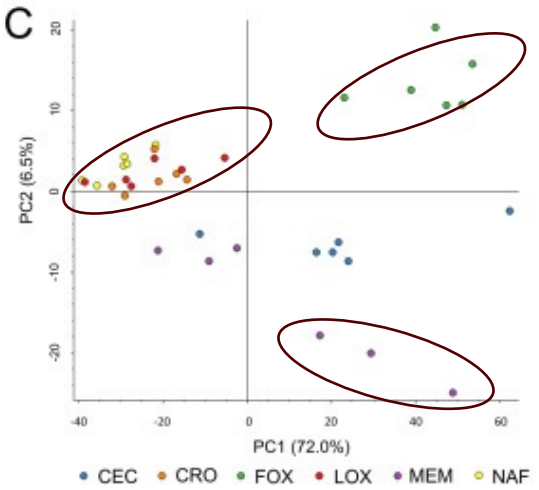
C. Lew











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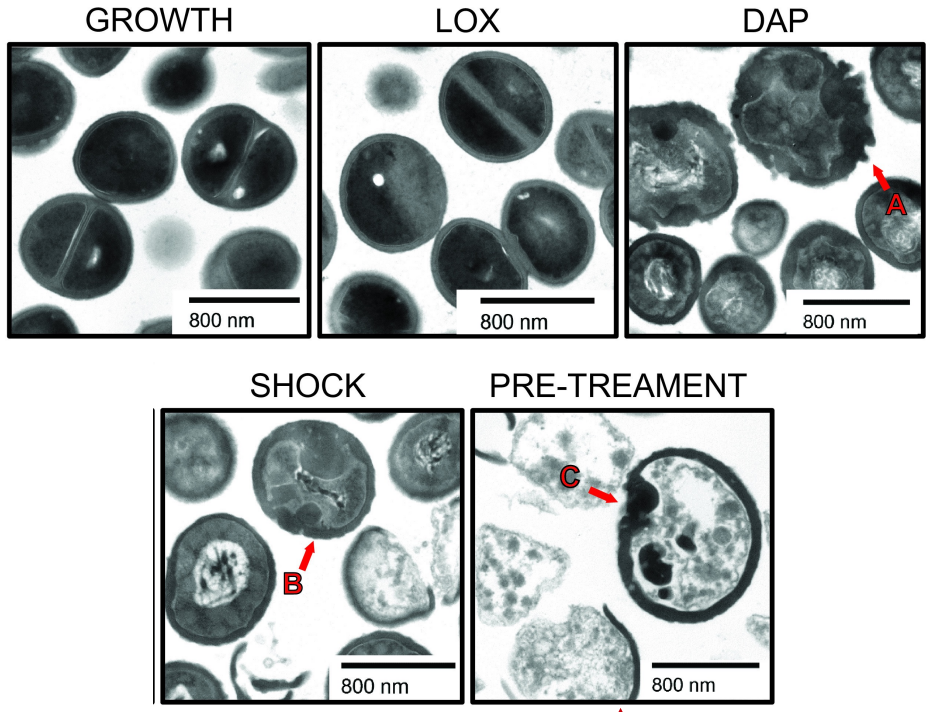
Lew C, Antimicrob Agents Chemother. 2022 Mar; 66(3): e02017-21.

Autolysins induced with B-lactam preconditioning

Identified autolysins with significant abundance ratios in different conditions[a]

Condition with or without D712	ATLA	ISAA	LYTM	SCEd	sLE1	sSAa
NAF	0.764	**0.344**	0.060	**0.167**	0.764	0.257
MEM	**0.224**	**0.138**	0.010	**0.083**	**0.140**	**0.211**
LOX	0.933	0.693	—	0.881	0.663	0.664
CRO	0.587	0.413	0.682	0.563	0.452	0.393
CEC	0.271	0.224	0.160	0.184	0.209	0.215
FOX	**0.222**	**0.133**	0.010	**0.040**	0.284	**0.178**

[a]Values of <1 indicate increased abundance with β-lactam pretreatment (boldface indicates a significant ratio). —, protein not identified.



