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Adaptive Metabolic Awakening of Staphylococcus Aureus Persisters: Mechanisms and Therapeutic Implications

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Abstract

Staphylococcus aureus is a major human pathogen responsible for acute and chronic infections, including endocarditis, osteomyelitis, and device-associated biofilm infections. A key contributor to treatment failure and infection relapse is the formation of persister cells—phenotypic variants that survive lethal antibiotic exposure without acquiring genetic resistance. Historically, persisters were considered metabolically dormant; however, accumulating evidence demonstrates that S. aureus persisters retain low-level or adaptable metabolic activity that can be therapeutically exploited. This review synthesizes current knowledge on the metabolic states of S. aureus persisters and explores strategies aimed at inducing metabolic reactivation ("awakening") to restore antibiotic susceptibility. We discuss regulatory pathways governing persistence, metabolic heterogeneity within persister populations, and emerging therapeutic approaches such as metabolite-enabled antibiotic potentiation, respiration modulation, and proteostasis disruption. Finally, we highlight experimental challenges and future directions required to translate metabolic awakening strategies into clinically effective anti-persister therapies.

Keywords: Staphylococcus aureus; persister cells; metabolic reactivation; antibiotic tolerance; biofilms; metabolic heterogeneity; anti-persister therapy

1. Introduction

Staphylococcus aureus is a Gram-positive opportunistic pathogen that colonizes approximately 30% of the human population and causes a wide spectrum of diseases, ranging from superficial skin infections to life-threatening systemic illnesses. Despite the availability of potent antibiotics, eradication of S. aureus infections often remains challenging due to antibiotic tolerance and persistence rather than classical genetic resistance.

Persister cells represent a small subpopulation of bacteria that survive bactericidal antibiotic treatment by entering a transient, non- or slow-growing physiological state. Unlike resistant mutants, persisters retain antibiotic susceptibility upon regrowth, making persistence a reversible and phenotypic phenomenon. In S. aureus, persisters are strongly associated with biofilms, intracellular survival, and chronic or relapsing infections.

For decades, persisters were assumed to be metabolically inactive. However, recent advances in single-cell analysis, metabolic profiling, and antibiotic-adjuvant studies reveal that persister metabolism is not completely shut down. Instead, persisters exhibit **adaptive metabolic downshifting**, which can be

reversed under specific conditions. This realization has given rise to the concept of **metabolic awakening**—deliberately reactivating persister metabolism to sensitize them to antibiotics.

2. Biology of Persister Cells in Staphylococcus aureus

2.1 Definition and Characteristics

Persister cells in S. aureus are characterized by:

- Transient antibiotic tolerance
- Lack of genetic resistance mutations
- Ability to regrow after antibiotic withdrawal
- Enrichment during stationary phase, stress conditions, and biofilm growth

These cells survive exposure to antibiotics such as β-lactams, fluoroquinolones, aminoglycosides, and glycopeptides.

2.2 Distinction Between Resistance and Persistence

Antibiotic resistance involves heritable genetic changes that increase MIC values, whereas persistence does not alter MIC but allows survival at concentrations far above MIC. This distinction is clinically critical because persisters can act as a reservoir from which resistant mutants may later emerge.

3. Metabolic States of S. aureus Persisters

3.1 Metabolic Downshifting Rather Than Dormancy

Contrary to earlier assumptions, S. aureus persisters are not metabolically inert. Studies measuring ATP levels, membrane potential, redox activity, and macromolecular turnover demonstrate that persisters maintain:

- Low but measurable ATP pools
- Residual respiration
- Minimal protein synthesis and proteostasis activity

This metabolic flexibility enables survival while avoiding antibiotic-mediated killing.

3.2 Energetic Adaptations

Low intracellular ATP levels correlate strongly with antibiotic tolerance in S. aureus. Reduced ATP diminishes the activity of antibiotic targets such as cell wall synthesis enzymes and DNA replication machinery. Importantly, restoring ATP levels has been shown to resensitize persisters to antibiotics.

