Robust, linear correlations between growth rates and β-lactam–mediated lysis rates

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It is widely acknowledged that faster-growing bacteria are killed faster by β-lactam antibiotics. This notion serves as the foundation for the concept of bacterial persistence: dormant bacterial cells that do not grow are phenotypically tolerant against β-lactam treatment. Such correlation has often been invoked in the mathematical modeling of bacterial responses to antibiotics. Due to the lack of thorough quantification, however, it is unclear whether and to what extent the bacterial growth rate can predict the lysis rate upon β-lactam treatment under diverse conditions. Enabled by experimental automation, here we measured >1,000 growth/killing curves for eight combinations of antibiotics and bacterial species and strains, including clinical isolates of bacterial pathogens. We found that the lysis rate of a bacterial population linearly depends on the instantaneous growth rate of the population, regardless of how the latter is modulated. We further demonstrate that this predictive power at the population level can be explained by accounting for bacterial responses to the antibiotic treatment by single cells. This linear dependence of the lysis rate on the growth rate represents a dynamic signature associated with each bacterium–antibiotic pair and serves as the quantitative foundation for designing combination antibiotic therapy and predicting the population-structure change in a population with mixed phenotypes.

beta-lactams | systems biology | quantitative biology | antibiotic resistance

As the oldest and most widely used antibiotics, β-lactams opened a new era of history in medicine (1) and became a foundation for subsequent development of antibiotics (2–5). In the past decade, however, there has been a rapid rise of resistance to β-lactams as well as to all other antibiotic classes (6). To address this emerging antibiotic crisis, there is an urgent need for developing treatment strategies to better use existing antibiotics, in addition to developing new ones (7). To this end, it is important to have a quantitative understanding of how bacterial populations respond to antibiotic treatment under diverse conditions (8–10); however, this understanding is often lacking.

In this study, we examine bacterial antibiotic response to β-lactams, which represent 65% of all antibiotics used to treat bacterial infections (11). It is well recognized that faster-growing bacteria are more susceptible to killing by β-lactams. In other words, the β-lactam–mediated lysis rate is expected to increase with the bacterial growth rate, all else being equal. In the extreme case, bacteria can switch into a phenotypically dormant state (with little or no growth) and exhibit drastically increased tolerance to β-lactams (12). Having the qualitative notion of the positive correlation, however, is insufficient for making quantitative predictions of bacterial population dynamics during antibiotic treatment. A positive correlation can imply many different things: It can take the form of any monotonic increasing function (linear, quadratic, exponential, and so on), and each specific form will have distinct consequences in the resulting population dynamics during antibiotic treatment. To date, the quantitative nature of the positive correlation between growth and lysis rates is far from being well established. Thorough quantifications are extremely lacking, considering the nearly universal acceptance of the correlation.

Over the last several decades, only a few studies attempted to examine this correlation (13–15). In one, Tuomanen et al. (13) provided direct evidence for a strict proportionality between growth rates and lysis rates for bacteria in a balanced growth environment, as maintained by using a chemostat. However, this pioneering study left open important questions regarding the generality and robustness of the observed proportionality. First, the direct proportionality was established based on measurements of two *Escherichia coli* strains against two antibiotics, each tested for three to four growth rates (13), which limited its scope and statistical robustness.

Second, the measurements were done for bacteria experiencing balanced growth, with the growth environment maintained at a steady state in a chemostat. In balanced growth, the cell physiology is, on average, constant over time. In general, however, as the growth environment changes over time, the growth rate changes slightly. As such, it is not as "balanced" as one that is maintained by a chemostat at a steady state. In unbalanced growth, the bacterial physiology is markedly different from that during balanced growth. During unbalanced growth, both the bacterial growth rate and the different aspects of bacterial physiology, as evident in intracellular gene expression (16) and metabolism (17–19), continually change over time due to the interplay among gene expression, bacterial growth, and modulation of the environment (20–22). Moreover, other environmental perturbations are also known to modulate bacterial physiology substantially (23). Third, still unknown is how a particular correlation between growth rate and lysis rate for a population emerges from how single cells respond to antibiotics.