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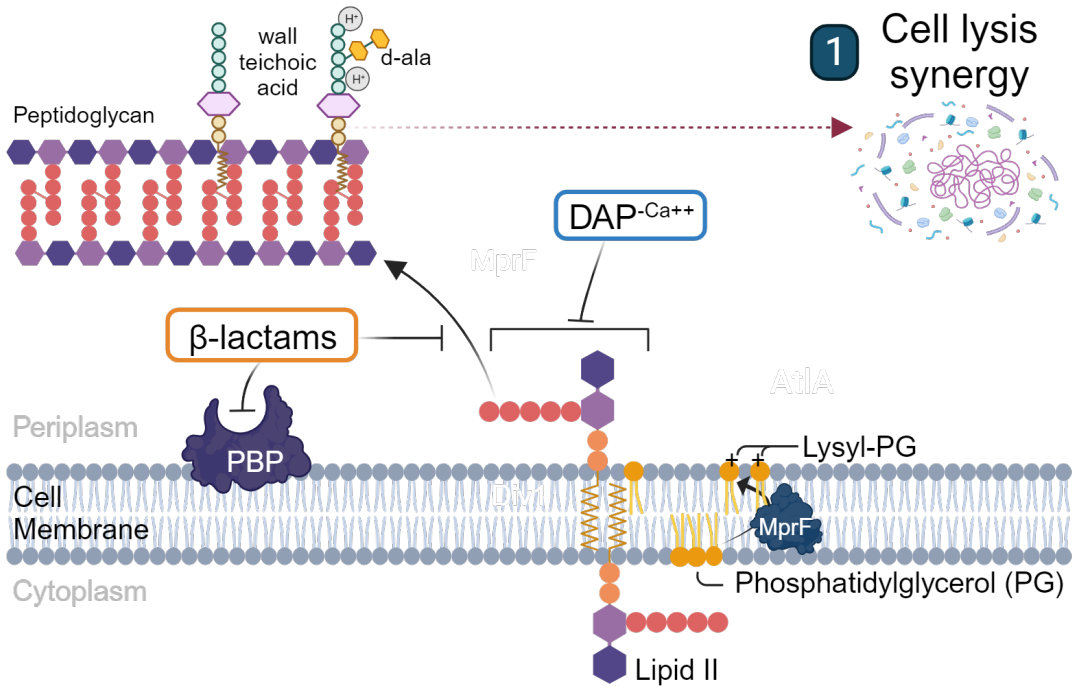
wall teichoic acid
d-ala
Peptidoglycan
1
Cell lysis synergy
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