Available online **www.ijpras.com**

International Journal of Pharmaceutical Research & Allied Sciences, 2026, 15(4):141-151

https://doi.org/10.51847/ShaLknJG4i

Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

Antibiotic Dosing as an Evolutionary Intervention: A Pharmacokinetic–Pharmacodynamic Theory Linking Bacterial Killing, Resistant Subpopulation Amplification, and Post-Treatment Ecological Recovery

Grace Wanjiru[1]*, Thomas Mwangi[1], Samuel Adeyemi[2], Rajiv Menon[3]

[1]Department of Pharmaceutical Sciences, University of Nairobi, Nairobi, Kenya.
[2]Department of Clinical Pharmacy, University of Ibadan, Ibadan, Nigeria.
[3]Department of Pharmacoinformatics, Indian Institute of Technology Bombay, Mumbai, India.

***Email:** grace.wanjiru@uonbi.ac.ke*

ABSTRACT

Antibiotic dosing is routinely evaluated through drug exposure, bacterial suppression, and clinical response, whereas evolutionary consequences may emerge across different populations and timescales. A regimen can reduce the susceptible pathogen burden while enriching resistant survivors, altering commensal competition, or leaving ecological opportunities that persist after drug clearance. This original pharmacological theory proposes that antibiotic outcomes arise from the relative timing of five partially coupled processes: active exposure at microbial niches, bactericidal or growth-suppressive response, phenotypic survival and memory, heritable resistance selection, and community reassembly. Evidence from antimicrobial pharmacokinetics, experimental evolution, bacterial physiology, and gut-microbiome studies supports the relevance of these processes individually, but does not validate their proposed integration as a universal dosing rule. The theory differentiates absolute killing from changes in resistance frequency and distinguishes microbiome structural return from functional or resistome recovery. It predicts that regimens with similar summary pharmacokinetic–pharmacodynamic indices may produce different evolutionary outcomes when tissue exposure, survivor-state duration, population bottlenecks, or recovery conditions differ. Longitudinal, compartment-resolved experiments are needed before these predictions can guide clinical dosing.

Key words: *Antibiotic pharmacokinetics–pharmacodynamics, Bacterial population dynamics, Heteroresistance, Evolutionary selection, Phenotypic persistence, Microbiome recovery*

Received: 08 August 2026; **Revised:** 11 September 2026; **Accepted:** 16 September 2026

INTRODUCTION

Conventional antimicrobial pharmacokinetic–pharmacodynamic (PK–PD) analysis relates an administered regimen to exposure and antibacterial response. Interindividual pharmacokinetic variation and the choice of exposure index remain central to dose optimization [1]. Yet therapeutic response, within-pathogen evolution, and selection among untargeted commensals are distinct outcomes. Following brief antibiotic exposure, human gut bacteria may acquire and maintain resistance under comparatively low fitness costs [2]. Drug discontinuation therefore cannot serve as an automatic endpoint for evolutionary observation.

Definitions matter. Genetically resistant organisms can grow under concentrations inhibiting susceptible comparators; tolerant populations survive prolonged exposure without necessarily acquiring a raised minimum inhibitory concentration (MIC); and persistence denotes a phenotypically surviving subpopulation assessed under specified conditions [3]. The resulting trajectories cannot be recovered from an aggregate kill curve or a single

© 2026 The International Journal of Pharmaceutical Research and Allied Sciences (IJPRAS). **Open Access** - This article is under the **CC BY NC SA** license (https://creativecommons.org/licenses/by-nc-sa/4.0).

post-treatment susceptibility test alone. A decline in total viable burden provides limited information about which lineages remain or which ecological niches will be recolonized.

The question developed here is how the *timing* of microbial killing, phenotype switching, resistant-lineage expansion, and community recovery interacts with the shape and site of drug exposure. The contribution is a falsifiable, nonempirical temporal theory. Its central claim is conditional: identical summary exposure targets need not imply identical evolutionary or ecological outcomes when the relevant biological processes begin, end, or recover at different times. No new experiment, quantitative efficacy estimate, or clinically validated dosing algorithm is claimed.

Antibiotic dosing as a therapeutic and evolutionary problem

The therapeutic clock measures how promptly a regimen suppresses infection at the relevant site; the evolutionary clock follows which cells or lineages survive, reproduce, and establish. Neither begins at a uniform physiological state. In infected mice and tissue-mimicking systems, nutrient starvation substantially constrained antibiotic clearance of *Salmonella*, challenging routine attribution of poor killing to generalized stress or persister mechanisms [4]. The biological conditions governing target activity can therefore matter as much as nominal dose intensity.

Selection can also occur outside exposures designed to eradicate infection. Laboratory evolution under low antibiotic concentrations demonstrated sequential routes toward high-level resistance [5]. This does not specify an equivalent threshold for human infection, because local concentration histories, population sizes, and transmission opportunities differ. Under sustained bacteriostatic exposure, *Escherichia coli* populations developed small-colony-associated heteroresistance [6]. A population-average MIC or early decrease in total cells could conceal the coexistence of differently susceptible states.

