




REVIEW

Antibiotic chemotherapy against heterogeneous pathogen populations in complex host tissues [version 1; peer review: 2 approved]

Dirk Bumann , Joseph Fanous, Jiagui Li, Frédéric Goormaghtigh

Research Area Infection Biology, Biozentrum, University of Basel, Basel, CH-4056, Switzerland

v1 First published: 21 Oct 2019, 8(F1000 Faculty Rev):1781 (<https://doi.org/10.12688/f1000research.19441.1>)

Latest published: 21 Oct 2019, 8(F1000 Faculty Rev):1781 (<https://doi.org/10.12688/f1000research.19441.1>)

Abstract

Antibiotic chemotherapy effectively cures many infections caused by susceptible bacterial pathogens. However, in some cases, even extended treatment duration does not completely eradicate the pathogenic bacteria from host tissues. A common model for underlying mechanisms assumes the stochastic formation of bacterial persisters similar to observations in laboratory cultures. However, alternative explanations related to the complexity of infected host tissues could also be relevant. We discuss several of these aspects and emphasize the need for integrated analysis as a basis for new control strategies.

Keywords

Antibiotics, Persistence, Heterogeneity

Open Peer Review

Reviewer Status  

	Invited Reviewers	
	1	2
version 1 published 21 Oct 2019		

F1000 Faculty Reviews are written by members of the prestigious F1000 Faculty. They are commissioned and are peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

- 1 **Ursula Theuretzbacher**, Center for Anti-Infective Agents (CEFAIA), Vienna, Austria
- 2 **Veronique Dartois**, Rutgers University, Newark, USA

Any comments on the article can be found at the end of the article.

Corresponding author: Dirk Bumann (dirk.bumann@unibas.ch)

Author roles: **Bumann D:** Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Fanous J:** Writing – Review & Editing; **Li J:** Writing – Review & Editing; **Goormaghtigh F:** Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by Swiss National Foundation grants 310030_156818 and 310030_18231
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2019 Bumann D *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Bumann D, Fanous J, Li J and Goormaghtigh F. **Antibiotic chemotherapy against heterogeneous pathogen populations in complex host tissues [version 1; peer review: 2 approved]** F1000Research 2019, 8(F1000 Faculty Rev):1781 (<https://doi.org/10.12688/f1000research.19441.1>)

First published: 21 Oct 2019, 8(F1000 Faculty Rev):1781 (<https://doi.org/10.12688/f1000research.19441.1>)

Antibiotics have been saving the lives of millions of people. For most bacterial pathogens, short-term treatments of a few days effectively cure infections and prevent relapses^{1,2}. However, in some cases such as tuberculosis and other chronic infections such as deep-seated abscesses with *Staphylococcus aureus*, severe typhoid fever, or polymicrobial infections of patients with cystic fibrosis, extended treatments over months and even years can fail to completely eradicate the pathogens from tissues, posing a risk for relapse (Figure 1A). Many treatment failures are due to inheritable antibiotic resistance. However, surprisingly, treatment failures also occur when the pathogen retains full susceptibility to the antibiotics of choice in laboratory tests. There is an urgent medical need to improve the efficacy and shorten the treatments for these patients on the basis of a detailed mechanistic understanding of the problem. Several different factors influence antibiotic activities against pathogenic bacteria in host tissues. Most research groups active in the field focus on one particular factor: the stochastic variation of pathogen cells. However, other factors could be at least as important. In this review, we discuss some of these aspects and stress the need for an integrated analysis (Figure 1B).

Stochastic variation of bacterial properties in laboratory cultures

Bacterial cultures show heterogeneous properties even under completely homogeneous laboratory conditions indicating important endogenous stochastic variation within bacterial cells³⁻⁵. Exposure of bacterial cultures to lethal concentrations of bactericidal antibiotics rapidly kills most bacterial cells, but a small fraction of cells can survive for extended periods. It is possible that similar processes occur also in infected host tissues where they could contribute to incomplete eradication under antimicrobial chemotherapy. Because this phenomenon is readily observable *in vitro*, it has attracted the attention of a large number of research groups.