Significance

How fast bacteria grow influences the efficacy of β-lactams, one of the most commonly used classes of antibiotics. However, the quantitative nature of this correlation is not well established. With precise measurements and analyses enabled by experimental automation, we found a robust relationship between growth and lysis rates that is generally applicable to diverse pairs of β-lactams and bacteria. That is, the growth rate of population serves as a reliable predictor for the lysis rate in response to a β-lactam. This quantitative correlation lays the foundation for predicting bacterial population dynamics during β-lactam treatments. This predictive capability is critical for designing effective antibiotic dosing protocols, in addressing the rising antibiotic resistance crisis.

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To address these questions, we used high-throughput, quantitative experiments to examine the predictive power of growth rates for lysis rates for multiple bacterium–antibiotic combinations, under diverse experimental conditions. Using stochastic modeling, we further provide a coarse-grained mechanistic interpretation of the population-level responses based on single-cell dynamics. Such a quantitative understanding is critical for reliable modeling of bacterial dynamics that involve the use of β-lactams, including the design of engineered gene circuits (24) and the rational design of antibiotic dosing protocols (25, 26). We illustrate this by examining the divergent population dynamics resulting from different types of correlations between growth rates and lysis rates.

Results

We measured bacterial growth/lysis dynamics using an automated robotic liquid handling system (*SI Appendix*, Fig. S1), which enables high-throughput collection of growth and lysis dynamics with high temporal resolution (see *Materials and Methods*). Fig. 1*A* shows time courses of the OD_{600} of an *E. coli* MG1655 culture before and after treatment with 50 μg/mL carbenicillin (added at 3.5 h). We controlled the growth conditions such that the OD values used in our study were linearly correlated to the true density, in terms of biomass (*SI Appendix*, Fig. S2). Typically, the culture grew approximately exponentially before the treatment (Fig. 1*A*). Upon the antibiotic addition, the OD continued to increase for about 1 h before crashing due to lysis. We define the growth rate as the rate of increase in total biomass of the culture, which includes contributions from an increase in cell number as well as bacterial elongation triggered by the antibiotic (27). Due to the morphological changes in single cells caused by β-lactam, an increase in biomass does not necessarily correspond to an increase in cell number. For the same reason, the change in the biomass due to growth and death provides more direct quantification of the bacterial population dynamics during β-lactam treatment. In this context, direct measurement of viable cell counts could be misleading, as well as being too technically tedious to allow generation of growth/lysis dynamics with high temporal resolution. Indeed, numerous studies have used OD measurements to quantify population and gene expression dynamics in bacteria or yeast (19, 27–30).

The growth and lysis of a bacterial population can be described by an ordinary differential equation: $dN/dt = G \cdot N - L \cdot N$, where N is the total biomass, G is the growth rate, and L is the lysis rate. The equation can be rearranged as: $d\ln(N)/dt = G - L$; that is, the time derivative of the log-transformed cell density reflects the combined effects of growth and lysis over time. As OD is proportional to N (*SI Appendix*, Fig. S2*D*), we have: $d\ln(N)/dt = d\ln(OD)/dt$; thus, all calculations of growth or lysis rates can be done with OD values. For each growth/lysis curve, we determined this derivative as a function of time by filtering the data using a median filter and then taking the linear regression of every data point obtained in a moving 1-h time window, to reduce the impact of variations caused by experimental operation.

Before antibiotic treatment, we assume that there is no intrinsic lysis ($L = 0$); thus, $d\ln(OD)/dt = G$, which corresponds to the instantaneous growth rate at the moment of antibiotic addition. After adding the β-lactam, the rate of change in OD is a combination of continued biomass accumulation and lysis: $d\ln(OD)/dt = G - L$. Based on previous observations, we assume that the instantaneous preantibiotic growth rate remained constant (31, 32) after the addition of the antibiotic: $L = G - (d\ln(OD)/dt)$; that is, L is determined as the difference between the OD curve and what we would have expected if the culture had continued to grow at the preantibiotic growth rate. In general, L is a function of time. In our analysis, we aimed to determine whether the instantaneous preantibiotic growth rate could predict the maximum lysis rate following treatment with an antibiotic. Even if all cells are lysed, the accumulation of their debris would provide a baseline OD value. If there were no background OD value, the lysis rate would asymptotically approach a constant. In the presence of the background OD value, the lysis rate exhibits a maximum, in general. This property makes the maximum lysis rate a unique metric to approximate the lysis dynamics after antibiotic treatment, as illustrated from our model simulation (*SI Appendix*, Fig. S8).

