Trends in Microbiology

CellPress REVIEWS

Opinion

Phagocytes, Antibiotics, and Self-Limiting Bacterial Infections

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Most antibiotic use in humans is to reduce the magnitude and term of morbidity of acute, community-acquired infections in immune competent patients, rather than to save lives. Thanks to phagocytic leucocytes and other host defenses, the vast majority of these infections are self-limiting. Nevertheless, there has been a negligible amount of consideration of the contribution of phagocytosis and other host defenses in the research for, and the design of, antibiotic treatment regimens, which hyper-emphasizes antibiotics as if they were the sole mechanism responsible for the clearance of infections. Here, we critically review this approach and its limitations. With the aid of a heuristic mathematical model, we postulate that if the rate of phagocytosis is great enough, for acute, normally self-limiting infections, then (i) antibiotics with different pharmacodynamic properties would be similarly effective, (ii) low doses of antibiotics can be as effective as high doses, and (iii) neither phenotypic nor inherited antibiotic resistance generated during therapy are likely to lead to treatment failure.

Trends

With a sufficiently high rate of phagocytosis: antibiotics with very different pharmacodynamic properties, bactericidal and bacteriostatic, can be similarly effective; phenotypic resistance (persistence) will have little effect on the rate of clearance; minority populations of genetically resistant bacteria will not ascend to dominance; and lower doses of antibiotics will be as effective as higher doses.

As the rate of phagocytosis declines, bactericidal drugs will be increasingly more effective than bacteriostatic ones, persistence will play an increasingly important role in the course of therapy, minority populations of resistant bacteria will ascend to dominance, and higher doses of antibiotics will be more effective than lower doses.

Antibiotics as Life-Style Rather than Life-Saving Drugs

The current concern about the future of antibiotic therapy due to the rampant evolution of antibiotic resistance in pathogenic bacteria and the dearth of drugs with novel targets [1–3] is well justified for life-threatening bacterial infections, particularly for those in immune-compromised patients in hospitals. We should not forget, however, that, in the developed world, some 90% of human use of antibiotics is in the community, not in hospitals (http://ecdc.europa.eu/), and is employed to treat common, normally self-limiting, acute bacterial infections in otherwise healthy, immune-competent, patients [4–6].

In the community, as well as in hospitals, we are continually confronted with bacteria capable of colonizing and replicating in or on human hosts, but, thanks to a variety of innate defenses [7], symptomatic infections with these bacteria that would prompt patients to seek medical attention are rare in immune-competent hosts. Particularly prominent among these innate defenses are the evolutionarily ancient ones: phagocytic cells that recognize invading microbes and engulf and destroy them [8–10]. In addition to this predatory response, the phagocytic leucocytes play a significant role in modulating the other host responses to infecting microbes and the homeostasis of the immune system [11].

Even when symptoms are apparent, in the vast majority of cases the innate immune defenses – responsible for maintaining the subclinical detente [7] between potentially invasive bacteria and humans – clear the infection. This applies to many common infections, including acute otitis

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media, acute rhinosinusitis, tonsillitis, periodontitis, mild skin and soft-tissue infections, conjunctivitis, acute exacerbations of chronic bronchitis, lower uncomplicated urinary tract infections, asymptomatic bacteriuria, vaginitis, vaginosis, urethritis, and proctitis. In many of these infections – acute otitis media providing a particularly pertinent example – there is a seemingly permanent controversy about the need for antibiotic therapy, clearly based on the many observations of high rates of spontaneous cure of the infection [12–19]. Be that as it may, antibiotics can certainly accelerate the rate of clearance of these normally self-limiting bacterial infections and thereby reduce the magnitude and term of morbidity and thus the amount of time out of work/school/daycare, thereby increasing productivity at home, at work, and in education [20–23], and, in general, improving personal comfort. In this sense, for common acute infections in immune-competent hosts, antibiotics can better be described as life-style preserving rather than life-saving drugs (Box 1).

The Under-Appreciated Collaboration

Despite the recognition of the central role of phagocytic leucocytes and the innate immune system in clearing bacterial infections, the development and design of treatment regimens for bacterial infections has been almost entirely antibiotic-centric. With few exceptions – largely, but not exclusively, in the theoretical literature, for example [24–28] – little consideration is given to the role of the host's phagocytic response in the clearance dynamics of infecting bacteria under antibiotic exposure. As noted by George Drusano [29], in the existing literature 'there is virtually no information about how phagocytic granulocytes interact with antimicrobial chemotherapy to kill bacterial cells'.

