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Review Article

The Fitness Cost of Antibiotic Resistance: A Critical Factor in Bacterial Adaptation

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Abstract

Antibiotic resistance often incurs fitness costs that can impair bacterial growth, competitiveness, or adaptability in drug-free environments. However, these disadvantages are frequently offset by compensatory mutations, ecological interactions, and horizontal gene transfer, enabling resistant strains to persist and spread. This review explores the molecular basis and evolutionary dynamics of resistance-associated fitness costs, with a focus on how genetic, physiological, and environmental factors shape bacterial adaptation. We highlight experimental approaches to quantify fitness costs, the role of microbial communities and host environments, and emerging strategies to exploit evolutionary trade-offs. Integrating fitness cost insights into antibiotic stewardship and predictive modeling offers a promising path to managing resistance more effectively and prolonging the efficacy of existing therapies.

Keywords: Antibiotic resistance; Compensatory evolution; Collateral sensitivity; Resistance persistence.

Introduction

The emergence and spread of antibiotic resistance represent a significant challenge to global healthcare systems. While the benefits of resistance for bacterial survival under antimicrobial pressure are clear, the consequences of resistance acquisition on bacterial fitness are often overlooked. In many cases, resistance mutations or the acquisition of resistance genes impose physiological burdens, leading to reduced growth rates, impaired competitiveness, or attenuated virulence in drug-free environments (Dhital *et al*., 2024; Andersson and Hughes, 2010). These costs, collectively referred to as the fitness cost of resistance, can influence the evolutionary dynamics of bacterial populations and the stability of resistance traits over time (Melnyk *et al.,* 2015).

Understanding the fitness costs of resistance is crucial for anticipating how long resistance traits might persist in both clinical and environmental settings, especially when antibiotics are no longer in use (Andersson and Hughes, 2010). Additionally, bacteria can sometimes overcome these fitness disadvantages through secondary mutations or changes in gene regulation, which makes managing resistance even more challenging (Hemez *et al*., 2022). To effectively mitigate the spread of antibiotic resistance, it is essential to thoroughly understand the factors that influence fitness costs, including the molecular mechanisms of resistance, the genetic background of the host, and environmental conditions (Vogwill and MacLean, 2015).

This review examines the biological basis of fitness costs associated with antibiotic resistance, the methods used to measure these costs, and their implications from both evolutionary and clinical perspectives. We also discuss recent findings on compensatory evolution and consider how these insights could help guide antibiotic use and the development of treatment strategies that take bacterial evolution into account.

Mechanisms of Antibiotic Resistance and Their Associated Fitness Costs

Enzymatic Degradation

One of the primary mechanisms by which bacteria evade the action of antibiotics is through the production of enzymes that degrade or modify the antimicrobial compound. A well-characterized example is the production of β-lactamases, which hydrolyze the β-lactam ring of penicillin and cephalosporins, thereby inactivating these antibiotics and conferring high-level resistance to the host bacterium (Bush and Bradford, 2016). Although enzymatic degradation is an effective mechanism of antibiotic resistance, it is often associated with fitness costs. The biosynthesis and secretion of resistance enzymes require significant cellular energy and metabolic resources, which can reduce bacterial growth rates in antibiotic-free environments (Melnyk *et al.,* 2015). Furthermore, the genes encoding these enzymes are frequently carried on plasmids or other mobile genetic elements, whose replication and maintenance can impose an additional metabolic burden, particularly in competitive environments (Andersson and Hughes, 2010; San Millan and MacLean, 2017).

Target Modification

Alterations in antibiotic target sites, typically arising through point mutations, constitute a major mechanism of resistance in many bacterial species. Notable examples include mutations in ribosomal proteins or rRNA that confer resistance to macrolides and aminoglycosides, as well as mutations in DNA gyrase (gyrA) or topoisomerase IV (parC) that lead to the resistance against fluoroquinolones (Vila-Sanjurjo *et al*., 2023). While these mutations can effectively reduce antibiotic binding and thus confer resistance, they often compromise the native function of essential cellular machinery. For instance, mutations in ribosomal components may impair the fidelity or efficiency of protein synthesis, whereas alterations in DNA gyrase can hinder the supercoiling and replication of DNA (Andersson and Hughes, 2010). These disruptions in core biological processes can impose fitness costs, commonly observed as reduced growth rates, impaired competitive ability, or decreased adaptability under fluctuating environmental conditions (Melnyk *et al.,* 2015; Durão *et al.,* 2018).

Efflux Pumps and Permeability Changes

Another common mechanism of antibiotic resistance involves either increased efflux or decreased permeability of the bacterial cell envelope. Multidrug efflux pumps, such as the AcrAB-TolC system in *Escherichia coli*, actively transport a wide range of antibiotics and toxic compounds out of the cell, thereby reducing intracellular drug concentrations and enhancing bacterial survival under antimicrobial pressure (Du *et al.,* 2018). However, the overexpression of these efflux systems can impose a fitness cost by disrupting cellular homeostasis and consuming significant amounts of energy, particularly in antibiotic-free environments (El Meouche and Dunlop, 2018). Similarly, reduced membrane permeability, often caused by mutations or loss of outer membrane porins in Gram-negative bacteria, limits antibiotic uptake but may also hinder the transport of essential nutrients, impairing bacterial growth under nutrient-limited conditions (Helmy *et al*., 2023). These resistance strategies, while effective against antimicrobial agents, are frequently associated with fitness trade-offs that may reduce bacterial competitiveness in the absence of selective pressure (Andersson and Hughes, 2010).