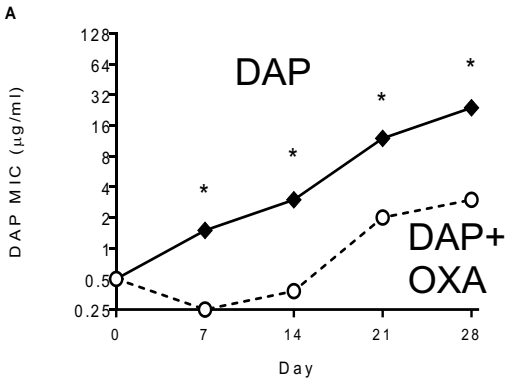
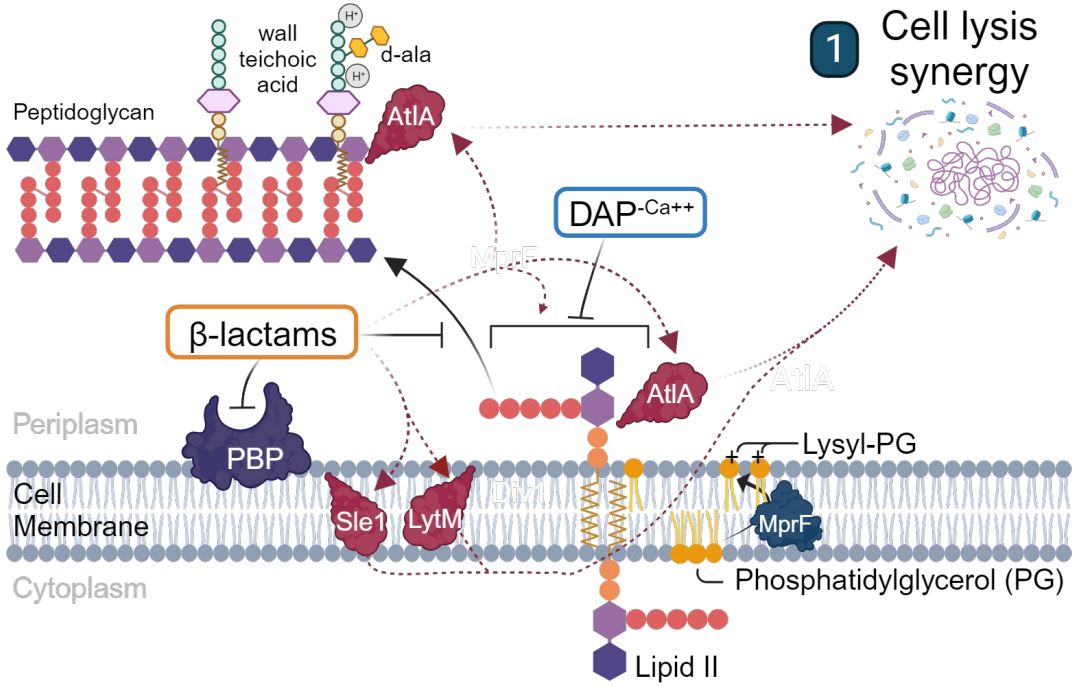
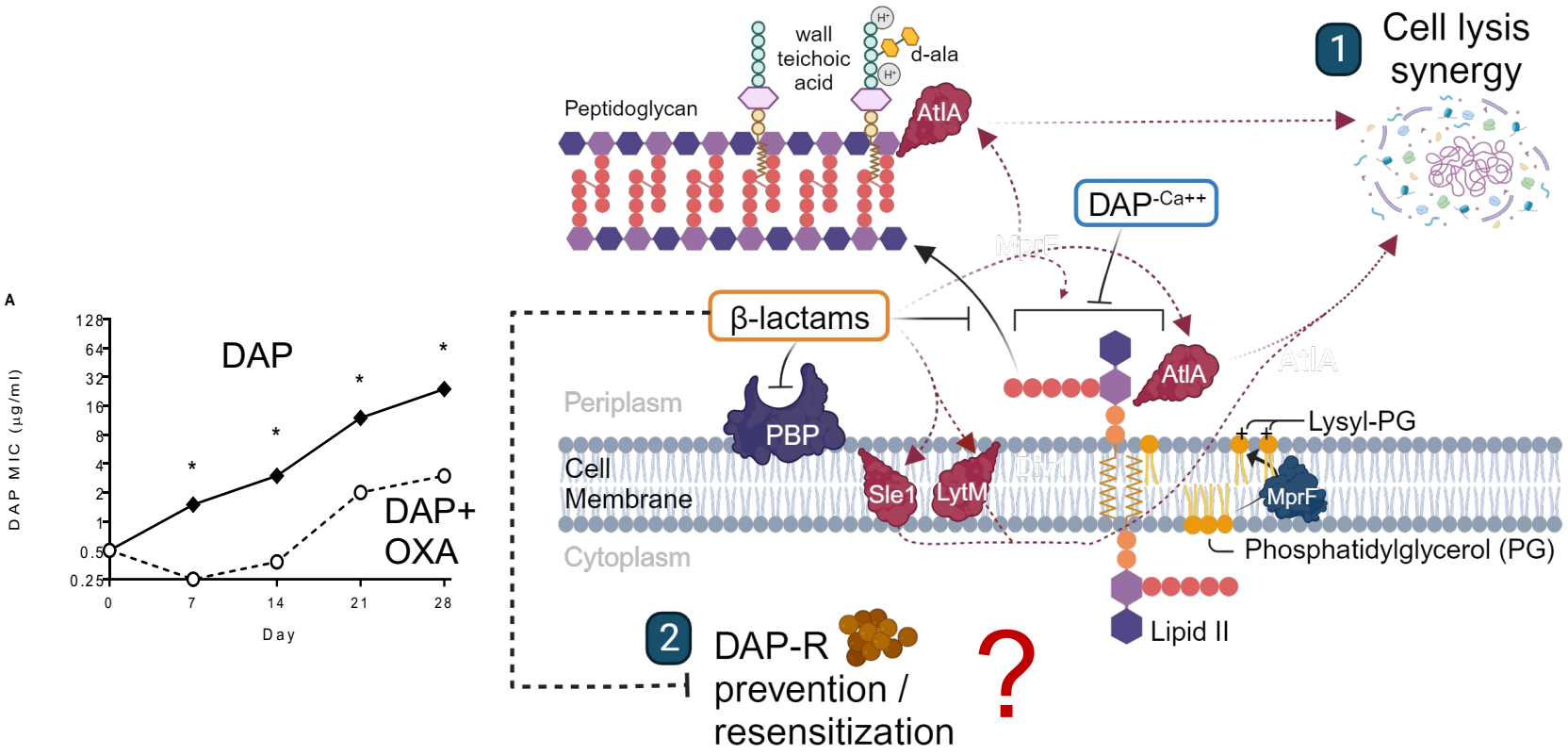
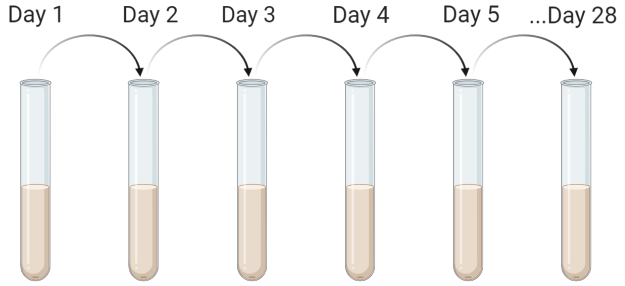
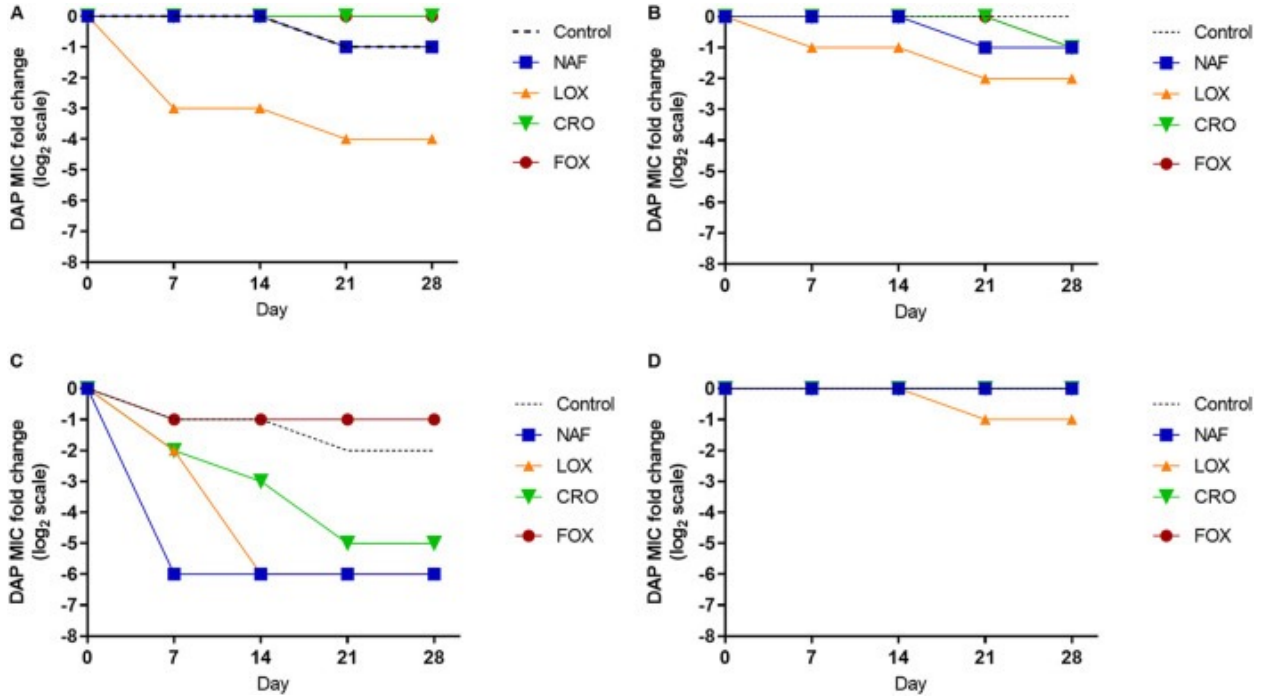

