Mutations that inactivate the Tricarboxylic Acid Cycle in *Staphylococcus aureus* arise during persistent MRSA bacteremia

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Invasive MRSA infections continues to be a present threat

Persistent MRSA bacteremia is common with high morbidity and mortality despite appropriate antibiotics.

There is great interest in understanding the mechanism(s) behind persistent MRSA infections.

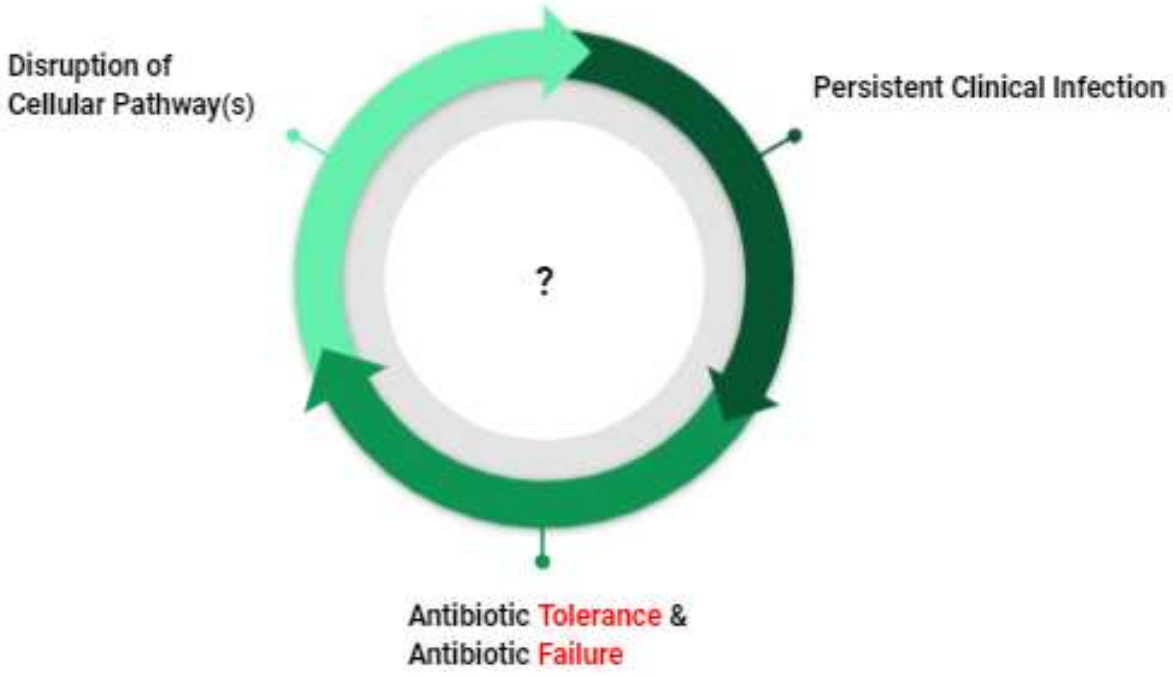
CDC

Persistent MRSA infections are associated with perturbations in cellular pathways and antibiotic tolerance

Clinically persistent *S. aureus* infections have been associated with alterations in cellular pathways and antibiotic tolerance.

These persistent infections have been linked to phenotypes associated with antibiotic failure despite appropriate antibiotic choice.

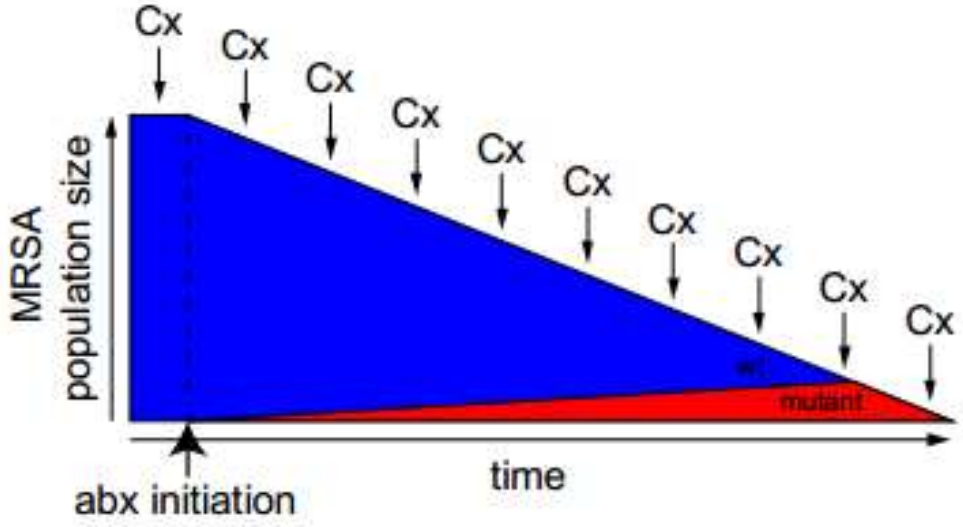
- Small Colony Variants (SCVs)
- Antibiotic “persister” sub-populations



