Isolated cell behavior drives the evolution of antibiotic resistance

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***Short Abstract* — Bacterial antibiotic resistance is typically quantified by the minimum inhibitory concentration (MIC). However, for β-lactam antibiotics such as cefotaxime, the cooperative inactivation of drugs by the bacterial population causes the measured MIC to depend strongly on the initial cell density. Here we demonstrate that the resistance of a single, isolated cell—which we call the Minimum Killing Concentration (MKC)—provides a superior metric for antibiotic resistance. We find that the MKC both predicts the direction of selection and specifies the antibiotic concentration at which selection begins to favor new mutants, and that the MIC is not reliable for these two properties. This study demonstrates that understanding the cooperative nature of bacterial growth in antibiotics is essential in predicting the evolution of antibiotic resistance.**

***Keywords* — Antibiotic resistance**

Predicting the evolution of antibiotic resistance in bacterial populations is a key challenge [1]. The level of antibiotic resistance of microbes is typically quantified via the minimum inhibitory concentration (MIC)[2-4], which is defined as the minimal concentration of antibiotic that will inhibit bacterial growth starting from a standard cell density over a 20 hour period [4]. The MIC is often used as a proxy for bacterial fitness in the presence of the antibiotic [5, 6], and in addition is thought to indicate the minimal antibiotic concentration at which there is selection for increased resistance [8,9]. Thus, the MIC plays a major role in our understanding of the evolution of antibiotic resistance in bacteria.

However, while the MIC is often considered a one-value proxy for fitness, its relationship to evolutionary fitness is often complicated. For β-lactam antibiotics (both the oldest and most widely-used class of antibiotics [10]), the MIC is subject to the "inoculum effect": its measured value is strongly dependent upon the initial density of the culture [11]. Because the standard initial cell density is essentially an arbitrarily chosen value, and given the sensitivity of the measured MIC on the starting initial cell density, any quoted MIC value lacks a clear connection to bacterial fitness.

In this work, we demonstrate experimentally that MIC is a flawed metric for quantifying the level of antibiotic resistance because it is not independent of the cooperative growth dynamics between cells. We show that instead, the characterization of the direct benefit conferred by resistance for a single isolated cell is a more robust, meaningful, and useful way to quantify the level of resistance of a bacterial strain. This single cell resistance is simply the measured MIC in the limit of low initial cell densities—what we call the Minimum Killing Concentration (MKC). This quantity predicts both the direction of selection and the antibiotic concentrations at which there is selection for increased resistance. Importantly, these two key properties of the MKC are independent of the experimental cell density. Our study demonstrates that understanding the cooperative nature of bacterial growth in antibiotics is essential in predicting the evolution of antibiotic resistance.

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