3.3 Metabolic Heterogeneity

Persister populations are heterogeneous. Even within a single biofilm, subsets of S. aureus cells exhibit different metabolic profiles, suggesting multiple persister states rather than a single uniform phenotype.

4. Regulatory Mechanisms Governing Persistence in S. aureus

4.1 Toxin–Antitoxin Systems

Although toxin–antitoxin (TA) systems are more extensively studied in Gram-negative bacteria, S. aureus possesses functional TA modules that contribute to growth arrest and persistence through inhibition of translation and replication.

4.2 Stringent Response and (p)ppGpp

The stringent response, mediated by the alarmone (p)ppGpp, plays a central role in S. aureus persistence. Elevated (p)ppGpp levels reprogram metabolism, suppress macromolecular synthesis, and promote survival under antibiotic stress.

4.3 Stress Responses and Global Regulators

Oxidative stress, nutrient limitation, and acidic pH induce transcriptional regulators that shift S. aureus into a tolerant state. Sigma factors and global regulators coordinate metabolic slowdown and stress adaptation.

5. Concept of Metabolic Awakening

Metabolic awakening refers to **controlled stimulation of persister metabolism** to re-enable antibiotic killing mechanisms. The strategy is based on two principles:

1. Many antibiotics require active cellular processes to exert bactericidal effects
2. Persisters retain the capacity to resume metabolic activity when provided appropriate stimuli

Rather than forcing full growth resumption, partial metabolic reactivation is often sufficient to restore antibiotic susceptibility.

6. Strategies for Metabolic Reactivation of S. aureus Persisters

6.1 Metabolite-Enabled Antibiotic Potentiation

Supplementation with carbon sources such as glucose, fructose, or pyruvate has been shown to increase ATP production and membrane potential in persisters, thereby enhancing antibiotic uptake and killing—particularly with aminoglycosides.

6.2 Respiration Modulation

Stimulating cellular respiration increases proton motive force and energizes transport systems. Conversely, complete respiratory shutdown promotes persistence. Fine-tuned modulation of respiration can shift persisters into vulnerable metabolic states.

6.3 Proteostasis Disruption

Activation of proteolytic machinery, especially through ClpP protease hyperactivation using acyldepsipeptides (ADEPs), induces uncontrolled protein degradation and kills S. aureus persisters independently of growth.

6.4 Combination Therapies

Combining metabolic activators with conventional antibiotics has shown synergistic effects, reducing persister survival in planktonic cultures and biofilms.

7. Metabolic Awakening in Biofilm-Associated Persisters

Biofilms provide a protective niche characterized by nutrient gradients, hypoxia, and slow growth. S. aureus biofilm persisters are particularly tolerant. Metabolic awakening strategies have demonstrated:

- Improved antibiotic penetration
- Reduction in viable biofilm cells
- Disruption of biofilm integrity

Targeting metabolic heterogeneity within biofilms remains a major research focus.

8. Experimental Approaches to Study Metabolic Reactivation

8.1 Persister Isolation

Common approaches include:

- Antibiotic challenge assays
- Stationary-phase enrichment

- Biofilm-derived persister models

8.2 Metabolic Measurements

ATP quantification, redox dyes, respirometry, and single-cell fluorescence techniques provide insights into persister metabolic states.

8.3 In Vivo and Ex Vivo Models

Animal infection models and host cell infection systems are essential to validate metabolic awakening strategies under clinically relevant conditions.

9. Therapeutic Implications and Clinical Potential

Metabolic awakening offers several advantages:

- Bypasses genetic resistance
- Enhances existing antibiotics
- Reduces treatment duration and relapse

However, challenges include targeted delivery, safety of metabolic adjuvants, and avoiding unintended stimulation of bacterial growth.

10. Conclusion

The paradigm of Staphylococcus aureus persisters as metabolically dormant cells is being replaced by a more nuanced view of adaptive metabolic suppression. Evidence strongly supports the feasibility of metabolically awakening persisters to restore antibiotic susceptibility. While significant translational challenges remain, metabolic reactivation represents a promising frontier in combating chronic and relapsing S. aureus infections and addressing the broader crisis of antibiotic tolerance.