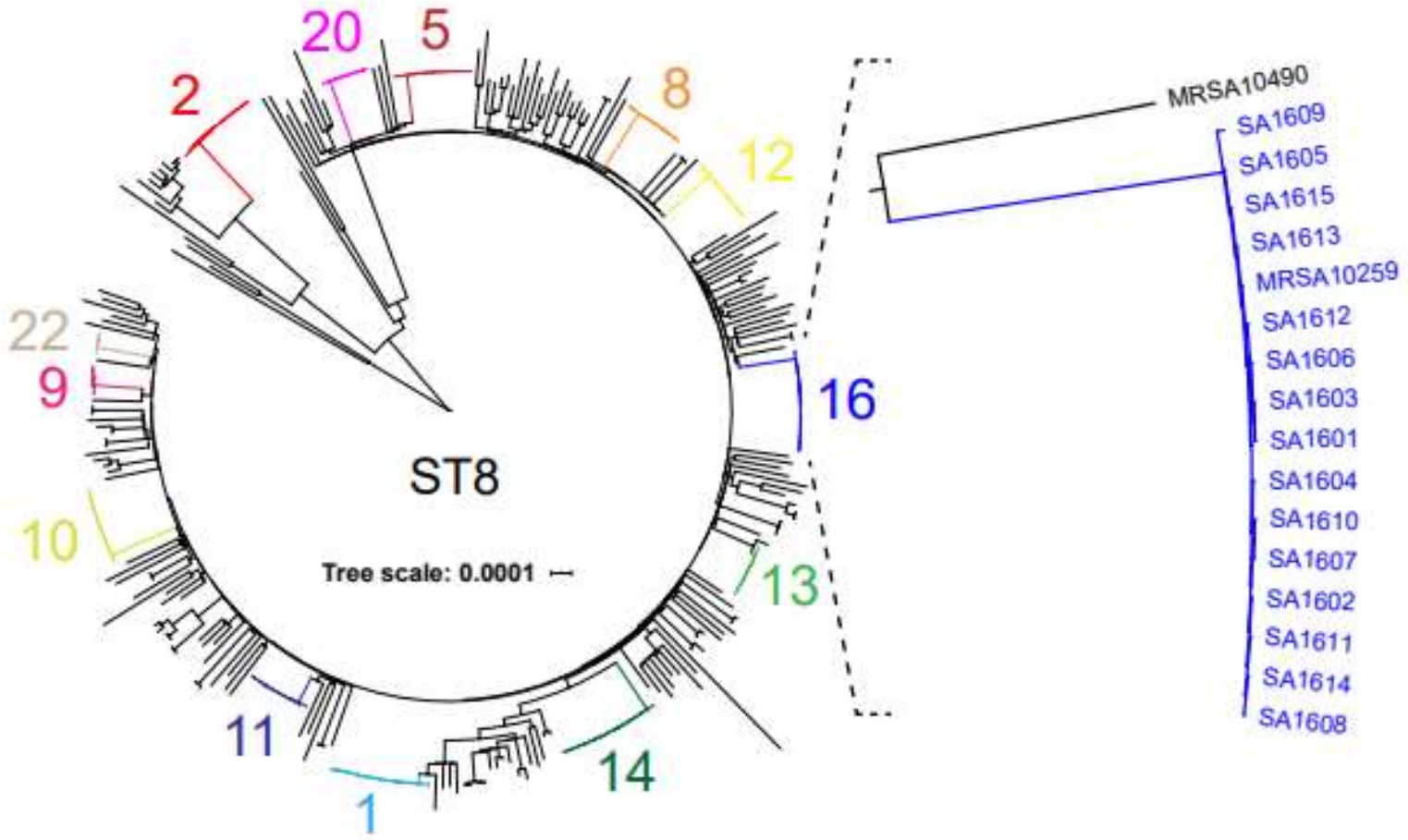
A forward genetic screen to search for within-host evolution of antibiotic tolerance