Persistence introduces a further interval between exposure and evolution. Survival without immediate division permits later regrowth, but subsequent genetic resistance is contingent on the environment and available adaptive routes; persistence is not inherently a resistance mutation [7]. Regulation involving an alarmone–GTP switch supplies a mechanistically specific example of how a physiological state may govern persister formation in studied bacteria [8]. Such mechanisms justify a *phenotypic-memory clock* whose duration need not match either the drug half-life or a resistance allele's persistence. Distinguishing these intervals prevents therapeutic failure, surviving susceptible cells, and heritable selection from being assigned the same explanation.

Current evidence on antimicrobial PK–PD and bacterial population dynamics

An antibiotic concentration is both a pharmacological input and an environmental condition for selection. Models of metabolic fitness landscapes demonstrate that resistance-associated growth effects can change across antibiotic concentrations [9]. Metabolism further shapes susceptibility and resistance-related physiology, complicating the idea of one fixed exposure–kill relationship across nutrient conditions or infection sites [10]. The same measured concentration can therefore encounter cells with different growth, drug-target engagement, and recovery capacities.

Population evolution is not a direct reading of kill-curve slope. Experimental threshold-like selection has been shown to coexist with neutral drift and variable phenotypic resistance [11]. Single-cell lineage experiments additionally identify inherited, nonpermanent susceptibility variation, implying that some population responses contain information about recent cellular ancestry [12]. These mechanisms change the expected timing of survivor re-emergence even when acquired resistance alleles are absent.

Heteroresistance can depend on dynamically changing genetic dosage. Copy-number flexibility has been implicated in resistance to increasing beta-lactam pressure [13]. Comparative laboratory evolution of ESKAPE pathogens demonstrates that resistance accessibility differs across organisms and antimicrobial candidates [14]. Resistance-gene duplication and horizontal transfer indicate further routes by which genomic states and their distribution may change [15]. High-throughput genotype–phenotype testing reveals extensive mutation-specific effects that must be interpreted within their experimentally tested drug and genetic backgrounds [16]. These observations support multiple evolutionary routes, not a common rate of resistance amplification.

For temporal interpretation, three measurements must be distinguished: the total number of viable bacteria, the absolute number of resistant bacteria, and the resistant fraction among survivors. The fraction may rise because susceptible cells die more rapidly, even while resistant numbers fall. Resistant absolute abundance can subsequently rise through reproduction or immigration. A mechanistic account must therefore follow genotype-specific abundance and phenotype-specific survival over time, together with actual active exposure at the site of

interest. Standard PK indices summarize useful exposure features but do not encode this complete population history.

Unresolved relationships among bacterial killing, resistance selection, and recovery

After treatment, the surviving community is not necessarily a scaled-down version of its pretreatment state. Antibiotic perturbations modify gut-microbiome composition and function, with recovery varying by organism, exposure, and ecological setting [17]. In longitudinal tuberculosis-treatment and experimental microbiome contexts, resistant commensals contributed to community resilience under antibiotic disruption [18]. This apparent benefit within one ecological function does not erase the independent concern that resistance determinants may remain in commensal populations.

The category *survivor* is similarly heterogeneous. In a mouse gut model using exposure intended to approximate human cefepime pharmacokinetics, genetically susceptible *E. coli* survived in intestinal tissue; some isolates exhibited altered host-cell invasion or persistence-associated behavior [19]. Hence the absence of killing is not evidence, by itself, of resistant growth. In another mouse system, beta-lactamase-mediated ampicillin degradation by *Klebsiella oxytoca* enabled commensal outgrowth, while dietary context modified subsequent colonization resistance [20]. Exposure can be transformed by community members even when the administered regimen is unchanged.

Post-antibiotic recovery has its own determinants. Dietary intervention strongly influenced reassembly in a mouse comparison involving microbial transplantation [21]. Human antimicrobial use also has measurable effects on bystander gut microbial populations and resistome composition, extending selection beyond the intended pathogen [22]. Conversely, a Gram-negative-selective experimental antibiotic showed microbiome-sparing properties in studied preclinical systems [23]. These results indicate the importance of drug spectrum, commensal susceptibility, and available ecological resources without establishing a universal rank order of clinical regimens. Collateral microbiota injury can increase vulnerability to later colonization or infection [24]. This may happen after cultured target pathogens have diminished or circulating concentrations have declined. The key unresolved relationship is the ordering of three endpoints: disappearance of susceptible target organisms, establishment or suppression of resistant survivors, and return of community-mediated protection. **Table 1** discriminates the biological units and observations involved in these endpoints; a favorable result on one cannot be substituted for the others.

Table 1. Exposure-dependent antibacterial responses and their non-equivalent implications for selection and ecological persistence

Observational unit	PK–PD or biological event	Supportable interpretation	Critical unresolved distinction	Temporal-theory implication
Susceptible pathogen cells	Concentration- and physiology-dependent killing	Viable susceptible burden may decline during active exposure	Killing rate versus residual refugial survival	Track site exposure and susceptible absolute abundance together
Genetically resistant subpopulation	Differential growth, survival, or copy-number adaptation	Resistant fraction can change during treatment	Relative enrichment versus net resistant-cell expansion	Separate change in resistant frequency from change in resistant count
Phenotypic survivor within a genotype	Transient tolerance, persistence, lineage memory, or altered localization	Cells may survive despite susceptibility in standard testing	Reversible state and host refuge versus stable MIC elevation	Observe survivor state and susceptibility after regrowth
Drug-modifying commensal community	Antibiotic degradation and competitive release	Local bioactive concentration and commensal regrowth may change	Community protection versus persistence of resistance determinants	Follow lumen exposure and community function after dosing
Post-treatment microbial ecosystem	Resource-dependent succession and altered colonization resistance	Taxonomic return may accompany functional recovery or resistome change	Community structure versus colonization resistance versus resistome	Keep recovery outcomes distinct until measured

Table note. Entries describe published mechanism classes and bounded conceptual inferences; they are not pooled effect estimates or recommended therapeutic thresholds. The temporal-theory implications are proposed measurement requirements. PK–PD, pharmacokinetics–pharmacodynamics; MIC, minimum inhibitory concentration.

