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On the Race to Starvation: How Do Bacteria Survive High Doses of Antibiotics?

Yusuf Talha Tamer[1] **and Erdal Toprak**[1,2,*]
[1]Green Center for Systems Biology
[2]Department of Pharmacology
University of Texas Southwestern Medical Center, Dallas TX, USA
*Correspondence: erdal.toprak@utsouthwestern.edu
https://doi.org/10.1016/j.molcel.2017.12.004

In this issue of *Molecular Cell*, Gutierrez et al. (2017) unravel a bacterial survival strategy that they term "density-dependent persistence" or DDP. The authors demonstrate that the majority of isogenic cells in bacterial populations survive lethal antibiotic doses once bacteria consume nutrients and enter stationary growth phase.

Bacteria are highly adaptive, facilitating survival in extreme environments and, not surprisingly, in nutrient-sparse niches in the human body. There is a continuous arms race between the human immune system and pathogenic bacteria (Capewell et al., 2015; Choy and Roy, 2013). For instance, in immunocompromised patients, bacteria thrive in the setting of an impaired immune system, resulting in morbidity and even mortality. Since the discovery of penicillin, antibiotics with bactericidal or bacteriostatic effects have been used to treat bacterial infections and help our immune system in its fight against pathogenic bacteria. Although this approach has been very successful and countless lives have been saved, pathogenic bacteria have evolved several resistance mechanisms, such as drug degradation or target modification, that render antibiotic molecules ineffective (Oz et al., 2014). However, acquiring resistance-conferring genetic changes is not the only means by which bacteria adapt to survive antibiotics. For example, several studies have reported that a small fraction within a bacterial population can switch to persistent or tolerant phenotypic states and become insensitive to antibiotic treatments (Levin-Reisman et al., 2017). Older studies posited that these phenotypes were not genetically inherited and that they were mostly due to a standing phenotypic variation (Balaban et al., 2004). More recent studies powered with next-generation sequencing approaches have demonstrated that antibiotic tolerance and persistence can be due to spontaneous mutations, and their emergence has been shown to potentiate evolution of antibiotic resistance (Andersson and Hughes, 2011; Levin-Reisman et al., 2017; Maisonneuve et al., 2011, 2013; Michiels et al., 2016; Van den Bergh et al., 2016). In their study in this issue of *Molecular Cell*, Gutierrez et al. (2017) report a novel persistence mechanism, which is environmentally induced and allows the majority of cells in bacterial populations to survive lethal doses of quinolones, which are DNA gyrase (topoisomerase II) inhibitors, without acquisition of any resistance conferring spontaneous mutations or horizontal gene transfer.

Bacteria grown *in vitro* follow a typical growth cycle: lag, exponential, and stationary phases (Figure 1). Interestingly, in this study, Escherichia *coli* cells were shown to become insensitive to an otherwise lethal dose of antibiotics once they enter the stationary phase. Gutierrez et al. (2017) hypothesized that the persistence phenotype observed during starvation was due to either a deficiency in drug uptake or extremely slow cellular activity. Indeed, the latter was the major cause of density-dependent persistence (DDP), as the starvation response of bacteria in stationary phase correlates well with diminished bactericidal activity. Thus, in dense cultures at stationary phase, cells transiently become insensitive to antibiotics when the metabolites that couple carbon catabolism to oxidative phosphorylation become unavailable. Strikingly, these bacteria can be re-sensitized to antibiotics when supplemented with glucose and terminal electron acceptors such as fumarate (Figure 1). These