To modulate the instantaneous growth rate, we varied the richness of the growth media (*SI Appendix*, Fig. S3*A*), changed growth temperatures (*SI Appendix*, Fig. S3 *A–C*), used sublethal concentrations of ribosome-inhibiting antibiotics to inhibit bacteria growth (*SI Appendix*, Fig. S3 *D and E*), and controlled the timing of antibiotic addition. Remarkably, regardless of how the growth rate was modulated, our results revealed a linear correlation between the growth rate and the corresponding lysis rate (Fig. 1*B*). Changing these parameters affects different aspects of bacterial physiology (16–23). However, despite the fundamental differences in how the growth rates of the population were varied, the correlation between growth and lysis rates was maintained. Additional analysis and the growth curves corresponding to each data point are shown in *SI Appendix*, Fig. S4 *B–D*.

In contrast to a previous observation (13), however, the linear correlation is not strictly proportional—the *y*-intercept is typically not zero. The slope becomes smaller and the *y*-intercept becomes larger with increasing antibiotic concentrations (Fig. 2). Our results suggest that, at a low concentration of the antibiotic, the growth rate must exceed a threshold to trigger lysis. At a higher antibiotic concentration, however, the cells would lyse, even at a very small growth rate, indicating basal-level killing by β-lactams. Consistent with this notion, our microscopy experiment (Movies S1–S3) shows that even extremely slow-growing cells were lysed at a high antibiotic concentration (Movie S2).

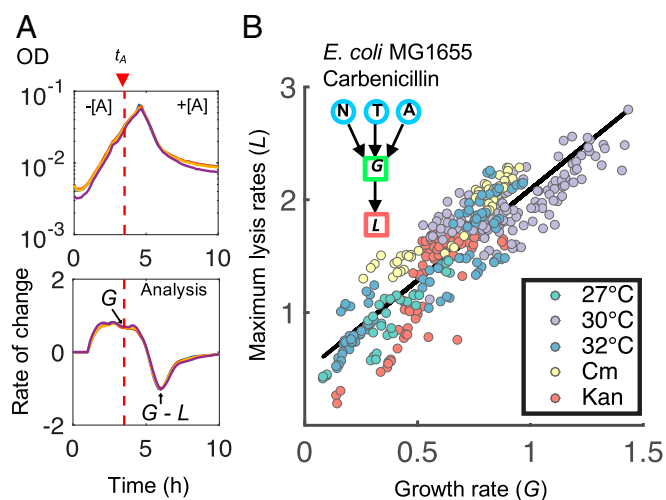**Fig. 1.** The growth rate predicted the β-lactam–mediated lysis rate, regardless of how the overall growth rate was modulated. (*A*, *Top*) Time courses of bacterial growth and lysis dynamics over time. t_A indicates the time when the antibiotic was added. Four replicates are shown. (*A*, *Bottom*) The rate of change calculated from the growth curves. Before antibiotic treatment, *G* is defined as the instantaneous growth rate. The lowest point of the curve corresponds to the sum of preantibiotic growth rate and maximum lysis rate (*L*). (*B*) Growth modulation with various parameters, including nutrient concentration (N), temperature (T), and second antibiotic concentration (A). A robust, linear correlation emerges between growth and lysis rates collected from various modes of growth-rate modulation. The linear fit has a slope of 1.62, a *y*-intercept of 0.48, and an R^2 value of 0.7903. CM, chloramphenicol; Kan, kanamycin.
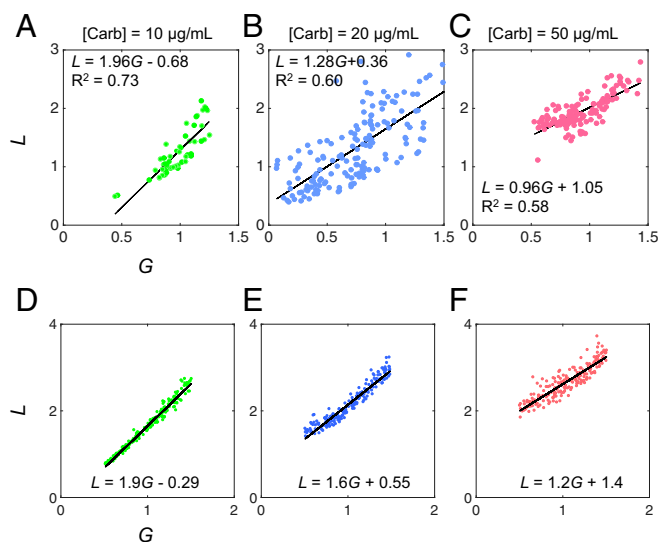
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Fig. 2. Antibiotic dose-dependent correlations between growth and lysis rates in *E. coli* MG1655. (*A–C*) *E. coli* MG1655 strain was treated with 10, 20, or 50 μg/mL carbenicillin at various time points. The growth condition was kept the same otherwise. The linear correlations shift according to specific antibiotic concentrations. At 10 μg/mL carbenicillin, there is a threshold for growth rate for lysis to occur. At 20 and 50 μg/mL carbenicillin, there is a basal-level lysis rate (by extrapolation), even when the growth rate is 0, which increased with the antibiotic concentration. (*D–F*) Simulation results capture the qualitative trends in the experimental data. The average growth rate varies from 0.5 to 1.5 per hour. Parameter *a* increases with increasing growth rates: $a' = a$, $b = 2.5$, $b' = 5b$, $\lambda = 0.2$, $\sigma_1 = 0.548$, $\sigma_1' = 5.0$, $\sigma_2 = 0.0344$, $\sigma_3 = 0.2$, $P_{high} = 0.1$, $P_{b1} = 0.1$. (*D*) $P_{b2} = 0$. (*E*) $P_{b2} = 0.02$. (*F*) $P_{b2} = 0.03$. See *SI Appendix, Model Development* for a detailed description of the stochastic model.