Theoretical challenges in connecting treatment exposure with evolutionary outcomes

Combination therapy adds an interaction clock: drug effects can depend on overlap, order, and bacterial physiology, so an identical single-agent exposure need not represent the same selective environment in a combination [25]. Population bottlenecks also alter which variants can survive chance loss and undergo selection [26]. A delayed resistant rebound could consequently reflect changing inoculum size or founder composition even if the dosage schedule itself is stable.

A second challenge concerns compartment identity. Plasma concentrations need not represent concentrations that a bacterium encounters. Dynamic positron emission tomography has revealed heterogeneous tissue exposure for tuberculosis drugs in the brain and lungs [27]. Such imaging describes drug distribution; it does not directly measure free active concentration adjacent to each bacterium, its survival, or heritable selection. Spatial localization and host-cell refuge therefore remain alternative explanations for apparent discrepancies between systemic exposure and culture response.

A third challenge is the state dependence of selection. Experimentally induced bioenergetic stress has been linked to resistance and persistence outcomes in studied *E. coli* systems [28]. Physiological adaptation may outlast a concentration pulse, while selection on newly viable descendants may occur later. A coherent account must distinguish the timing of (i) active drug arrival and removal; (ii) growth arrest, death, and nonheritable survival; (iii) reproductive selection; and (iv) ecological resource restoration. These intervals overlap but are not interchangeable.

Figure 1 shows two conceptual trajectories under the same nominal treatment window. Its contrasted pathways illustrate why viable-cell counts, resistant fractions, and protected niche survival cannot be read as an identical population curve. The curves are qualitative hypotheses, not patient-level estimates or extrapolated empirical time courses.

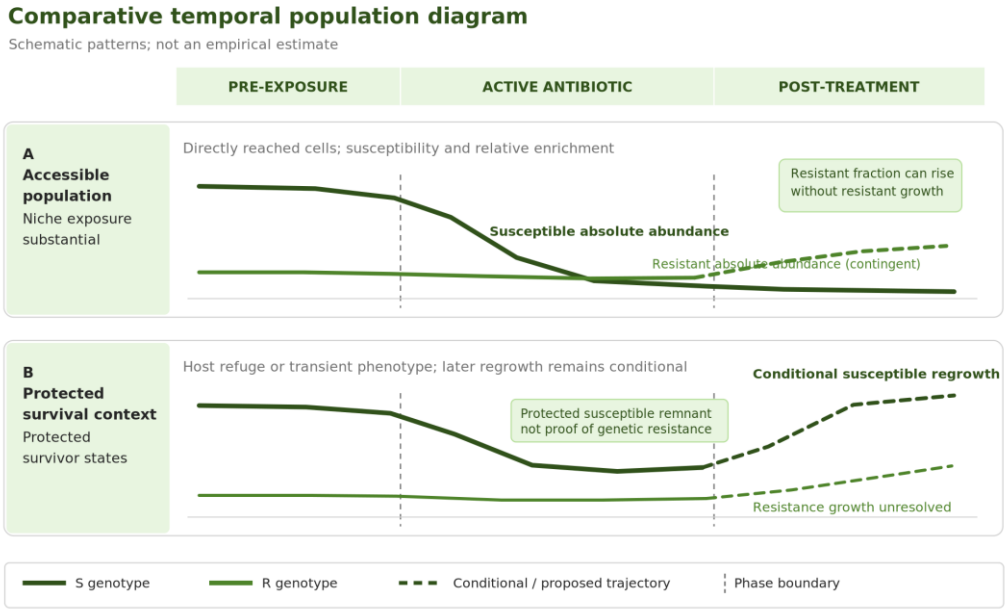


Figure 1. Bacterial population trajectories diverge when exposure, refuge, and regrowth clocks become asynchronous

***Figure 1** legend.* Left-to-right time runs from the pre-exposure baseline through dosing to post-treatment observation, separated by vertical phase boundaries. The upper lane schematizes an accessible population with pronounced susceptible suppression and a potentially changing resistant fraction; the lower lane depicts context-dependent persistence of susceptible survivors in spatial or physiological refuges, followed by regrowth. Solid paths indicate conceptual population trajectories consistent with documented mechanism classes; dashed segments mark contingent regrowth or enrichment hypotheses. No plotted height represents an experimentally observed count, MIC, dose, or rate. Resistant *fraction* is not equated with resistant *absolute count*. Outcome depends on genotype, niche exposure, physiological state, and ecological context. MIC, minimum inhibitory concentration; PK–PD, pharmacokinetics–pharmacodynamics.