References

1. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. Bacterial persistence as a phenotypic switch. Science. 2004. PubMed
2. Lewis K. Persister cells. Annu Rev Microbiol. 2010. PubMed
3. Allison KR, Brynildsen MP, Collins JJ. Metabolite-enabled eradication of bacterial persisters by aminoglycosides. Nature. 2011. PMC+1
4. Van den Bergh B, Fauvart M, Michiels J. Formation, physiology, ecology, evolution and clinical importance of bacterial persisters. FEMS Microbiol Rev. 2017. OUP Academic
5. Maisonneuve E, Shakespeare LJ, Jørgensen MG, Gerdes K. (p)ppGpp controls bacterial persistence by stochastic induction of toxin–antitoxin activity. Cell (Cell). 2013. PubMed
6. Orman MA, Brynildsen MP. Establishment of a method to rapidly assay bacterial persister metabolism and discover metabolic inhibitors. Antimicrob Agents Chemother. 2013. ASM Journals
7. Radzikowski JL, Vedelaar S, Qiu Y, et al. Bacterial persistence from a system-level perspective. Curr Opin Microbiol. 2017. ScienceDirect
8. Keren I, Shah D, Spoering A, Kaldalu N, Lewis K. Specialized persister cells and the mechanism of multidrug tolerance. J Bacteriol. 2004. ASM Journals
9. Kaldalu N, Hauryliuk V, Tenson T. Persisters—as elusive as ever. Appl Microbiol Biotechnol. 2016. Springer Link
10. Orman MA, Brynildsen MP. Inhibition of stationary phase respiration impairs persister formation in Escherichia coli. Nat Commun. 2015. Nature

11. Mohiuddin SG, Carey JN, Brynildsen MP. Identifying metabolic inhibitors to reduce bacterial persistence. Scientific Reports / PMC. 2020. PMC
12. Conlon BP, Nakayasu ES, Fleck LE, et al. Killing persister cells and eradicating a biofilm infection by activating ClpP protease. Nature. 2013. PMC
13. Helaine S, Thompson JA, Watson KG, Liu M, Boyle C, Holden DW. Internalization of Salmonella by macrophages induces formation of nonreplicating persisters. Science. 2014. PubMed
14. Stapels DAC, Hill PWS, Westermann AJ, et al. Salmonella persisters undermine host immune defenses during antibiotic treatment. Science. 2018. Science
15. Dörr T, Lewis K, Åkerman D. Ciprofloxacin causes persister formation by inducing the TisB toxin. PLoS Biology. 2010. PLOS
16. Kaspy I, Rotem E, Weiss N, Ronin I, Balaban NQ, Glaser G. HipA-mediated antibiotic persistence via phosphorylation of the glutamyl-tRNA synthetase. Nat Commun. 2013. Nature
17. Rotem E, Loinger A, Ronin I, et al. Regulation of phenotypic variability by a threshold-based mechanism underlies bacterial persistence. PNAS. 2010. PNAS
18. Germain E, Castro-Roa D, Zenkin N, Gerdes K. Stochastic induction of persister cells by HipA through (p)ppGpp. PNAS. 2015. PNAS
19. Maisonneuve E, Gerdes K. Molecular mechanisms underlying bacterial persisters. Cell. 2014. ScienceDirect
20. Fauvart M, de Groote VN, Michiels J. Role of persister cells in chronic infections. J Med Microbiol. 2011. microbiologyresearch.org
21. Harms A, Brodersen DE, Mitarai N, Gerdes K. Toxin–antitoxin biology and persister formation. Nat Rev Microbiol / Mol Microbiol review (2018). ScienceDirect
22. Wood TL, McLean RJC. Combatting bacterial persister cells. Trends Biotechnol. 2016. Wiley Online Library
23. Cabral DJ, Podell S, Brynildsen MP. Antibiotic persistence as a metabolic adaptation: stress responses and mechanisms. Pharmaceutics/MDPI review (2018). MDPI
24. Van den Bergh B, Fauvart M, Michiels J. Persisters: formation, physiology, ecology and evolution. FEMS Microbiol Rev. 2017. OUP Academic
25. Amato SM, Orman MA, Brynildsen MP. Persister heterogeneity arising from a single metabolic stress. Curr Biol. 2015. Cell
26. Orman MA, Brynildsen MP. Persister formation in Escherichia coli can be inhibited by nitric oxide. Free Radic Biol Med. 2016. BioRxiv
27. Nguyen D, Joshi-Datar A, Lepine F, et al. Active starvation responses mediate antibiotic tolerance in biofilms and nutrient-limited bacteria. Science. (Representative studies on biofilm/persister physiology.) Springer Link
28. Keren I, Shah D, Spoering A, et al. Persister cells and tolerance to antimicrobials. FEMS Microbiol Lett. 2004. PubMed
29. Fauvart M, De Groote VN, Michiels J. Persister cells in chronic infections and antibiotic treatment outcomes. J Med Microbiol. 2011. microbiologyresearch.org
30. Kędzierska B, Hayes F. Emerging roles of toxin–antitoxin modules in bacterial physiology. MDPI Molecules review. 2016. MDPI
31. Gelens L, Hill L, Vandervelde A, Danckaert J, Loris R. A general model for toxin–antitoxin dynamics that explains persister formation. PLoS Comput Biol. 2013. ScienceDirect

