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Collective antibiotic tolerance: Mechanisms, dynamics, and intervention

Hannah R. Meredith^{1,a}, Jaydeep K. Srimani^{1,a}, Anna J. Lee¹, Allison J. Lopatkin¹, and Lingchong You^{1,2,3,*}

¹Department of Biomedical Engineering, Duke University, NC

²Center for Systems Biology, Duke University, Durham, NC

³Center for Genomic and Computational Biology, Duke University, Durham, NC

Abstract

Bacteria have developed resistance against every antibiotic at an alarming rate, considering the timescale at which new antibiotics are developed. Thus, there is a critical need to use antibiotics more effectively, extend the shelf life of existing antibiotics, and minimize their side effects. This requires understanding the mechanisms underlying bacterial drug responses. Past studies have focused on survival in the presence of antibiotics by individual cells, as genetic mutants or persisters. In contrast, a population of bacterial cells can collectively survive antibiotic treatments lethal to individual cells. This tolerance can arise by diverse mechanisms, including resistance-conferring enzyme production, titration-mediated bistable growth inhibition, swarming, and inter-population interactions. These strategies can enable rapid population recovery after antibiotic treatment, and provide a time window for otherwise susceptible bacteria to acquire inheritable genetic resistance. Here, we emphasize the potential for targeting collective antibiotic tolerance behaviors as an antibacterial treatment strategy.

Introduction

Antibiotic-resistant bacteria have emerged as a global crisis, resulting in increased morbidity rates, mortality rates, and healthcare costs. The time interval between the introduction of a new antibiotic and the emergence of resistance has rapidly decreased since the 1930s, largely due to antibiotic overuse and misuse¹. Concurrently, a lack of financial incentive has led pharmaceutical companies to decrease antibiotic research and development². Given the drying antibiotic pipeline, it is critical to develop a detailed understanding of how bacteria survive antibiotic treatment. Doing so can uncover new treatment strategies and enable us to use existing antibiotics more effectively³.

Bacteria can survive antibiotics via many different mechanisms (Figure 1a). Genetic resistance can arise from *de novo* mutations or horizontal gene transfer⁴. Expression of

*Correspondence should be addressed to L.Y. (you@duke.edu).

^aThese authors contributed equally

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resistance proteins can allow individual bacteria to survive antibiotic treatment by deactivating the antibiotic, altering the antibiotic's target, or preventing its intracellular accumulation^{1, 4}.

Bacteria can also exhibit *phenotypic tolerance*, whereby they survive antibiotic treatment without acquiring new mutations⁵. For example, a predominantly sensitive population often has a very small fraction of non- or slow-growing bacteria (persisters). These persisters may emerge stochastically, in response to stress, or due to errors in replication and cell metabolism^{6, 7}. They are genetically identical to susceptible cells but are tolerant to antibiotics^{7, 8}. In the absence of antibiotics, persisters can switch back to the growing state, leading to population recovery. As a result, persisters can cause post-treatment relapses and enable development of genetic resistance⁸. Unlike genetic resistance, persistence is non-inheritable, though the frequency of persisters in a population can be genetically determined^{8, 9}.

In contrast, a population at a sufficiently high density can survive an antibiotic dose that is lethal to a low-density population (Figure 1b&c). This *collective antibiotic tolerance* (CAT) can arise from diverse mechanisms, including collective synthesis of resistance-conferring enzymes, antibiotic titration, and social interactions within and between populations^{10–12}. CAT enables a population to recover faster than persisters, and provides a time window for otherwise susceptible bacteria to acquire genetic resistance¹² (Box 1, Figure 2). Here, we review mechanisms underlying CAT, their characteristic dynamics, and potential strategies to inhibit or exploit these mechanisms for antibacterial treatment.