In many cases, the surviving small subset of bacteria represents non-growing remnant cells of a previous stationary culture that have an extended lag phase⁶. Such persisters are thus one particular instance of the widely characterized extensive heterogeneity of stationary-phase cultures⁷. A variety of other stress conditions also lead to increased antibiotic tolerance, including low ATP levels⁸, over-expression of toxins or unrelated proteins⁹, translation arrest^{10,11}, oxidative stress¹², and pre-exposure of cells to sub-MIC levels of bactericidal antibiotics¹³. Importantly, even growing bacteria show widely heterogeneous kill rates indicating that dormancy is not absolutely required for survival during antibiotic exposure¹⁴⁻¹⁷, implying alternative mechanisms leading to heterogeneous killing rates in clonal populations. These include asymmetric cell division^{4,18,19} with uneven partitioning of efflux pumps among daughter cells²⁰, heterogeneous expression of prodrug-activating enzymes¹⁴, transient gene amplifications²¹, and heterogeneous induction of specific stress responses¹⁷.

Although endogenous stochastic variation in bacteria often is assumed to play a major role in impairing antibiotic efficacy, empirical evidence is surprisingly scarce in infected tissues²². Some clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli* from antibiotic-treated patients showed increased persister frequencies^{23,24}, but this could also reflect fitness advantages of persisters under hostile host conditions or phage attacks. One argument against a general clinical relevance of persisters is the effectiveness of short-term antibiotic chemotherapy against many pathogens, which readily form refractory persisters in laboratory cultures^{1,2}. Persisters thus might arise in patients but the host immune system seems to be capable of eradicating them quickly²². Moreover, bacteriostatic antibiotics, which cause population-wide growth arrest in bacteria and make them tolerant against other antibiotics, are as effective as bactericidal antibiotics for the treatment of most infectious diseases²⁵. However, the distinction between

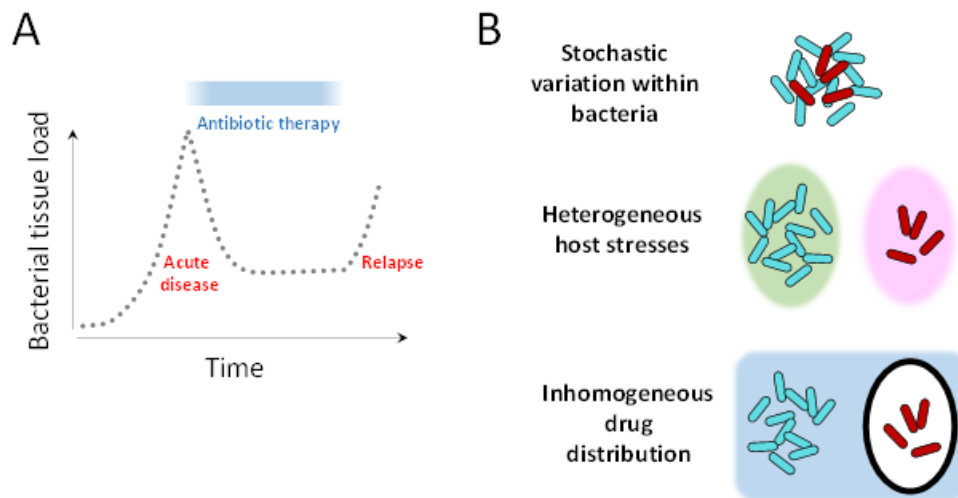


Figure 1. Treatment failures and potential causes. (A) Incomplete eradication of bacterial pathogens during extended antimicrobial chemotherapy causes a risk for relapses after termination of therapy. (B) Possible mechanisms that enable a bacterial subset (red) to survive during treatment while the rest of the bacterial population (blue) is successfully eradicated.

bacteriostatic and bactericidal antibiotics is not absolute, as many “bacteriostatic” drugs can kill bacteria at higher exposure levels or during extended exposure times or both²⁵. In addition, translation arrest by bacteriostatic antibiotics might impair survival in host tissues due to an inability to produce essential virulence factors or stress defenses. Finally, non-growing persisters can also occur in biofilms where they could escape clearance by host phagocytes²⁶.

Although the general relevance of bacterial persisters in infectious diseases remains unclear²⁷, they might be crucial for infections with frequent relapses even after extended treatment durations^{28,29}. However, other much less studied mechanisms could also contribute to infection relapse.

