The American Journal of Applied Sciences
ISSN 2689-0992 Volume 08 - 2026

Proceedings of Women in Academia, Research and Management for Work-life Initiatives for Sustainable Health & Empowering Safety (WARM-WISHES 2026)

Persister Cell–Mediated Phenotypic Antibiotic Tolerance in Biofilm-Forming Bacteria: Mechanisms and Clinical Implications

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Received: 05 Apr 2026 | Received Revised Version: 22 Apr 2026 | Accepted: 03 May 2026 | Published: 09 May 2026

Volume 08 Issue 05 2026 | DOI: 10.37547/tajas/warm-03

Abstract

The persistence of bacterial infections despite antibiotic susceptibility represents a major challenge in modern medicine, largely driven by phenotypic antibiotic tolerance mediated by persister cells within biofilm-forming populations. Basically, persister cells represent a temporary, non-inheritable subpopulation of bacteria whose dormancy or low metabolism makes them capable to evade the killing effect of antibiotics that normally eliminate the bacteria. By forming biofilms, bacteria not only protect persister cells through physical barriers to antibiotics but also further strengthen this tolerance, since biofilms create a nutrient-limited microenvironment with oxygen gradients and make it difficult for antibiotics to penetrate. In fact, the three factors together lead to a lifestyle of persister cells, which explains why the treatment is ineffective and the infection comes back. The pathways that lead to the production of persister cells are quite complex, as they involve several different cellular mechanisms and processes, such as toxin-antitoxin modules, generation of the stringent response through (p)ppGpp signaling, drop in the levels of ATP, and different stress-response routes. What is more, the system of quorum sensing leading to cell-cell communication and signalling inside biofilms is of utmost importance in the regulation of persistence and the survival of the community at the same time. In contrast to antibiotic resistance, which is the result of genetic mutations, the state of tolerance mediated by persisters is transient and capable of reversal, so when the antibiotic pressure is not there, cells can grow again. From a clinical perspective, persister cells cause infections that continue over a long time or keep coming back, like urinary tract infections, tuberculosis, and infections related to medical devices. They make it very difficult to treat with medicines. Unfortunately, the treatment methods we have today still fall short of eliminating these cells. However, new techniques are being researched that may improve clinical outcomes, including bacteriophage therapy, anti-persister medications, biofilm destruction, stimulation of persister cell metabolism, and nanotechnology-based treatments. Therefore, developing novel treatments for persistent bacterial infections requires a deep understanding of the biology of persister cells.

Keywords: Persister cells, Biofilms, Antibiotic tolerance, Chronic infections, Toxin-antitoxin systems, Quorum sensing

Cite This Article: Singh, K., & Singh, A. (2026). Persister Cell–Mediated Phenotypic Antibiotic Tolerance in Biofilm-Forming Bacteria: Mechanisms and Clinical Implications. The American Journal of Applied Sciences, 19–25. https://doi.org/10.37547/tajas/warm-03.

1. Introduction

Bacteria can live in the presence of antibiotics for a brief period of time without developing genetic resistance thanks to a reversible phenotypic shift known as antibiotic tolerance. Tampering of cells persists in this process. Persister cells are a tiny dormant subpopulation that resists bactericidal antibiotics and after treatment is stopped, they repopulate (Balaban et al. 2019; Lannes-Costa et al. 2024). Persistent cell-mediated antibiotic tolerance is a major cause of chronic infections that flare up again and again and are difficult to treat (Kunnath et al. 2024). Biofilms further increase tolerance by supplying a protective microenvironment that is featured by lower metabolic activity, penetration of antibiotics is limited, and physiological heterogeneity (Liu et al. 2024). Such conditions are conducive to the induction and support of persister cells, which is the reason why infections related to biofilms are virtually impossible to eradicate. The spatial heterogeneity of biofilms, including gradients in oxygen, nutrients, and antibiotic penetration that promote persister cell formation, is illustrated in **Figure 1**.