Bioenergetic and Metabolic Consequences

Many antibiotic resistance mechanisms exert direct or indirect effects on bacterial metabolism and energy production. For example, mutations that confer resistance to aminoglycosides frequently disrupt components of the electron transport chain, resulting in a reduced proton motive force and impaired ATP synthesis (Lopatkin *et al.,* 2021). Similarly, resistance-associated alterations in membrane composition or function, such as changes in lipid structure or porin loss, can interfere with ion gradients and membrane potential, key elements of cellular bioenergetics (Ghai, 2023). These disruptions not only compromise bacterial growth and survival under specific environmental conditions but may also diminish the cell's capacity to manage additional stresses, such as oxidative damage or nutrient limitation, thereby exacerbating the overall fitness cost associated with resistance (Melnyk *et al.,* 2015; Durão *et al.,* 2018).

Quantifying Fitness Costs: Experimental Approaches

Growth Kinetics and Competition Assays

Different experimental approaches for quantifying fitness costs of antibiotic resistance are summarized in Table 1. One of the most widely used approaches to quantify the fitness costs associated with antibiotic resistance involves the analysis of bacterial growth kinetics. Traditional growth curve assays, typically conducted in nutrient-rich media, enable the measurement of parameters such as lag phase duration, exponential growth rate, and maximum cell density. Resistant strains often display reduced growth rates or prolonged lag phases relative to their susceptible counterparts in the absence of antibiotics, reflecting the physiological and metabolic burdens imposed by resistance mechanisms (Andersson and Hughes, 2010; Melnyk *et al.,* 2015). A more sensitive and ecologically relevant method involves competition assays, where resistant and susceptible strains are co-cultured under identical conditions. Changes in their relative abundances over time are used to calculate competitive indices or selection coefficients, providing precise estimates of relative fitness (Durão *et al.,* 2018). These assays can uncover subtle fitness differences that are not evident in monoculture and are especially useful for evaluating the evolutionary dynamics of resistance in heterogeneous environments.

Animal Models and Ecological Settings

Although *in vitro* assays provide controlled conditions to assess the fitness costs of antibiotic resistance, they often do not reflect the multifactorial complexity of natural or host-associated environments. *In vivo* models, such as murine infection systems, offer valuable insights into how resistance impacts bacterial fitness within a host context. Host-related factors, including immune responses, tissue-specific nutrient availability, and competition with resident microbiota, can significantly influence the expression and magnitude of fitness costs (Diard and Hardt, 2017; Melnyk *et al.,* 2015). These models help elucidate how resistance affects colonization, virulence, and transmission dynamics in ways not captured by laboratory media. Similarly, ecological studies conducted in more natural environments, such as soil, aquatic systems, or host-associated microbiomes, have demonstrated that the fitness effects of resistance are often context-dependent (Santamaria de Souza *et al*., 2025; Allen *et al.,* 2010). Resistance mutations that impose a growth disadvantage under laboratory conditions may be neutral or even advantageous in certain ecological niches due to environmental pressures such as nutrient limitation, microbial competition, or co-selective factors like heavy metals or biocides. These findings underscore the importance of integrating diverse experimental systems to fully characterize the evolutionary and ecological consequences of antibiotic resistance (Herren and Baym, 2022).

Table 1: Experimental approaches for quantifying fitness costs of antibiotic resistance

Experimental approach	Main advantages	Main limitations
Growth kinetics assays	Simple, high-throughput; Quantitative measures (e.g., doubling time, growth yield).	May miss subtle fitness effects; Artificial laboratory conditions.
Competition assays	Sensitive to small fitness differences; ecologically relevant.	Requires precise strain labeling (e.g., fluorescent markers); interpretation can be complex.
Animal infection models	Captures host-pathogen interactions; clinically relevant.	High variability; ethical and technical challenges.
Environmental/ecological studies	Real-world relevance; incorporates community interactions.	Difficult to control confounding factors; complex data interpretation.
Single-cell analysis (e.g., microfluidics, microscopy)	Reveals population heterogeneity; detects rare subpopulations.	Technically demanding; often lower throughput.
Fitness landscape mapping (e.g., barcoded libraries, deep mutational scanning)	High-resolution fitness profiling across many variants.	Requires large datasets and computational analysis.