Figure 1 *alt text.* Two stacked horizontal panels share three chronological phases: baseline, antibiotic exposure, and recovery. Each panel contains differentiated susceptible and resistant population paths drawn across the phase boundaries. In the upper panel the susceptible path falls during dosing, while the resistant path remains low and may subsequently change. In the lower panel an annotated sheltered susceptible remnant persists during dosing and can rebound after treatment; a separate resistant path stays uncertain. Vertical boundary lines and dashed recovery segments indicate that timing and outcomes are conditional, with no numerical scale.

Development of the proposed evolutionary PK–PD theory

The central theoretical unit is a *niche-specific exposure–population history*, rather than a dose or an isolated kill curve. For niche *i*, let $E_i(t)$ denote locally bioactive antibiotic exposure; $S_i(t)$ and $R_i(t)$ the absolute viable abundances of drug-susceptible and heritably resistant genotypes; and $M_i(t)$ their time-dependent phenotypic states, including dormancy, tolerance, and memory. Let $C_i(t)$ represent measurable community functions such as colonization resistance or antimicrobial degradation. These quantities refer to different biological and observational levels, and neither $M_i(t)$ nor $C_i(t)$ is inferred automatically from $S_i(t)$ or $R_i(t)$.

An exposure clock records when $E_i(t)$ becomes microbiologically relevant and when it falls; a response clock records genotype- and state-dependent killing and regrowth; a heredity clock records selective changes that persist after regrowth; and an ecological clock records the loss and restoration of community functions. Adaptation is constrained by drug mechanism and available evolutionary pathways: laboratory comparisons of antibiotics with limited observable resistance provide an important counterweight to assuming inevitable selection [29]. Consequently, the clocks need not all advance during every course of treatment.

The theory's distinctive prediction concerns *temporal mismatch*. Two regimens achieving the same summary exposure index could generate different trajectories when one leaves susceptible survivors active during community depletion, or when locally protected resistant lineages encounter nutrient release only after concentrations subside. Human-microbiome-derived experiments show that growth of introduced resistant clinical strains depends on multiple community interactions [30]. Their findings support ecological conditioning of invasion, while the specific timing-and-dosing counterfactual remains to be tested.

Three linked propositions follow. First, the interval between local antibiotic decline and resumption of growth will modify whether an initial resistant-frequency increase becomes sustained resistant abundance. Second, the persistence of phenotypic survivor states will alter later population composition only when survival permits subsequent reproduction or transfer. Third, microbiome recovery may reduce colonization opportunity while maintaining antibiotic-resistance carriage, producing divergent clinical and evolutionary endpoints. Eco-evolutionary treatment literature offers a basis for examining such feedback, although it does not establish the present temporal coupling as a validated intervention [31].

These propositions operate under explicit safeguards. Natural recovery, bacterial immigration, mutation supply, antibiotic inactivation, host immunity, and dose adherence can create similar curves by different causes. Direct model discrimination therefore requires simultaneously observed tissue or luminal exposure, genotype-resolved absolute burden, survivor phenotype, and longitudinal community function. **Figure 2** maps the proposed cross-timescale dependencies and indicates where observation ends and theoretical inference begins.

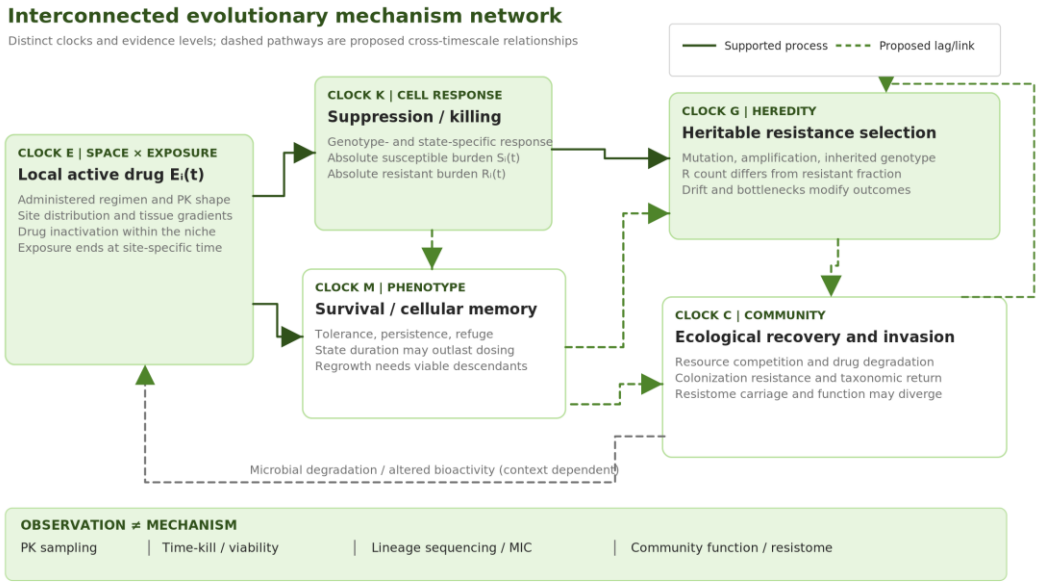


Figure 2 *legend.* The figure separates local active exposure, drug-target response, phenotypic survival, heritable lineage selection, and community succession into distinct but interacting domains. Solid connections denote mechanism classes supported by the approved literature; dashed arrows indicate this paper's unvalidated predictions about phase lags and cross-domain consequences. Local metabolism and compartment distribution moderate exposure; ecological degradation can feed back on bioactivity; resource-dependent succession moderates post-treatment expansion. Observation bands distinguish drug measurements, cell-level viability assays, genomic/lineage tracking, and microbiome-function measurements. The diagram asserts no shared quantitative rate across clocks and no validated clinical dosing rule. PK–PD, pharmacokinetics–pharmacodynamics; MIC, minimum inhibitory concentration.