For the most part, the recommendations made by proponents of the 'rational' (as opposed to purely empirical) approach to antibiotic therapy [30,31] regarding doses and dosing regimens are founded on: (i) pharmacokinetics (PK), almost exclusively estimates of the changes in the concentrations of antibiotics in the serum of experimental animals and/or human volunteers (albeit rarely in infected patients), (ii) *in vitro* studies of the pharmacodynamics (PD) of the antibiotics and the target bacteria, almost exclusively based on estimates of a single parameter, the minimum inhibitory concentration (MIC) of the drug, and informal consideration of the relative contributions of time and dose to the change in the viable cell density of bacteria exposed to these drugs [32–38], and (iii) experiments with laboratory animals, commonly neutropenic laboratory mice treated shortly after infection (e.g., [31,39,40]). Eventually the schedules for antibiotic use derived from these studies are refined in clinical trials, or in phase IV studies, but the PK/PD data, and particularly the main composite parameters – namely, (i) the time during which the antibiotic concentration in serum exceeds the MIC for the offending organism and drug, and/or (ii) the ratio of the area under the time–concentration curve (AUC) to the MIC – remain the main predictors of success with different types and dosages of drugs [41].

This 'rational' approach to the design of treatment protocols has the virtue of addressing the worst-case situations, infections in immune-compromised patients where antibiotics are indeed life-saving drugs. This approach has been considered successful [32,42,43], and it

Box 1. Antibiotics as Life-Style Drugs

By the phrase 'life-style-preserving' to describe the use of antibiotics to treat normally self-limiting bacterial infections, we are not suggesting that we believe their use in this way is medically or socially trivial. We certainly do not. We do, however, believe that the clinical value of life-style-preserving applications of antibiotics should be far better evaluated than they are currently. For example, there is evidence that symptomatic treatment of uncomplicated urinary-tract infections with Ibuprofen can be as effective as treatment with ciprofloxacin [112]. And, symptomatic treatment of self-limiting infections with anti-inflammatory compounds rather than antibiotics will be an effective strategy to limit the spread of antibiotic resistance [113]. On the other side, there is evidence that countries with low rates of antibiotic use for treating common infections, such as acute otitis media, have higher rates of acute mastoiditis compared to countries with higher rates of antibiotic use [114], but also see [115] for an alternative perspective of this observation.

seems reasonable to assume that 'what works for the worse cases should also work for the milder ones'. Not clear, however, is whether these worst-case protocols are optimal for the treatment of acute, normally self-limiting infections in immune-competent hosts. By considering the contribution of the host's antibacterial system in the design of antibiotic treatment protocols, could these infections be successfully treated with lower doses of antibiotics used for shorter terms than currently recommended? It is very likely that lower doses and shorter terms of treatment, possibly with older and less-expensive drugs, would reduce the likelihood of adverse sequelae of treatment, like those due to the disturbance of the microbiome [44], and decrease the intensity of selection for the ascent and dissemination of resistance in the commensal flora as well as the target bacteria [45].

A Heuristic Model

To explore and illustrate the contribution of the host's antibacterial defenses to the course of antibiotic treatment and generate hypotheses about this process, we use a simple mathematical model. A diagram and description of this model is presented in Figure 1 and its legend. This model combines the PD and PK of antibiotic treatment developed in previous studies [46–48] with the dynamics of phagocytosis considered in [24]. The equations for this model, the definitions of the variables, and the parameters and values used for numerical solutions are