A
DAP MIC (µg/ml)
128
64
32
16
8
4
2
1
0.5
0.25
0
7
14
21
28
Day
DAP
DAP+
OXA
*
*
*
*


wall
teichoic
acid
d-ala
H+
H+
Peptidoglycan
AtlA
1
Cell lysis
synergy
DAP-Ca++
MprF
β-lactams
AtlA
Periplasm
PBP
Lysyl-PG
Cell
Membrane
Sle1
LytM
MprF
Phosphatidylglycerol (PG)
Cytoplasm
Lipid II


A
DAP MIC (µg/ml)
128
64
32
16
8
4
2
1
0.5
0.25
0
7
14
21
28
Day
DAP
DAP+
OXA
*
wall
teichoic
acid
d-ala
H+
Peptidoglycan
AtlA
1
Cell lysis
synergy
DAP-Ca++
MprF
β-lactams
PBP
Periplasm
Cell
Membrane
Cytoplasm
Sle1
LytM
AtlA
Lysyl-PG
MprF
Phosphatidylglycerol (PG)
Lipid II
2
DAP-R
prevention /
resensitization
?

B-lactam induced DAP-resensitization





Isolate	Passage	Replicate	DAP MIC (mg/liter)	β-lactam MIC (mg/liter)[b]	*mprF* SNP	MprF domain	*div1b* mutation
J01	None		0.5				
J03	None		2		$T_{345}I$	Bifunctional	
	Media	i	2		None		
	Media[a]	ii	1		$Y_{325}H$	Bifunctional	
	Media[a]	iii	1		$R_{437}P$	Synthase	
	CRO[c]	ii	0.75	512	$V_{152}G$	Translocase	
	LOX[c]	ii	0.125	32	$R_{788}L$	Synthase	Q_{425}[d]
	LOX[c]	iii	0.125	32	$R_{788}L$	Synthase	Q_{415}[d]

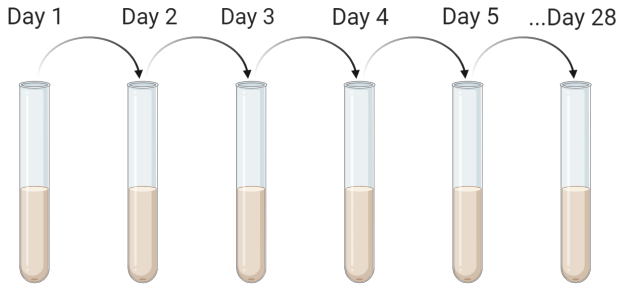
Rachel Jenson, PharmD

Benjamin Howden, MD, PhD,

School of Pharmacy
UNIVERSITY OF WISCONSIN-MADISON

Jenson, R. Antimicrob Agents Chemother 2020 Aug 20;64(9):e00890-20.