Mechanisms underlying CAT

Antibiotic-mediated altruistic death

Genetic antibiotic resistance arises from the expression of resistance-conferring proteins, which often degrade or modify the antibiotic⁴. However, expression levels of the resistance protein may be insufficient to provide single-cell level protection, depending on the antibiotic concentration. If so, the fate of a bacterial population will depend on its density (Figure 3a): population survival is determined by the relative rates of antibiotic-mediated killing and population-mediated antibiotic degradation. The population will initially decline due to antibiotic-mediated killing of some bacteria. This death is altruistic because the subsequent release of resistance proteins will benefit the surviving cells by contributing to antibiotic degradation^{13, 14}. If the initial population density is too low, the population will be eradicated before the antibiotic is degraded to a sub-lethal level. Conversely, if the initial population density is sufficiently high, the total resistance protein can clear the antibiotic before complete eradication of the population, allowing the survivors to repopulate. The interplay between cell growth, antibiotic-mediated killing, and antibiotic degradation by the resistance protein from cells (live or dead) will result in CAT. Indeed, smaller inocula of pathogens expressing β -lactamases (Bla) are more susceptible to β -lactams than larger inocula¹⁵. Such CAT often arises from constitutive expression of Bla and other resistance proteins¹⁶. Alternatively, many pathogens' Bla expression is up-regulated through the induction of *ampC* in the presence of β -lactam antibiotics¹⁶.

Partial population death is a natural consequence of bactericidal antibiotic action. In other contexts, antibiotic-mediated death is genetically programmed¹³. For example, some antibiotics affect bacterial death by interfering with toxin-antitoxin systems¹⁷. The *mazEF* system can result in programmed cell death (PCD) when an antibiotic, such as chloramphenicol, inhibits the transcription/translation of the MazE antitoxin¹⁸. Without the labile MazE antitoxin, the stable MazF toxin accumulates and triggers PCD. Other antibiotics, such as fluoroquinolones, trigger PCD by inducing an SOS response¹⁹. During a stress response, PCD can benefit survivors by directly or indirectly relieving stress^{13, 14}. When this occurs, the use of antibiotic can promote the survival of a pathogen and aggravate its virulence. For instance, antibiotics induce PCD in Shiga toxin (Stx)-producing *Escherichia coli* (STEC) O157:H7. Encoded in a bacteriophage²⁰, Stx is assembled and released upon cell lysis. Stx kills nearby eukaryotic predators, thereby enabling STEC growth and survival²¹. Death here is altruistic and required for the release of Stx. The population must be large enough to afford the cost of lysing the bacteria necessary to release sufficient Stx for survival.

Quorum Sensing and Biofilm Formation

If a resistance protein's production is costly, it may be advantageous to delay synthesis until the cell density is high enough so the overall benefit of the protein outweighs its production cost. This delay can be realized by quorum sensing (QS), which enables bacteria to coordinate gene expression in a density-dependent manner^{22, 23}. In QS, individual cells produce a signaling molecule that increases in extracellular concentration with cell density. By sensing the signal concentration, bacteria can coordinate gene expression according to their density²². Using a synthetic gene circuit, QS has been demonstrated to represent an optimal strategy for controlling the expression of a costly protein that benefits the entire population²⁴.

QS could control the expression of resistance-conferring enzymes (Figure 3b). In *Providencia stuartii*, a Gram-negative pathogen that causes urinary catheter infections, QS controls the expression of an acetyltransferase, which inactivates aminoglycoside antibiotics^{25,26}. Low-density populations express minimal levels of acetyltransferase, rendering them sensitive to aminoglycosides; high cell density triggers acetyltransferase expression, yielding CAT.

QS can also contribute to CAT by modulating biofilm formation, which enhances antibiotic tolerance²⁷. Although the specifics of biofilm formation vary by species, the general steps are similar^{23, 28}. Planktonic bacteria first attach to a surface at a low density and begin to form microcolonies, while producing basal levels of QS signals. Once the population reaches a sufficiently high density, the accumulated QS signals activate genes involved in biofilm formation²². Depending on the antibiotic and bacterial species, the surface layer of biofilms may serve as a protective barrier for resident bacteria by reducing the penetration rate of some antibiotics^{29, 30}. Moreover, some antibiotics, such as piperacillin or ampicillin, can induce antibiotic-deactivating enzymes in biofilms, which can further reduce antibiotic penetration and the killing of biofilm-residing bacteria^{31, 32}. Biofilms also have limited

nutrients at their core, which diminishes internal cell growth. These slow growing cells tend to be less sensitive to certain antibiotics³³.