Figure 2 *alt text.* A left-side antibiotic-exposure domain leads into separate cell-response and heritable-lineage domains in the center, alongside a lower community domain. An upper phenotypic-survival module connects conditionally to later regrowth; a middle heritable-selection module leads to resistant abundance, while community recovery and colonization resistance sit lower right. A feedback arrow returns from microbial drug degradation to the exposure domain. Solid arrows indicate supported process classes, dashed arrows proposed timing dependencies, and horizontal measurement bands identify distinct assays. Module grouping and connectors distinguish population, cellular, and ecosystem levels without relying on color.

Implications for antibiotic dosing and resistance interpretation

The temporal account changes which outcomes are inspected together when a regimen appears microbiologically successful. In an experimental mixed-species community, evolution of imipenem resistance in *Pseudomonas aeruginosa* was accompanied by competitive exclusion of a beta-lactam-degrading protective species [32]. Protection supplied by commensals is therefore contingent on the subsequent population history of the protected pathogen. A drug regimen may modify its own effective ecological exposure through feedback between resistance, interspecies competition, and local antibiotic degradation. Such feedback cannot be assumed in every infection or translated directly from an experimental community into patient-level dosing advice.

An additional delayed consequence follows from resource release. Following antibiotic perturbation, nutrient-enriched intestinal environments supported growth of vancomycin-resistant *Enterococcus* in experimental microbiome systems, while depleted inhibitory metabolites also shaped the available niche [33]. Resistant-colonizer expansion here involves a distinct event from a newly arising resistant mutation: an already-resistant lineage can increase when community resources become permissive. Its growth may occur after the dosing interval, at a time when conventional pathogen killing endpoints are no longer being sampled.

Interpretation of recovery requires independent measurements. A critical review found that proposed probiotic-mediated restoration of an antibiotic-perturbed microbiota lacked a demonstrated causal link between reported clinical benefits and microbiota protection or recovery [34]. Neither taxonomic similarity to baseline nor improvement in a single symptom establishes restoration of colonization resistance, clearance of resistance

determinants, or resolution of all ecological consequences. The exposure history, genotype-resolved bacterial burden, viable survivor states, and functional community state consequently require different observation schedules.

The practical implication is a conditional measurement strategy, pending validation, not a substitute dosing target. A study reporting improved target-pathogen suppression should state whether resistant absolute abundance declined, whether resistance frequency changed, and whether colonization resistance returned after treatment. For matched summary PK–PD targets, sampling should extend beyond the time of apparent microbiological cure whenever persistence, bystander selection, or ecological displacement is central to the research question. Treatment changes remain governed by established clinical evidence, toxicity constraints, patient characteristics, and site-specific PK–PD guidance.

Table 2 separates possible temporal orderings of exposure, population response, and community function. Its interpretive contrasts expose different mechanisms that could yield superficially similar short-term antibacterial outcomes.

Table 2. Temporally separated exposure, survivor, and community events produce distinct evolutionary interpretations

Temporal comparison unit	Observable trajectory	Mechanism compatible with evidence	Competing interpretation	Proposed discriminating observation
Active exposure versus susceptible killing	Local bioactive drug declines while susceptible viability remains low or later rises	Pharmacological suppression coupled to growth state and compartment access	Incomplete tissue exposure or immigration mimicking cellular tolerance	Concurrent niche concentration and viable genotype-resolved counts
Resistant fraction versus resistant abundance	Fraction rises during susceptible-cell loss	Differential survival or growth among extant genotypes	Denominator shrinkage without net resistant expansion	Absolute resistant count and allele identity at successive intervals
Drug removal versus survivor regrowth	Susceptible descendants return after measured exposure subsides	Phenotypic persistence, reversible memory, or sheltered survival	New resistance or renewed inoculation	Regrowth susceptibility, lineage tracking, and spatial localization
Commensal degradation versus pathogen suppression	Local antibiotic activity changes as drug-modifying species expand or disappear	Community-mediated protection or competitive exclusion	Ordinary pharmacokinetic clearance without microbial mediation	Bioactive luminal drug, degradation activity, and species-resolved abundance
Ecological recolonization versus resistome persistence	Community functions recover while resistance carriage remains detectable	Resource-dependent reassembly and differential lineage retention	Taxonomic resemblance mistaken for restored protection	Functional colonization assays, longitudinal resistome and absolute loads

Table note. The comparison units combine experimentally described mechanism classes with proposed tests; they do not assert a universal sequence, causal estimate, exposure threshold, or clinical prescription. PK–PD, pharmacokinetics–pharmacodynamics.

Falsifiable predictions and mechanism-discriminating research

A multiple-clocks account should be discarded or constrained when temporal separation fails to add predictive information beyond a simpler exposure–response explanation. First, use matched regimens with similar total exposure indices but different schedules, then measure site-specific active concentrations, viability, minimum inhibitory concentrations, and genotype frequencies before, during, and after treatment. If genotype-resolved absolute abundances and later community endpoints remain invariant across well-controlled exposure shapes, the proposed temporal-mismatch contribution weakens. Survival phenotypes must follow operational definitions that distinguish persistence or tolerance from heritable resistance [3].