Advances in Single-Cell Analysis and Fitness Landscapes

Recent technological advancements have significantly enhanced the resolution at which fitness costs associated with antibiotic resistance can be studied. Single-cell approaches, including time-lapse fluorescence microscopy and microfluidic-based tracking, enable the direct observation of individual bacterial cells, revealing phenotypic heterogeneity in growth, division, and survival among resistant populations (Hare *et al*., 2021; El Meouche and Dunlop, 2018). These methods uncover population-level dynamics that are often masked in bulk measurements, providing deeper insight into how resistance influences cell-to-cell variability and adaptive potential. In parallel, high-throughput genomic tools such as transposon sequencing (Tn-seq) and CRISPR interference (CRISPRi) have facilitated the construction of comprehensive fitness landscapes. These approaches allow systematic evaluation of the relative fitness effects of resistance mutations across a range of environmental conditions (Lopatkin *et al.,* 2021). Mapping such landscapes not only refines our understanding of resistance-associated fitness costs but also elucidates the potential evolutionary pathways accessible to resistant strains, including those involving compensatory mutations that ameliorate initial fitness burdens (Durão *et al.,* 2018). Together, these technologies provide powerful tools for predicting resistance dynamics and informing evolution-informed treatment strategies.

Genetic and Environmental Modulators of Fitness

Compensatory Mutations: Offsetting the Costs of Resistance

Antibiotic resistance frequently incurs a fitness cost, particularly in antibiotic-free environments. However, bacterial populations can often mitigate these costs through the acquisition of compensatory mutations (Hinz *et al*., 2024; Andersson and Hughes, 2010). These adaptations allow resistant strains to persist and even thrive in the absence of selective antibiotic pressure, complicating efforts to reverse resistance through withdrawal strategies alone. Well-documented examples include mutations that modify ribosomal assembly to offset the translational defects associated with aminoglycoside resistance in *Escherichia coli* (Björkman *et al.,* 2000), as well as regulatory mutations that fine-tune the expression of multidrug efflux pumps to reduce metabolic burden (Sun *et al.,* 2014). The rate and success of compensatory evolution are influenced by factors such as population size, genetic background, mutation supply, and environmental context (Maeda and Furusawa, 2024). Understanding the genetic mechanisms and physiological pathways involved in compensatory adaptation is essential for predicting the persistence and evolutionary stability of resistance traits.

Influence of Microbial Community Context and Host Environment

The fitness costs associated with antibiotic resistance are not fixed properties but are highly context-dependent, varying significantly across microbial communities and host environments. In polymicrobial ecosystems, interspecies interactions such as competition, mutualistic cross-feeding, or cohabitation in biofilms can modulate the expression and magnitude of fitness costs. For example, metabolic cooperation among species may alleviate the resource burden of resistance mechanisms, while competitive interactions may intensify selective pressures against less fit resistant strains (Stubbendieck *et al.,* 2016; Vega and Gore, 2014). Within host organisms, additional factors such as immune system activity, spatial heterogeneity, and fluctuating nutrient levels further influence the cost-benefit balance of resistance traits. Resistance mutations that reduce bacterial growth *in vitro* may provide a net advantage *in vivo* by enhancing survival against host-derived stressors such as antimicrobial peptides, oxidative bursts, or phagocytosis (Melnyk *et al.,* 2015; Gifford *et al.,* 2020). Tissue-specific microenvironments, including differential oxygen tension or pH, may also reshape the physiological impacts of resistance-associated alterations (Hancock *et al.,* 2017). Similarly, Tu *et al*. (2025) demonstrated that the expression of metallo-β-lactamase VIM-2 imposes a significant fitness cost on bacterial pathogen under zinc-limited conditions lead to increased membrane vulnerability and greater reliance on envelope stress-response pathways. These weaknesses can be exploited using antibiotics that target membrane integrity, thereby helping to overcome resistance. These ecological and host-mediated influences underscore the importance of evaluating resistance fitness within biologically relevant contexts.

Role of Horizontal Gene Transfer and Mobile Genetic Elements

Horizontal gene transfer (HGT) plays a pivotal role in the dissemination of antibiotic resistance genes (ARGs) across bacterial populations and species. These genes are frequently embedded within mobile genetic elements (MGEs) such as plasmids, transposons, and integrons, which facilitate their rapid horizontal spread in clinical, environmental, and agricultural settings (Partridge *et al.,* 2018). While the acquisition of MGEs often imposes a fitness cost due to the metabolic burden of replicating and expressing additional genetic material, bacteria have evolved multiple strategies to mitigate these costs and stabilize the elements within their genomes. Stabilization mechanisms include plasmid addiction systems (e.g., toxin-antitoxin modules), which ensure plasmid retention by killing plasmid-free segregants, and genetic linkage with other advantageous traits such as heavy metal or disinfectant resistance, a phenomenon known as co-selection (San Millan and MacLean, 2017). Additionally,

the insertion of resistance genes into low-cost chromosomal loci or integration into genomic islands can reduce the physiological burden and promote long-term persistence (Porse *et al.,* 2018). Moreover, HGT can introduce not only resistance determinants but also compensatory elements such as regulatory genes or stress response factors that offset the fitness costs of resistance. This genetic co-transfer may enhance the adaptive potential of bacterial hosts, allowing resistance to persist even in the absence of selective antibiotic pressure (Dimitriu *et al.,* 2021). The dynamic relationship between MGEs, fitness modulation, and resistance stability underscores the complexity of resistance evolution. It highlights the need to consider mobile genetic ecology in antibiotic stewardship and surveillance strategies.