Pathogen physiology in host tissues

Clinical microbiology relies largely on standard *in vitro* assays in rich media to assess antibiotic susceptibility of pathogens. The results often are predictive of therapeutic efficacy, but pathogen physiology is significantly different under assay conditions compared with infected host tissues³⁰. Genetic screens in diverse pathogens typically have revealed many hundreds of virulence genes that are specifically required in host tissues but not in rich broth cultures, indicating large-scale relevant functional differences. Several parameters that differ between tissues and broth cultures also have a major impact on antibiotic activities. This includes³¹ oxygen tension³², carbon dioxide tension^{33,34}, metabolite concentrations³⁵, pH^{36,37}, and antimicrobial effector molecules of the host immune system such as cationic antimicrobial peptides (CAMPs)³⁸ and nitric oxide³⁹. Limited nutrient supply and stress conditions can result in slow pathogen proliferation, which strongly affects the activity of most antibiotics^{40–42}. Finally, pathogens can also adapt to the antibiotic exposure⁴³ and this adaptation might be more successful when antibiotics gradually penetrate into the infection site, compared with abrupt exposure in standard *in vitro* assays. All of these effects might lead to poor antibiotic efficacy, requiring extended treatment times.

Pathogen heterogeneity in host tissues

As an additional complexity, pathogens show significantly increased single-cell heterogeneity in tissues and body fluids of human patients and infected animals, compared with homogeneous laboratory cultures^{3,44–46}. This includes wide variation in bacterial growth rates, aggregation state, drug-efflux pumps, metabolism, and stress responses. All of these parameters influence the activity of antimicrobials, and it is possible that pathogen subsets with favorable properties tolerate antimicrobial exposure much better than their conspecifics, making eradication more difficult. Host-induced pathogen heterogeneity can reflect inhomogeneous host microenvironments triggering differences in bacterial physiology^{45–47} but also host-induced pathogen activities that change local microenvironments⁴⁸.

An astonishing early finding was the differential recovery of *Mycobacterium tuberculosis* from open and closed (that is,

no connection to airways) cavities in the lung of patients with tuberculosis⁴⁹. Whereas drug-resistant *M. tuberculosis* recovered from open cavities formed visible colonies on plates within a few weeks, *M. tuberculosis* from closed cavities of the same patients appeared only after a lag of many months. Strikingly, these colonies showed full drug susceptibility, suggesting limited selection for resistance development despite extended antimicrobial treatment. Sputum of patients with tuberculosis also contains mycobacteria with a wide range of cultivation phenotypes⁵⁰. A recent example that directly reveals heterogeneous bacteria physiology in tissues comes from *Salmonella* mouse infection models. *Salmonella* shows local and transient adaptations to divergent nutrient supply and disparate antimicrobial host attacks with reactive oxygen and nitrogen species⁵¹. The resulting heterogeneity in *Salmonella* growth rates has a major impact on *Salmonella* eradication^{52–54}.

Drug concentration at the site of infection

Antimicrobials must reach bacterial cells to execute their bactericidal/bacteriostatic activities. Bacterial subsets hiding in tissue microenvironments with poor drug penetration might delay complete eradication during antimicrobial chemotherapy. Indeed, host anatomy and biochemistry might provide physical or chemical barriers (or both) for drug penetration. This includes the blood–brain barrier, bones, and serum proteins that bind antimicrobials, thereby decreasing their free concentration. Host inflammation increases tissue heterogeneity by altering endothelial permeability and the formation of lesions and abscesses. On the other hand, certain drugs such as fluoroquinolones, azithromycin, and bedaquiline accumulate in host phagocytes^{55–57}, which might lead to elevated drug concentrations around the bacteria. Antimicrobial availability at the site of infection depends on the intestinal absorption of orally administered drugs, the distribution to the infected tissue, metabolism, and excretion. All of these processes depend on the physicochemical properties of the drug as well as the physiology of the patient, which might vary during bacterial infection. Drug penetration into accessible tissues can be measured by using microdialysis⁵⁸, but analysis of body fluids such as serum, cerebrospinal fluid, tracheal secretions, or urine is more common. It is unclear how representative these values are for drug availability around the bacterial cells during infection. Indeed, novel methods such as matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging demonstrate remarkable differences in local drug concentrations in lesions compared with surrounding lung tissue in tuberculosis patients and animals infected with *M. tuberculosis*^{59,60}. The emerging data suggest that inhomogeneous drug exposure could be a crucial factor for difficulties in eradicating *M. tuberculosis*. Unfortunately, experimental data are lacking for most other infectious diseases.