Sequenced whole genomes of 206 serially positive MRSA blood cultures from 20 patients with persistent clinical infections.

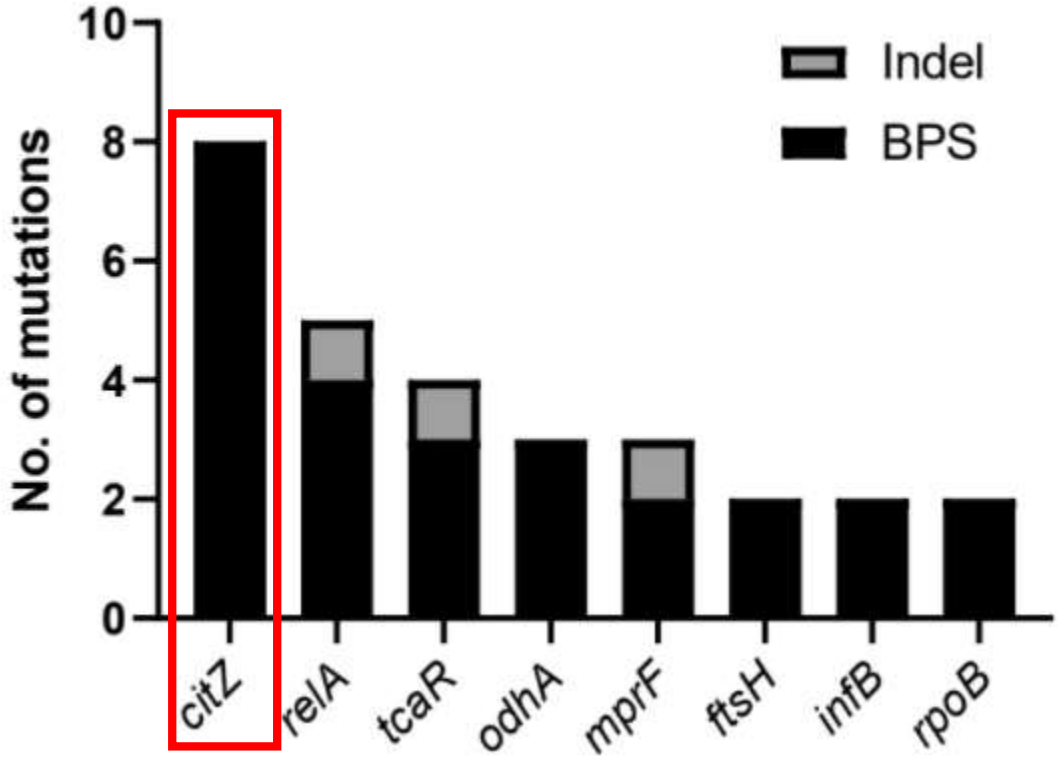
Hypothesis that continuous antibiotic exposure will give rise to mutations that convey antibiotic tolerance through within-host evolution.