Evolutionary Outcomes and Persistence of Resistance

Dynamics in the Presence and Absence of Antibiotic Pressure

The evolutionary dynamics of antibiotic resistance are profoundly influenced by the presence or absence of selective pressure. In environments with sustained antibiotic exposure, resistant strains are positively selected, enabling their proliferation despite any associated fitness burdens (Andersson and Hughes, 2010). Conversely, when antibiotic use is discontinued, resistant populations may experience a competitive disadvantage relative to susceptible strains due to the metabolic or physiological costs of resistance (Melnyk *et al.,* 2015). However, the rate and extent of resistance decline in the absence of antibiotics are highly variable and depend on multiple factors, including the magnitude of the fitness cost, the ecological niche, and the genetic context. Importantly, resistance can persist even without ongoing antibiotic pressure. This persistence is often facilitated by compensatory mutations that restore fitness while maintaining resistance (Hinz *et al*., 2024), or by genetic linkage between resistance genes and other advantageous traits, such as virulence factors or stress response elements (San Millan and MacLean, 2017). Additionally, resistance determinants located on stable plasmids or within integrative elements may persist due to low fitness costs or the presence of co-selective agents such as heavy metals and disinfectants (Pal *et al.,* 2015). These evolutionary outcomes underscore the importance of evaluating not only the acquisition of resistance but also its long-term stability and reversibility under varying environmental conditions.

Cost Amelioration over Evolutionary Time

Over prolonged evolutionary periods, bacterial populations can acquire mutations that alleviate the fitness costs associated with antibiotic resistance. This phenomenon, referred to as cost amelioration, may occur through the accumulation of compensatory mutations, global regulatory changes, or epistatic interactions that mitigate the deleterious effects of resistance determinants (Chowdhury and Findlay, 2023). These genetic adaptations can restore cellular functions impaired by resistance mutations without compromising the resistance phenotype itself. Experimental evolution studies have shown that even resistance mechanisms associated with substantial initial fitness costs can become nearly cost-free following successive rounds of adaptive evolution in the absence of antibiotics (Pal and Andersson, 2024). Such compensatory evolution enhances the evolutionary stability of resistance, enabling resistant strains to persist and compete effectively even in antibiotic-free environments. Consequently, the long-term fixation of ameliorated resistance traits complicates efforts to reverse resistance trends solely through reduced antibiotic use or stewardship programs. Understanding the mechanisms and dynamics of cost amelioration is therefore critical for predicting the durability of resistance in microbial populations.

Reversibility of Resistance and Evolutionary Constraints

Although theoretical models predict that antibiotic resistance alleles carrying significant fitness costs should decline in frequency when selective pressure is removed, empirical evidence demonstrates that the reversion to full susceptibility is uncommon in natural and clinical settings (Andersson and Hughes, 2010). Several evolutionary constraints limit the reversibility of resistance. Genetically, some resistance-conferring mutations may be irreversible or unlikely to revert due to low mutation rates or deleterious effects of back-mutations. Ecologically, low-level or intermittent antibiotic exposure commonly found in clinical and agricultural environments can sustain selective pressure that favors resistant strains (Sundqvist *et al.,* 2010; Pal *et al.,* 2015). Additionally, the accumulation of compensatory mutations can restore fitness without reversing resistance, allowing resistant bacteria to persist even in the absence of antibiotics (Hinz *et al*., 2024). Moreover, resistance alleles can become genetically linked to other advantageous traits through pleiotropy or genetic hitchhiking, further stabilizing resistance within bacterial populations (Trindade *et al.,* 2009; Bustamante *et al*., 2024; Hall *et al.,* 2011). These evolutionary dynamics highlight the limitations of relying on antibiotic withdrawal as a resistance reversal strategy and underscore the necessity of integrated, proactive resistance management approaches that consider the potential for long-term persistence of resistance determinants.

Clinical and Therapeutic Implications

Designing Treatments to Maintain or Amplify Fitness Costs

One approach involves the use of combination therapies in which a conventional antibiotic is paired with an adjuvant that disrupts compensatory pathways. For instance, the co-

administration of antibiotics with efflux pump inhibitors or agents targeting key metabolic functions can exacerbate the physiological stress experienced by resistant bacteria, thereby increasing their susceptibility to host immune defenses and subsequent antimicrobial interventions (Blair *et al.,* 2015). Such synergistic treatments may prevent the evolutionary optimization that typically reduces the fitness burden of resistance over time. The evolutionary and ecological dynamics that shape resistance development and persistence propose that understanding fitness trade-offs is crucial for designing more effective antimicrobials strategies (Fuzi, 2025). In addition, adaptive dosing strategies such as intermittent or pulsed antibiotic regimens have been proposed to destabilize resistance by periodically removing selective pressure. These dynamic treatment protocols can prevent the fixation of resistance traits and delay the emergence of compensatory mutations, thereby prolonging the clinical utility of existing antimicrobials (Day and Read, 2016; Hansen *et al.,* 2020). By integrating insights from microbial physiology, evolutionary biology, and pharmacodynamics, these approaches offer a rational framework for designing treatments that not only eliminate pathogens but also manage resistance evolution more effectively.

