Post-fluoroquinolone treatment molecular events and nutrient availability modulate *Staphylococcus aureus* antibiotic persistence

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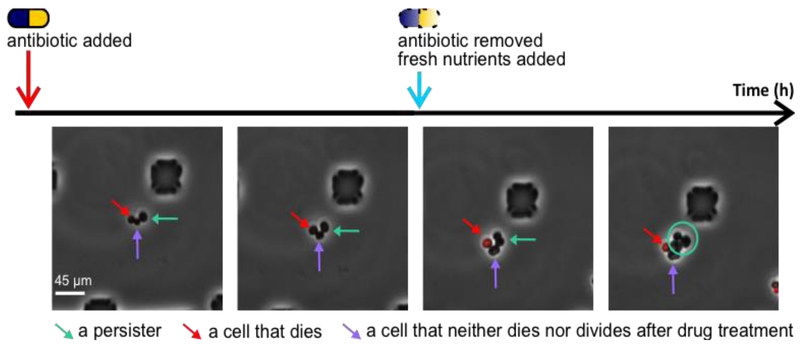
UCONN HEALTH

INTRODUCTION

What are persisters?



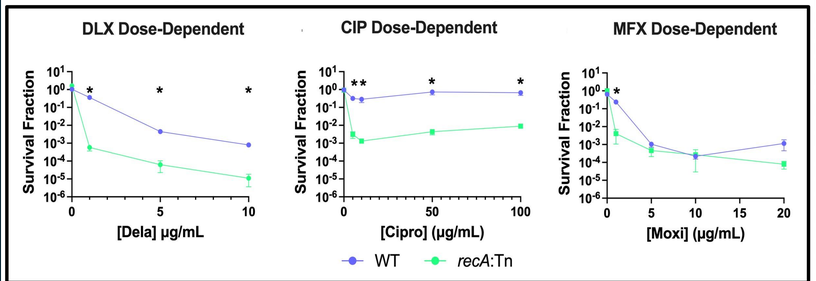
- Persisters are antibiotic-tolerant phenotypic variants that contribute to chronic and relapsing bacterial infections, posing a significant challenge to effective treatment; therefore, it is critical to study bacterial persisters.

Our Hypothesis

- FQs cause topoisomerases to stall, which leads to double-stranded breaks (DSBs) that must be repaired for the bacteria to survive[2].
- In *Escherichia coli*, *recA* and *recB,* and other genes involved in DNA recombination and DSB repair, are shown to be important for FQ persisters stemming from stationary-phase cultures[3].
- We investigate whether DSB repair enzymes are important for persisters from starved *S. aureus* cultures following FQ treatment and probe the effect of nutrient availability before, during, and after FQ therapy on persistence.

RESULTS

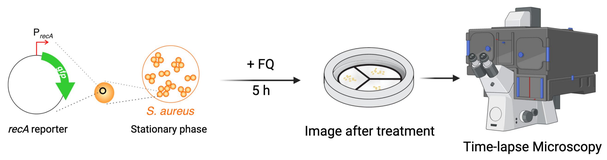
Is *recA* required for *S. aureus* FQ persister survival?

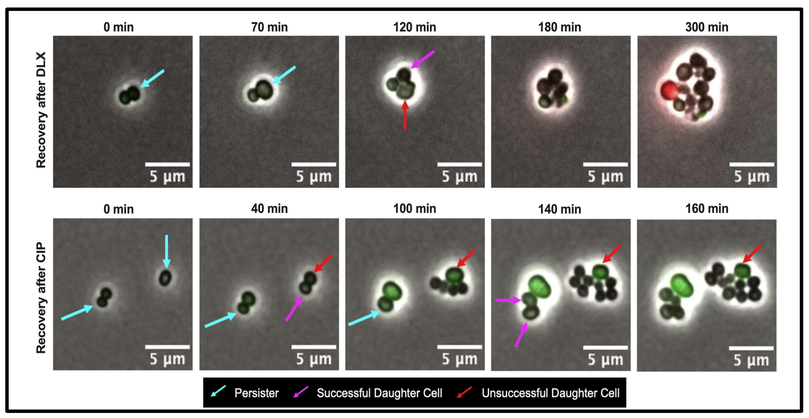


Conclusions

Loss of *recA* decreases FQ persistence of stationary-phase *S. aureus*.