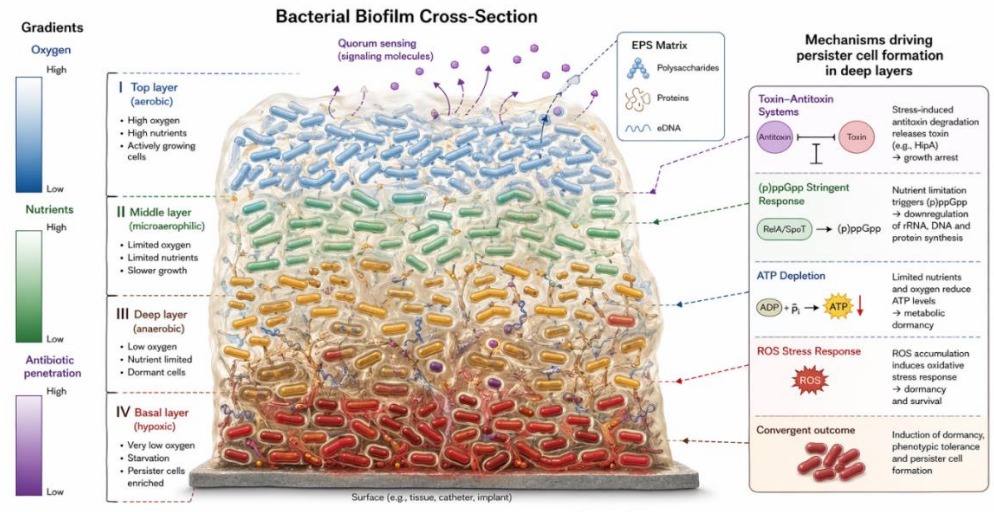

Figure 1-Schematic of a biofilm cross-section showing gradients of oxygen, nutrients, and antibiotic penetration. Deeper hypoxic layers harbor dormant persister cells, with mechanisms such as toxin–antitoxin systems, (p)ppGpp response, ATP depletion, R

2. Biofilm Formation and Structure

Bacterial cells first adhere to a surface, then proliferate and sense their population to form microcolonies. Mature biofilms with intricate three-dimensional structures are formed and metabolic heterogeneity is observed, and finally dispersion takes place, which results in the formation of new colonies indefinitely (Rumbaugh & Sauer, 2020; Liu et al. 2024). In addition to scaffolding the biofilm, the extracellular polymeric substance (EPS) matrix, which is mostly composed of proteins, polysaccharides, and extracellular DNA, inhibits antibiotics by reducing their efficacy and decreasing their diffusion (Flemming et al. 2021; Almatroudi, 2025). Biofilms create gradients in pH, nutrients, and oxygen as a result of limited diffusion. The innermost layers of cells experience deprivation and hypoxia, which cause metabolic dormancy and the production of persister cells (Niu et al. 2024). The sequential stages of biofilm formation—from initial attachment to dispersion—along with the structural role of the EPS matrix are depicted in **Figure 2**.

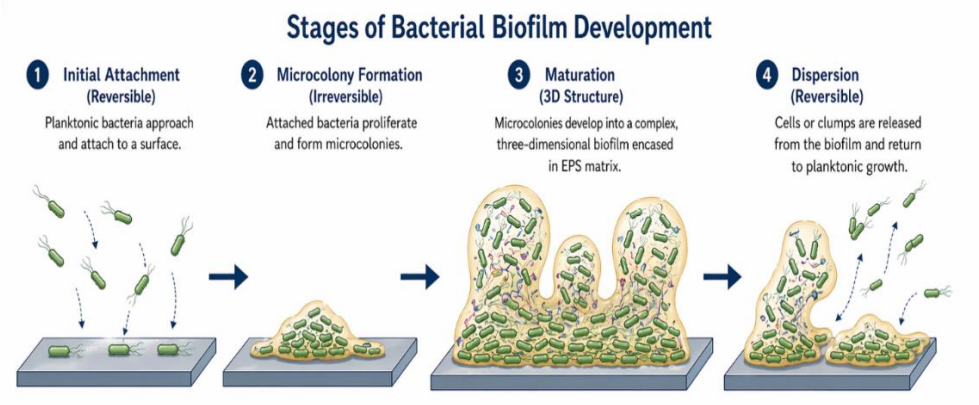

Figure 2-Sequential stages of biofilm formation: initial attachment, microcolony formation, maturation into a 3D structure, and dispersion. The EPS matrix—composed of polysaccharides, proteins, and extracellular DNA—provides structural support and prot