Exploiting Collateral Sensitivity and Evolutionary Trade-offs

Collateral sensitivity, where the evolution of resistance to one antibiotic increases susceptibility to another, represents a promising strategy to counteract multidrug resistance by exploiting evolutionary trade-offs inherent to bacterial adaptation (Baym *et al.,* 2016). This phenomenon typically arises when resistance-conferring mutations incur structural or regulatory changes that simultaneously compromise defense mechanisms against alternative drugs. By systematically identifying these collateral networks, clinicians can devise sequential or cycling antibiotic therapies that maintain therapeutic efficacy while limiting the emergence of broadly resistant strains (Barbosa *et al.,* 2019). Furthermore, a comprehensive understanding of the evolutionary trade-offs between resistance and other bacterial functions such as virulence, metabolic efficiency, and stress tolerance can inform the design of interventions that target the unintended consequences of resistance evolution (Melnyk *et al.,* 2015; Lázár *et al.,* 2013). For instance, drugs that exploit metabolic bottlenecks or attenuate virulence in resistant strains could synergize with conventional antimicrobials to enhance treatment outcomes and suppress resistance maintenance. Integrating collateral sensitivity into treatment design thus offers a dynamic, evolution-informed approach to resistance management, with the potential to extend the lifespan of existing antibiotics.

Integrating Fitness Cost Data with Antibiotic Stewardship Programs

Incorporating insights into the fitness costs associated with antibiotic resistance into stewardship programs presents a promising avenue for enhancing the precision and sustainability of antimicrobial use. Quantifying the fitness burden of resistance across different bacterial taxa enables more accurate predictions regarding the persistence, dissemination, and reversibility of resistant strains under various clinical and environmental contexts (Melnyk *et al.,* 2015; Andersson and Hughes, 2010). By integrating such data into surveillance systems, clinicians and public health officials can monitor the trajectory of resistance evolution, including the emergence of compensatory mutations that may stabilize resistance even in the absence of antibiotics (San Millan and MacLean, 2017). This knowledge can directly inform treatment strategies by identifying regimens that maintain or amplify fitness costs, thereby limiting the competitive advantage of resistant strains (Baym *et al*., 2016). Additionally, fitness cost metrics can support the development of antibiotic cycling protocols and narrow-spectrum therapies tailored to local resistance dynamics, helping to prolong the clinical utility of existing antibiotics (Muteeb *et al*., 2023). At the policy level, integrating evolutionary and ecological principles into stewardship frameworks may aid in reducing the selection and spread of multidrug-resistant pathogens, promoting a more evolutionarily informed and globally coordinated response to antibiotic resistance.

Future Perspectives and Research Directions

Predictive Models of Resistance Spread Based on Fitness Landscapes

Recent advances in computational biology and systems modeling have opened new avenues for forecasting the dynamics of antibiotic resistance. Predictive frameworks that incorporate fitness landscapes quantitative maps of the fitness effects of resistance mutations across various genotypic and environmental contexts offer a powerful tool for understanding and anticipating resistance evolution (Lässig *et al.,* 2017). By integrating genomic data with information on mutation effects, epistatic interactions, population structure, and ecological variability, such models can simulate the trajectories of resistant clones under differing selective regimes. These models have the potential to identify high-risk evolutionary pathways, forecast the emergence of resistance under specific treatment regimens, and pinpoint geographic or clinical hotspots of resistance emergence (Farrokhian *et al.,* 2022). Incorporating dynamic environmental variables, such as fluctuating antibiotic concentrations, microbial competition, and host immune pressures, can further enhance their predictive accuracy and relevance to real-world scenarios (Levin-Reisman *et al.,* 2017). Future efforts should prioritize the calibration of these models using

empirical data from clinical, environmental, and experimental evolution studies to improve their translational utility in informing antimicrobial stewardship and public health interventions.

Novel Strategies to Prevent Compensatory Evolution

While compensatory mutations serve as an adaptive mechanism by which resistant bacteria alleviate the fitness costs of antibiotic resistance, they also complicate efforts to reverse resistance and restore antimicrobial efficacy. Preventing or delaying compensatory evolution is therefore a critical goal in resistance management. Emerging strategies focus on disrupting the molecular pathways that facilitate compensation, including regulatory networks involved in stress responses, energy metabolism, and protein homeostasis (Njenga *et al*., 2023). Targeting these pathways pharmacologically could prevent the restoration of fitness in resistant strains, thereby maintaining their competitive disadvantage in antibiotic-free environments. Additionally, the development of small molecules or RNA-based therapeutics that selectively interfere with compensatory mutations represents a promising frontier. For instance, antisense RNA approaches have been explored to inhibit the expression of genes involved in resistance maintenance or fitness restoration (Jia *et al.,* 2022). Moreover, exploiting synthetic lethality or collateral vulnerabilities that emerge during compensatory adaptation may provide a means to selectively eliminate compensated resistant bacteria (Lopatkin *et al.,* 2021).

