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Microbial phenotypic heterogeneity and antibiotic tolerance

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Phenotypic heterogeneity, defined as metastable variation in cellular parameters generated by epigenetic mechanisms, is crucial for the persistence of bacterial populations under fluctuating selective pressures. Diversity ensures that some individuals will survive a potentially lethal stress, such as an antibiotic, that would otherwise obliterate the entire population. The refractoriness of bacterial infections to antibiotic therapy has been ascribed to antibiotic-tolerant variants known as 'persisters'. The persisters are not drug-resistant mutants and it is unclear why they survive antibiotic pressure that kills their genetically identical siblings. Recent conceptual and technological advances are beginning to yield some surprising new insights into the mechanistic basis of this clinically important manifestation of phenotypic heterogeneity.

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Introduction

In a classic study, Bigger [1] coined the term 'persisters' to describe a subpopulation of bacteria within a genetically homogeneous culture of *Staphylococcus aureus* that survived, despite prolonged exposure to bactericidal concentrations of penicillin. This phenomenon has since been observed in a broad range of microbial species exposed to different classes of antibiotics, both *in vitro* and *in vivo* [2–12]. The persisters are not antibiotic-resistant in the traditional (genetic) sense. Their refractoriness to antibiotics is transient and has been attributed to 'phenotypic tolerance' or 'non-inherited antibiotic resistance', in contrast to the more stable genetic changes that are responsible for antibiotic resistance [13,14].

When an isogenic culture of bacteria is treated with a bactericidal antibiotic, the majority of the population is killed rapidly, with log-linear kinetics (Figure 1a). Following this initial period, the rate of cell death slows markedly and a small fraction of cells (the persisters) survives, resulting in biphasic population-decay kinetics (Figure 1a). The characteristic biphasic kinetics of bacterial killing by antibiotics can be modeled on the basis of the emerging hypothesis (discussed later) that the pre-antibiotic population comprises subpopulations that are killed with fast kinetics (the non-persisters) or slow kinetics (the persisters; Figure 1b). The persister trait appears to be epigenetic, because the trait is not inherited by the progeny of persisters, which are equally as antibiotic-sensitive as the original population after subculture in the absence of antibiotics [1].

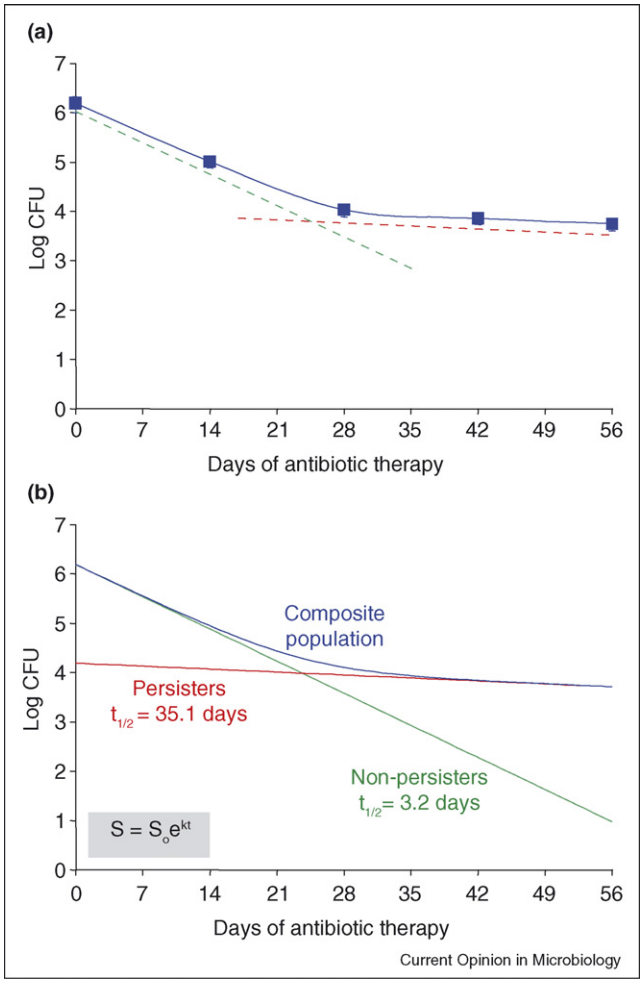
In the intervening decades since Bigger first described persisters in *S. aureus*, there has been surprisingly little progress in understanding the phenomenon and its potential medical implications. This neglect might be due in part to the technical challenges involved. The persister trait has been difficult to study because of the low frequency of persisters in naïve populations of bacteria (10^{-4} to 10^{-6} in *Escherichia coli*) [13] and because phenotypic tolerance is metastable and is rapidly lost on subculture. Recently, there has been a revival of interest in the subject because of the recognition that persisters might be responsible for the recalcitrance of biofilms to antibiotics [15•] and the potential contribution of persisters to the difficulty in treating certain bacterial infections, such as tuberculosis [16,17]. Also, new technologies that permit the detailed analysis of bacterial behavior at single-cell resolution [18] have made the persister problem more accessible to researchers.