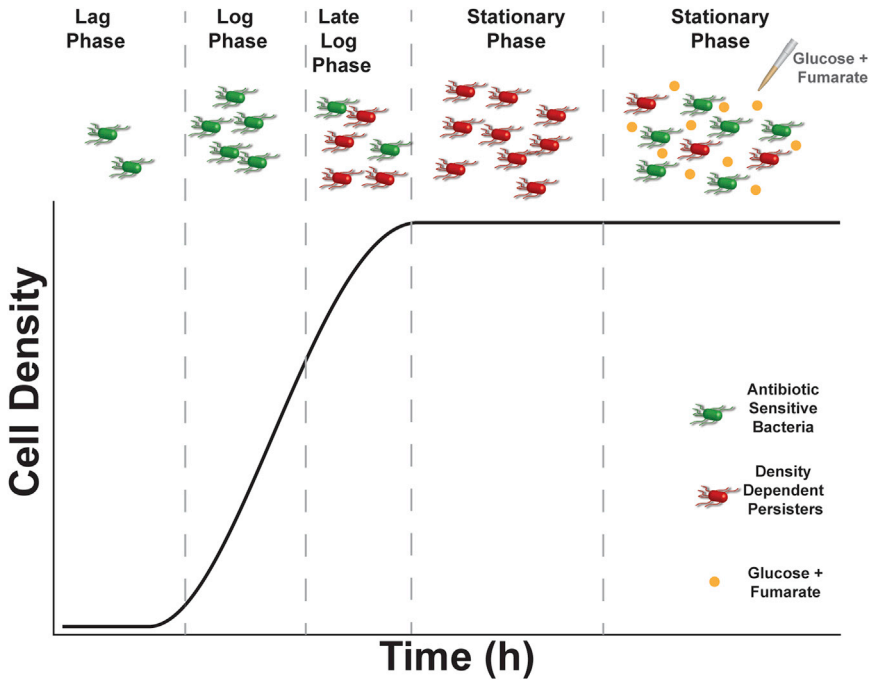

Figure 1. Pathogenic Bacteria Develop Immunity to Antibiotic Molecules Once They Consume Nutrients and Enter Stationary Growth Phase

Bacteria grown *in vitro* follow a typical growth cycle: lag, log (exponential), and stationary phases. Cells that are in the lag or log phase are sensitive to antibiotics (green cells), and they gradually switch to an antibiotic-insensitive (red cells) phenotype as cell density increases. Persistent cells in the stationary phase can be re-sensitized to antibiotics if glucose and terminal electron acceptors, such as fumarate, are provided.

findings challenge conventional antibiotic sensitivity quantification methods where minimum inhibitory concentrations (MICs) for antibiotics are measured by inoculating low numbers of bacteria that are typically in their lag phase. This phenomenon was not limited to *E. coli*. Both *Staphylococcus aureus* and *Mycobacterium smegmatis* populations in stationary phase also displayed sensitization to ciprofloxacin if glucose and fumarate are provided. These findings clearly suggest that antibiotic efficacy can be enhanced if pathogenic bacteria can be transiently sensitized to antibiotics prior to antibiotic administration. Whether this approach can be successfully implemented in a human host (by providing carbon sources and electron acceptors) without significant toxicity and/or unintended expansion of the targeted pathogen is not clear at the moment.

Much of the scientific and physiologic knowledge about pathogenic bacteria were generated in nutrient-rich laboratory settings. There is limited knowledge regarding the natural life cycle of pathogenic bacteria, particularly within a mammalian host. For example, bacterial fitness is often defined as how fast bacterial cells divide when grown in well-aerated, nutrient-rich media. However, even then, bacteria briefly stay in the exponential growth phase and spend most of their time in the stationary phase after nutrients are consumed. In nature, a continuous flux of nutrients is a rarity, and dense bacterial populations are more likely to have limited access to metabolites and oxygen. Therefore, if density-dependent antibiotic persistence is a generalizable phenomenon across pathogenic bacteria, antibiotic molecules will have limited or no inhibitory effects against bacteria, unless bacterial cells are in the lag phase or dividing. Therefore, it is absolutely crucial to design antibiotic treatment strategies that can kill pathogenic bacteria regardless of their growth phase.

One straightforward approach to increase antibiotic efficacy is "jump starting" the metabolism of pathogenic bacteria by providing nutrients, as demonstrated by Gutierrez et al. (2017). The potential negative consequences of this approach, however, are concerning. First, providing a carbon source to "wake up" pathogenic bacteria might increase the number of pathogens and create extra disease burden. Second, if antibiotic molecules more efficiently kill these sensitized bacterial cells, selection pressure may favor those mutants that stay dormant or have lower uptake of metabolites. Therefore, it will be important to further investigate the potential evolutionary outcomes of antibiotic treatment strategies that increase antibiotic efficacy by transiently sensitizing persistent bacteria.

DDP is an intriguing observation that further complicates the definition of fitness. It might be necessary to revisit the definition of fitness in bacteria (and possibly other organisms) to more deeply understand the evolution of bacterial populations and the ecological interactions between them. Growing fast and quickly depleting the available resources certainly help bacteria to outcompete their competitors. However, quickly depleting available resources also means a longer starvation time, which is often considered a bad thing for cells. Conversely, the findings from Gutierrez et al. (2017) suggest that cells that grow faster will not only have higher numbers compared to their competitors, but they might also have better protection against antibiotics and other environmental factors. Laboratory evolution experiments and the systematic study of bacterial survival strategies will clarify whether rapid growth in bacteria is being selected because it confers immunity to environmental stress factors despite the cost of longer starvation.

ACKNOWLEDGMENTS

Authors would like to thank Dr. Miriam Osterfield and Dr. Andrew Y. Koh for their critical reading of the manuscript.

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