Role of Fitness Costs in Multi-Drug Resistance

The global rise of MDR and co-resistant bacterial pathogens poses a critical challenge to public health. Understanding the role of fitness costs in the evolution and persistence of resistance to multiple antibiotics is essential for addressing this threat. MDR often imposes cumulative or synergistic fitness burdens, resulting from the simultaneous expression of multiple resistance mechanisms such as efflux pumps, target modifications, and enzymatic inactivation (Andersson and Hughes, 2010; San Millan and MacLean, 2017). These costs may reduce bacterial competitiveness in drug-free environments, but can be mitigated through compensatory adaptations or favorable genetic backgrounds. In co-resistance scenarios where resistance determinants to different antibiotics are genetically linked, often on mobile genetic elements, fitness trade-offs become more complex. For instance, plasmids carrying multiple resistance genes can impose a substantial metabolic burden, yet their persistence is frequently supported by co-selection with other beneficial traits (Alonso-del Valle *et al.,* 2021). Investigating how such fitness constraints influence the evolutionary trajectories of MDR bacteria may reveal vulnerabilities that can be exploited using rationally designed combination therapies or antibiotic cycling strategies. Future research should prioritize the identification of genetic interactions and ecological pressures that govern the stability of MDR and co-resistance phenotypes. Integrating fitness cost data into these studies will improve our ability to forecast the spread of resistant strains and inform strategies for curbing the expansion of MDR pathogens.

Conclusions

Antibiotic resistance, often accompanied by fitness costs, represents a highly dynamic evolutionary process influenced by complex genetic, physiological, and ecological factors. Although resistance mutations frequently hinder bacterial growth or competitiveness without antibiotics, these disadvantages rarely suffice to eliminate resistant strains from clinical or environmental contexts. The rapid emergence of compensatory adaptations, the effects of microbial communities, host environments, and the mobilization of resistance genes through horizontal gene transfer all play a role in the persistence and spread of resistance. Understanding the nuances of fitness costs, how they arise, how they are mitigated, and how they influence bacterial evolution offers critical insights into resistance management. Emerging strategies that exploit collateral sensitivity, evolutionary trade-offs, and metabolic vulnerabilities have the potential to enhance treatment efficacy and delay the stabilization of resistance. Moreover, incorporating fitness cost data into stewardship programs and predictive models can help design more informed, adaptive interventions. Continued research into compensatory mechanisms, ecological contexts, and multi-drug resistance dynamics is essential to counter the persistence and spread of resistance.

Authors' Contribution

Bigya Dhital conceptualized and designed the present review, Prepared the first draft manuscript; Rameshwor Pudasaini and Hsin-I Chiang critically analysed the data & revised the manuscript. Final version of manuscript was approved by all authors.

Conflict of interest

The authors declare that they have no conflict of interest with the present publication.

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References

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, and Handelsman J (2010) Call of the wild: antibiotic resistance genes in natural environments. *Nat Rev Microbiol* **8**: 251–259. DOI: 10.1038/nrmicro2312

Alonso-del Valle A, León-Sampedro R, Rodríguez-Beltrán J, DelaFuente J, Hernández-García M, Ruiz-Garbajosa P, and San Millan A (2021) Variability of plasmid fitness effects contribute to plasmid persistence in bacterial

communities. *Nat Commun* **12**: 2653. DOI: 10.1038/s41467-021-22958-w

Andersson DI, and Hughes D (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat Rev Microbiol* **8**: 260–271. DOI: 10.1038/nrmicro2319

Barbosa C, Römhild R, Rosenstiel P, and Schulenburg H (2019) Evolutionary stability of collateral sensitivity to antibiotics in the model pathogen *Pseudomonas aeruginosa*. *eLife* **8**: e51481. DOI: 10.7554/eLife.51481

Baym M, Stone LK, and Kishony R (2016) Multidrug evolutionary strategies to reverse antibiotic resistance. *Science* **351**: aad3292. DOI: 10.1126/science.aad3292

Björkman J, Nagaev I, Berg OG, Hughes D, and Andersson DI (2000) Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. *Science* **287**: 1479–1482. DOI: 10.1126/science.287.5457.1479

Blair JMA, Webber MA, Baylay AJ, Ogbolu DO and Piddock LJV (2015) Molecular mechanisms of antibiotic resistance. *Nat Rev Microbiol* **13**: 42–51. DOI: 10.1038/nrmicro3380

Bush K, and Bradford PA (2020) Epidemiology of β-lactamase-producing pathogens. *Clin Microbiol Rev* **33**: e00047-19. DOI: 10.1128/CMR.00047-19

Bustamante M, Mei S, Daras IM, van Doorn GS, Falcao Salles J, and de Vos MGJ (2024) An eco-evolutionary perspective on antimicrobial resistance in the context of One Health. *iScience* **28**(1): 111534. DOI: 10.1016/j.isci.2024.111534

Chowdhury FR, and Findlay BL (2023) Fitness costs of antibiotic resistance impede the evolution of resistance to other antibiotics. *ACS Infect Dis* **9**(10):1834-1845. DOI: 10.1021/acsinfecdis.3c00156

Day T, and Read AF (2016) Does high-dose antimicrobial chemotherapy prevent the evolution of resistance? PLoS Comput Biol 12: e1004689. DOI: 10.1371/journal.pcbi.1004689