Second, independently manipulate antibiotic degradation and community resources. Prior mouse work implicates both beta-lactam modification by commensals and diet-dependent recovery [20]. A factorial design could track local drug bioactivity, microbial enzyme activity, resistant and susceptible counts, and colonization resistance after withdrawal. If resource restoration alone explains delayed colonizer expansion without measurable changes

in drug activity or survivor history, an ecological-resource account would supersede the suggested exposure-coupling pathway in that context.

Third, change population bottleneck size while keeping antibiotic exposure approximately matched. Experimental evolution has established that bottlenecks and selection levels affect resistance trajectories [26]. Replicate lineages should be sequenced longitudinally and assessed for absolute burden, not just final resistance frequency. If founder sampling alone explains the variation, an additional phenotypic-memory mechanism need not be invoked. Conversely, repeated delayed regrowth within the same genotype after standardized bottlenecks would motivate closer study of reversible physiological states.

Fourth, separate plasma-matched dosing from niche-specific exposure. Dynamic imaging has demonstrated heterogeneous tissue distribution of tuberculosis drugs [27]; imaging must be paired with methods appropriate to estimating microbiologically relevant active exposure. Models should distinguish tissue delivery, protected cellular localization, and altered susceptibility. Failure of plasma summaries to predict site-level survival, coupled with successful prediction using local exposure histories, would support a compartment-specific interpretation without establishing the larger ecological theory.

Finally, introduce identical resistant lineages into communities characterized before and after antibiotic perturbation. Experiments with human-derived microbiomes already show interactions that condition growth of clinical resistant strains [30]. Measure time to establishment, available substrates, inhibitory metabolites, and community function while holding inoculum and bacterial genotype constant. If post-antibiotic community state does not alter establishment or its timing, the ecological-clock proposition would require narrowing. Across all tests, the primary endpoint is discrimination among mechanisms, with uncertainty intervals and prespecified comparator models, not merely correspondence between curves.

Discussion, competing theories, and future directions

A parsimonious PK-only account may be sufficient when measured local exposure consistently explains killing and regrowth. Nutrient-dependent physiological limitation provides a serious counterexample to attributing survival to an autonomous persister clock: *Salmonella* clearance work found limits to the contribution of stress and persisters under the studied conditions [4]. In such cases, the response timescale may be governed primarily by changes in substrate availability and bacterial growth. The multiple-clocks interpretation earns value only if it improves discrimination beyond this explanation.

Spatial refuge supplies another rival mechanism. Genetically susceptible *E. coli* survived antibiotic treatment in mammalian intestinal models without acquired resistance [19]. If poor accessibility or host association explains their persistence, adding a special inherited survival state may be unnecessary. Further, bioenergetic stress has been experimentally associated with resistance and persistence [28]; cellular metabolism can jointly alter kill susceptibility and the opportunity for adaptive change. Measured time lags alone cannot distinguish these possibilities. Compartment sampling, controlled physiological perturbations, and verified post-regrowth susceptibility are needed.

A selection-only model offers a third comparison: altered resistant frequency may result entirely from known growth or death differences plus demographic bottlenecks. The evolutionary theory adds a claim only where a distinct memory, local-exposure, or community-recovery variable improves prediction of subsequent absolute resistant load or establishment. This is consistent with eco-evolutionary treatment thinking, which recognizes feedback between ecological conditions and resistance evolution but does not validate any universal temporal dosing prescription [31]. Falsification therefore requires models fitted prospectively to the same longitudinal compartments, with withheld trajectories and explicit assessments of parameter identifiability.

Future work should prioritize stratified mechanistic studies before clinical intervention trials: single-cell measurements of phenotypic survival, genotype-aware absolute counts, metabolite and drug-activity assays, and repeated ecological-function testing. A useful study reports when each signal becomes observable, what constitutes baseline recovery, and whether alternative causal pathways remain consistent with the data. A theory that cannot distinguish reversible survival from mutation or colonizer immigration should be revised rather than presented as mechanistic confirmation.

Limitations and theoretical applicability boundaries

This theory integrates heterogeneous evidence from in-vitro evolution, animal infection models, imaging, human microbiome observations, and reviews. It was not validated against a unified longitudinal dataset. The resulting temporal propositions may apply differently to bactericidal and bacteriostatic drugs, slow- and fast-growing

organisms, polymicrobial niches, intracellular infection, and different host immune states. Single-cell lineage memory is context-dependent and should not be generalized as heritable resistance [12]. The conceptual variables remain incompletely observable in routine care.

Bystander resistome measurements reflect community-level genetic potential and carriage, not necessarily mutation arising in the treated pathogen. Findings from adult gut microbiome studies do not directly quantify selection in lung, urinary, or bloodstream infection compartments [22]. In addition, metabolite shifts and taxonomic recovery can occur without proven restoration of colonization resistance or clinical benefit. The evidence on antibiotic-perturbed microbiota and probiotics illustrates this causal-inference limitation [34].