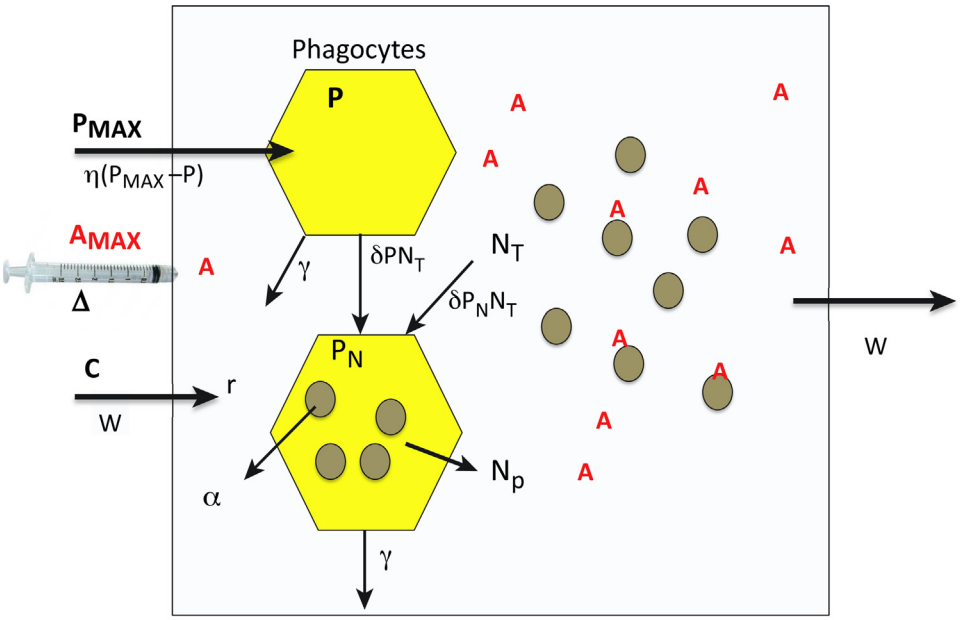

Figure 1. A Model for the Combined Action of Antibiotics and Phagocyte Leukocytes in Controlling the Proliferation of Bacteria at the Site of an Infection. Fresh phagocytes, P per ml, enter the site of the infection at a rate that is directly proportional to their maximum density within the reservoir, P_{MAX}, and the existing density at the site of the infection, $\eta(P_{MAX} - P)$. Viable bacteria are of two states, free and engulfed by phagocytes. The total density of free bacteria, N_T, includes antibiotic-susceptible, N, phenotypically resistant cells, persisters, N_E, and genetically resistant cells, N_R. The total density of bacteria of these three types within the phagocytes is N_{TP}. A limiting resource, r, μg/ml, enters the site of infection from a reservoir where it is maintained at a concentration C μg/ml fresh phagocytes, P_{MAX} cells/ml. Antibiotics at a dose of A_{MAX} μg/ml are introduced at defined intervals, Δ hours, and decay at a rate of d_A/h. Free and bacteria-populated phagocytes, P and P_N, engulf free bacteria at a rate proportional to the product of their densities, that of the free bacteria and a rate constant, δ, which is the same for free bacteria of all states and both P and N_P phagocytes. Both P and P_N phagocytes die at a rate γ/cell/h. We assume that when they die, or are removed from the site of the infection, the bacteria within the N_{PT} phagocytes die and are removed as well. Bacteria within the phagocytes die at a rate of α/cell/h. Bacteria, phagocytes, excess resources, and antibiotics are removed from the site of the infection at a rate of w/unit volume/h.

Box 2. The Model

Variable and parameter definitions, parameter values used for the simulations, and equations for the model of the joint action of antibiotics and phagocytic for the treatment of acute infections depicted and described in Figure 1.

Variable	Definition	
N	Free antibiotic-sensitive bacteria, cells/ml	
N_R	Free antibiotic-resistant bacteria, cells/ml	
N_E	Free persisters, cells/ml	
P	Free phagocytes/ml	
P_N	Phagocytes with one or more bacteria/ml	
N_{TP}	Bacteria within phagocytes, per cell	
A	Concentration, antibiotic, μg/ml	
R	Concentration, limiting resource, μg/ml	
N_T	Total density of free bacteria/ml	

Parameters	Definition	Values used for the simulations
Ψ_{MAX}	Maximum growth rate/cell/h	1.2
k	Monod constant, μg	1
e	Resource conversion efficiency, μg	5×10^{-7}
η	Phagocyte input parameter/h	0.5
γ	Phagocyte engulfment parameter	10^{-5}, 5×10^{-6}, 10^{-7}
Ψ_{MIN}	Minimum growth rate (antibiotic-mediated)/h	−5, −1.0, −0.1
κ	Hill coefficients	3.5, 1
P_{MAX}	Phagocytes in reservoir/ml	10^5
C	Reservoir resource concentration, μg/ml	1000
A_{MAX}	Maximum dose of the antibiotic, μg/ml	0.1 to 5
Δ	Dosing interval, hours	12 or 18
g	Persister transition rate $N \rightarrow N_E$/cell/h	0 or 10^{-6}
h	Persister transition rate $N_E \rightarrow N$/cell/h	0 or 10^{-6}
α	Rate of kill of bacteria with phagocytes/h	0.5
d_p	Rate of loss phagocytes cell/h	0.1
d_a	Rate of decay of the antibiotic/h	0.1

Equations

$$\frac{dR}{dt} = w(C-R) - e\left(\frac{R}{(R+k)}\right)\left(N\psi(A) + N_R\psi_{MAX}\right)$$

$$\frac{dA}{dt} = -d_A A - wA$$

$$\frac{dN}{dt} = \psi(A)N\left(\frac{R}{R+k}\right) - \gamma N(P+P_N) - gN + hN_E - wN$$

$$\frac{dN_E}{dt} = gN - hN_E - \gamma N_E(P+P_N) - wN_E$$

$$\frac{dN_R}{dt} = \psi_{MAX}N_R - \gamma N_R(P+P_N) - wN_R$$

$$\frac{dP}{dt} = \eta(P_{MAX}-P) - \gamma(N+N_E)P - P(d_P+w)$$

$$\frac{dP_N}{dx} = \gamma(N+N_E+N_R)P - P_N(d_P+w)$$

$$\frac{dN_{TTP}}{dt} = \gamma(N+N_E+N_R)(P+P_N) - N_{TP}(\alpha+w)$$

where

$$\psi(A) = \psi_{MAX} - (\psi_{MAX} - \psi_{MIN})\left[\frac{\left(\frac{A}{MIC}\right)^{\kappa}}{\left[\left(\frac{A}{MIC}\right)^{\kappa} - \frac{\psi_{MIN}}{\psi_{MAX}}\right]}\right]$$