Conclusions

Antibiotic chemotherapy of most bacterial infections is highly effective if the causative pathogen is susceptible to the antibiotic of choice. However, some infections require extended

treatments to prevent relapses. Antibiotic-tolerant bacterial subsets (“persisters”) as observed in laboratory cultures might contribute to this problem. Alternatively, the host tissue environment could be decisive by providing inhomogeneous stress conditions and limiting drug distribution. To clarify these issues, we need more integrated *in vivo* research exploiting

recent single-cell approaches and complementary techniques such as mass spectrometry imaging and three-dimensional high-resolution whole-organ microscopy^{61,62}. A better understanding of the real problems impairing the chemotherapy of such infections is critically important to devise novel strategies for more effective and rapid treatments.

References



- Dawson-Hahn EE, Mickan S, Onakpoya I, *et al.*: **Short-course versus long-course oral antibiotic treatment for infections treated in outpatient settings: a review of systematic reviews.** *Fam Pract.* 2017; **34**(5): 511–9. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hanretty AM, Gallagher JC: **Shortened Courses of Antibiotics for Bacterial Infections: A Systematic Review of Randomized Controlled Trials.** *Pharmacotherapy.* 2018; **38**(6): 674–87. [PubMed Abstract](#) | [Publisher Full Text](#)
- Davis KM, Isberg RR: **Defining heterogeneity within bacterial populations via single cell approaches.** *Bioessays.* 2016; **38**(8): 782–90. [PubMed Abstract](#) | [Publisher Full Text](#)
- Aldridge BB, Fernandez-Suarez M, Heller D, *et al.*: **Asymmetry and aging of mycobacterial cells lead to variable growth and antibiotic susceptibility.** *Science.* 2012; **335**(6064): 100–4. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Santi I, Dhar N, Bousbaine D, *et al.*: **Single-cell dynamics of the chromosome replication and cell division cycles in mycobacteria.** *Nat Commun.* 2013; **4**: 2470. [PubMed Abstract](#) | [Publisher Full Text](#)
- Balaban NQ, Helaine S, Lewis K, *et al.*: **Definitions and guidelines for research on antibiotic persistence.** *Nat Rev Microbiol.* 2019; **17**(7): 441–8. [PubMed Abstract](#) | [Publisher Full Text](#)
- Ryall B, Eydallin G, Ferenci T: **Culture history and population heterogeneity as determinants of bacterial adaptation: the adaptomics of a single environmental transition.** *Microbiol Mol Biol Rev.* 2012; **76**(3): 597–625. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Zalis EA, Nuxoll AS, Manuse S, *et al.*: **Stochastic Variation in Expression of the Tricarboxylic Acid Cycle Produces Persister Cells.** *MBio.* 2019; **10**(5): pii: e01930-19. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Vazquez-Laslop N, Lee H, Neyfakh AA: **Increased persistence in *Escherichia coli* caused by controlled expression of toxins or other unrelated proteins.** *J Bacteriol.* 2006; **188**(10): 3494–7. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kwan BW, Valenta JA, Benedik MJ, *et al.*: **Arrested protein synthesis increases persister-like cell formation.** *Antimicrob Agents Chemother.* 2013; **57**(3): 1468–73. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Amato SM, Orman MA, Brynildsen MP: **Metabolic control of persister formation in *Escherichia coli*.** *Mol Cell.* 2013; **50**(4): 475–87. [PubMed Abstract](#) | [Publisher Full Text](#)
- Wu Y, Vulić M, Keren I, *et al.*: **Role of oxidative stress in persister tolerance.** *Antimicrob Agents Chemother.* 2012; **56**(9): 4922–6. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Dörr T, Lewis K, Vulić M: **SOS response induces persistence to fluoroquinolones in *Escherichia coli*.** *PLoS Genet.* 2009; **5**(12): e1000760. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wakamoto Y, Dhar N, Chait R, *et al.*: **Dynamic persistence of antibiotic-stressed mycobacteria.** *Science.* 2013; **339**(6115): 91–5. [PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
- Orman MA, Brynildsen MP: **Dormancy is not necessary or sufficient for bacterial persistence.** *Antimicrob Agents Chemother.* 2013; **57**(7): 3230–9. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bhaskar A, De Piano C, Gelman E, *et al.*: **Elucidating the role of (p)ppGpp in mycobacterial persistence against antibiotics.** *IUBMB Life.* 2018; **70**(9): 836–44. [PubMed Abstract](#) | [Publisher Full Text](#)