3. Persister Cells: Characteristics and Identification

Mersister cells are phenotypic variants that survive antibiotic treatment by going dormant and stopping their metabolism without having genetic resistance to the drugs (Balaban et al. 2019; Niu et al. 2024). Their main characteristics are a halt in growth, lower ATP content, and a transient tolerance that is not inherited, which enables them to wake up after the antibiotic is gone. Persisters are usually detected with the help of biphasic killing curves, in which a tiny portion of the population remains alive after the speedy killing phase. More sophisticated techniques like using fluorescent metabolic markers and single-cell analysis allow for the identification of cellular heterogeneity and dormancy (Orman & Brynildsen, 2020). Persisters are divided into two types according to their way of forming: Type I (formed during the stationary phase under stress) and Type II (generated continuously during growth). Type I persisters are predominantly found in biofilms while Type II happen randomly at low rates. as shown in **Figure 3**

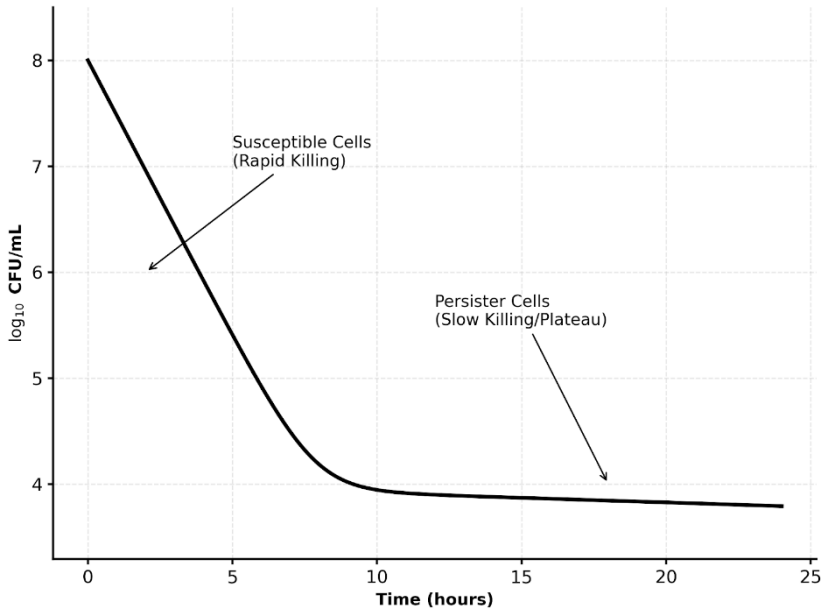

Figure 3 - Biphasic killing curve showing rapid decline of antibiotic-sensitive cells followed by a plateau representing surviving persister cells. These cells exhibit transient metabolic dormancy and antibiotic tolerance rather than genetic resistance and can regrow once the antibiotic is removed.

4. Molecular Mechanisms of Persister Cell Formation

4.1 Toxin–Antitoxin (TA) Systems

Toxin-antitoxin systems are a major mechanism for the regulation of persister cell formation. When cells face stress, the antitoxin is degraded and the toxin is freed; the toxin then targets the translation process which leads to a growth arrest of the cell (Ronneau & Helaine, 2021). The HipAB system is a good example that has been well characterized. In this system, the HipA toxin stops cells from growing by blocking the translation process and inducing persistence (Niu et al. 2024).