Plasma PK indices may inadequately describe tissue microenvironments, but imaging distributions also cannot establish bacterium-adjacent free-drug activity [27]. Unobserved adherence, immigration, host immunity, mutation supply, and ecological baseline can generate competing explanations for the same measured trajectory. A general clinical optimization rule, resistance-prevention threshold, or recommended antibiotic schedule cannot be deduced from these theoretical relationships. Applications must remain explicitly drug-, organism-, compartment-, assay-, and population-specific until prospectively tested.

CONCLUSION

Antibiotic dosing can influence evolutionary outcomes through the relative timing of local active exposure, bacterial killing, reversible survival, resistant-lineage reproduction, and ecological reassembly. These processes can separate after a regimen achieves its immediate therapeutic objective. The theory's discriminating contribution is the prediction that similar aggregate PK–PD exposures may produce different later resistant burdens or colonization outcomes because physiological, population, and community clocks are misaligned. The crucial tests will measure local exposure alongside absolute genotype-specific counts, survivor identity, and functional microbiome recovery, and compare those results with simpler pharmacological, demographic, and ecological explanations. Until those tests succeed, temporal coupling remains a bounded scientific hypothesis, not clinical dosing guidance.

ACKNOWLEDGMENTS: None

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

REFERENCES

1. Rawson TM, Wilson RC, O'Hare D, Herrero P, Kambugu A, Lamorde M, et al. Optimizing antimicrobial use: Challenges, advances and opportunities. Nat Rev Microbiol. 2021;19(12):747-58. doi:10.1038/s41579-021-00578-9
2. Yaffe E, Dethlefsen L, Patankar AV, Gui C, Holmes S, Relman DA. Brief antibiotic use drives human gut bacteria towards low-cost resistance. Nature. 2025;641(8061):182-91. doi:10.1038/s41586-025-08781-x
3. Balaban NQ, Helaine S, Lewis K, Ackermann M, Aldridge B, Andersson DI, et al. Definitions and guidelines for research on antibiotic persistence. Nat Rev Microbiol. 2019;17(7):441-8. doi:10.1038/s41579-019-0196-3
4. Fanous J, Claudi B, Tripathi V, Li J, Goormaghtigh F, Bumann D. Limited impact of Salmonella stress and persisters on antibiotic clearance. Nature. 2025;639(8053):181-9. doi:10.1038/s41586-024-08506-6
5. Wistrand-Yuen E, Knopp M, Hjort K, Koskiniemi S, Berg OG, Andersson DI. Evolution of high-level resistance during low-level antibiotic exposure. Nat Commun. 2018;9(1):1599. doi:10.1038/s41467-018-04059-1
6. Gil-Gil T, Berryhill BA, Manuel JA, Smith AP, McCall IC, Baquero F, et al. The evolution of heteroresistance via small colony variants in Escherichia coli following long term exposure to bacteriostatic antibiotics. Nat Commun. 2024;15(1):7936. doi:10.1038/s41467-024-52166-z
7. Bakkeren E, Diard M, Hardt WD. Evolutionary causes and consequences of bacterial antibiotic persistence. Nat Rev Microbiol. 2020;18(9):479-90. doi:10.1038/s41579-020-0378-z