- Goormaghtigh F, Van Melderen L: **Single-cell imaging and characterization of *Escherichia coli* persister cells to ofloxacin in exponential cultures.** *Sci Adv.* 2019; **5**(6): eaav9462. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Shan Y, Brown Gandt A, Rowe SE, *et al.*: **ATP-Dependent Persister Formation in *Escherichia coli*.** *MBio.* 2017; **8**(1): pii: e02267-16. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Rego EH, Audette RE, Rubin EJ: **Deletion of a mycobacterial divisome factor collapses single-cell phenotypic heterogeneity.** *Nature.* 2017; **546**(7656): 153–7. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Bergmiller T, Andersson AMC, Tomasek K, *et al.*: **Biased partitioning of the multidrug efflux pump AcrAB-ToIC underlies long-lived phenotypic heterogeneity.** *Science.* 2017; **356**(6335): 311–5. [PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
- Nicoloff H, Hjort K, Levin BR, *et al.*: **The high prevalence of antibiotic heteroresistance in pathogenic bacteria is mainly caused by gene amplification.** *Nat Microbiol.* 2019; **4**(3): 504–14. [PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
- Levin BR, Baquero F, Ankomah PP, *et al.*: **Phagocytes, Antibiotics, and Self-Limiting Bacterial Infections.** *Trends Microbiol.* 2017; **25**(11): 878–92. [PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
- Mulcahy LR, Burns JL, Lory S, *et al.*: **Emergence of *Pseudomonas aeruginosa* strains producing high levels of persister cells in patients with cystic fibrosis.** *J Bacteriol.* 2010; **192**(23): 6191–9. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Schumacher MA, Balani P, Min J, *et al.*: **HipBA-promoter structures reveal the basis of heritable multidrug tolerance.** *Nature.* 2015; **524**(7563): 59–64. [PubMed Abstract](#) | [Publisher Full Text](#)
- Nemeth J, Oesch G, Kuster SP: **Bacteriostatic versus bactericidal antibiotics for patients with serious bacterial infections: systematic review and meta-analysis.** *J Antimicrob Chemother.* 2015; **70**(2): 382–95. [PubMed Abstract](#) | [Publisher Full Text](#)
- Conlon BP, Rowe SE, Lewis K: **Persister cells in biofilm associated infections.** *Adv Exp Med Biol.* 2015; **831**: 1–9. [PubMed Abstract](#) | [Publisher Full Text](#)
- McCall IC, Shah N, Govindan A, *et al.*: **Antibiotic Killing of Diversely Generated Populations of Nonreplicating Bacteria.** *Antimicrob Agents Chemother.* 2019; **63**(7): pii: e02360-18. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Sarathy JP, Via LE, Weiner D, *et al.*: **Extreme Drug Tolerance of *Mycobacterium tuberculosis* in Caseum.** *Antimicrob Agents Chemother.* 2018; **62**(2): pii: e02266-17. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Meylan S, Andrews IW, Collins JJ: **Targeting Antibiotic Tolerance, Pathogen by Pathogen.** *Cell.* 2018; **172**(6): 1228–38. [PubMed Abstract](#) | [Publisher Full Text](#)
- Shi D, Mi G, Wang M, *et al.*: ***In vitro* and *ex vivo* systems at the forefront of infection modeling and drug discovery.** *Biomaterials.* 2019; **198**: 228–49. [PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
- Gold B, Nathan C: **Targeting Phenotypically Tolerant *Mycobacterium tuberculosis*.** *Microbiol Spectr.* 2017; **5**(1). [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Malik M, Hussain S, Drlca K: **Effect of anaerobic growth on quinolone lethality with *Escherichia coli*.** *Antimicrob Agents Chemother.* 2007; **51**(1): 28–34. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Ersoy SC, Heithoff DM, Barnes L 5th, *et al.*: **Correcting a Fundamental Flaw in the Paradigm for Antimicrobial Susceptibility Testing.** *EBioMedicine.* 2017; **20**: 173–81. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
- Farha MA, French S, Stokes JM, *et al.*: **Bicarbonate Alters Bacterial Susceptibility to Antibiotics by Targeting the Proton Motive Force.** *ACS Infect Dis.* 2018; **4**(3): 382–90. [PubMed Abstract](#) | [Publisher Full Text](#) | [F1000 Recommendation](#)
- Yang JH, Bering SC, Collins JJ: **Antibiotic efficacy-context matters.** *Curr*