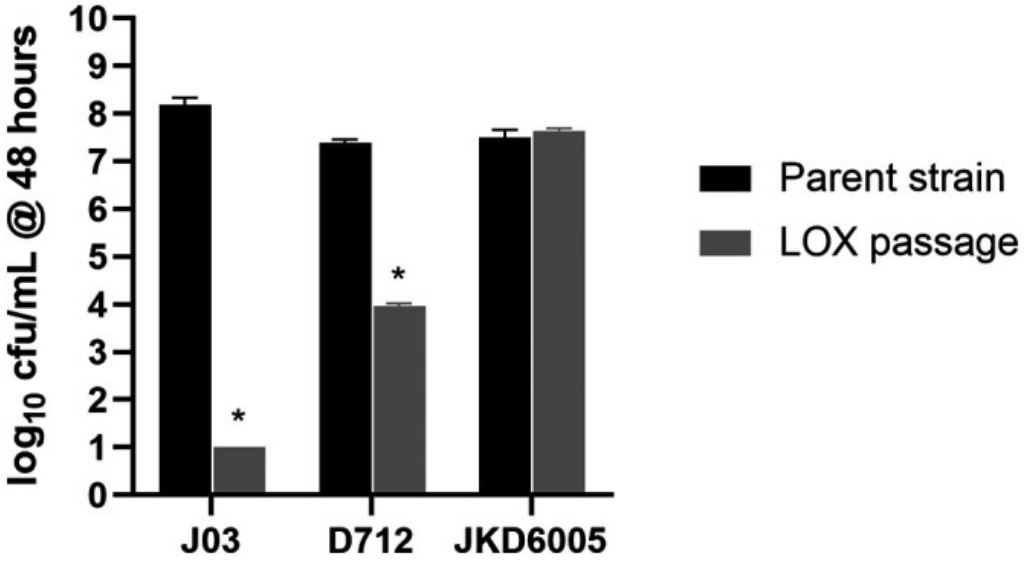
B-lactam induced DAP-resensitization



Rapid conversion to DAP-S

Isolate	Passage	Replicate	Additional *mprF* SNP frequency at day:			
			7	14	21	28
J03[b]	LOX	i	0.58	0.74	0.11	0.00[c]
J03[b]	LOX	ii	0.11	0.08	0.78	0.98
J03[b]	LOX	iii	0.51	0.61	0.79	0.99
D712[d]	LOX	i	0.91	0.67	0.86	1.00
D712[d]	LOX	ii	1.00	1.00	1.00	0.99
D712[d]	LOX	iii	0.00	0.00	0.00	0.00

Highly susceptible to DAP killing



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Jenson, R. Antimicrob Agents Chemother 2020 Aug 20;64(9):e00890-20.

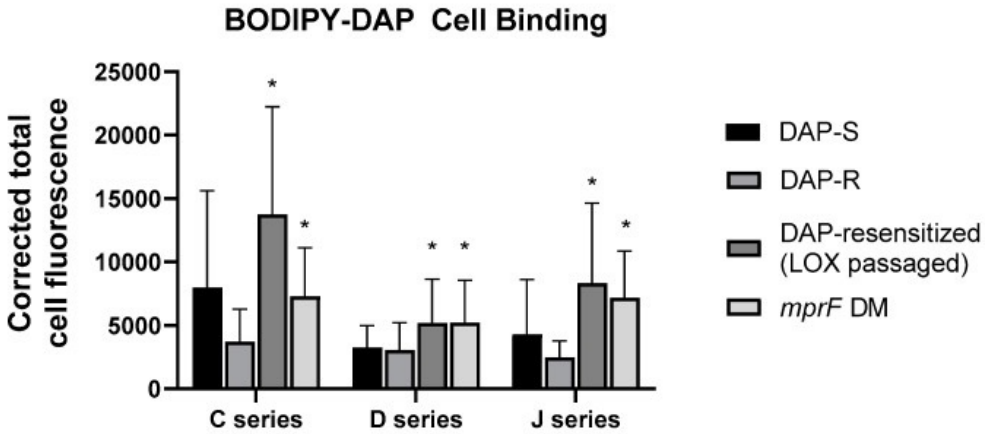
Secondary mutations in *mprF* lead to DAP-resensitization

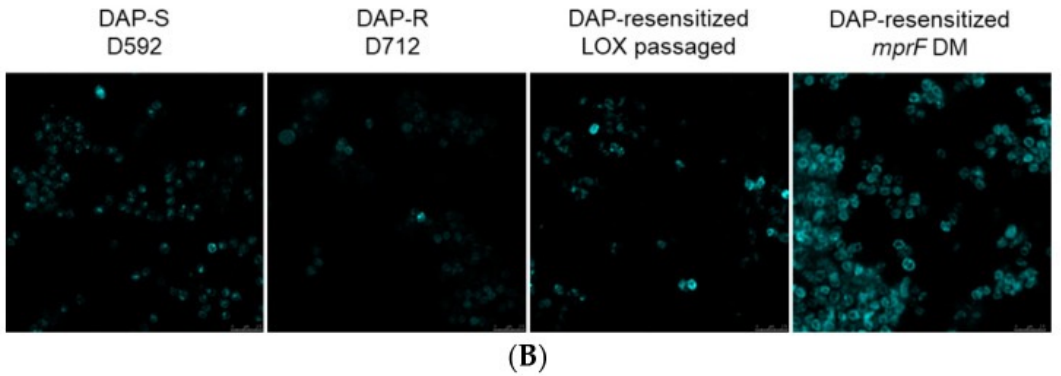
Nagendra Mishra PhD

B. Howden

Strain Set [a]	Strain Name	Strain Description	DAP MIC [b] (μg/mL)	LOX MIC [c] (μg/mL)	SNPs in *mprF* [d]
I	C24	DAP-S	0.5	8	WT
	C25	DAP-R	2	4	S295L
	C25-LOX	DAP-resensitized (LOX passaged)	<0.125	8	S295L + L84 (Translocase domain)
	C25, *mprF* DM	Secondary *mprF* mutation (L84 [e]) introduced into C25	0.125	16	S295L + L84 [e]
II	D592	DAP-S	0.5	512	WT
	D712	DAP-R	2	512	L341S
	D712-LOX	DAP-resensitized (LOX passaged)	0.5	1024	L341S + S136L (Translocase domain)
	D712, *mprF* DM	Secondary *mprF* mutation (S136L) introduced into D712	0.5	1024	L341S + S136L
III	J01	DAP-S	0.5	16	WT
	J03	DAP-R	2–4	2	T345I
	J03-LOX	DAP-resensitized (LOX passaged)	0.125	32	T345I + R788L Synthase domain
	J03, *mprF* DM	Secondary *mprF* mutation (R788L) introduced into J03	0.125	16	T345I + R788L

[a] Sets of isolates are represented by alternative shading and no shading, with the first strain in each set being the DAP-S parental strain, the second in each set being the DAP-R or allelic exchange, respectively; [b,c,d] Data in this table have been previously published (41); [e] nonsense mutation (41).