Bistable inhibition of the replication machinery

When bacterial populations produce a resistance-conferring protein, density-dependence may be intuitive: increasing cell density increases total protein production, thus increasing the likelihood that a population will survive treatment³⁴. However, CAT has also been observed in response to antibiotics that inhibit ribosome activity and protein synthesis (e.g. gentamicin) without requiring production of degrading enzymes³⁵.

A previous study has established a mechanism for CAT in the absence of active antibiotic degradation: intra-population antibiotic titration coupled with bistable ribosome inhibition¹⁰ (Figure 3c). In each cell, ribosome synthesis is controlled by positive feedback: ribosomes translate mRNA into proteins, including ribosome components, resulting in ribosome synthesis³⁶. This feedback can be inhibited by intracellular antibiotic, resulting in a bistable response³⁷.

Within a population, the extracellular antibiotic is allocated to individual cells according to the import and export rates across the cell membrane. When import is faster than export, the intracellular antibiotic will be higher than the extracellular concentration, and will decrease slightly with increasing cell density. This decrease is amplified by ribosomal positive feedback, leading to bistable growth inhibition: at high cell densities, the average intracellular antibiotic concentration is relatively low (below minimum inhibitory concentrations), positive feedback overcomes antibiotic inhibition and enables population survival. Modeling suggests that density dependence can be modulated by altering various processes, such as drug uptake and binding. However, the critical condition required for CAT in this scenario is the rapid degradation of ribosomes, which can be triggered by antibiotics that target ribosomal components. These antibiotics induce heat-shock response³⁸, which leads to upregulation of proteases and faster degradation of ribosomal components³⁹. If the ribosome degradation rate remains slow, as with chloramphenicol treatment, the population fate is density-independent: populations either grow (low antibiotic concentrations) or die (high antibiotic concentrations). When coupled with direct induction of heat shock, however, these antibiotics can also cause bistable inhibition of population growth, leading to CAT.

Mixed strategies

In nature, CAT often arises from traits that are controlled by combinations of the mechanisms discussed above. For example, when PCD is coupled with density-dependent gene expression in *Staphylococcus aureus*, lysis is tightly controlled and can result in CAT through biofilm formation. CidA and LrgA expressions turn lysis ON and OFF at specified stages of cell growth, respectively⁴⁰. Lysis releases genomic DNA, resulting in enhanced biofilm formation^{41, 42}. These genes have been linked to regulation of antibiotic tolerance: mutations disrupting CidA or LrgA enhance or reduce population survival, respectively^{40, 43}.

Another strategy to survive antibiotic treatment involves a combination of phenotypically shifting from a "swimming" state (planktonic growth) to a "swarming" state (surface growth), QS, and altruistic death (Figure 3d). Swarming is flagella-driven group-migration of bacteria over a semi-solid surface and happens exclusively at the leading edge of a population^{44, 45}. Although the underlying mechanisms are species dependent and not fully elucidated, some swarming species have been observed to rely on QS to upregulate the production of biosurfactants, such as rhamnolipids, which are necessary for reducing surface tension⁴⁴. Swimmers exhibit increased tolerance to a variety of antibiotics, including aminoglycosides and fluoroquinolones^{45, 46}. Altruistic death may contribute to this survival advantage. It is speculated that cells dying from prolonged direct contact with the antibiotic form a protective platform that prevents other swimmers from coming into direct contact⁴⁵. Thus, the population has to have a high enough density to upregulate QS-mediated swarming genes⁴⁵ and afford the cost of individuals that are directly exposed to the antibiotic. While swarming and biofilm formation involve similar mechanisms, such as altruistic death and QS, the two states are inversely regulated^{47, 48}. As a result, biofilms contain a population of sessile cells while swarms disperse a population of active cells⁴⁸.