- Opin Microbiol.* 2017; **39**: 73–80.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
36. Stubbings W, Leow P, Yong GC, *et al.*: **In vitro** spectrum of activity of flaxloxacina, a novel, pH-activated fluoroquinolone, under standard and acidic conditions. *Antimicrob Agents Chemother.* 2011; **55**(9): 4394–7.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
37. **F** Vulin C, Leimer N, Huemer M, *et al.*: **Prolonged bacterial lag time results in small colony variants that represent a sub-population of persisters.** *Nat Commun.* 2018; **9**(1): 4074.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
38. Sakoulas G, Kumaraswamy M, Kousha A: **Interaction of Antibiotics with Innate Host Defense Factors against Salmonella enterica Serotype Newport.** *mSphere.* 2017; **2**(6): pii: e00410-17.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
39. **F** Gusarov I, Shatalin K, Starodubtseva M, *et al.*: **Endogenous nitric oxide protects bacteria against a wide spectrum of antibiotics.** *Science.* 2009; **325**(5946): 1380–4.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
40. Eng RH, Padberg FT, Smith SM, *et al.*: **Bactericidal effects of antibiotics on slowly growing and nongrowing bacteria.** *Antimicrob Agents Chemother.* 1991; **35**(9): 1824–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
41. Sufya N, Allison DG, Gilbert P: **Clonal variation in maximum specific growth rate and susceptibility towards antimicrobials.** *J Appl Microbiol.* 2003; **95**(6): 1261–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
42. Smirnova GV, Oktyabrsky ON: **Relationship between Escherichia coli growth rate and bacterial susceptibility to ciprofloxacin.** *FEMS Microbiol Lett.* 2018; **365**(1): fnx254.
[PubMed Abstract](#) | [Publisher Full Text](#)
43. **F** Dörr T, Vulić M, Lewis K: **Ciprofloxacin causes persister formation by inducing the TisB toxin in Escherichia coli.** *PLoS Biol.* 2010; **8**(2): e1000317.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
44. Kreibich S, Hardt WD: **Experimental approaches to phenotypic diversity in infection.** *Curr Opin Microbiol.* 2015; **27**: 25–36.
[PubMed Abstract](#) | [Publisher Full Text](#)
45. Bumann D: **Heterogeneous host-pathogen encounters: Act locally, think globally.** *Cell Host Microbe.* 2015; **17**(1): 13–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
46. Cadena AM, Fortune SM, Flynn JL: **Heterogeneity in tuberculosis.** *Nat Rev Immunol.* 2017; **17**(11): 691–702.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
47. Mitchison DA: **The action of antituberculosis drugs in short-course chemotherapy.** *Tubercle.* 1985; **66**(3): 219–25.
[PubMed Abstract](#) | [Publisher Full Text](#)
48. **F** Davis KM, Mohammadi S, Isberg RR: **Community behavior and spatial regulation within a bacterial microcolony in deep tissue sites serves to protect against host attack.** *Cell Host Microbe.* 2015; **17**(1): 21–31.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
49. Vandiviere HM, Loring WE, Melvin I, *et al.*: **The treated pulmonary lesion and its tubercle bacillus. II. The death and resurrection.** *Am J Med Sci.* 1956; **232**(1): 30–7; passim.
[PubMed Abstract](#) | [Publisher Full Text](#)
50. Chengalroyen MD, Beukes GM, Gordhan BG, *et al.*: **Detection and Quantification of Differentially Culturable Tubercle Bacteria in Sputum from Patients with Tuberculosis.** *Am J Respir Crit Care Med.* 2016; **194**(12): 1532–40.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
51. Burton NA, Schürmann N, Casse O, *et al.*: **Disparate impact of oxidative host defenses determines the fate of Salmonella during systemic infection in mice.** *Cell Host Microbe.* 2014; **15**(1): 72–83.