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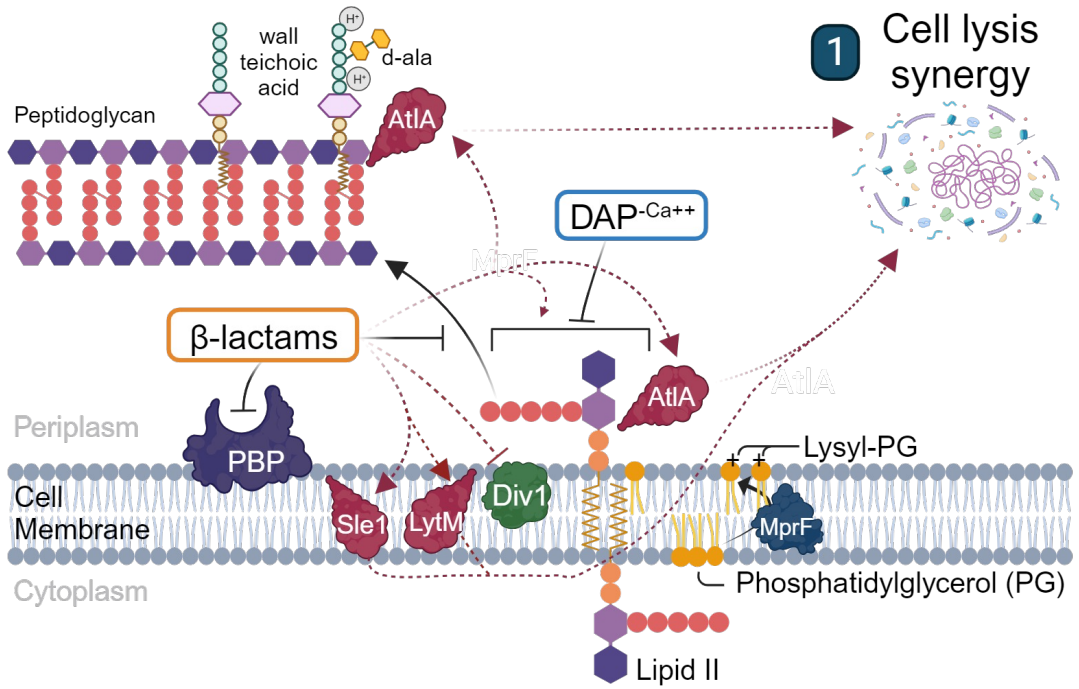