We are assuming that neither the persisters, N_E, nor the bacteria within phagocytes, N_{TP}, consume resources.

presented in Box 2. For our numerical solutions of these equations, computer simulations, we use Berkeley Madonna™. Copies of these programs and instructions for their use can be found on www.eclf.net.

Wonder Drugs Are Not So Wondrous on Their Own

Antibiotics vary profoundly in the rates at which they kill bacteria. This can be seen below in the figure in the Mendeley site in the Resource section preceding the references, where we consider the rates at which *Staphylococcus aureus* is killed with 10× the MIC of nine antibiotics with different PD properties. Drugs like gentamicin kill at a high rate, others, like vancomycin, at a relatively low rate, and still others, like linezolid, inhibit replication but have minimal cidal effect on *S. aureus* at all, and would be considered bacteriostatic. On first consideration, it would seem that antibiotics that rapidly kill the target bacteria would be more effective for treating infections than those that kill them slowly or just prevent their replication. There is indeed a bias prioritizing bactericidal drugs by clinicians and pharmaceutical companies, which is reflected in the guidelines for antibiotic use [49]. However, as measured by clinical efficacy of the treatment of Gram-positive infections, there is no distinction between antibiotics that are designated bactericidal and those designated bacteriostatic based on their *in vitro* PD properties . As Pankey and Sabath state 'the presumptive superiority of *in vitro* bactericidal over bacteriostatic action in the treatment of Gram-positive infections is intuitive rather than based on rigorous scientific research' [50]. This interpretation is also supported by a meta-analysis of the relative efficacy of antibiotics deemed bactericidal and bacteriostatic for the treatment of immuno-competent patients with 'serious bacterial' infections (endocarditis and meningitis were not introduced in this study) [51]; antibiotics with very different PD properties, bacteriostatic and bactericidal, were similarly effective.

If antibiotics were the unique factor responsible for the clearance of infections, and the bacteria were susceptible to the treating drug, contrary to these observations, the rate of clearance of an infection would be proportional to the rate at which these drugs kill the bacteria. Figure 2A illustrates the Hill function PD relationship between antibiotic concentration and rate of growth/death of bacteria for three hypothetical antibiotics: a strongly bactericidal drug, denoted S, a weakly bactericidal drug W, and a bacteriostatic antibiotic, B. Under conditions where, in the absence of any drugs, the bacterial population would maintain a constant density, 10^9 cells/ml, we illustrate what occurs with simulations of the model when antibiotics are the sole mechanism responsible for the eradication of the bacteria (Figure 2B–D). The highly bactericidal antibiotic, S, clears the infection more rapidly than the weakly bactericidal drug, W, and far more rapidly than the bacteriostatic agent, B (Figure 2B). The dynamics of treatment are different if we allow for phenotypic resistance, the phenomenon of persistence [52–55]. This can be seen in Figure 2C where susceptible bacteria enter the phenotypically resistant state and return to the susceptible state, $N \leftrightarrow N_{E,}$ at a rate of 10^{-6}/cell/h. Even the most bactericidal antibiotic is unable to clear the infection in the 10 days of simulated treatment (Figure 2C).

Inherited antibiotic resistance is another reason that antibiotics alone will not be able to clear an infection. Even when the population of bacteria responsible for the infection is fully susceptible to the treating antibiotic, by mutation or acquisition by horizontal transfer of genetic elements with resistance genes, minority populations of bacteria genetically resistant to the treating drug may well be present or generated during the course of therapy. If that were the case, our model predicts that, within short order, these resistant cells will ascend to dominance during therapy and continue to be maintained at a density that is limited only by the availability of resources (Figure 2D). While the rate of decline in densities of the susceptible bacteria is proportional to the rate of killing by the drug, the rate at which the resistant subpopulation ascends is almost independent of the rate of killing by the antibiotics.

