Bacterial Tolerance and Persisters: Overcoming the Antibiotic Storm

Introduction

In the era of antimicrobial resistance (AMR), bacterial tolerance and persisters are increasingly recognized as one of the important factors in treatment failures, prolonged illness, and increased risk of complications, even in cases where bacteria are deemed susceptible based on standard susceptibility testing. The tenacity and ingenuity of tolerance and persisters shown by bacteria to combat and overcome the antibiotic storm is a remarkable strategy.

Bacterial infections have long posed a significant threat to human health, and the discovery of antibiotics revolutionized treatment in the past century. However, in recent decades, antibiotic resistance has become a growing concern, diminishing the effectiveness of traditional treatments.[1] The emergence of "superbugs" in clinical settings signals the declining efficacy of conventional antibiotics.[2] Alongside resistance, antibiotic tolerance and persistent infections are now major challenges in treating bacterial diseases.[3] Individuals with persistent infections often face ongoing or recurrent bacterial episodes that resist standard treatments.[4] Examples include tuberculosis,[5] typhoid fever,[6] Lyme disease,[7] and recurrent urinary tract infections (UTIs),[8] with bacterial persister cells playing a key role in treatment failure and relapse.

Bacterial populations exposed to antibiotics can consist of different subtypes: Sensitive (green), persister (red), tolerant (yellow), and resistant (purple) cells, each showing distinct responses [Figure 1a] and antibiotic-killing patterns [Figure 1b]. Sensitive bacteria are typically eradicated, showing a steady decline in the kill curve and unable to regenerate once the antibiotic is removed. In contrast, resistant bacteria survive and continue to grow despite antibiotic exposure, leading to an upward trend in their population curve.[9,10]

Definition

Although bacterial tolerance and persister cells are distinct phenomena, they appear superficially related, as both involve the survival of growth-restricted bacteria during exposure to bactericidal antibiotics. The key difference lies in their scope within a population. Persister cells represent a transient, antibiotic-tolerant subpopulation that is typically slow-growing or growth-arrested. In contrast, antibiotic tolerance affects the entire bacterial population, usually as a result of genetic mutations or environmental factors that lead to reduced growth rates.[11]

Bacterial Tolerance

"Tolerant bacteria" refer to an entire bacterial population that exhibits persister-like tolerance, characterized by slower killing rates compared to actively growing cells, yet retaining the ability to regrow once the antibiotic is removed.

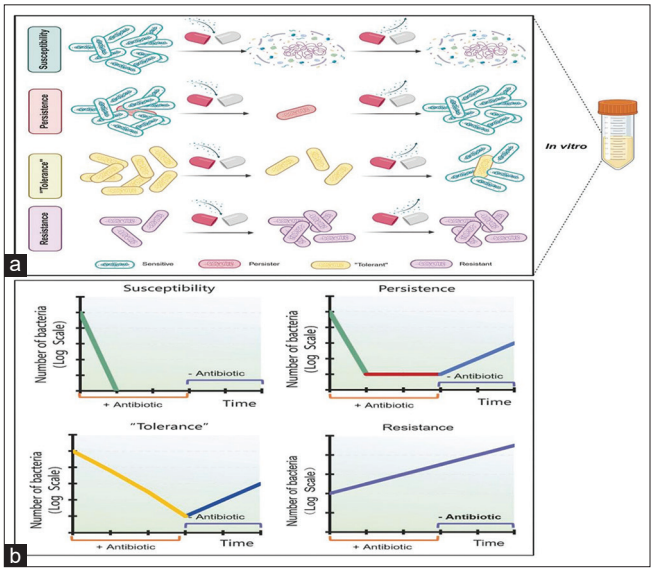

Figure 1: (a) Comparative features of persister, resistant, and tolerant bacterial cells. (b) Depicts the *in vitro* models showing persister cells, tolerance, and resistant bacteria along with the antibiotic killing curves[20]

They have the ability to survive transient exposure to antibiotics and exhibit a reduced sensitivity to antibiotics, often due to slowed or nongrowing states. In contrast to persisters, it is a broader phenomenon, this tolerance operates at the whole-population level, typically arising from genetic mutations or environmental factors that restrict bacterial growth. Tolerant cells are not actively dividing during antiobiotic stress, making them less susceptible to drugs that target growth-related process. Tolerance can be induced by various stress conditions, including antibiotic exposure. Other characteristics are:[12]

- Tolerance does not involve increased Minimum Inhibitory Concentration (MIC)
- It typically results in delayed killing not prevention of killing
- Commonly seen in biofilms and nutrient-limited environments
- Mechanisms of tolerance include
- Slow metabolism
- Stress pathways
- Altered cell wall permeability.

Persister cells

Persister cells – are rare phenotypically distinct subset or small fraction of dormant cells from within a clonal bacterial

population that can survive exposure to high concentrations of bactericidal antibiotics without undergoing genetic changes. While the bulk of growing bacteria are killed rapidly, the persisters are still alive. Once the antibiotic is removed, persisters can resuscitate and resume growth. They enter a dormant state allowing them to survive antibiotic treatment.[13] They are characterized by:

- Their slow or non-growing state and their ability to resume growth or 'wake up" after antibiotic stress is removed and lead to a relapse of infection
- Often found in biofilms and stationary-phase cultures, where they can constitute a significant proportion of the population
- They are not Mutants and do not pass on their state
- Major contributors to chronic and relapsing infections.

Known triggers

- Toxin – antitoxin system
- Starvation
- Oxidative stress
- Quorum sensing and biofilm signalling.