The mechanisms underlying swarming-mediated motility and density-dependent survival appear to vary between species; QS has been implicated in *Pseudomonas aeruginosa*⁴⁴, but not in *Salmonella*⁴⁵. Other studies have examined the impact of biofilm creation⁴⁵ and increased efflux pump expression⁴⁹. Swarming motility has been observed to strongly correlate with the expression of the *pmrHFIJKLM* operon⁴⁶. This operon encodes the lipid A portion of LPS, which reduces the binding strength of various antimicrobial peptides⁵⁰. A previous study shows *pmrK* expression is upregulated in conditions that promote swarming. Expression decreases as cells dedifferentiate from the swarming phenotype, and knockout results in the loss of swarming ability⁴⁶.

Social interactions

In a mixed population, CAT can be facilitated by "charitable" actions, wherein one subpopulation produces a product that helps another subpopulation, without receiving any benefit from the latter (Figure 3e). For example, a recent study demonstrated how a population of drug sensitive *E. coli* can tolerate antibiotic with the aid of a few highly resistant population members⁵¹. In the absence of antibiotic, the entire population produces indole, a signaling molecule generated by growing bacteria. In the presence of an antibiotic, however, only a subpopulation with mutated efflux pumps has the resistance necessary to continue producing large amounts of indole. The released indole upregulates efflux pump expression and oxidative-stress mechanisms, thus enabling the entire population to tolerate the antibiotic. This survival tactic will fail if the fraction of mutant indole producers is too small to support a large population of sensitive cells or if the metabolic cost of production is too high.

CAT can also arise from *mutualistic* interactions, where two or more bacterial populations depend on one another to survive. For instance, an engineered system can demonstrate how two populations can collaborate to survive different antibiotics¹². Each engineered population produced the QS signal molecule needed by the other to upregulate the

production of a resistance protein. Increasing either population's initial density increased QS signal accumulation, resulting in higher final densities following antibiotic treatment.

Similarly, multispecies swarms can arise due to the communal nature of QS signals. For example, *Serratia ficaria* do not produce biosurfactants, but they do produce QS signals necessary for *S. liquefaciens* QS-deficient mutants to upregulate their own biosurfactant production. Once sufficient signaling molecules accumulate, *S. liquefaciens*' biosurfactant production facilitates the swarming⁵² and, presumably, the CAT of both populations.

Another example of mutualism-mediated CAT can be found in multi-species biofilms⁵³. For example, species can work together to decrease antibiotic penetration of biofilms. *Burkholderia cepacia* and *P. aeruginosa*, both associated with cystic fibrosis, produce polysaccharides that interact to increase biofilm viscosity and decrease antibiotic activity⁵⁴.

Implications for antimicrobial treatment strategies

The diverse mechanisms underlying CAT have implications for developing new treatment strategies. In particular, different mechanisms can lead to unique population and evolutionary dynamics that can be exploited for better treatment efficacy or serve as a cautionary tale against simplistic treatment strategies.

Timing in antibiotic dosing

A common property of CAT mechanisms is that a bacterial population is most vulnerable at low densities. This suggests the potential to inhibit bacterial growth by controlling the timing of antibiotic doses. One method would be to detect and treat infections at earlier stages of development, when insufficient bacteria are present to realize CAT (Figure 4a). A mouse study on treating STEC infections showed early treatment (1–3 days after infection) with a lysis-inducing antibiotic resulted in zero mortality, while late treatment (3–5 days) resulted in 95% mortality⁵⁵. The improved outcome by early treatment may have resulted from suppression of bacterial growth or virulence factor production. Targeting early stage infections is also effective in treating biofilms; studies have demonstrated that immature biofilms are more susceptible to antibiotic treatment^{56, 57}.

Timing control is intrinsic in the design of periodic antibiotic dosing protocols, which can be determined by the dynamic response of a population exhibiting CAT. A previous study demonstrated that different periodic dosing frequencies have drastically different outcomes when treating a population capable of generating CAT due to bistable ribosome inhibition¹⁰. When antibiotic doses were delivered at high frequency, populations had insufficient time to recover. Conversely, when doses were delivered at low frequency, the antibiotic eradicated the population to such an extent that it could not recover. For intermediate frequencies, however, treatment was ineffective; populations recovered between doses. Furthermore, the frequency range over which populations were able to survive can be modulated by changing the fraction of each period spent at a high antibiotic concentration. In general, the optimal dosing protocol will likely depend on the specific types of mechanisms by which CAT arises, representing a significant direction for quantitative biology research.