8. Fung DK, Barra JT, Yang J, Schroeder JW, She F, Young M, et al. A shared alarmone–GTP switch controls persister formation in bacteria. Nat Microbiol. 2025;10(7):1617-29. doi:10.1038/s41564-025-02015-6
9. Pinheiro F, Warsi O, Andersson DI, Lässig M. Metabolic fitness landscapes predict the evolution of antibiotic resistance. Nat Ecol Evol. 2021;5(5):677-87. doi:10.1038/s41559-021-01397-0
10. Ahmad M, Aduru SV, Smith RP, Zhao Z, Lopatkin AJ. The role of bacterial metabolism in antimicrobial resistance. Nat Rev Microbiol. 2025;23(7):439-54. doi:10.1038/s41579-025-01155-0
11. Erdoğan AN, Dasmeh P, Socha RD, Chen JZ, Life BE, Jun R, et al. Neutral drift upon threshold-like selection promotes variation in antibiotic resistance phenotype. Nat Commun. 2024;15(1):10813. doi:10.1038/s41467-024-55012-4
12. Stine W, Akiyama T, Weiss D, Kim M. Lineage-dependent variations in single-cell antibiotic susceptibility reveal the selective inheritance of phenotypic resistance in bacteria. Nat Commun. 2025;16(1):4655. doi:10.1038/s41467-025-59807-x
13. Choby JE, Ozturk T, Abbott CN, Nnabuife C, Colquhoun JM, Satola SW, et al. Copy number flexibility facilitates heteroresistance to increasing antibiotic pressure and threatens the beta-lactam pipeline. Nat Commun. 2025;16(1):5721. doi:10.1038/s41467-025-60828-9
14. Daruka L, Czikkely MS, Szili P, Farkas Z, Balogh D, Grézal G, et al. ESKAPE pathogens rapidly develop resistance against antibiotics in development in vitro. Nat Microbiol. 2025;10(2):313-31. doi:10.1038/s41564-024-01891-8
15. Maddamsetti R, Yao Y, Wang T, Gao J, Huang VT, Hamrick GS, et al. Duplicated antibiotic resistance genes reveal ongoing selection and horizontal gene transfer in bacteria. Nat Commun. 2024;15(1):1449. doi:10.1038/s41467-024-45638-9
16. Jago MJ, Soley JK, Denisov S, Walsh CJ, Gifford DR, Howden BP, et al. High-throughput method characterizes hundreds of previously unknown antibiotic resistance mutations. Nat Commun. 2025;16(1):780. doi:10.1038/s41467-025-56050-2
17. Fishbein SRS, Mahmud B, Dantas G. Antibiotic perturbations to the gut microbiome. Nat Rev Microbiol. 2023;21(12):772-88. doi:10.1038/s41579-023-00933-y
18. Bhattarai SK, Du M, Zeamer AL, Morzfeld BM, Kellogg TD, Firat K, et al. Commensal antimicrobial resistance mediates microbiome resilience to antibiotic disruption. Sci Transl Med. 2024;16(730):eadi9711. doi:10.1126/scitranslmed.adi9711
19. Rodrigues M, Sabaeifard P, Yildiz MS, Lyon A, Coughlin L, Ahmed S, et al. Susceptible bacteria can survive antibiotic treatment in the mammalian gastrointestinal tract without evolving resistance. Cell Host Microbe. 2024;32(3):396-410.e6. doi:10.1016/j.chom.2024.01.012
20. Almási ÉdH, Eisenhard L, Osbelt L, Lesker TR, Vetter AC, Knischewski N, et al. Klebsiella oxytoca facilitates microbiome recovery via antibiotic degradation and restores colonization resistance in a diet-dependent manner. Nat Commun. 2025;16(1):551. doi:10.1038/s41467-024-55800-y
21. Kennedy MS, Freiburger A, Cooper M, Beilsmith K, St George ML, Kalski M, et al. Diet outperforms microbial transplant to drive microbiome recovery in mice. Nature. 2025;642(8068):747-55. doi:10.1038/s41586-025-08937-9
22. Cunningham-Oakes E, Price V, Mphasa M, Mallewa J, Darby AC, Feasey NA, et al. Quantifying the bystander effect of antimicrobial use on the gut microbiome and resistome in Malawian adults. Nat Commun. 2026;17(1):954. doi:10.1038/s41467-025-67677-6
23. Muñoz KA, Ulrich RJ, Vasan AK, Sinclair M, Wen PC, Holmes JR, et al. A Gram-negative-selective antibiotic that spares the gut microbiome. Nature. 2024;630(8016):429-36. doi:10.1038/s41586-024-07502-0
24. de Nies L, Kobras CM, Stracy M. Antibiotic-induced collateral damage to the microbiota and associated infections. Nat Rev Microbiol. 2023;21(12):789-804. doi:10.1038/s41579-023-00936-9
25. Roemhild R, Bollenbach T, Andersson DI. The physiology and genetics of bacterial responses to antibiotic combinations. Nat Rev Microbiol. 2022;20(8):478-90. doi:10.1038/s41579-022-00700-5
26. Mahrt N, Tietze A, Künzel S, Franzenburg S, Barbosa C, Jansen G, et al. Bottleneck size and selection level reproducibly impact evolution of antibiotic resistance. Nat Ecol Evol. 2021;5(9):1233-42. doi:10.1038/s41559-021-01511-2
27. Chen X, Arun B, Nino-Meza OJ, Sarhan MO, Singh M, Jeon B, et al. Dynamic PET reveals compartmentalized brain and lung tissue antibiotic exposures of tuberculosis drugs. Nat Commun. 2024;15(1):6657. doi:10.1038/s41467-024-50989-4

28. Li B, Srivastava S, Shaikh M, Mereddy G, Garcia MR, Chiles EN, et al. Bioenergetic stress potentiates antimicrobial resistance and persistence. Nat Commun. 2025;16(1):5111. doi:10.1038/s41467-025-60302-6
29. Maharramov E, Czikkely MS, Szili P, Farkas Z, Grézal G, Daruka L, et al. Exploring the principles behind antibiotics with limited resistance. Nat Commun. 2025;16(1):1842. doi:10.1038/s41467-025-56934-3
30. Leon-Sampedro R, Boumasmoud M, Reichlin M, Pfrunder-Cardozo KR, Noll N, Egli A, et al. Multi-layered ecological interactions determine growth of clinical antibiotic-resistant strains within human microbiomes. Nat Commun. 2025;16(1):9733. doi:10.1038/s41467-025-64714-2
31. Sanz-García F, Gil-Gil T, Laborda P, Blanco P, Ochoa-Sánchez LE, Baquero F, et al. Translating eco-evolutionary biology into therapy to tackle antibiotic resistance. Nat Rev Microbiol. 2023;21(10):671-85. doi:10.1038/s41579-023-00902-5
32. Quinn AM, Bottery MJ, Thompson H, Friman VP. Resistance evolution can disrupt antibiotic exposure protection through competitive exclusion of the protective species. ISME J. 2022;16(10):2433-47. doi:10.1038/s41396-022-01285-w
33. King OG, Yip AYG, Horrocks V, Miguéns Blanco J, Marchesi JR, Mullish BH, et al. Vancomycin-resistant enterococci utilise antibiotic-enriched nutrients for intestinal colonisation. Nat Commun. 2025;16(1):6376. doi:10.1038/s41467-025-61731-z
34. Szajewska H, Scott KP, de Meij T, Forslund-Startceva SK, Knight R, Koren O, et al. Antibiotic-perturbed microbiota and the role of probiotics. Nat Rev Gastroenterol Hepatol. 2025;22(3):155-72. doi:10.1038/s41575-024-01023-x