There was negligible growth or death in the absence of antibiotic (Movie S3). In contrast, fast-growing cells were quickly killed (Movie S1). This property is consistent with the observation that, at a sufficiently high concentration, a β-lactam can still kill persisters, albeit at a much reduced rate (12).

We then measured the correlation using combinations of different β-lactams and different bacterial species or strains, including clinical isolates of bacterial pathogens that express extended-spectrum β-lactamases (ESBLs). When testing with ESBL-producing pathogens, we supplemented the media with 20 μg/mL clavulanic acid to inhibit β-lactamases in order to prevent enzyme-mediated degradation of the antibiotic (33). We verified that ESBL-producing pathogens became sensitive to β-lactams in the presence of the Bla inhibitor (*SI Appendix*, Fig. S7). For each antibiotic–bacterium combination, the linear correlation remained (Fig. 3), indicating its general applicability. However, for different combinations, the linear correlation varied in terms of slope and intercept, which can be considered a quantitative signature for a specific antibiotic–bacterium combination.

Given the generality of the linear correlation, its core underlying mechanism is likely insensitive to molecular mechanisms associated with specific strains/species or growth conditions. Instead, it is likely due to general dynamic features associated with a variety of β-lactams and gram-negative bacteria. For individual cells, it has been shown that the biomass continues to increase for some time at the same rate upon addition of a β-lactam, before the cell lyses (31, 32). Moreover, the lysis requires the assembly of the cell division machinery (34), which implies preferential lysis just before a cell would otherwise divide.

To examine whether such single-cell responses could account for the population-level linear correlations between the growth rate and the lysis rate, we developed a parsimonious stochastic

model (*SI Appendix, Model development*). Similar approaches have been adopted to map single-cell growth dynamics and population fitness in other contexts (35, 36). Our model accounts for the effects of cell-to-cell variability in size and growth rate in the absence or presence of an antibiotic. Briefly, we adopted a noisy linear map (37) to generate the initial size distribution before adding the antibiotic. Upon antibiotic addition, the cell size will typically become larger than usual before lysis (*SI Appendix*, Fig. S6 *A* and *B*). In addition, we assume that the cells continue to elongate upon antibiotic addition (38, 39), that the rates at which cells elongate remain the same as pre–antibiotic-treatment rates (38), and that the probability of lysis is higher when cells are about to divide (34). This simple stochastic model predicts a strict proportionality between the growth rate and the lysis rate (*SI Appendix*, Fig. S6C). This prediction provides a parsimonious interpretation of strict proportionality observed for bacteria during balanced growth but does not account for the results under more general conditions (Figs. 1 and 3). Instead, assuming a basal-level probability of lysis independent of biomass accumulation or cell division can lead to a linear (but nonproportional) dependence, as observed in experiments with increasing antibiotic concentrations (*SI Appendix*, Figs. S4 and S6 *D–F*). Specifically, increasing antibiotic concentration further increases the probability that a cell exhibits lysis at a slow growth rate and, thus, results in a linear correlation different from previously observed strict proportionality (13). The necessity to invoke this additional assumption underscores the complexity of potential molecular mechanisms underlying the response of single cells to β-lactams. This additional assumption also constrains the molecular mechanisms that underlie the single-cell response to a β-lactam.