32. Fasani RA, Savageau MA. Molecular mechanisms of multiple toxin–antitoxin systems coordinate the persister phenotype. PNAS. 2013. PNAS
33. Keren I, Shah D, Spoering A, et al. Characterization and transcriptome analysis of persister cells. mBio / J Bacteriol (2011 sample). PMC
34. Hossain T, et al. Antibiotic tolerance, persistence and resistance review (2021). iScience. Cell
35. Niu H, Zhang J, et al. Bacterial persisters: molecular mechanisms and anti-persister strategies. Signal Transduction and Targeted Therapy (2024 review). Nature
36. Shultis MW, et al. Are all antibiotic persisters created equal? Front Cell Infect Microbiol. 2022. Frontiers
37. Orman MA, Brynildsen MP. Aminoglycoside-enabled elucidation of bacterial persister metabolism (methods). Curr Protoc Microbiol. 2015. currentprotocols.onlinelibrary.wiley.com
38. Conlon BP, Rowe SE, Gandt AB, et al. Persister formation and eradication in Staphylococcus aureus. (ADEP and ClpP studies) PMC. 2013. PMC
39. Mabanglo MF, et al. ClpP protease structural dynamics and ADEP activation. Communications Biology. 2019. Nature
40. Malik IT, Maupin-Furlow J, et al. Conformational control of Clp protease by small-molecule activators. Nat Prod Rep. 2017. RSC Publishing
41. Stapels DAC, et al. Intracellular persister biology and host reprogramming. Science (2018). PubMed
42. Fauvart M, et al. Clinical relevance of persisters in infection relapse. J Med Microbiol. 2011. microbiologyresearch.org
43. Helaine S, et al. Macrophage internalization drives Salmonella persister formation. Science. 2014. PubMed
44. Korch SB, et al. HipA/HipB and persister formation. (HipA foundational studies and follow-ups.) PLoS Genetics / other sources. ScienceDirect
45. Germain E, Castro-Roa D, et al. HipA–(p)ppGpp–TA axis and persistence. PNAS 2015. PNAS
46. Orman MA, Brynildsen MP. Persister metabolic heterogeneity and glycerol/glucose uptake mapping. AAC / 2013. ASM Journals
47. Mohiuddin SG, et al. Metabolic inhibitors and persister suppression screening. Sci Rep / PMC. 2020. PMC
48. Dörr T. Mechanisms of antibiotic persistence and DNA damage–dependent toxin induction. PLoS Biol. 2010. PLOS
49. Van den Bergh B, Michiels J. Ecology and evolution of persisters and persistence. FEMS Microbiol Rev (2017). OUP Academic
50. Definitions & guidelines for persistence/tolerance research — Balaban et al. Nat Rev Microbiol. 2019. Nature