When is *recA* expressed in FQ-treated *S. aureus*?





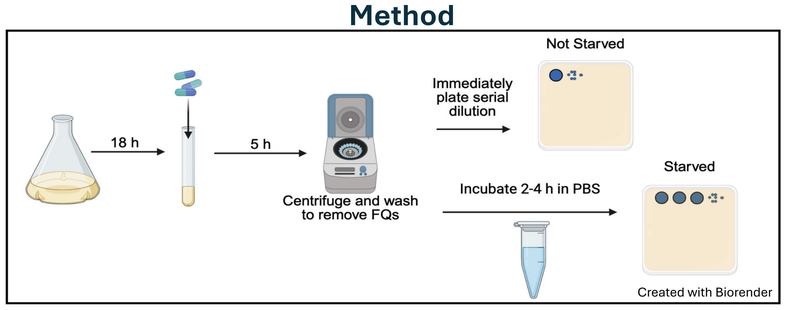
Conclusions

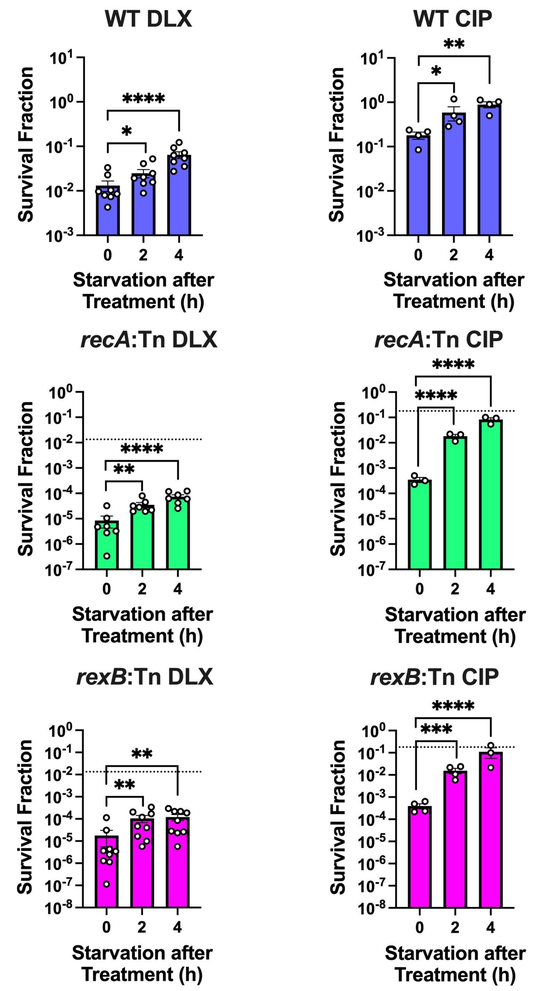
- *S. aureus* expresses *recA* during the post-treatment recovery period
- *S. aureus* FQ persisters do not all survive simply because they have escaped antibiotic-induced damage
- *S. aureus* persisters may not completely repair antibiotic-induced damage before dividing, and the damage may be partitioned to the unsuccessful daughter.

RESULTS

How does the nutrient environment post-FQ treatment recovery period modulate DNA repair activities?

Method





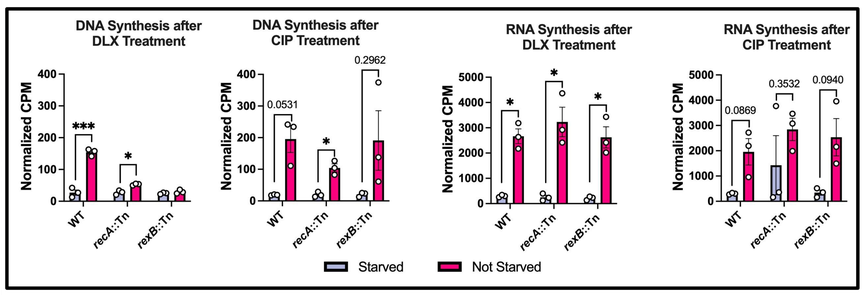
Conclusions

Starvation after FQ treatment increases persistence even in mutants lacking DSB repair enzymes

How do post-FQ treatment starvation effects nucleic acid synthesis?