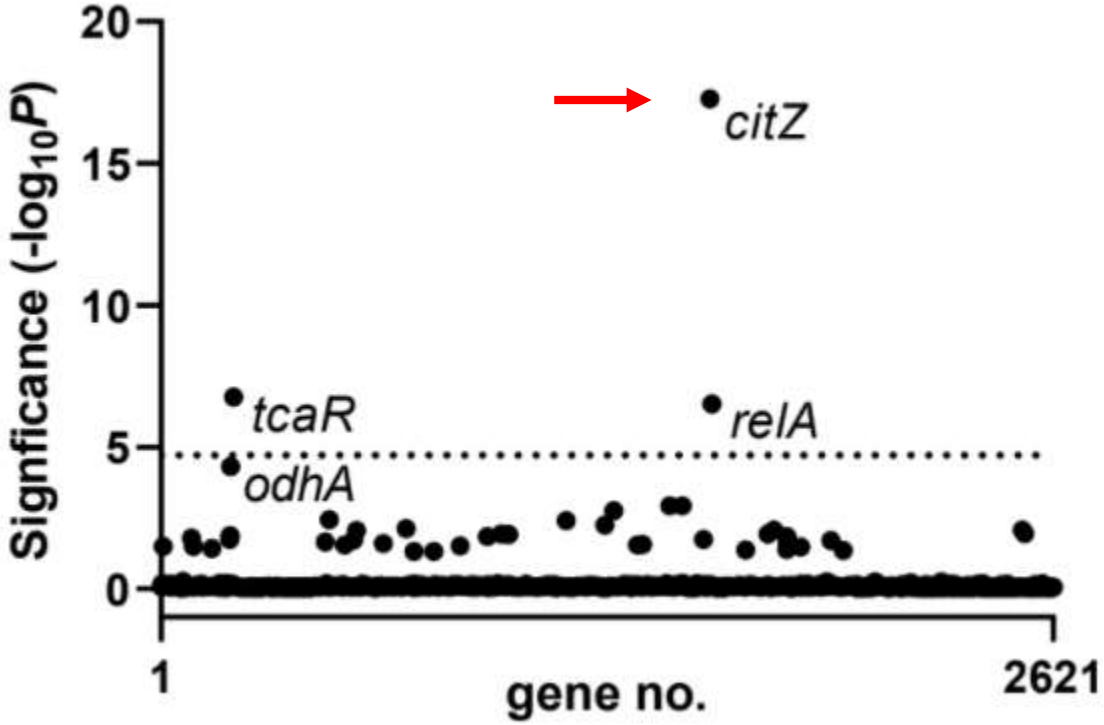


MRSA from different patients are genetically distinct



Convergent evolution of TCA cycle gene *citZ*