Molecular interventions to Disrupt CAT

Different mechanisms underlying CAT also suggest potential targets for molecular-level intervention (Figure 4b). CAT facilitated by enzyme expression can be reduced by inhibiting either the production or the action of the enzyme. These strategies are often adopted for treating bacteria that produce Bla. Weak inducers of AmpC Bla production, e.g. cefepimes and piperacillin, have been shown to have lower MICs when treating inducible pathogens⁵⁸. Alternatively, a Bla-inhibitor, such as clavulanic acid, sulbactam, and tazobactam, is frequently used in conjunction with a β -lactam antibiotic to treat Bla-producing bacteria⁵⁹. It should be noted that some bacteria have already evolved resistant mechanisms to Bla inhibitors⁶⁰.

This strategy can also be applied to target CAT resulting from PCD. Studies have begun to screen antimicrobial peptides that have increased efficacy in activating killing by triggering bacterial toxin-antitoxin systems^{61, 62}. For some pathogens, however, activation of PCD alone may be undesirable as it can enhance bacterial survival and virulence. Instead, PCD inhibition can reduce virulence by suppressing the release of effectors that promote bacterial survival or virulence. For example, in *Streptococcus pneumoniae*, release of a virulence factor to extracellular space is mediated by autolysis of a subpopulation of cells⁶³, which in turn can benefit survivors. Serum from mice immunized with autolysin inhibited autolysis and reduced bacterial virulence⁶³. It will be interesting to examine how such strategies will impact overall bacterial survival. As demonstrated using synthetic gene circuits⁶⁴, reduced lysis can paradoxically lead to overall worse bacterial survival.

Similar strategies that inhibit either the production or the action of an enzyme have been applied to target QS pathogens. Targeting QS is appealing as it can reduce virulence without directly targeting the immediate survival of cells. For example, azithromycin has been shown to inhibit QS signaling, leading to inhibition of biofilm formation and virulence in *P. aeruginosa*^{65,66}. Likewise, enzymes, such as AiiA that hydrolyze QS signal molecules⁶⁷ and synthetic molecules that prevent AHL receptor activation⁶⁸, can also block QS signaling and lead to decreased virulence and increased susceptibility to antibiotic treatment in QS pathogens⁶⁹. Drugs that target the function of QS-mediated proteins have also been studied. Oseltamivir is a drug originally developed to inhibit viral production of neuraminidase. As neuraminidase is also a QS-mediated enzyme essential for *P. aeruginosa*'s initial colonization process and subsequent biofilm formation, oseltamivir has been tested for its ability to inhibit biofilm formation. Indeed, a dose-dependent decrease in biofilm formation was observed when oseltamivir was applied⁷⁰.

Rapid ribosome turnover is a prerequisite to generate CAT against antibiotics that target ribosomes¹⁰. As the rate of ribosome turnover increases, the range of antibiotic concentrations over which CAT occurs shrinks and shifts towards lower values. To reduce or eliminate CAT, future drug development could focus on alternative avenues to disrupt ribosome efficacy, such as interfering with subunit assembly⁷¹. Targeting pathways involved in the stringent response could also eliminate CAT. In response to various stresses, many bacterial species reduce rRNA transcription and ribosome synthesis while up-regulating amino acid synthesis⁷². These metabolic shifts can promote bacterial survival and

virulence⁷³. Inhibition of such metabolic shifts could prevent CAT at high population densities. These pathways may thus represent potential targets for new drugs⁷⁴.

A prior study observed that a fundamental requirement for swarming is the production of extracellular surfactants, particularly lipopolysaccharides (LPS), which lubricate cells' local environment so they can "slide" past each other⁴⁶. Therefore, the increased antibiotic tolerance exhibited by swimmers could be avoided by inhibiting LPS production⁷⁵. Previous studies have shown that LPS inhibition by antimicrobial peptides results in the loss of swarming motility⁵⁰. A caveat is that inhibiting swarming motility could lead to increased biofilm formation⁷⁶, as these phenotypes are often linked.

