An Experimentally Driven Multiscale Study of Bacterial Efflux Machinery

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***Short Abstract* — Bacterial multi-drug resistance efflux pumps are complex molecular machines that expel multiple drugs and antibiotics. They also influence important genetic and cellular processes to confer additional drug resistance. We develop a multi-scale mathematical model that integrates structural, genetic, and cellular processes to understand how efflux pumps work. To test our model, we carried out measurements on the time-kill behavior of wild-type *Pseudomonas aeruginosa* as well a mutant strain with a deficient efflux system lacking the pumps (ΔMexAB- OprM). The integrated model predicts the same quantitative behavior as the measurements and suggests specific experimentally testable mechanisms that are important for drug efflux.**

I. Purpose

BACTERIA can detect the presence of antibiotic inside the cell. Responding to this signal, they increase the production of efflux pumps that expel the drug [1] and decrease the production of porins that allow entry of the drug [2]. The production of efflux pumps is controlled both by local regulator proteins that turn on expression of the pump genes, and by global regulator proteins that affect expression of many genes including porins. The detailed mechanisms of how drugs interact with and control drug efflux systems will allow an understanding of how the bacteria respond to the threat of antibiotics. Efflux pumps can also transport quorum-sensing molecules which, when exported from one cell, induce neighboring cells to produce a new set of proteins for biofilm production allowing bacterial growth, virulence, and survival in the presence of antibiotics [3-4].

It is our hypothesis that an experiment-based model that integrates structural, genetic, and cellular mechanisms to predict drug uptake and survival will provide a quantitative understanding of how efflux pumps and the associated genetic and cellular systems defend bacteria against drugs and antibiotics.

II. Methodology

We have constructed a mathematical model using coupled ordinary differential equations that integrate structural, genetic, and cellular processes. These equations relate the growth and death of freely growing or biofilm bacteria in the presence of antibiotics to experimentally observable quantities such as concentrations and Hill coefficients. The model includes mechanisms of antibiotic uptake and efflux, control of efflux pump activity by antibiotics, and the effects of antibiotics on cell growth and death.

To test our model, we carried out measurements on the killing of *P. aeruginosa* cells by the antibiotic, ciprofloxacin, in liquid culture. We studied both wild-type cells and mutant cells lacking the MexAB-OprM pump which is of particular interest because it has been shown to transport both ciprofloxacin and quorum sensing acetyl homoserine lactones [5].

III. Conclusion

Compared to the mutant cells, the wild-type cells showed increased antibiotic uptake at short times. The mutant cells accumulated higher antibiotic levels at long times, however, and were killed at a higher rate. The model captures these qualitative behaviors and agrees quantitatively with the measurements on both wild-type and mutant cells. Our model is a mathematical representation of specific drug efflux mechanisms that can be targeted for more detailed experimental studies. We conclude that our integrated model coupling structural, genetic and cellular levels can describe key experimentally testable aspects of drug resistance in bacteria.

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