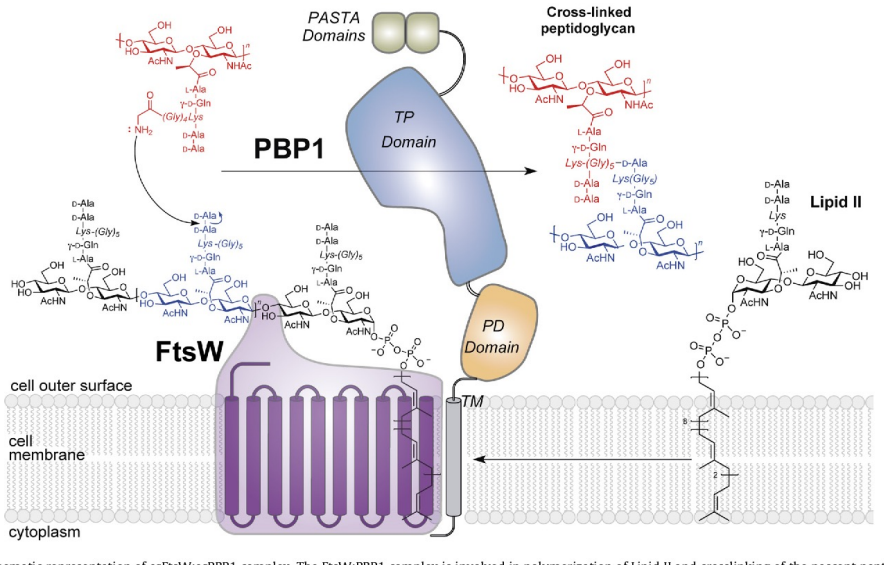
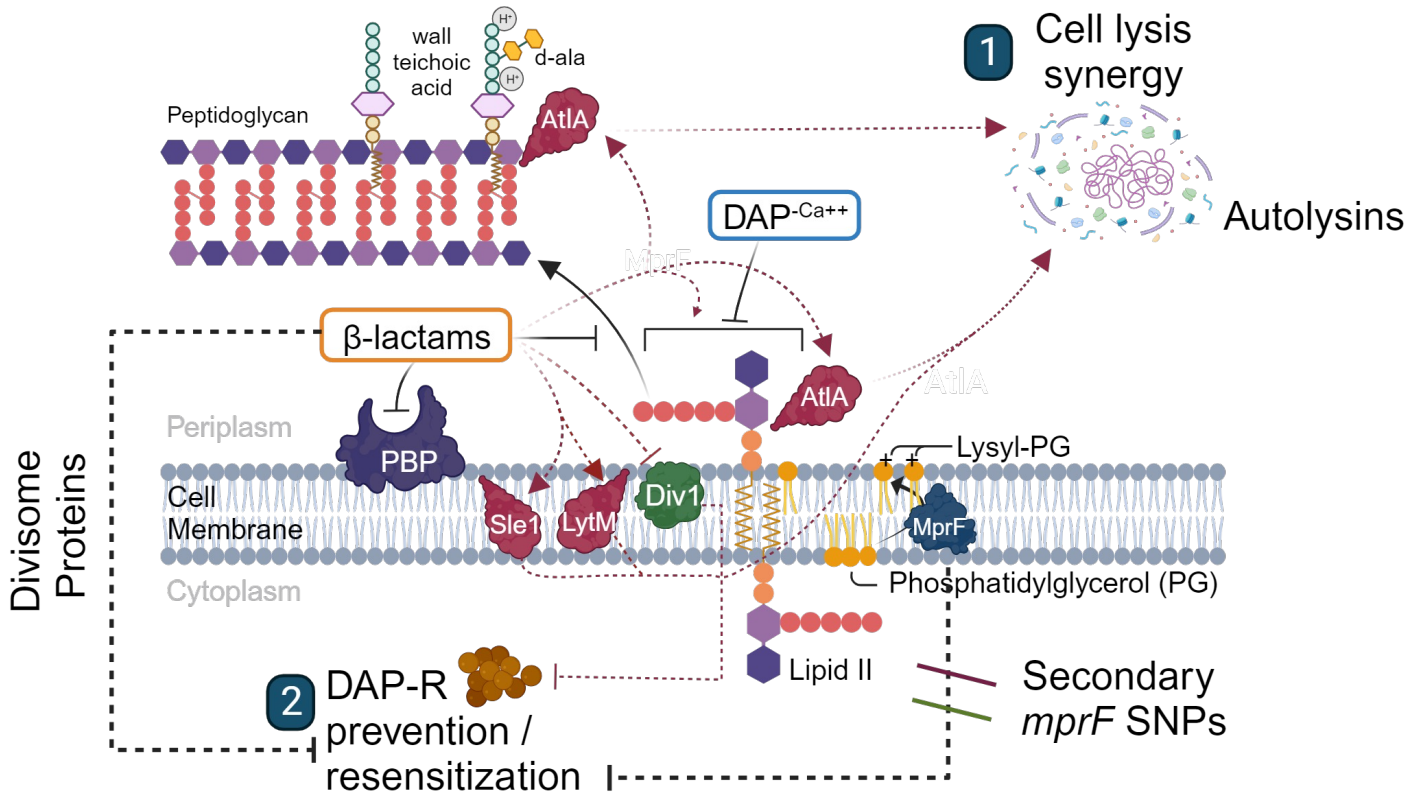
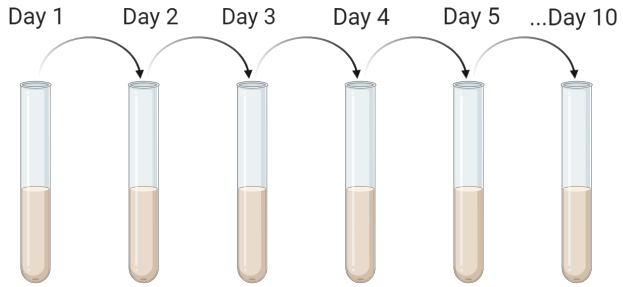