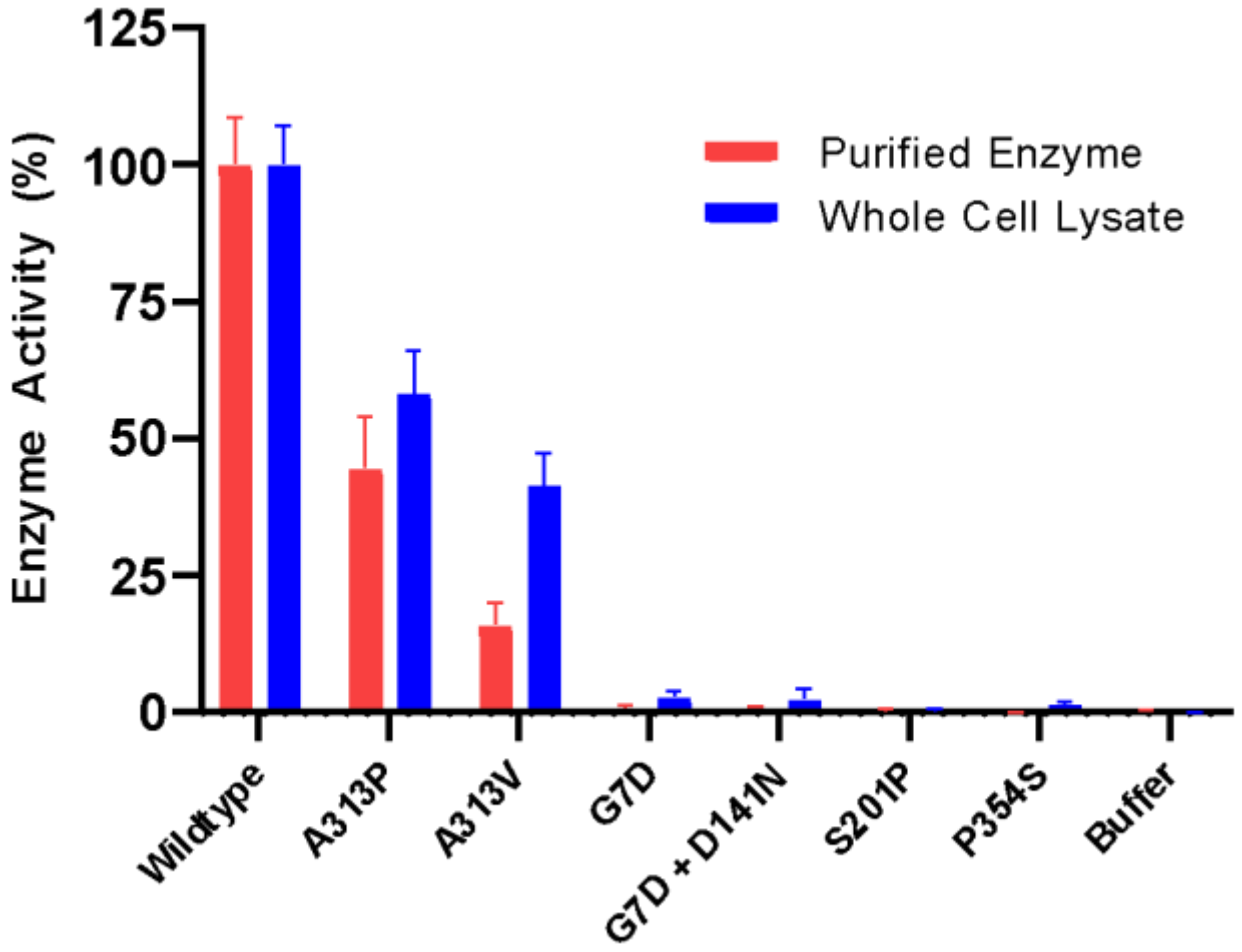


Key Questions

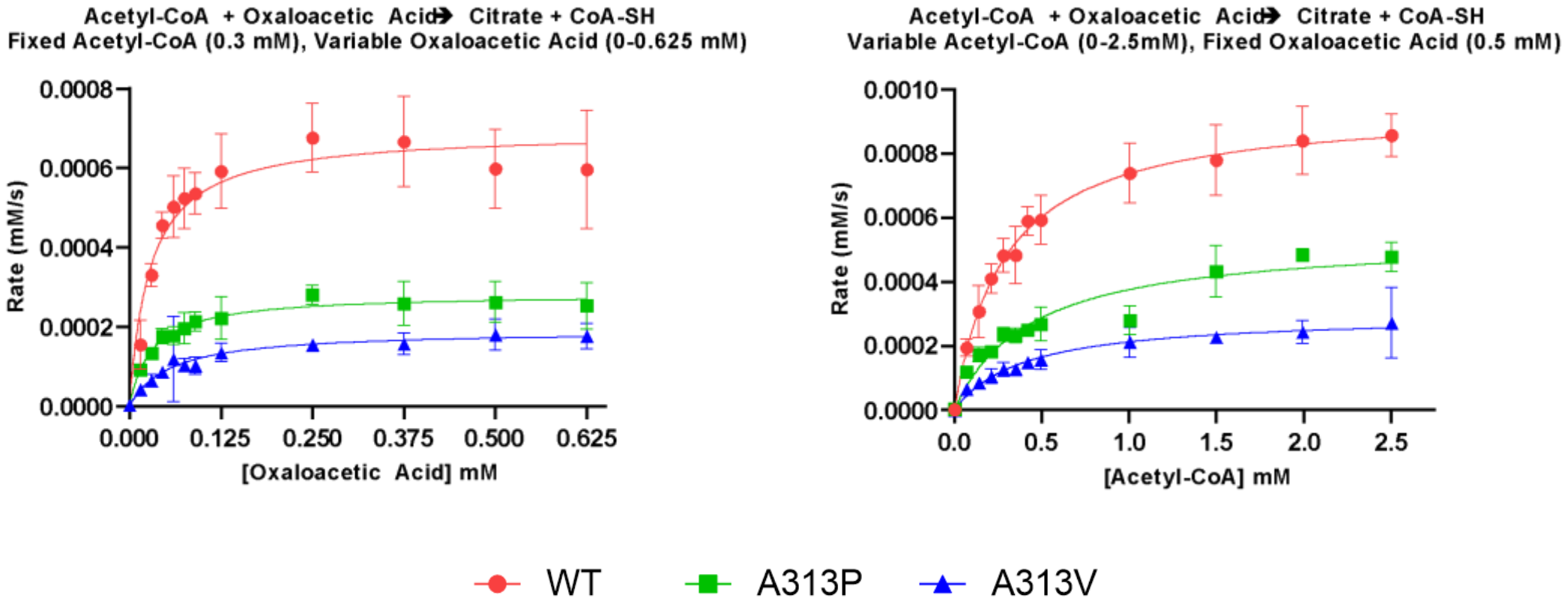
1. What are the effects of the *citZ* mutations on enzyme function?

