Population Dynamics of Cooperative Resistance in *E. faecalis*

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***Short Abstract* — *E. faecalis* is a gram-positive bacterial species among the leading causes of nosocomial infections. In this work, we investigate the dynamics of enzyme-mediated cooperative, drug resistance in *E. faecalis* populations exposed to temporally varying influx of β-lactam antibiotics. By combining experiments in computer-automated bioreactors with mathematical models, we show that drug-treated populations exhibit a range of dynamic behaviors, including bistability between population extinction and survival depending on the flow rate of antibiotic, the initial population density, and ratio of sensitive and resistant cells.**

***Keywords* — Population Dynamics, Cooperation, Antibiotic Resistance, Modeling, Chemostat**

I. Background

Antibiotic resistance is an urgent threat to public health and has garnered significant research interest. While the molecular mechanisms of resistance are increasingly understood, the picture of how resistance determinants are spread in complex microbial populations remains incomplete. While selection driven fixation of a single resistance phenotype is perhaps the simplest route to a resistant population, recent work has highlighted that resistance may also be a collective phenomenon depending in complex ways on the competition and cooperation between sensitive and resistant cells [1-4].

In this work, we investigate the population dynamics of β-lactamase mediated drug resistance in *E. faecalis*, a common source of hospital-acquired infections. β-lactamase is responsible for the degradation of antibiotics of the β-lactam class [5-6]. Recent work has shown that drug deactivation, even if intracellular [4], can lead to counterintuitive population dynamics by promoting the survival of drug sensitive cells [1-4]. For example, adding an enzyme inhibitor to disrupt β-lactamase function may lead to populations increasingly dominated by enzyme-producing resistant cells [3]. In addition, we've recently shown [7] that the efficacy of β-lactams increases with population density in *E. faecalis*, a surprising pH-mediated effect that occurs in the absence of β-lactamase production. Taken altogether, these results indicate that β-lactamase resistance may induce rich dynamical behavior in bacterial populations, particularly in the presence of time-dependent flows of antibiotics.

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II. Results

Here we combine experiments using computer-automated bioreactors and flow cytometry with mathematical models to investigate β-lactamase resistance in mixed populations of sensitive and resistant (enzyme-producing) cells in the presence of time-dependent influx of antibiotic. We observe a wide range of dynamical behavior, including bistability between population extinction and survival, that depends on flow rate of drug, the initial (total) population density, and the ratio of sensitive to resistant cells. Using a simple mathematical model, we derive and experimentally validate a full phase diagram that predicts regimes of population survival, extinction, and bistability that arise from the interplay between drug degradation, temporal dosing dynamics, and density-dependent efficacy of the antibiotic. In addition, we experimentally modulate pH using different growth media to tease apart contributions to these dynamics from density-dependent drug activity (modulated by pH), and density-dependent drug degradation (modulated by enzyme production). Finally, we discuss ongoing work to optimize dynamical drug dosing strategies for maximizing population extinction.

III. Conclusion

Our findings uncover rich dynamics of *E. faecalis* populations exposed to β-lactams. These results underscore the need for quantitative understanding of cooperative resistance in the systematic optimization of antimicrobial treatment strategies.

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