[PubMed Abstract](#) | [Publisher Full Text](#)
52. **F** Helaine S, Cheverton AM, Watson KG, *et al.*: **Internalization of Salmonella by macrophages induces formation of nonreplicating persisters.** *Science.* 2014; **343**(6167): 204–8.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
53. Kaiser P, Regoes RR, Dolowtschiak T, *et al.*: **Cecum lymph node dendritic cells harbor slow-growing bacteria phenotypically tolerant to antibiotic treatment.** *PLoS Biol.* 2014; **12**(2): e1001793.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
54. Claudi B, Spröte P, Chirkova A, *et al.*: **Phenotypic variation of Salmonella in host tissues delays eradication by antimicrobial chemotherapy.** *Cell.* 2014; **158**(4): 722–33.
[PubMed Abstract](#) | [Publisher Full Text](#)
55. Panteix G, Guillaumond B, Harf R, *et al.*: **In-vitro concentration of azithromycin in human phagocytic cells.** *J Antimicrob Chemother.* 1993; **31** Suppl E: 1–4.
[PubMed Abstract](#) | [Publisher Full Text](#)
56. Vallet CM, Marquez B, Ngabirano E, *et al.*: **Cellular accumulation of fluoroquinolones is not predictive of their intracellular activity: studies with gemifloxacin, moxifloxacin and ciprofloxacin in a pharmacokinetic/pharmacodynamic model of uninfected and infected macrophages.** *Int J Antimicrob Agents.* 2011; **38**(3): 249–56.
[PubMed Abstract](#) | [Publisher Full Text](#)
57. Greenwood DJ, Dos Santos MS, Huang S, *et al.*: **Subcellular antibiotic visualization reveals a dynamic drug reservoir in infected macrophages.** *Science.* 2019; **364**(6447): 1279–82.
[PubMed Abstract](#) | [Publisher Full Text](#)
58. Marchand S, Chauzy A, Dahyot-Fizelier C, *et al.*: **Microdialysis as a way to measure antibiotics concentration in tissues.** *Pharmacol Res.* 2016; **111**: 201–7.
[PubMed Abstract](#) | [Publisher Full Text](#)
59. Prideaux B, Lenaerts A, Dartois V: **Imaging and spatially resolved quantification of drug distribution in tissues by mass spectrometry.** *Curr Opin Chem Biol.* 2018; **44**: 93–100.
[PubMed Abstract](#) | [Publisher Full Text](#)
60. **F** Blanc L, Daudelin IB, Podell BK, *et al.*: **High-resolution mapping of fluoroquinolones in TB rabbit lesions reveals specific distribution in immune cell types.** *eLife.* 2018; **7**: pii: e41115.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)
61. Cronan MR, Rosenberg AF, Oehlers SH, *et al.*: **CLARITY and PACT-based imaging of adult zebrafish and mouse for whole-animal analysis of infections.** *Dis Model Mech.* 2015; **8**(12): 1643–50.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
62. **F** Fung C, Tan S, Nakajima M, *et al.*: **High-resolution mapping reveals that microniches in the gastric glands control Helicobacter pylori colonization of the stomach.** *PLoS Biol.* 2019; **17**(5): e3000231.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#) | [F1000 Recommendation](#)

Open Peer Review

Current Peer Review Status:  

Editorial Note on the Review Process

F1000 Faculty Reviews are written by members of the prestigious F1000 Faculty. They are commissioned and are peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

The reviewers who approved this article are:

Version 1

1 **Veronique Dartois**

Public Health Research Institute, New Jersey Medical School, Rutgers University, Newark, New Jersey, USA

Competing Interests: No competing interests were disclosed.

2 **Ursula Theuretzbacher**

Center for Anti-Infective Agents (CEFAIA), Vienna, Austria

Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research