2. What role do these mutations play in clinically persistent MRSA infections?

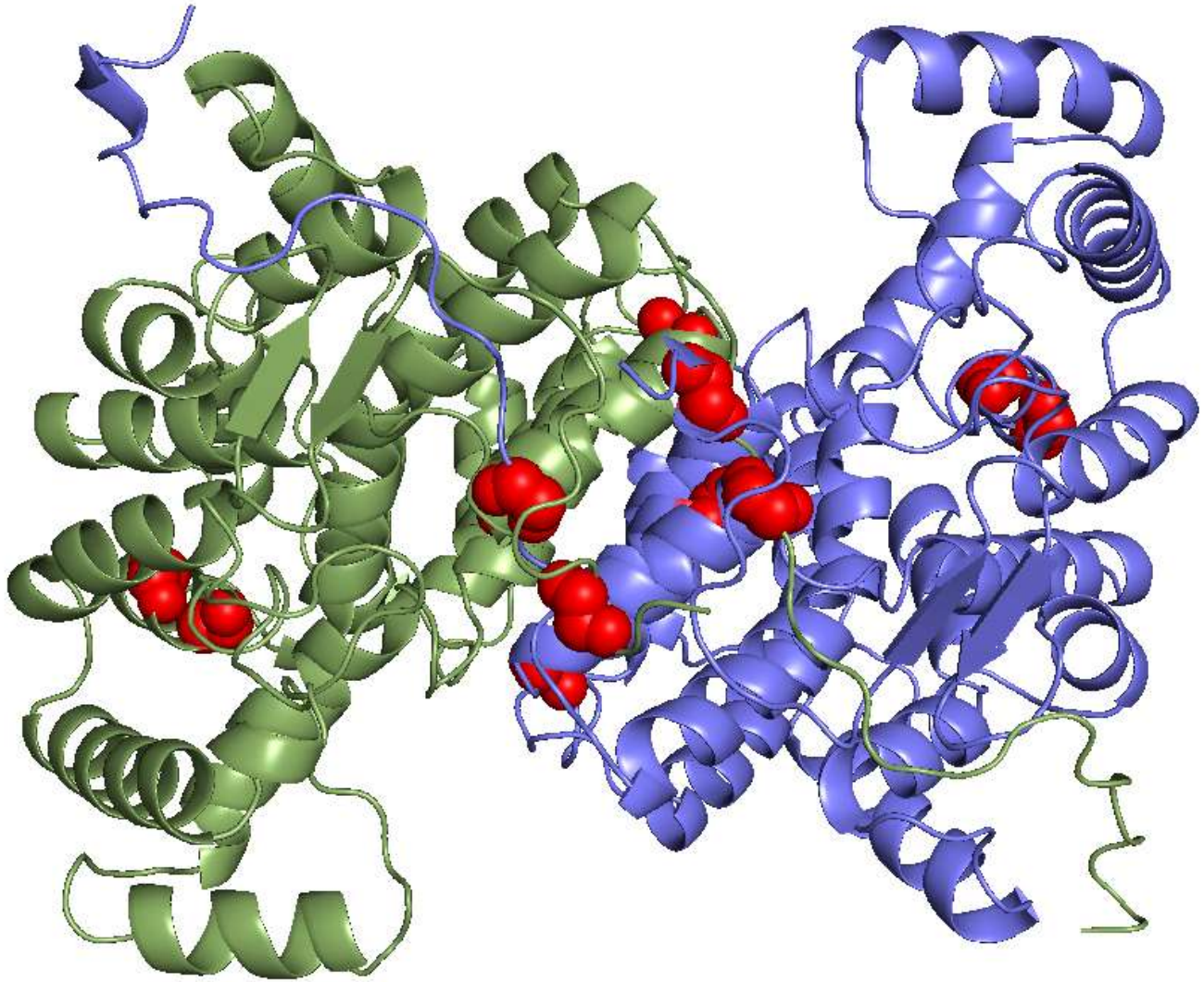
citZ mutations decrease citrate synthase enzyme activity



citZ mutations decrease citrate synthase enzyme activity



Citrate synthase mutations disrupt functional dimerization and destabilize α-helix packing