Finally, mixed populations can be inhibited by targeting the keystone members. In the case of charitable interactions, treatments should target the subpopulation producing the resistant protein that protects producers and non-producers alike. For instance, penicillin treatment of tonsillitis is often ineffective due to mixed populations of Bla producing pathogens that protect *Streptococci*. However, the infections were effectively treated upon the introduction of a Bla inhibitor, clavulanic acid⁷⁷. In a mutual relationship, such as in the aforementioned synthetic system¹², survival depends on both subpopulations being present. For such systems, a viable treatment strategy could be to disable one subpopulation such that all participating subpopulations are more susceptible to treatment. To this end, Clustered Regulatory Interspaced short palindromic repeats (CRISPR)-Cas systems could represent an ideal antimicrobial strategy to directly target the specific sub-populations that promote CAT. This genome-editing tool utilizes targeted guide RNA sequences that can be designed to recognize desired bacterial DNA and induce nucleic acid breaks, which can disrupt gene function in the target. Indeed, recent studies demonstrate the ability to customize antimicrobial treatments by the targeted inactivation or killing of specific bacteria within a population through CRISPR delivery^{78, 79}.

Treatment Considerations

Depending on the underlying mechanisms, complex treatment responses could arise from populations capable of CAT. One counterintuitive response is the Eagle effect, a phenomenon wherein increasing antibiotic concentration promotes bacterial recovery⁸⁰. Whether and to what extent the Eagle effect happens in the clinical setting remains to be tested; however, the Eagle effect has been observed to result from PCD-mediated tolerance *in vitro*⁶⁴.

Another example is the counterintuitive effect of inhibiting different aspects of QS. Inhibiting QS signal synthesis effectively increases the cell density threshold required to trigger downstream gene expression, such as virulence factor production⁸¹. However, QS inhibition also alleviates the metabolic burden placed on each cell, resulting in faster growth²⁴. Despite this caveat, the overall outcome of QS signaling inhibition ultimately depends on bacterial survival and virulence development. The treatment would likely be considered successful if the target bacteria are rendered non-pathogenic⁸².

While QS inhibition can attenuate virulence in the short term, it can promote the selection of more virulent pathogens in the long term⁸³. For example, long-term inhibition of QS

signaling in *P. aeruginosa* by azithromycin can select for more cooperative and virulent individuals^{66, 83}. Azithromycin inhibits general protein synthesis; however, it has been shown that QS-regulated genes are among the most severely inhibited by this drug⁸⁴. Over an 11 day course of azithromycin in patients, a slight decrease in the frequency of QS-deficient mutants was observed; the QS-deficient mutant frequency in the placebo group increased approximately 2-fold⁸³. One possible explanation for this counterintuitive response is, when uninhibited, the cooperative nature of QS is prone to cheaters: cells that do not produce virulence factors exploit the benefit while avoiding the cost of public good production⁸⁵. Cheaters, therefore, are less virulent, but have a fitness advantage. For instance, *P. aeruginosa* cheaters that are QS-deficient due to LasR mutations have a growth advantage over the wildtype^{66, 86}. When the QS signal is inhibited and no public goods are synthesized, the fitness advantage by the cheaters will be reduced, making it more difficult for them to outcompete cooperators. The negative consequences of direct inhibition of enzyme production may be avoided by inhibiting the enzyme after it is produced⁸⁷. This strategy aims to maintain the cost of enzyme production while eliminating the benefits, leaving virulent bacteria at a metabolic disadvantage and more vulnerable to treatment. This method may also reduce the selective pressure induced by antibiotic treatment, slowing pathogens' adaptive response.