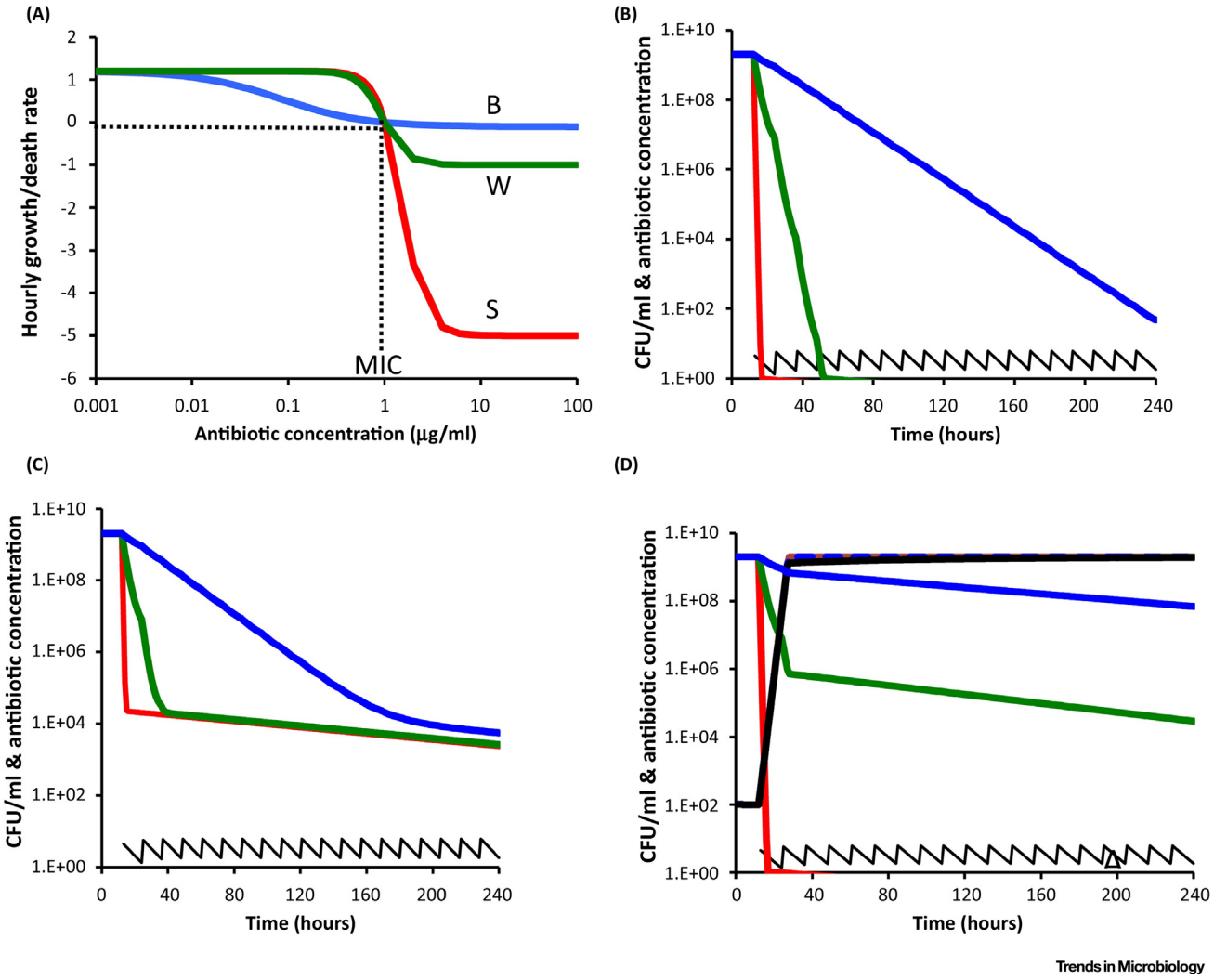

Figure 2. Simulation Results: Pharmacodynamics of Antibiotics and Bacteria and the Population Dynamics of Treatment When These Drugs Are the Sole Mechanism for Controlling the Proliferation of the Bacteria. (A) Hill function pharmacodynamics for a strongly bactericidal, S, a weakly bactericidal, W, and a 'bacteriostatic' antibiotic, B, all of which have a minimum inhibitory concentration (MIC) of 1 μg/ml. The maximum bacterial growth rate, ψ_{MAX}, is a property of the bacteria, culture conditions, and media. For *Staphylococcus aureus* Newman, growing in MHII at 37 °C, $\psi_{MAX} = 1.2\ h^{-1}$ [97], which is the value we use in the above simulations. For the strongly bactericidal antibiotic, S, $\psi_{MIN} = -5.0\ h^{-1}$ and $\kappa = 3.5$. For the weakly bactericidal antibiotic, W, $\psi_{MIN} = -1.0\ h^{-1}$ and $\kappa = 1.0$. For the bacteriostatic antibiotic, B, $\psi_{MIN} = -0.1\ h^{-1}$ and $\kappa = 3.5$. To simulate antibiotic treatment, starting after the first 12 h, a maximum dose, $A_{MAX} = 5$ μg/ml, is introduced every $\Delta = 12$ h. The antibiotic decays at a rate of $da = 0.1/h$ and is washed out of the site of the infection at a rate of $w = 0.01/h$. The oscillating black lines in B, C, and D are the changes in the concentration of the antibiotic during the course of treatment. (B) Change in the viable cell density of bacteria in the absence of phenotypic or inherited resistance. (C) Change in the viable cell density of bacteria with phenotypic but no inherited resistance. (D) Changes in the viable cell density of bacteria with an initial minority population of genetically resistant cells.