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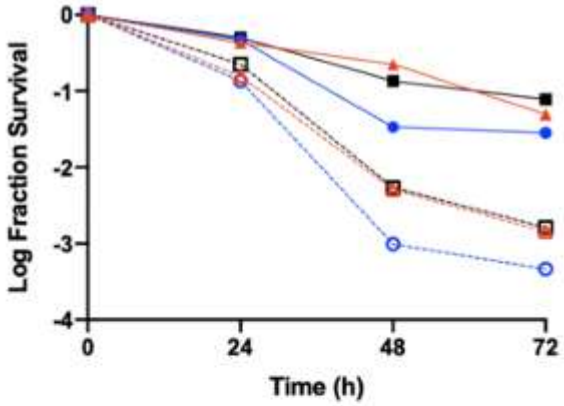
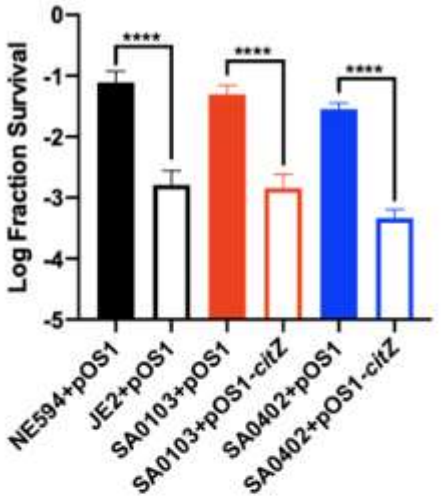
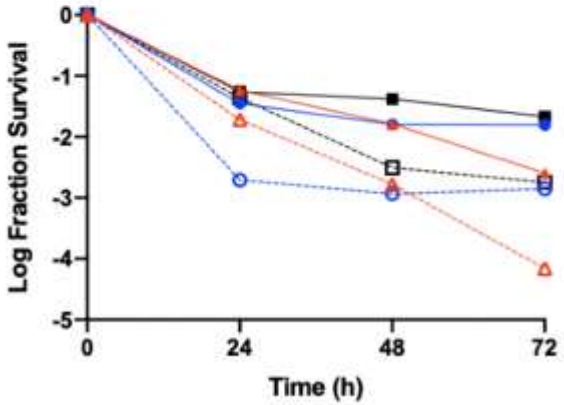
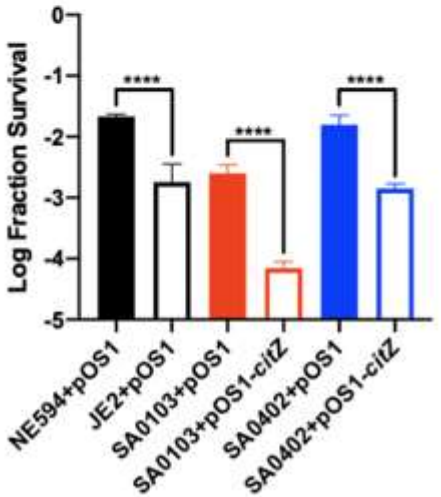
Clinical strains with *citZ* mutations have increased antibiotic tolerance





Ceftaroline





Daptomycin

Conclusions

- Convergent evolution of TCA cycle (*citZ*, *odhA*) and Stringent Response (*rel*) genes occur during persistent MRSA bacteremia.
- The strongest signal occurred in *citZ*, where the mutations caused loss of citrate synthase activity.
- Clinical strains harboring *citZ* mutations demonstrate increased antibiotic tolerance.

∴ Convergent evolution of antibiotic tolerance occurs frequently in MRSA bacteremia.

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