Relationship between Tolerance, Persisters, and Resistant Bacteria

Persister cells are bacteria that are either nongrowing or slow-growing, allowing them to survive under stressful conditions such as antibiotics, reactive oxygen species, acidic pH, or nutrient deprivation. Once the stress is removed, persisters can resume growth and remain sensitive to the same stressors.[13]

The survival of otherwise susceptible bacteria in the presence of bactericidal antibiotics is commonly attributed to two closely related phenomena. The first is antibiotic persistence, where a subpopulation of bacteria within an otherwise antibiotic-sensitive population undergoes a transient phenotypic shift, entering a dormant state with reduced activity or uptake of the drug. The second is antibiotic tolerance, in which the entire bacterial population survives longer exposure to antibiotics due to genetic mutations or environmental factors that inhibit growth in all cells. While both persistence and tolerance are defined by a lack of bacterial proliferation, they differ in penetrance, meaning the extent to which the phenotype is present across the population.[14]

However, genetic resistance is not the only way bacteria can withstand antibiotic treatment. Persister cells, while capable of surviving in the presence of antibiotics, are distinct from resistant bacteria. Persisters show strong antibiotic tolerance, including multidrug tolerance, but this is purely phenotypic, not driven by genetic mutations or resistance genes. In contrast to resistant bacteria, which exhibit a higher MIC, persisters typically have the same or even lower MICs. Despite these differences, persisters and resistant bacteria are not completely separate; they can interconvert or overlap under certain conditions.[15]

Both states pose a big challenge in the appropriate treatment of bacterial infections. The paradox of therapy leads to increased survival, and treatment failure and acts as a reservoir for the development of antibiotic resistance.

Key Differences between Resistance versus Tolerance versus Persistence

The differences between resistant, tolerance, and persistence, as shown in the table highlight the very high reinfection opportunity with persisters [Table 1].[16]

Clinical relevance

- Chronic relapsing infections-prolonged treatment
- Biofilms-communities of bacteria encased in a protective matrix harbor high persisters as in prosthetics, and catheters
- Tuberculosis and UTI are classic examples where persisters contribute to relapse
- Traditional susceptibility tests (like MIC) fail to detect tolerance or persisters, leading to misinterpretation of treatment efficacy
- Tolerance and persistence even in the absence of resistance can cause Antibiotic failure making treatment less effective.[17]

Therapeutic strategies

- Metabolic re-activation-waking up persisters to make them vulnerable to antibiotics
- Combination of therapy-targetting different metabolic states
- Anti persister agents-compounds to kill dormant cells
- Phage therapy and host-directed therapies.

Methods for Detecting Tolerance and Persister Cells

The Kirby-Bauer disk-diffusion assay is commonly used in clinical settings to determine bacterial antibiotic resistance, but it is not effective for detecting persistent bacteria. Several simple laboratory tests can identify tolerant and persistent bacteria, especially in cases of chronic infections that do not resolve even when the bacteria are susceptible

Table 1: Differences between Resistant, Tolerance & Persisters

Feature	Resistance	Tolerance	Persistence
Basis	mutation	Genetic adaptation	Phenotypic variation	Phenotypic
Cell survival with drug	Active growth	Survival without growth	Dormancy
Duration	Permanent	Reversible	Reversible
Population	Whole population	Whole population	Subpopulation
Reinfection	High	High	Very high risk

to antibiotics. Therefore, along with assessing antibiotic susceptibility, detecting bacterial persistence in clinical environments is essential for determining the most effective treatment options.

Methods for Detecting Tolerant or Persister Bacteria

- Time kill assay: This involves exposing bacterial cells to lethal doses of antibiotics and measuring the survival rate using colony-forming units
- Scan lab and Col Tapp Method: This method monitors the two-dimensional distribution of lag times and growth patterns of each colony on an agar plate.
- Tolerance Disk Test: A two-step process using glucose disks to observe re-growth in the antibiotic-sensitive zone, which helps identify surviving bacteria[18]
- Replica Plating Tolerance Isolation System: A two-step approach designed to both isolate and calculate the number of tolerant cells.[19]

Discussion

Antibiotic tolerance and persistence are often mistaken for similar phenomena, as both involve the survival of growth-restricted bacteria in the presence of bactericidal antibiotics. Both persistence and tolerance are marked by a lack of bacterial proliferation, with the main difference being penetrance – that is, the proportion of the bacterial population that exhibits the phenotype.[11] Despite their similarities, the physiological states and molecular mechanisms that enable tolerant bacteria and persisters to survive antibiotic treatment are often considered to be the same.

While antibiotic resistance remains a major concern, bacterial tolerance and persister cells represent equally significant challenges. Addressing these survival mechanisms is crucial for developing more effective therapies. A key unresolved question is whether the nature of antibiotic persistence and tolerance results in different clinical outcomes, particularly when pathogens can trigger relapse after antibiotic treatment is discontinued.

Conclusion

Although persistence and tolerance are distinct, they are closely related, with the primary difference being a matter of degree. Tolerant bacteria can be considered shallow persisters within a broader continuum of persistence, which includes both type I persisters and slow-growing type II persisters. Persistent infections, in addition to antibiotic resistance, present a significant challenge in bacterial infection management. One often overlooked issue is the role of persister cells, which are resistant to antibiotic treatment. Addressing persistence is challenging due to the difficulty of studying these cells, and much is still unknown about their physiology and the factors that contribute to their formation. Overall, tolerance and persisters can be seen as two related survival strategies within bacterial populations, allowing them to endure the "antibiotic storm."

Kunal K. Lahiri

Department of Microbiology, Heritage Institute of Medical Sciences Varanasi, Uttar Pradesh, India

Address for correspondence: Dr. Kunal K. Lahiri, Department of Microbiology, HeritageInstitute of Medical Sciences, NH2 By Pass Bhadwar, Varanasi - 221 311, Uttar Pradesh, India.
E-mail: lahirikunal@hotmail.com

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