Despite the prevalence of ESBL-producing pathogens, β-lactams can be inadvertently used as first-line antibiotics before full diagnosis reveals the composition of an infection consisting of these pathogens. This practice could influence the structure of an infecting population consisting of subpopulations with different growth rates (35, 40). To gain insight into such selection dynamics, we developed a kinetic model to examine the response to β-lactam treatment by a mixed population consisting of 100
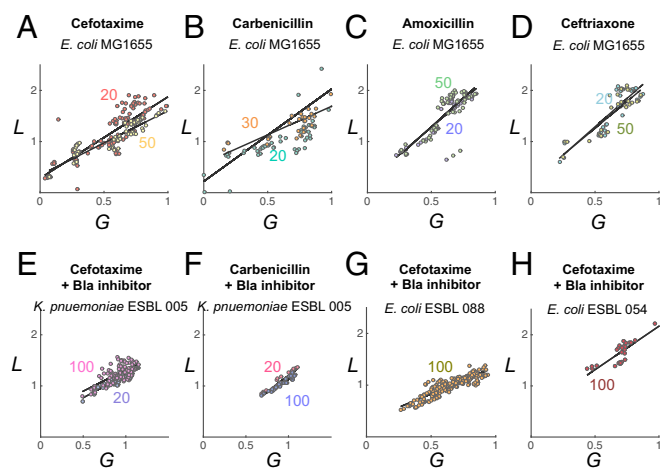

Fig. 3. Generality of the linear correlation between the growth rate and the lysis rate. (*A–H*) We examined the correlations between growth and lysis rates in different drug–bacterial species pairs. The degree of correlation was different for each pair. The numbers in the panels indicate the concentrations (in micrograms per milliliter) of the drug used for each condition. In general, increasing concentrations of antibiotic increased the basal lysis level (*y*-intercept) and decreased the slope of the correlation, except for *E. coli* ESBL 008 and cefotaxime pairs. For ESBL strains, either 10 or 20 μg/mL clavulanic acid was supplemented in the media to inhibit ESBLs, making these strains sensitive to cefotaxime.

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Bla-producing subpopulations, each with a different growth rate (Fig. 4*A*; also see *SI Appendix, Model development*). Upon addition of a β-lactam, each subpopulation would lyse at a rate depending on its growth rate. The released Bla would collectively degrade the β-lactam, allowing surviving cells to recover (Fig. 4*B*). Our simulations indicate that this interplay among differential lysis rates, β-lactam degradation, and subsequent competition could drastically affect the final population structure, depending on how the lysis rate depends on the growth rate (Fig. 4 *C–E*). In general, treatment with a β-lactam leads to selection of a subpopulation with intermediate growth rates. However, a low antibiotic concentration, which only lyses cells growing at a sufficiently high rate (Fig. 2), would lead to the strongest enrichment of slow-growing cells, but not nongrowing cells (Fig. 4*C*). A higher antibiotic concentration would lead to selection against both slow-growing and very fast growing cells (Fig. 4 *D* and *E*). Especially, in the case where there is a high basal lysis rate, there is a sharp selection for subpopulations with a narrow range of intermediate growth rates (Fig. 4*E*). These simulation results underscore the critical importance of having precise quantification between the lysis and growth rates for different drug–bacterium combinations for predicting population change upon antibiotic treatment.