The Virtue of Collaboration: Phagocytes and Antibiotics

If phagocytic leucocytes enter the site of infection and phagocytose free bacteria at a sufficiently high rate ($\delta = 10^{-5}$), within short order the planktonic population of bacteria will be cleared (Figure 3A), but viable bacteria will continue to persist within the phagocytes. How long they persist in this state would depend on the rate at which they are killed within the phagocytes. With a somewhat lower rate constant of phagocytosis, $\delta = 5 \times 10^{-6}$, viable planktonic and phagocytosed bacteria will continue to be maintained, but the total density of bacteria is approximately four orders of magnitude less than that in the absence of phagocytosis.

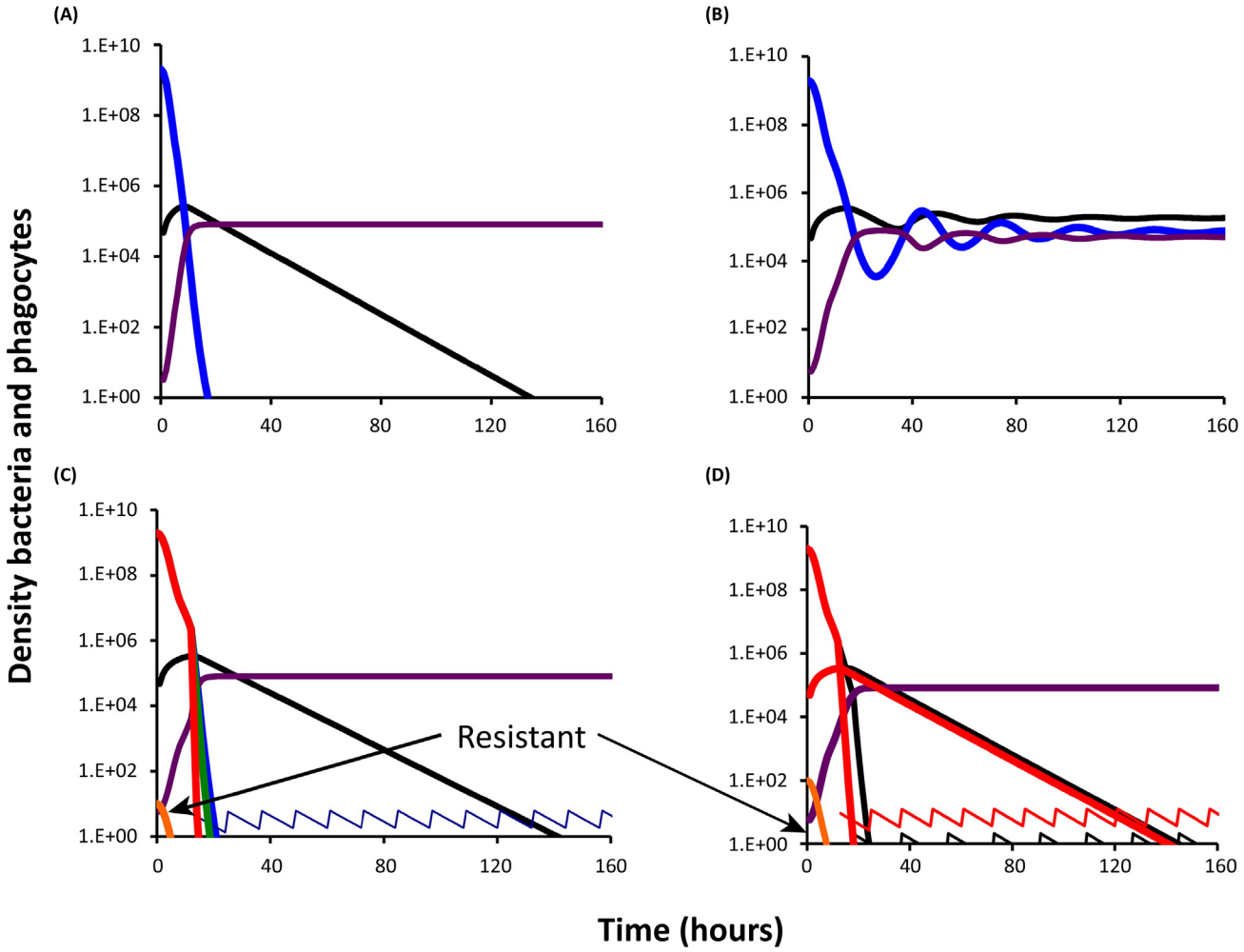

Figure 3. Simulation Results: The Population Dynamics of the Control of a Bacterial Population by Phagocytic Leucocytes Alone, and in Combination with Antibiotics. (A,B) Changes in the densities of viable bacteria and phagocytes in the absence of antibiotics. Blue, total free bacteria, N_T; purple, free phagocytes, P; black, bacteria within phagocytes, N_{TP}. Save for the rate constant of phagocytosis, the phagocytosis parameters are identical in all of these simulation, $\eta = 0.5$, $P_{MAX} = 10^5$, $\gamma = 0.5$, $\alpha = 0.1$. (A) Phagocytosis rate constant, $\delta = 10^{-5}$. (B) Phagocytosis rate constant $\delta = 5 \times 10^{-6}$. (C,D) Changes in the viable cell density of bacteria and phagocytes with the joint action of phagocytes and antibiotics, $\delta = 5 \times 10^{-6}$. (C) Treatment with antibiotics of the three different class, changes in density of free bacteria with: red, highly bactericidal agent, S; green, weakly bactericidal, W; blue, bacteriostatic, B; purple, free phagocytes, P; black, density of viable bacteria within phagocytes, N_{PT}. Persistent bacteria, N_E, are generated at the same rate as in Figure 2C, and there is a minority population of bacteria genetically