Dhital B, Chuang S-T, Hsieh J-C, Hsieh M-H, and Chiang H-I (2024) Prevalence, virulence, and antimicrobial resistance of major mastitis pathogens isolated from Taiwanese dairy farms. *Antibiotics* **13**(1): 36. 10.3390/antibiotics13010036

Diard M, and Hardt WD (2017) Evolution of bacterial virulence. *FEMS Microbiol Rev* **41**: 679–697. DOI: 10.1093/femsre/fux023

Dimitriu T, Matthews AC, and Buckling A (2021) Increased horizontal gene transfer accelerates evolution and shifts ecological structure. *Nat Ecol Evol* **5**: 612–622. DOI: 10.1038/s41559-021-01388-5

Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV, and Luisi BF (2018) Multidrug efflux pumps: structure, function and regulation. *Nat Rev Microbiol* **16**: 523–539. DOI: 10.1038/s41579-018-0048-6

Durão P, Balbontín R, and Gordo I (2018) Evolutionary mechanisms shaping the maintenance of antibiotic resistance. *Trends Microbiol* **26**: 677–691. DOI: 10.1016/j.tim.2018.01.005

El Meouche I, and Dunlop MJ (2018) Heterogeneity in efflux pump expression predisposes antibiotic-resistant cells to mutation. *Science* **362**: 686–690. DOI: 10.1126/science.aap9346

Farrokhian N, Lopatkin AJ, and You L (2022) Predictive modeling of antibiotic resistance evolution: integrating molecular mechanisms with evolutionary dynamics. *Curr Opin Microbiol* **67**: 102148. DOI: 10.1016/j.mib.2022.102148

Fuzi M (2025) The fitness connection of antibiotic resistance. *Front Microbiol* **16**:1556656. DOI: 10.3389/fmicb.2025.1556656

Ghai I (2023) A barrier to entry: examining the bacterial outer membrane and antibiotic resistance. *Appl. Sci* **13**(7): 4238. 10.3390/app13074238

Gifford DR, MacLean RC, and Ramiro RS (2020) Fitness costs and benefits of antibiotic resistance in bacterial pathogens. *Curr Opin Microbiol* **57**: 90–96. DOI: 10.1016/j.mib.2020.08.002

Hall AR, MacLean RC and Gandon S (2011) Evolutionary consequences of resistance diagnostics for antibiotic resistance management. *Evol Appl* **4**: 290–302. DOI: 10.1111/j.1752-4571.2010.00159.x

Hancock REW, Haney EF and Gill EE (2016) The immunology of host defence peptides: beyond antimicrobial activity. *Nat Rev Immunol* **16**: 321–334. DOI: 10.1038/nri.2016.29

Hansen E, Woods RJ and Read AF (2020) How to use a chemotherapeutic agent when resistance to it threatens the patient. *PLoS Biol* **18**: e3000989. DOI: 10.1371/journal.pbio.3000989

Hare PJ, LaGree TJ, Byrd BA, DeMarco AM, and Mok WWK (2021) Single-cell technologies to study phenotypic heterogeneity and bacterial persisters. *Microorganisms*. **9**(11): 2277. DOI: 10.3390/microorganisms9112277

Helmy YA, Taha-Abdelaziz K, Hawwas HAE-H, Ghosh S, AlKafaas SS, Moawad MMM, Saied EM, Kassem II, and Mawad AMM (2023) Antimicrobial resistance and recent alternatives to antibiotics for the control of bacterial pathogens with an emphasis on foodborne pathogens. *Antibiotics*. **12**(2): 274. 10.3390/antibiotics12020274

Hemez C, Clarelli F, Palmer AC, Bleis C, Abel S, Chindelevitch L, Cohen T, Abel Zur and Wiesch P (2022) Mechanisms of antibiotic action shape the fitness landscapes of resistance mutations. *Comput Struct Biotechnol J* **20**: 4688-4703. DOI: 10.1016/j.csbj.2022.08.030.

Herren CM, and Baym M (2022) Decreased thermal niche breadth as a trade-off of antibiotic resistance. *ISME J* **16**: 1843–1852. DOI: 10.1038/s41396-022-01235-6m

Hinz A, Amado A, Kassen R, Bank C, and Wong A (2024) Unpredictability of the fitness effects of antimicrobial resistance mutations across environments in *Escherichia coli*. *Mol. Biol. Evol* **41**(5): msae086. DOI: 10.1093/molbev/msae086

Hooper DC, and Jacoby GA (2015) Mechanisms of drug resistance: quinolone resistance. *Ann N Y Acad Sci* **1354**: 12–31. DOI: 10.1111/nyas.12830

Jia B, Zhang Y, Zhou M, and Liu M (2022) Antisense RNA-based strategies for combating bacterial antibiotic resistance. *Front Microbiol* **13**: 862893. DOI: 10.3389/fmicb.2022.862893

Lässig M, Mustonen V, and Walczak AM (2017) Predicting evolution. *Nat Ecol Evol* **1**: 77. DOI: 10.1038/s41559-017-0077