Discussion

In general, biological processes are incredibly complex due to the vast number and diversity of interactions among biomolecules in
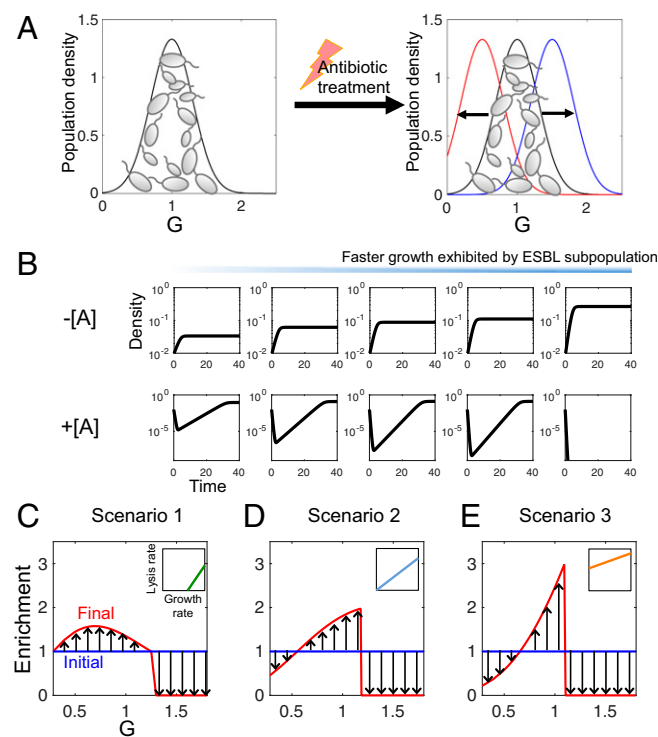
Fig. 4. Predicting the change in the population structure in a Bla-producing population consisting of subpopulations with varying growth rates. Bacterial subpopulations exhibit a wide range of growth rates due to genetic and phenotypic variations. The change in the population structure has a particular importance when populations exhibit collective tolerance against β-lactams (8, 25). (*A*) Schematic of the growth-rate (G) distribution of the bacterial population. (*B*) Growth/death dynamics of sample subpopulations, each with a different maximum growth rate. A, antibiotic. (*C–E*) Enrichment of each bacterial subpopulation when assuming different correlations between growth and lysis rates. This simulation result indicates that the quantitative property of the correlation can drastically influence the effects of antibiotic treatment on the population structure of an infecting population.

a cell, among different cells in a community, and between cellular communities and their environments. Despite this complexity, however, simple, quantitative rules often emerge when biological systems are examined at appropriate levels of abstraction. Examples include linear correlations underlying cell-size homeostasis in bacteria (37, 41, 42), scaling properties of the drug responses by bacteria and cancer cells (43), correlation between the "entropy of population" and the potential for emergence of order in space (44), ranking of quorum sensing modules according to their sensing potential (45, 46), and the growth laws resulting from dynamic partitioning of intracellular resources (47, 48). A key property of these simple rules is that they lump together contributions of multiple lower-level interactions and yet have predictive power for higher-level dynamics (49, 50).

The linear correlation between growth rates and β-lactam–mediated lysis rates represents another example of such simple rules. Although this correlation was partially established previously, until it was examined for broad applicability, as we have demonstrated here, its utility for quantitative reasoning was limited. Given the drastic differences in bacterial physiology among different growth conditions—balanced or unbalanced growth and modulation of growth rates by nutrients, stressors, or temperature—it is remarkable that this linear correlation is maintained. Our measurements provide the concrete foundation for the qualitative statement that faster bacteria are killed faster, and reveal apparently subtle differences that have drastic dynamic consequences. For instance, as illustrated in Fig. 4, the exact nature of the linear correlation can substantially affect the final structure of a population exposed to an antibiotic. Indeed, the quantitative mapping between single-cell growth dynamics and population fitness for different biological problems has been highlighted previously (35). This point has implications for the quantitative prediction of population or evolutionary dynamics of mixed populations (due to genetic or phenotypic variability) (25, 51–54) when treated by β-lactams. At the lower level, the quantitative property of the linear correlations imposes a fundamental constraint on the nature of the biophysical processes underlying the response of single bacteria to β-lactams. For instance, whether the linear correlation has a positive *y*-intercept dictates the probability by which nongrowing and growing cells are killed (*SI Appendix,* Fig. S6).