resistant to the antibiotic, N_R, orange, as in 2D. (D) Two regimens of treatment with a weakly bactericidal antibiotic, W. Oscillating light black line: treatment every $\Delta = 16$ h, maximum dose 2 μg/ml. Oscillating red line: treatment every 12 h, maximum dose 10 μg/ml. Thick red and black lines: corresponding changes in the densities of free bacteria and bacteria within phagocytes. Thick orange line: change in the density of genetically resistant bacteria. Purple line: change in the density of free phagocytes.

When antibiotics are administered, in Figure 3C, with the phagocytosis parameters in Figure 3B, the free bacteria are rapidly cleared and the viable bacteria present remain in the phagocytes. In this simulation, the rate of persistence is the same as that in Figure 2C and there is a minority population of genetically resistant cells, as in Figure 2D. These resistant bacteria do not ascend to dominance. While there are perceptible differences in the rate at which the highly bactericidal, weakly bactericidal, and bacteriostatic antibiotics clear the infection, the differences are negligible. In Figure 3D, we consider the contribution of the dose and frequency of administration of the weakly bactericidal antibiotic, W. The rate of clearance of the free bacteria with the aggressive treatment regimen – maximum dose 10 μg/ml (10X MIC)

administered every 12 h – is greater than when the dose is 2 μg/ml (2× MIC) administered every 16 h. The difference is, however, small.

When the efficacy of phagocytosis is lower, $\delta = 1 \times 10^{-6}$, in the absence of antibiotic treatment, the density of free bacteria is about an order of magnitude less than that which obtains in the absence of treatment (Figure 4A). The antibiotics are able to clear the infection, but the rate of clearance is now more dependent on the PD of the drug. The highly bactericidal antibiotic clears infection at a greater rate than the weakly bactericidal drug, which, in turn, is more