4.2 Stringent Response and (p)ppGpp Signaling

When bacteria face a shortage of nutrients and other stress factors, the stringent response comes into play and causes the buildup of alarmone molecules like (p)ppGpp. These molecules make significant changes in the metabolism of cells by suppressing the processes related to growth and stimulating the pathways of survival (Irving et al. 2021). As a result, this defense mechanism leads to inactivity and boosts the generation of persister cells, especially in the nutrient-deprived biofilm habitats (Niu et al. 2024).

4.3 Energy Depletion and ATP Reduction

Reduced intracellular ATP levels are assumed to be the main cause of persister formation. Lesser availability of energy leads to lower antibiotic uptake and decreased antibiotic targets turning antibiotics ineffective (Shan et al. 2021). Therefore, the primary mechanism linking medication tolerance and inactive metabolism is energy deprivation.

4.4 Stress Reactions and Reactive Oxygen Species (ROS)

When subjected to oxidative stress, bacteria activate defence mechanisms that improve their chances of survival. They can accomplish this by creating inactive cells, which are shielded from ROS-induced damage. Besides, these ROS can activate the pathways mentioned above that are responsible for inducing dormancy and protecting the cellular components from damage (Zhang et al. 2023). The ability of these potential bacteria to resist antibiotics by undergoing oxidative stress is one of their characteristics which these adaptive responses allow them to have enable persistence.

4.5 Quorum Sensing and Cell–Cell Communication

In fact, quorum sensing controls the expression of genes that is dependent on the cell density and contribute greatly to the coordination of the persistence of the biofilms. Signalling molecules impact the stress responses, virulence, and metabolic states and as a result, lead to persister cell formation (Liu et al. 2024). Integrating quorum sensing with other pathways helps at the community-level survival during antibiotic treatment.

5. Antibiotic Tolerance Mechanisms in Biofilms

Biofilms help bacteria tolerate antibiotics by both limiting the physical access of the drugs and by slowing down the physiological processes needed for drugs to be effective (Liu et al. 2024). The bacterial community in biofilms is embedded in a matrix made of extracellular polymeric substances (EPS), which blocks the diffusion of antibiotics, leading to their lower concentration in the cells situated in the inner layers (Almatroudi, 2025). Besides, the gradients in oxygen and nutrients that are characteristic of biofilms lead to decreased metabolic activity, which in turn results in bacteria going into a dormant state and becoming less susceptible to antibiotics that target only active processes (Niu et al. 2024). Besides, bacteria residing in biofilms make use of several active defence mechanisms like efflux pumps and enzymatic inactivation to keep the antibiotic concentration inside cells at a low level (Ronneau & Helaine, 2021). Most notably, the synergistic effect of EPS-mediated protection and the emergence of persister cells results in a highly tolerant bacterial population, thereby making the bacteria much more resistant to antibiotics (Hashemi, 2025). This can be understood more clearly by **Table 1**.

Table 1: Antibiotic Tolerance Mechanisms in Biofilms

Mechanism	Description	Effect	Reference
EPS barrier	Limits drug penetration	Reduced antibiotic exposure	Flemming et al., 2016
Microenvironment	Nutrient/oxygen gradients	Dormancy induction	Stewart & Franklin, 2008
Efflux pumps	Active drug removal	Lower intracellular drug levels	Mah & O'Toole, 2001
Enzymatic inactivation	Antibiotic degradation	Reduced efficacy	Hall & Mah, 2017

6. Clinical Consequences

In a clinical context, persister cells and biofilm-associated tolerance are significant problems, particularly when dealing with persistent and recurrent infections (Kunnath et al. 2024). They play a role in illnesses such cystic fibrosis, urinary tract infections, and tuberculosis, where some bacteria persist even after therapy and lead to the disease's recurrence (Lannes-Costa et al. 2024). Additionally, biofilms that form on medical devices, such as implants and catheters, frequently impede treatment, necessitating the removal of the device in the majority of cases (Almatroudi, 2025). Persister cells cause antibiotic treatments to fail and patients to relapse, necessitating the administration of prolonged or combination regimens (Hashemi, 2025). In addition to increasing healthcare expenses, chronic infections contribute to the development of antimicrobial resistance by prolonging the bacterial exposure to antibiotics (Liu et al. 2024).

