Antimicrobial Efficacy Against Antibiotic-Tolerant *Staphylococcus aureus* Depends on the Mechanism of Antibiotic Tolerance

Meredith EM[1], Harven LT[1], Patterson SK[1], Berti AD[1,2]

[1]Wayne State University College of Pharmacy, [2]Wayne State University School of Medicine

WAYNE STATE
Eugene Applebaum College of Pharmacy and Health Sciences

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ABSTRACT

Background
Bacteria can adopt an alternate metabolic state favoring small molecule synthesis over growth. In *Staphylococcus aureus* this is induced by factors present during infection including nutritional limitation, host responses and competition with other bacteria. Isogenic "tolerant" populations have variable responses to antibiotics and can remain viable. Survivors resume growth upon cessation of antibiotics and cause relapse or recurrent infection. In this study we compare the capability of antibiotics to reduce viability of *S. aureus* made tolerant by different mechanisms.

Methods
Overnight *S. aureus* SH1000 cultures were diluted to 10^7 cfu/mL. Tolerance was induced with mupirocin (nutritional), HQNO (competitive), peroxynitrite (oxidative) or serum (humoral). Tolerant cultures were exposed to ceftaroline, daptomycin, gentamicin, levofloxacin, oritavancin or vancomycin at physiological concentrations and viability assessed by dilution plating. Minimum duration for 3-log viability reduction ("bactericidal activity", $MDK_{99.9}$) and 24h viability reduction were calculated independently for each of three biological replicates. Significance ($P < 0.05$) was determined using Student's t-test.

Results
Each antibiotic is ineffective against at least one type of induced tolerant staphylococci. Only daptomycin remains effective against humoral-tolerant staphylococci. Oritavancin remains effective against all forms of induced tolerance except for humoral tolerance.

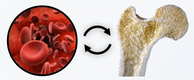
Conclusions
Antibiotic potency against tolerant staphylococci depends on the mechanism of tolerance. Each tolerance mechanism renders at least one antibiotic ineffective and each antibiotic is rendered ineffective by at least one mechanism of tolerance. Further studies to evaluate additional antibiotics, combination therapy and different tolerance inducers are warranted

Research Question:

Does the Mechanism of Tolerance Matter?

EXPERIMENTAL DESIGN

1 Nutritional Tolerance induced by mupirocin



- Abrupt nutritional downshift can result in acute ATP depletion and microbial cell death
- Bacteria respond to nutritional downshift by producing the alarmone ppGpp to induce a stringent response
- Mupirocin is a potent inducer of ppGpp production through inhibition of isoleucyl tRNA synthetases.
- *S. aureus* experiences abrupt nutrition downshifts as it moves from one infectious niche to another (*e.g.* blood to bone).

2 Competitive Tolerance induced by HQNO

- Bacteria in the same niche can induce tolerance
- *Pseudomonas aeruginosa* and *S. aureus* coexist in the cystic fibrosis lung
- *P. aeruginosa* produces several factors to impair *S. aureus* growth
- Heptyl-hydroxyquinoline *N*-oxide (HQNO) inhibits staphylococcal cytochrome bc1
- Impaired electron transport results in tolerance

3 Oxidative Tolerance induced by peroxynitrite

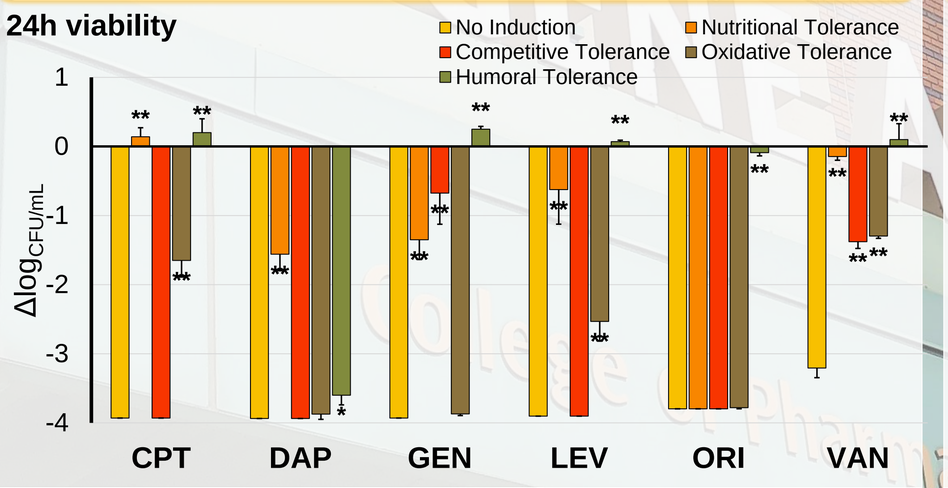
- Phagocytic immune cells kill foreign pathogens by producing reactive oxygen species
- Peroxynitrite is produced by macrophages during the oxidative burst
- Peroxynitrite uniquely induces tolerance by selectively inactivating bacterial aconitase

4 Humoral Tolerance induced by serum

- Neutrophils respond to invasive and endovascular staphylococcal infection by producing LL-37
- LL-37 induces rapid cell wall thickening by modulating the *graSR* pathway and reducing cell wall hydrolase production.
- Incubation with LL-37 levels present in normal human serum is sufficient for cell wall thickening and antimicrobial tolerance induction.

RESULTS

Change in Culture Viability 24 hours after antibiotic exposure



Time needed to induce a 3-log reduction in viability (hours)

Antibiotic	No induction	Nutritional Tolerance	Competitive Tolerance	Oxidative Tolerance	Humoral Tolerance
Ceftaroline	11.1 ± 0.19	†	11.1 ± 1.05	47.6 ± 0.34**	†
Daptomycin	1.5 ± 0.25	†	4.4 ± 0.09**	17.7 ± 0.95**	12.9 ± 0.72**
Gentamicin	1.3 ± 0.17	23.4 ±0.02**	†	21.9 ± 1.95**	†
Levofloxacin	1.7 ± 0.13	†	4.9 ± 0.56**	31.8 ± 3.00**	†
Oritavancin	1.2 ± 0.08	5.3 ± 0.41**	1.2 ± 0.02	1.4 ± 0.12	†
Vancomycin	25.8 ± 6.12	†	†	45.2 ± 1.39**	†

** - $P < 0.01$ vs. uninduced control
† - 3-log reduction in viability was not achieved within 48h

CONCLUSIONS

- Tolerance alters **both** the time to bactericidal effect and the extent of killing.
- Both the **antibiotic** and **mechanism of tolerance** impact time to bacterial effect and extent of killing.
- Humoral tolerance delays daptomycin bactericidal activity but does not change extent of killing.
- Oritavancin remains potent against most tolerant staphylococci except for humoral tolerance.
- Further studies to evaluate additional antistaphylococcal antibiotics and different inducers of tolerance are warranted

REFERENCES

- Brauner *et al*. 2016. Nat Rev Microbiol. 14(5):320-330.
- Ledger *et al*. 2022. Nat Commun. 13:2041.
- Beam *et al.* 2021. Infect Immun. 89(10):e0028621.
- Miller *et al.* 2021. PLoS One. 16(10):e0258592.
- Berti *et al.* 2020. Antibiotics (Basel). 9(11):789
- Berti *et al*. 2020. Science. 367(6474):141-142.