1
Cell lysis synergy
wall teichoic acid
d-ala
Peptidoglycan
AtlA
DAP-Ca++
MprF
β-lactams
AtlA
Periplasm
PBP
Lysyl-PG
Cell Membrane
Sle1
LytM
Div1
MprF
Phosphatidylglycerol (PG)
Cytoplasm
Lipid II





Martínez-Caballero, S et al. Comput Struct Biotechnol J. 2021 Sep 17:19:5392-5405.

Select rapid DAP-R emergence: implications for combination β-lactams therapy?



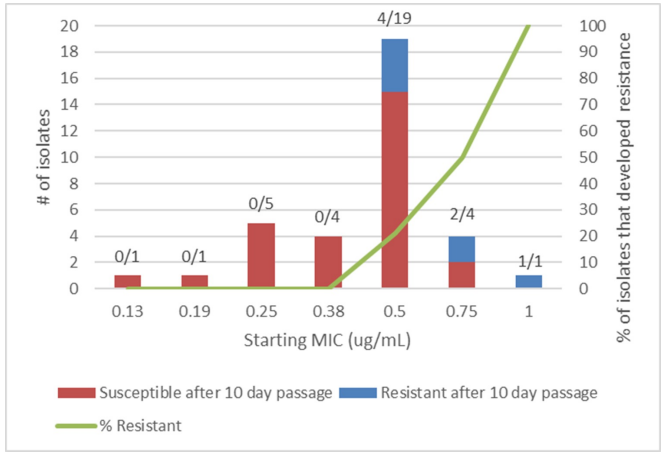
antibiotics

MDPI

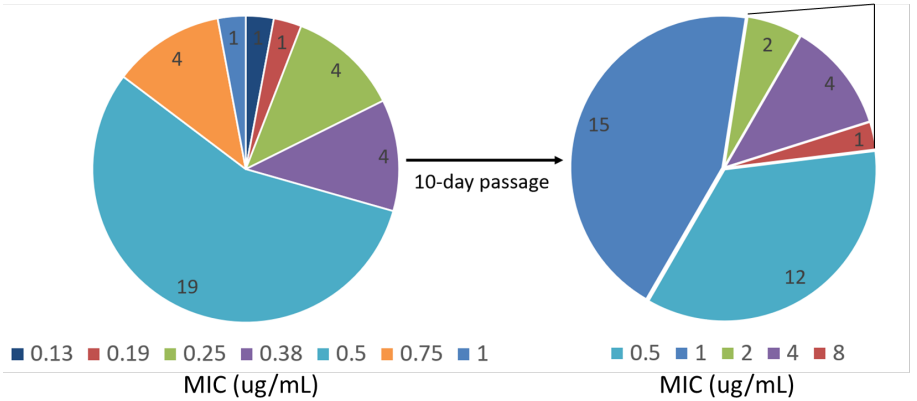
Article

Membrane Phenotypic, Metabolic and Genotypic Adaptations of *Streptococcus oralis* Strains Destined to Rapidly Develop Stable, High-Level Daptomycin Resistance during Daptomycin Exposures

Nagendra N. Mishra [1,2,*], Rodrigo de Paula Baptista [3,4,5], Truc T. Tran [3,4,5], Christian K. Lapitan [1], Cristina Garcia-de-la-Maria [6,7], Jose M. Miró [7], Richard A. Proctor [8] and Arnold S. Bayer [1,2]



20% developed DAP resistance



Cecilia Volk, PharmD

mprF mutations

In Vivo Mutation (occurrences)	*In Vitro Mutation (occurrences)*
L826F (10) L341S (4) S295L (3) T345I (3) M347R (2) S337L (2)	S295L (2) L341S (1)

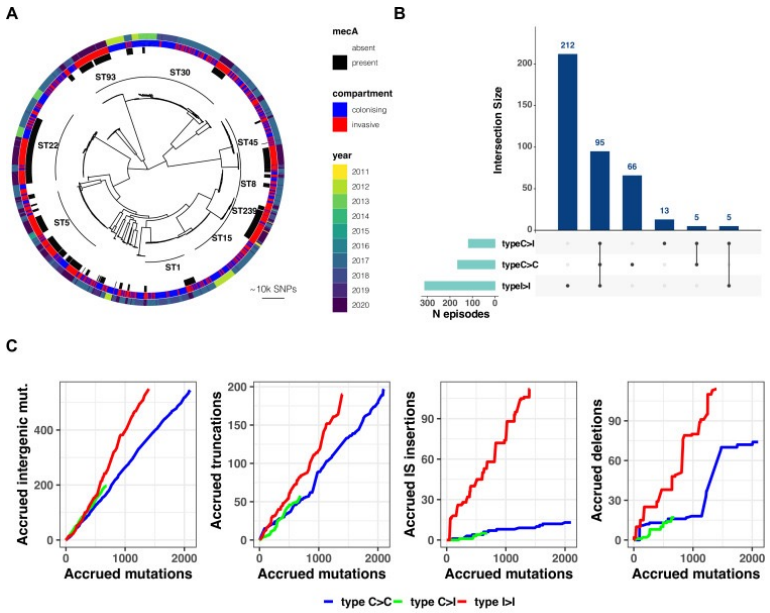
Future Directions

B. Howden

eLife RESEARCH ARTICLE

Niche-specific genome degradation and convergent evolution shaping *Staphylococcus aureus* adaptation during severe infections

Stefano G Giulieri[1,2,3], Romain Guérillot[1], Sebastian Duchene[1], Abderrahman Hachani[1], Diane Daniel[1,4], Torsten Seemann[4], Joshua S Davis[5,6], Steven YC Tong[6,7], Bernadette C Young[8], Daniel J Wilson[9], Timothy P Stinear[1]*, Benjamin P Howden[1,2,4]*



DAP-β-lactam Combinatorial Effects

Determine the convergent genetic pathway(s) for PBP-1 targeting that can both temporally prevent DAP-R, as well as potentially revert DAP-R-to-DAP-S with β-lactam exposures.

Giulieri SG et al. Elife. 2022;11. Epub 2022/06/15. doi: 10.7554/eLife.77195.

Considerations for Future Clinical Trials of MRSA Bacteremia

Initial Therapy:

- Use prognostic model to identify patients at risk for persistent MRSAB (e.g., using key biomarkers)
- Randomize high risk patients to:
  - Option 1: TEE; Abdominal-pelvic CT or PET/CT to find metastatic foci; vs usual care
  - Option 2: Combination vs standard IV monotherapy (vancomycin; daptomycin)

Adapted from Holland T et al. Clin Infect Dis, 2022 ciac364

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ROSE Lab

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- George Sakoulas and Victor Nizet – Univ. CA San Diego
- Sanjay Shukla - Marshfield Clinic
- Benjamin Howden and Sarah Baines – Univ. of Melbourne

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