# Evolutionary dynamics of combined antibacterial treatment

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***Short Abstract* — β-lactam antibiotics are administered in combination with β-lactamase (Bla) inhibitors to address Bla-mediated resistance. However, the factors governing the evolutionary consequences of Bla inhibitor use are still poorly understood. Using mathematical modeling and an engineered experimental system, we identify strain- and drug-specific factors that predict the evolutionary response of strains treated with β-lactam/Bla inhibitor combinations. Our results can guide clinicians in treating infections while minimizing resistance.**

## I. Introduction

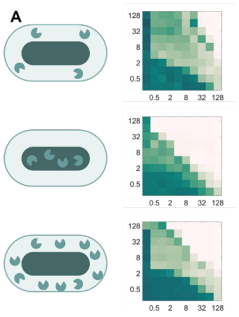
β-LACTAM antibiotics are the most commonly prescribed antibiotic in the United States [1]. Resistance to β-lactams is also becoming prevalent: the CDC estimates a 50% increase between 2013 and 2019 in infections caused by extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae [2]. Given the economic disincentives constraining development of new antibiotics, it is necessary to use available therapies in ways that 1) extend their useful lifetime and 2) minimize and slow resistance development.

Bla inhibitors are the prototypical example of adjuvants that can potentiate antibiotics against resistant bacteria [3]. However, due to the cooperative but partially privatized [4] benefits of Bla production, in which antibiotic degradation can incidentally protect a sensitive subpopulation, the evolutionary consequences of Bla inhibitor use are complex and poorly understood. It may reduce the protection of sensitive cells, selecting for resistant cells [5]; or it may reduce the private benefit of Bla production, giving metabolically unburdened sensitive cells a competitive advantage [6]. Here, we explore factors governing the evolutionary effects of β-lactam/Bla inhibitor combinations.

## II. Results and Discussion

### *A. Dose responses in synthetic strains*

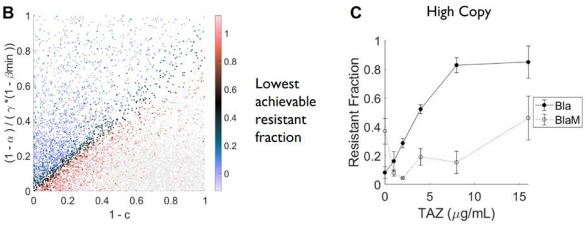
We designed plasmids with differing copy numbers and cytoplasmic and periplasmic versions of Bla. The cytoplasmic version BlaM requires cell lysis for release and provides less private benefit to the producing cell. Starting from an equal mixture of the resistant strain and the plasmid-free sensitive strain, we measured dose responses to different concentrations of amoxicillin and Bla inhibitors (tazobactam results in **Figure A**). Both privatization and burden influence survival.




### *B. Modeling analysis and experimental results*

Our modeling shows that three key parameters (high metabolic burden, low private benefit, and high intracellular Bla inhibition), favor selection against resistance. We find that a simplified criterion can predict the evolutionary responses of simulated strains with randomized parameters (**Figure B**).

Using fluorescence microscopy, we measure changes in the resistant fraction of a mixed population in response to a low dose of amoxicillin and increasing doses of different Bla inhibitors (selected results for high copy plasmids responding to tazobactam shown in **Figure C**). Across conditions, we find that the periplasmic Bla with higher private benefit results in higher resistance than cytoplasmic BlaM.




## III. Conclusion

Our results suggest that diagnostics to identify strains with low private benefit, flexibility in antibiotic to inhibitor ratio, and the development of adjuvants to increase intracellular inhibition may all help clinicians use β-lactam/Bla inhibitor combinations in a way that minimizes resistance selection.

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