This review examines some of the recent advances in our understanding of phenotypic heterogeneity and antibiotic tolerance in bacteria and current ideas about the cellular and molecular mechanisms that might be responsible for triggering the 'persister switch'.

Phenotypic heterogeneity

The persistence of microbial populations in fluctuating environments is linked to phenotypic heterogeneity [19,20••]. Random, spontaneous mutations contribute to bacterial population variation, as do mechanisms for genetic exchange between individuals of the same or different species. Traditionally, studies of antibiotic resistance in bacteria have focused on the heritable mechanisms of resistance. Well-characterized examples include chromosomal point mutations that alter the interaction of an antibiotic with its target or horizontally acquired DNA elements encoding antibiotic inactivation or efflux mechanisms. However, stable genetic changes of

Figure 1



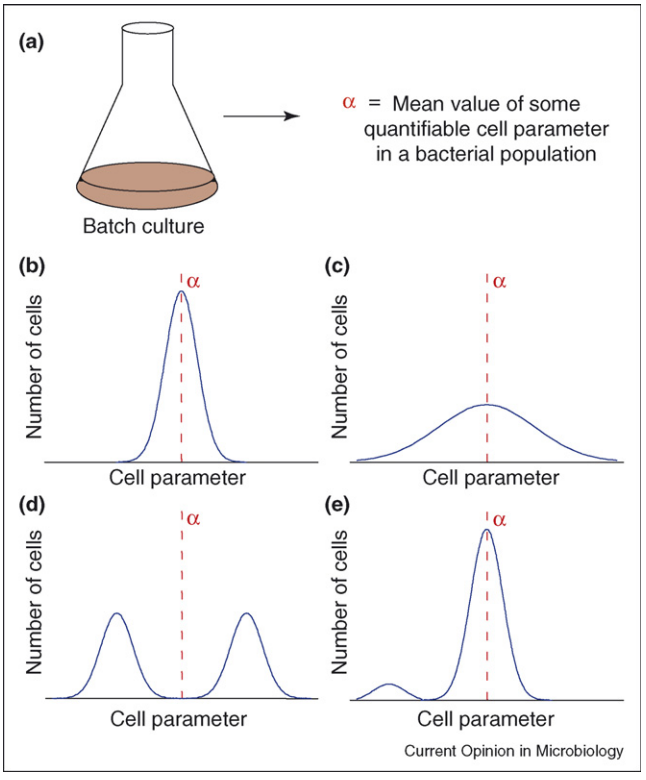
Biphasic killing of bacteria by antibiotics. **(a)** Bacterial colony-forming units (CFUs) in the lungs of C57BL/6 mice intravenously infected with 10^6 CFU of *M. tuberculosis* and treated with isoniazid, a frontline anti-tuberculosis antibiotic. The rate of killing slows dramatically after 4 weeks of isoniazid therapy [2]. **(b)** The biphasic kinetics of bacterial cell death in isoniazid-treated mice can be modeled on the basis of the hypothesis that the initial population comprises two distinct subpopulations that are killed with fast (green line) or slow (red line) kinetics [54••]. The composite of these two log-linear kill curves generates a biphasic curve (blue line) that mimics the experimental data depicted in (a), which supports (but does not prove) the persister switch hypothesis [54••].

this type are insufficient to explain the extent of phenotypic heterogeneity and the refractoriness to antibiotic-mediated killing exhibited by isogenic populations of bacteria [14].

The terms 'phenotypic variation' and 'phenotypic heterogeneity' refer to epigenetic sources of population variation that do not involve changes in the genome [21,22•]. These changes are usually unstable or metastable, and are typically not inherited by the variant's progeny. Phenotypic heterogeneity between individual bacteria in clonal populations is always present, to varying degrees, and can be quantified for almost any phenotype that can be scored at the single-cell level. Well-characterized examples include the differential induction of the *E. coli* lactose operon [23,24], competence induction in *Bacillus subtilis* [25••], and the elongation rates and generation times of individual bacterial cells [26–28].

Cell-to-cell variation is masked by traditional 'batch culture' experimental approaches that derive mean population values for a quantifiable cellular trait by pooling large numbers of cells (Figure 2a). Heterogeneity in bacterial populations can occur as a monomodal Gaussian with a narrow (Figure 2b) or broad (Figure 2c) distribution, or as a multimodal distribution comprising subpopulations of similar (Figure 2d) or vastly different (Figure 2e) numbers of individuals. The differences

Figure 2



Bacterial individuality and population structure are masked by methods that measure the average behavior of bacterial populations. **(a)** Quantification of a cellular parameter in a batch culture yields a mean value (α). This value could reflect very different underlying population distributions, including: **(b)** Gaussian with a narrow distribution around the mean, **(c)** Gaussian with a broad distribution around the mean, **(d)** multimodal distribution comprising subpopulations of equal size, or **(e)** multimodal distribution comprising populations of unequal sizes. In example (e) the smaller subpopulation makes a negligible contribution to the mean value of the measured parameter in a population. Nonetheless, as the example of persisters illustrates, small subpopulations can have a profound impact on the overall behavior and survival of a population.