# Evolution of antibiotic resistance in chronic *Pseudomonas aeruginosa* infections

David Ritz[1] and Daniel Schultz[1].

***Short Abstract* — Aminoglycosides are used to treat *Pseudomonas aeruginosa (Pa)* infections, but success is limited due to the MexXY-OprM multidrug efflux mechanism. During chronic infections, *Pa* commonly acquires mutations disabling MexXY-repressor MexZ. However, it is still unclear why *mexZ* mutations are favorable since these mutants do not show higher levels of resistance by common measures. Here we use single-cell and population-level experiments to show that that *mexZ* mutations increase fitness not by increasing drug resistance in steady state, but by delivering a faster response upon drug exposure.**

***Keywords* — Antibiotic, resistance, dynamics**

## I. Antibiotic response dynamics affect cell fate

*Pseudomonas aeruginosa* (*Pa*) is a leading cause of mortality for cystic fibrosis (CF) patients [1].

Aminoglycosides are often used in the treatment of chronic infections, but success is limited mostly due to high expression of a multidrug efflux mechanism MexXY [2]. About half of the lineages evolved in CF lungs eventually acquire mutations modifying MexXY-repressor MexZ, which are predominantly loss-of-function mutations [3]. This convergent evolution suggests the importance of these mutations during *Pa*'s adaptation to the human lung. However, it is not clear why *mexZ* mutants have a fitness advantage in the CF environment and which CF-relevant stressors favor the emergence of loss-of-function *mexZ* mutations, since the change in the mutant strain's susceptibility to aminoglycosides is negligible according to standard MIC tests. While both Δ*mexZ* and WT express *mexXY* to similar levels in the presence of aminoglycosides, Δ*mexZ* cells have a higher basal level of *mexXY* expression compared to WT [2], suggesting that higher resistance may be linked to increased basal levels of *mexXY* expression prior to drug exposure. Here, we test the hypothesis that *mexZ* mutations increase antibiotic resistance by delivering a fast response when challenged by the drug.

To study the dynamics and heterogeneity of aminoglycoside responses, we have constructed continuous culturing systems and microfluidic devices to follow induction of resistance during drug exposures, in both single-cells and mixed populations of mutants. We have determined the relative fitness between WT, Δ*mexZ*, and Δ*mexY* cells to provide a quantitative description of how *Pa* populations adapt their responses to new ecological niches.

## II. Fast expression of resistance is crucial upon exposure to high doses of antibiotics

### *A. Appropriate mexXY expression is important for survival*

To determine if *mexXY* expression can predict cell fate, we used a custom-built microfluidic device to image *Pa* transformed with a pMexXY reporter plasmid following abrupt exposure to an aminoglycoside. This experiment revealed heterogeneity in *mexXY* expression at the single-cell level. Furthermore, we found that high levels of *mexXY* expression upon drug exposure favored cell growth, while too low *mexXY* expression resulted in cell arrest or death, suggesting that the dynamics of *mexXY* expression upon drug exposure is crucial for cell survival.

### *B. Deletion of mexZ increases survival upon drug exposure*

We developed a simple assay to determine the proportion of cells that survive initial drug exposure, as well as the steady-state growth rate of the surviving subpopulation. We exposed growing cultures to aminoglycosides and measured the resulting delay in growth recovery to infer the size of the surviving subpopulation. This measure of resistance considers the dynamics of the response and is complementary to traditional steady-state minimum inhibitory concentration measurements. From this assay, we determined the steady-state growth of WT and Δ*mexZ* populations to be similar, but Δ*mexZ* has more surviving cells after drug exposure than WT.

### *C. ΔmexZ has a brief fitness advantage after drug exposure*

To determine the effect of having a greater number of cells survive a drug exposure, we competed Δ*mexZ* and WT populations in a custom-built continuous culture system as the strains are exposed to different aminoglycoside regimens. This culturing system allows the long-term propagation and detection of diverse microbial populations under defined metabolic states, which allows us to discern even small differences in fitness. After an abrupt drug exposure during the competition culture, Δ*mexZ* cells are briefly in higher abundance than WT cells, suggesting that *mexZ* mutants will be favored in fluctuating environments.

## III. Conclusion

This work helps reveal principles underlying the dynamics of antibiotic responses and the evolution of gene regulation controlling antibiotic responses.

## References

[1] Cramer, N. *et al.* Microevolution of the major common *Pseudomonas aeruginosa* clones C and PA14 in cystic fibrosis lungs: *Pseudomonas aeruginosa* microevolution in cystic fibrosis. *Environmental Microbiology* **13**, 1690–1704 (2011).

[2] Morita, Y., Tomida, J. & Kawamura, Y. MexXY multidrug efflux system of *Pseudomonas aeruginosa*. *Frontiers in Microbiology* **3**, (2012).

[3] Marvig, R. L., Sommer, L. M., Molin, S. & Johansen, H. K. Convergent evolution and adaptation of *Pseudomonas aeruginosa* within patients with cystic fibrosis. *Nat Genet* **47**, 57–64 (2015).

[1]Department of Microbiology and Immunology, Dartmouth College. E-mail: david.ritz.gr@dartmouth.edu