7. Strategies to Target Persister Cells and Biofilms

Persisting cells and biofilms resistant to killing need the use of drugs that can wake up the cells from their dormancy and, at the same time, disrupt the protective layers around them (Hashemi, 2025; Liu et al., 2024). Anti-persister drug development is mainly based on metabolism stimulation for awakening cells that are not dividing or the use of drugs that are specific to persisters and target the very mechanisms that keep ribosomes and cell survival (Niu et al. 2024). Methods to reduce biofilm consist of degrading the polysaccharide matrix by enzymatic action (e.g. DNases) and physical approaches like ultrasound, which promote penetration of antibiotics (Flemming et al. 2021; Almatroudi, 2025). Treatment combining antibiotics and adjuvants (e.g. efflux pump inhibitors) increases effectiveness in killing tolerant subpopulations (Ronneau & Helaine, 2021). Bacteriophage therapy, delivery of nanotechnology-based systems, and CRISPR-based targeting are some interesting possible strategies for efficiently and selectively managing bacteria (Liu et al. 2024).

8. Future Directions and Challenges

Despite advancements, persister cell research still faces significant challenges., Persisters are transient and heterogeneous, making them super difficult to study, plus no one standardized method for detecting persisters means limited reproducibility (Niu et al. 2024). On top of that, many anti-persister breakthroughs in labs still have not been turned into real-world/patient treatments (Hashemi, 2025). For instance, single-cell omics - one of the emerging technologies, can reveal the diversity of persister cells in detail while AI-based drug discovery can pinpoint the potential drugs for persister cells (Liu et al. 2024).

9. Conclusion

Persistent cell-mediated antibiotic tolerance is a key reason why infections by bacteria keep coming back and are hard to treat, especially when they are linked with biofilms. This article summarises the mechanism by which persister cells, through dormancy, metabolic inactivity, and by being genetically constant to the passed antibiotic-tolerant state, lead bacterial populations survive antibiotic exposure and cause chronic and recurrent infections (Niu et al. 2024; Kunnath et al. 2024). On top of the biofilm formation, the tolerance is boosted even more by the creation of less accessible microenvironments and the hindrance of antibiotic

effectiveness (Liu et al. 2024). Consequently, it is critical to target persister cells if we want to get better results from the application of therapies. Most of the time, antibiotics cannot really do the work of getting rid of the bacteria, so it is essential to come up with those methods that would disrupt the biofilms, wake up cells that are dormant, or kill the persisters directly (Hashemi, 2025). Dealing with this problem warrants a combination of different disciplines like microbiology, molecular biology, bioinformatics along with novel technologies like nanotechnology and artificial intelligence. It is through such a mix of endeavours that we could move forward and be able to offer efficient drug treatments and fight against persistent infections and antimicrobial resistance.

Author's contribution: KS: Data curation, Literature study, Analysis, Writing original draft, Illustrations. AS: Conceptualization, Supervision, Reviewing & editing the original draft. Both authors approved the final version.

10. Author Declaration Statements

Declaration: The authors hereby declare that the manuscript submitted for consideration is an original work and has not been published or submitted elsewhere for publication. The authors take full responsibility for the integrity, accuracy, and ethical compliance of the work presented in the manuscript.

Conflict of Interest: All authors confirm that:

i. Any potential conflicts of interest, whether financial or non-financial, have been fully disclosed. – ~~**Yes**~~ / **Not Applicable**√

ii. All sources of funding and financial support received for the conduct of the study have been appropriately acknowledged. – ~~**Yes**~~ / **Not Applicable**√

iii. Necessary ethical approvals have been obtained from the relevant institutional or regulatory bodies for studies involving human participants, animals, or sensitive data, wherever applicable. – ~~**Yes**~~ / **Not Applicable**√

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