Method

Labelled newly synthesized *S. aureus* RNA and DNA with ^{3}H-uridine during incubation in PBS (starved condition) or cation-adjusted Mueller-Hinton broth (non-starved condition) following DLX or CIP treatment.



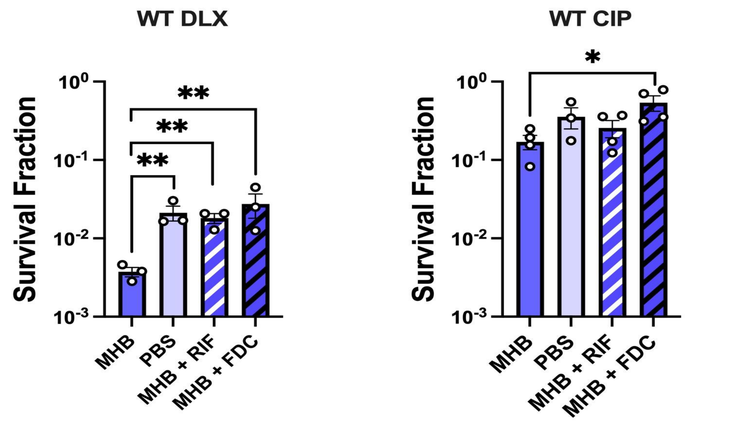
Conclusions

Starvation after FQ treatment decreased de novo nucleic acid synthesis

Is decreased nucleic acid synthesis responsible for the increased survival of persisters?

Method

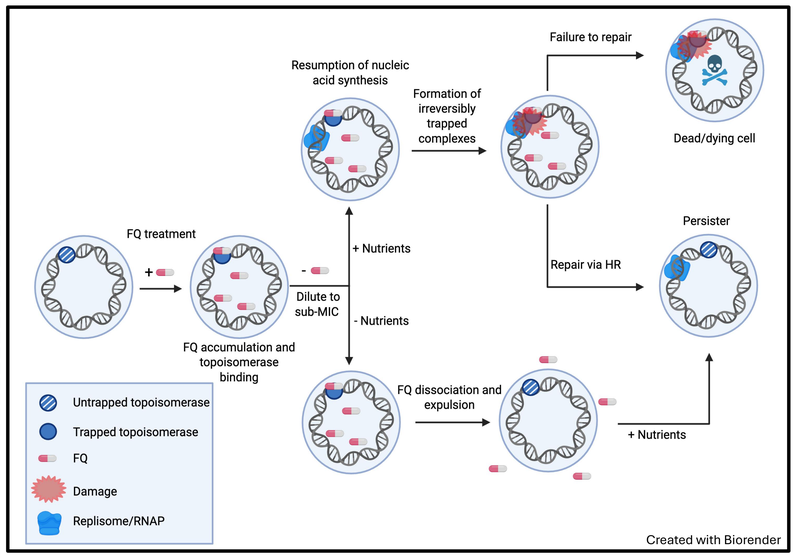
To determine whether decreased nucleic acid synthesis was responsible for the increased survival of starved cells, we used chemical inhibitors to halt *de novo* DNA (FDC; 5-fluoro-2'-deoxycytidine)or RNA (RIF; Rifampicin) synthesis after FQ treatment.



Conclusions

Inhibiting nucleic acid synthesis after FQ treatment increases survival even in the presence of nutrients.

SUMMARY



Acknowledgments

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References

1. Hooper, D. C.; Jacoby, G. A. Topoisomerase Inhibitors: Fluoroquinolone Mechanisms of Action and Resistance. *Cold Spring Harb. Perspect. Med.* **2016**, *6* (9), a025320.
2. Amarh, V.; Arthur, P. K. DNA Double-Strand Break Formation and Repair as Targets for Novel Antibiotic Combination Chemotherapy. *Future Sci. OA 5* (8), FSO411.
3. Mok, Wendy WK, and Mark P. Brynildsen. "Timing of DNA damage responses impacts persistence to fluoroquinolones." *Proceedings of the National Academy of Sciences* 115.27 (2018): E6301-E6309.
4. Jonathan I. Batchelder*, Nisha Mahey*, Wendy W. K. Mok. Post-fluoroquinolone treatment molecular events and nutrient availability modulate *Staphylococcus aureus* antibiotic persistence bioRxiv 2025.06.26.661800; doi: https://doi.org/10.1101/2025.06.26.661800