In addition to understanding how bacterial populations respond to β-lactams alone, our measurements can help evaluate the efficacy of combination treatment involving β-lactams. A rationale for combination therapy is that it would target different machineries of the bacterial cell and result in a more synergistic efficacy, with reduced chance for emergence of resistance (55). Indeed, a proper combination using a β-lactam with a Bla inhibitor would be effective in treating ESBL-producing bacteria (*SI Appendix,* Fig. S7), one of the World Health Organization's antibiotic-resistant priority pathogens. However, the outcomes of combination therapy are often variable (56). For example, a combination consisting of a β-lactam and an antibiotic inhibiting growth (e.g., aminoglycoside and macrolide) has demonstrated limited effectiveness (57). This lack of efficacy might be due to the antagonistic interaction between the two types of antibiotics: a sublethal concentration of the non–β-lactam could protect the population against killing by a β-lactam, by reducing the population growth rate.

Materials and Methods

Bacterial Strains, Growth Media, and Culturing Conditions. A single colony of *E. coli* cells (MG1655 unless noted otherwise) was inoculated from an agar plate into 4 mL of LB media in a test tube that was placed in a 37 °C shaker overnight. The overnight cell culture was washed twice in M9 media without glucose or casamino acid. The OD_{600} of the washed culture was calibrated to achieve 0.5 in fresh M9 media without glucose or casamino acid. For each

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growth and lysis experiment, cells were diluted into fresh M9 media with varying concentrations of limiting nutrient to modulate their growth rates. For our base case, we varied the amount of casamino acids from 0.002 to 0.5% as the limiting nutrient, with a fixed amount of glucose of 0.4% (wt/vol). Cells were diluted 100-fold into fresh growth media before the start of each experiment.

Automated Liquid Handling System. A liquid handling system (Freedom EVO100; Tecan) and a plate reader (Infinite 200; Tecan) allowed us to conduct semiautomated, high-throughput measurements of growth and lysis dynamics of bacterial cultures. Custom computer codes based on the Tecan software were developed to dispense growth media and bacterial culture into designated wells on a 96-well plate. A typical automated experimental protocol would conduct the following tasks:

i)   Distribute fresh M9 media supplemented with 0.4% glucose into 15-mL tubes.
ii) Add varying amounts of casamino acids or other variable nutrients or antibiotics to the 15-mL tubes.
iii) Add cells to the 15-mL tubes.
iv) Aliquot the media from 15-mL tubes to a 96-well plate.
v)   Move the 96-well plate into a plate reader (Infinite 200).
vi) Incubate at 30 °C and take OD measurements at 10-min intervals for ~3 to 5 h.
vii) Move the 96-well plate back onto the stage and add antibiotics to each sample on the plate.
viii) Move the 96-well plate back into the plate reader.
ix) Continue making OD measurements for ~14 h.

Cell Growth/Lysis Observed Under Microscope. One milliliter of overnight cell culture (MG1655, constitutively expressed GFP reporter on chromosome) was collected and washed three times with PBS. Next, we resuspended the cells in 1 mL of M9 media without glucose or casamino acids (diluted 1:50 into the same medium) and loaded the culture onto the microfluidic device. M9 salts were supplemented continuously at 12 μL/h. We monitored the cells under a microscope (Nikon Ti-E microscope with 100× objective) for 150 min to ensure cells are not growing. Next, we changed the medium to the M9 salts with 150 μg/mL carbenicillin using a programmable syringe pump (Pump 11 Elite; Harvard Apparatus; flow rate, 12 μL/h). For the first control, cells were washed, resuspended in M9 medium supplemented with 0.4% glucose and 2% casamino acids, diluted 1:500 into same medium, and loaded onto the microfluidic device. The M9 medium was flowed continuously at 12 μL/h. The cell growth was monitored 150 min before medium was changed to the M9 medium with 150 μg/mL carbenicillin (flow rate, 12 μL/h). For the second control, cells were washed, resuspended in M9 media without glucose or casamino acids, diluted 1:50 into same medium, and loaded onto the microfluidic device. The M9 medium was supplemented continuously at 12 μL/h. The design of the microfluidic device was published previously (58). The device was kept in a 37 °C chamber. The scale bars in Movies S1–S3 represent 10 μm, and the unit of time is hours.

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