Although resistance has been well studied, predicting long term consequences on bacterial dynamics has proved elusive. Bacteria have already developed strategies to survive QS inhibition⁸³ and combination treatments involving Bla inhibitors⁶⁰ and will likely develop strategies to survive other inhibition methods. A better understanding of bacterial population and evolutionary dynamics under different treatment strategies can help us optimize treatment strategy. More quantitative studies are needed to better establish the evolutionary dynamics involved in QS-mediated cooperation and the impact of inhibition on different aspects of QS. For instance, the discussion above only considers QS effectors that are public goods. The evolutionary outcomes can be significantly different if the effectors are private goods, which only benefit the cell that produces them,⁸⁸ or species-specific goods, which only benefit a particular population of cells⁸⁹. The same challenge is applicable for the population and evolutionary dynamics of other mechanisms leading to CAT. To this end, the use of well-defined model systems⁹⁰, both natural^{10, 88, 91, 92} and engineered^{24, 64, 89}, will be valuable for the development of better quantitative understanding of a population's response to antibiotics. This understanding can serve as the critical foundation to design 'evolution proof' treatment strategies that select against resistance⁹³.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box 1

The mechanism by which bacteria survive antibiotic treatment can significantly influence the speed of their recovery. Here, we use previously published models to compare the recoveries of populations dependent on different forms of CAT or persistence^{9, 10, 24, 64}. Figure 2a shows the three CAT populations recover significantly faster than the persister-dependent population. The recovery times decrease as the initial population density increases (Figure 2b). Populations surviving by enzyme-dependent CAT actively degrade antibiotic; thus, the antibiotic concentration is quickly reduced to sub-inhibitory levels, enabling recovery. In the case of bistable ribosome inhibition, at high cell densities, the intracellular antibiotic concentration is insufficient to overcome the positive feedback in ribosome synthesis, again leading to rapid recovery. Conversely recovery via persisters requires a relatively long time period due to the dependence on the slow, intrinsic removal of the antibiotic and the low frequency of persister formation.

Figure 1. Bacterial survival modes

a. Bacterial survival modes can be at the individual or population level. Populations can use different mechanisms to survive antibiotic collectively.

b. Collective antibiotic tolerance emerges when a population at a sufficiently high density can survive an antibiotic dose that would be lethal to a low density population.

c. Initial cell density determines the outcome of a population treated by an antibiotic. For each antibiotic concentration, there is a critical initial density above which the population will recover; below this, the population will die.

Figure 2. [within Box 1]. Comparison of population level responses due to different forms of CAT or persistence

a. Time course simulations. After being treated with an antibiotic, the bacteria actively tolerating antibiotics through bistable ribosome inhibition, PCD, or QS recover much faster than persisters.

b. Recovery times. For persisters and CAT bacteria alike, increasing the initial cell density decreases the time it takes for a population to recover from antibiotic treatment.

Figure 3. Underlying mechanisms of CAT

a. Antibiotic mediated altruistic death involves a subpopulation lysing to release effector proteins that benefit the remainder of the population. CAT can only emerge if the population is large enough to generate a collective effector protein concentration sufficient to inhibit the antibiotic before the antibiotic kills all of the population.

b. Quorum sensing is often involved in regulating the expression of effector proteins or the maturation of biofilm formation, both of which can lead to CAT.

c. Bistable inhibition of bacterial growth arises as a function of intracellular antibiotic concentration and bistable ribosome inhibition. Sufficiently dense populations can titrate the antibiotic such that the intracellular concentration does not inhibit ribosome synthesis. However, low density populations cannot titrate the antibiotic to a sublethal level, thus, ribosome synthesis will be inhibited.

d. Multiple mechanisms can be used by a clonal population to survive antibiotic treatment. For example, swarming is a function of phenotype, QS, and PCD that confers antibiotic tolerance.

e. Social interactions can facilitate the survival of multiple subpopulations (denoted by different colored cell outlines). For instance, one subpopulation can produce a signaling molecule necessary to upregulate a second subpopulation's resistance mechanism and vice versa. There are also situations where one subpopulation donates a signaling molecule that protects a second population, without deriving any benefit in return.

Figure 4. Inhibition strategies

a. Timing control of antibiotic dosing is critical for optimizing efficacy of periodic antibiotic treatments. By timing the application of antibiotic doses with when the population is at a low density, the amount of public good and/or toxin released would be insufficient to allow the population to recover or harm the host.

b. Molecular-level interventions of CAT could be used to trigger PCD and inhibit the production and/or the function of an effector protein.