Lázár V, Pal Singh G, Spohn R, Nagy I, Horváth B, Hrtyan M, ... and Pál C (2013) Bacterial evolution of antibiotic hypersensitivity. *Mol Syst Biol* **9**: 700. DOI: 10.1038/msb.2013.57

Levin-Reisman I, Ronin I, Gefen O, Braniss I, Shoresh N and Balaban NQ (2017) Antibiotic tolerance facilitates the evolution of resistance. *Science* **355**: 826–830. DOI: 10.1126/science.aaj2191

Lopatkin AJ, Bening SC, Manson AL, Stokes JM, Kohanski MA, Badran AH and Collins JJ (2021) Clinically relevant mutations in core metabolic genes confer antibiotic resistance. *Science* **371**: eaba0862. DOI: 10.1126/science.aba0862

Maeda T, and Furusawa C (2024) Laboratory evolution of antimicrobial resistance in bacteria to develop rational treatment strategies. *Antibiotics (Basel)* **13**(1):94. DOI: 10.3390/antibiotics13010094

Martinez JL and Rojo F (2011) Metabolic regulation of antibiotic resistance. *FEMS Microbiol Rev* **35**: 768–789. DOI: 10.1111/j.1574-6976.2010.00246.x

Melnyk AH, Wong A and Kassen R (2015) The fitness costs of antibiotic resistance mutations. *Evol Appl* **8**: 273–283. DOI: 10.1111/eva.12196

Muteeb G, Rehman MT, Shahwan M, and Aatif M (2023) Origin of Antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. *Pharmaceuticals* (Basel). **16**(11): 1615. DOI: 10.3390/ph16111615

Njenga R, Boele J, Öztürk Y, and Koch HG (2023) Coping with stress: How bacteria fine-tune protein synthesis and protein transport. *J Biol Chem* **299**(9): 105163. DOI: 10.1016/j.jbc.2023.105163

Pal A, and Andersson DI (2024) Bacteria can compensate the fitness costs of amplified resistance genes via a bypass mechanism. *Nat Commun* **15**: 2333. DOI: 10.1038/s41467-024-46571-7

Pal C, Bengtsson-Palme J, Kristiansson E and Larsson DGJ (2015) Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. *BMC Genomics* **16**: 964. DOI: 10.1186/s12864-015-2153-5

Partridge SR, Kwong SM, Firth N and Jensen SO (2018) Mobile genetic elements associated with antimicrobial resistance. *Clin Microbiol Rev* **31**: e00088-17. DOI: 10.1128/CMR.00088-17

Porse A, Schønning K, Munck C, and Sommer MOA (2018) Survival and evolution of a large multidrug resistance plasmid in new clinical bacterial hosts. *Mol Biol Evol* **35**: 286–298. DOI: 10.1093/molbev/msx273

San Millan A and MacLean RC (2017) Fitness costs of plasmids: a limit to plasmid transmission. *Microbiol Spectr* **5**: MTBP-0016-2017. DOI: 10.1128/microbiolspec.MTBP-0016-2017

Santamaria de Souza N, Cherrak Y, Andersen TB et al. (2025) Context-dependent change in the fitness effect of (in)organic phosphate antiporter glpT during Salmonella Typhimurium infection. *Nat Commun* **16**: 1912. DOI: 10.1038/s41467-025-56851-5

Stubbendieck RM, Vargas-Bautista C and Straight PD (2016) Bacterial communities: interactions to scale. *Front Microbiol* **7**: 1234. DOI: 10.3389/fmicb.2016.01234

Sun J, Deng Z and Yan A (2014) Bacterial multidrug efflux pumps: mechanisms, physiology and pharmacological exploitations. *Biochem Biophys Res Commun* **453**: 254–267. DOI: 10.1016/j.bbrc.2014.05.090

Sundqvist M, Geli P, Andersson DI, Sjölund-Karlsson M, Runehagen A, Cars H, Abelson-Storby K and Cars O (2010) Little evidence for reversibility of trimethoprim resistance after a reduction in trimethoprim use. *J Antimicrob Chemother* **65**: 350–360. DOI: 10.1093/jac/dkp387

Tu MM, Carfrae LA, Rachwalski, K. et al. (2025) Exploiting the fitness cost of metallo-β-lactamase expression can overcome antibiotic resistance in bacterial pathogens. *Nat Microbiol* **10**: 53–65. DOI: 10.1038/s41564-024-01883-8

Vega NM and Gore J (2014) Collective antibiotic resistance: mechanisms and implications. *Curr Opin Microbiol* **21**: 28–34. DOI: 10.1016/j.mib.2014.09.003

Vila-Sanjurjo A, Mallo N, Atkins JF, Elson JL, and Smith PM (2023) Our current understanding of the toxicity of altered mito-ribosomal fidelity during mitochondrial protein synthesis: What can it tell us about human disease? *Front Physiol* **14**: 1082953. DOI: 10.3389/fphys.2023.1082953

Vogwill T and MacLean RC (2015) The genetic basis of the fitness costs of antimicrobial resistance: a meta-analysis approach. *Evol Appl* **8**: 284–295. DOI: 0.1111/eva.12202