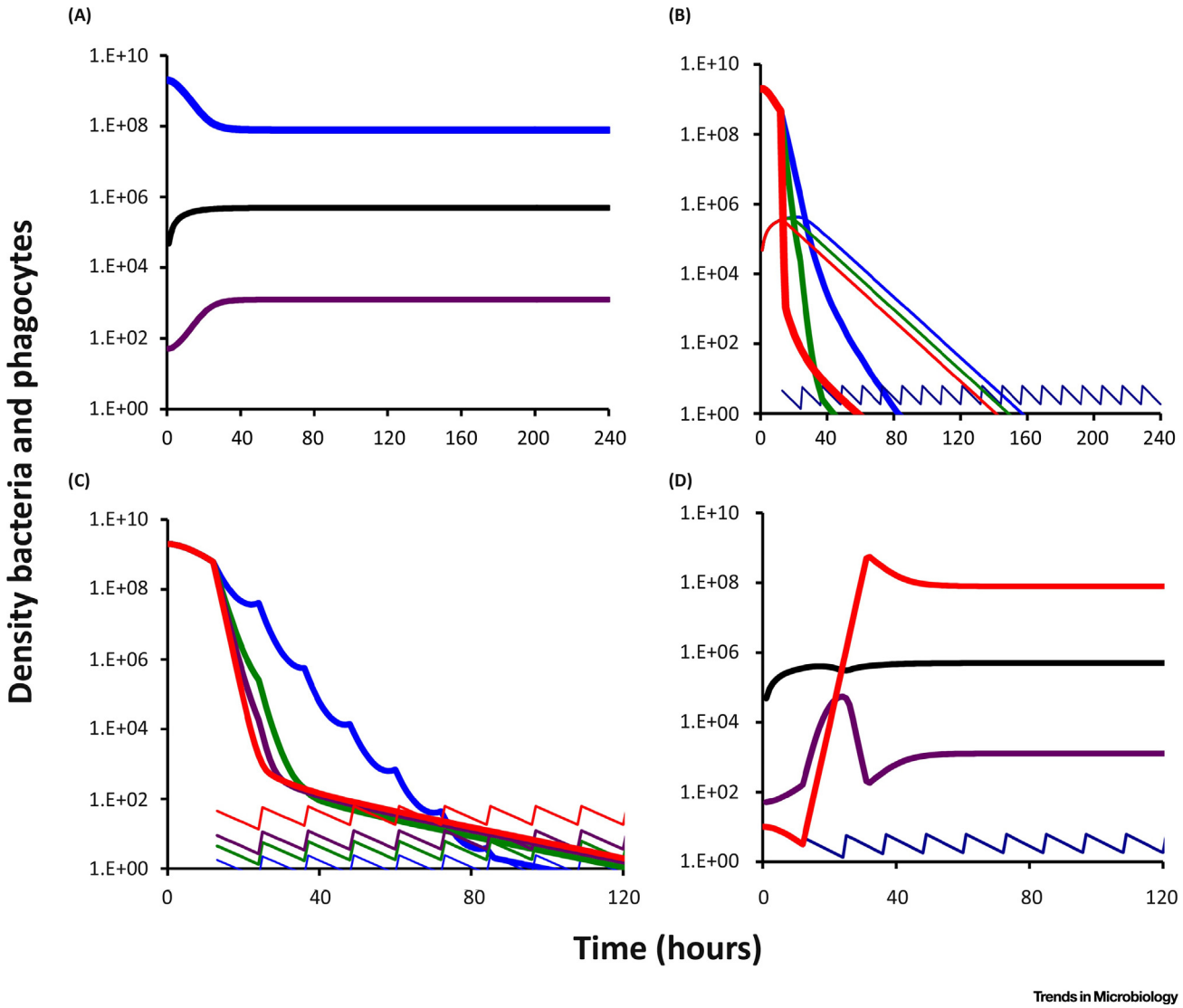

Figure 4. Simulation Results: The Population Dynamics of the Treatment of a Bacterial Population by Phagocytic Leucocytes Alone, and in Combination with Antibiotics. Changes in the densities of viable bacteria and phagocytes with an intermediate rate of phagocytosis $\delta = 10^{-6}$. (A) No antibiotics. Blue, total free bacteria, N_T; purple, free phagocytes, P; black, bacteria within phagocytes, N_{TP}. (B) Treatment with different antibiotics. Red, highly bactericidal; green, weakly bactericidal; blue, bacteriostatic. The wide lines are the respective densities of free bacteria, and the thin colored lines are the densities of viable bacteria within phagocytes. (C) The effect of different doses of a weakly bactericidal antibiotic on the rate of clearance. Thin lines represent antibiotic concentrations (ranging from 2X to 50X MIC), thick lines corresponding to the densities of free bacteria. (D) Treatment with a weakly bactericidal antibiotic and the ascent of a genetically resistant subpopulation. Purple, free phagocytes, P; black, bacteria within phagocytes; orange, resistant subpopulation, N_R.

effective than the bacteriostatic antibiotic (Figure 4B). With this intermediate level of phagocytosis, the rate of clearance is now more dependent on the maximum concentration of the drug administered, although, save for the lowest dose, 2× MIC, there is little difference in the rate of clearance with higher concentrations of the antibiotic (Figure 4C). Unlike the situation with the higher rate of phagocytosis (Figure 3D), the minority population of genetically resistant cells ascends and dominates the population (Figure 4D).

Phagocytosis with a lower rate constant, $\delta = 10^{-7}$, still reduces the viable density of free bacteria by nearly an order of magnitude relative to that of a population limited solely by resources (Figure 5A). However, with persistence, the course of the infection is more similar to that which obtains in the absence of phagocytosis (compare Figure 5B with Figure 2C). The more bactericidal antibiotic reduces the density of free bacteria at a higher rate than the bacteriostatic antibiotic. Also, although the density of the free bacteria is reduced to a lower level than what occurs in the absence of phagocytosis (Figure 5B), many bacteria are located within the phagocytes, and the infection is not cleared in the 10 days of simulated treatment irrespective of the type of antibiotic used for treatment.

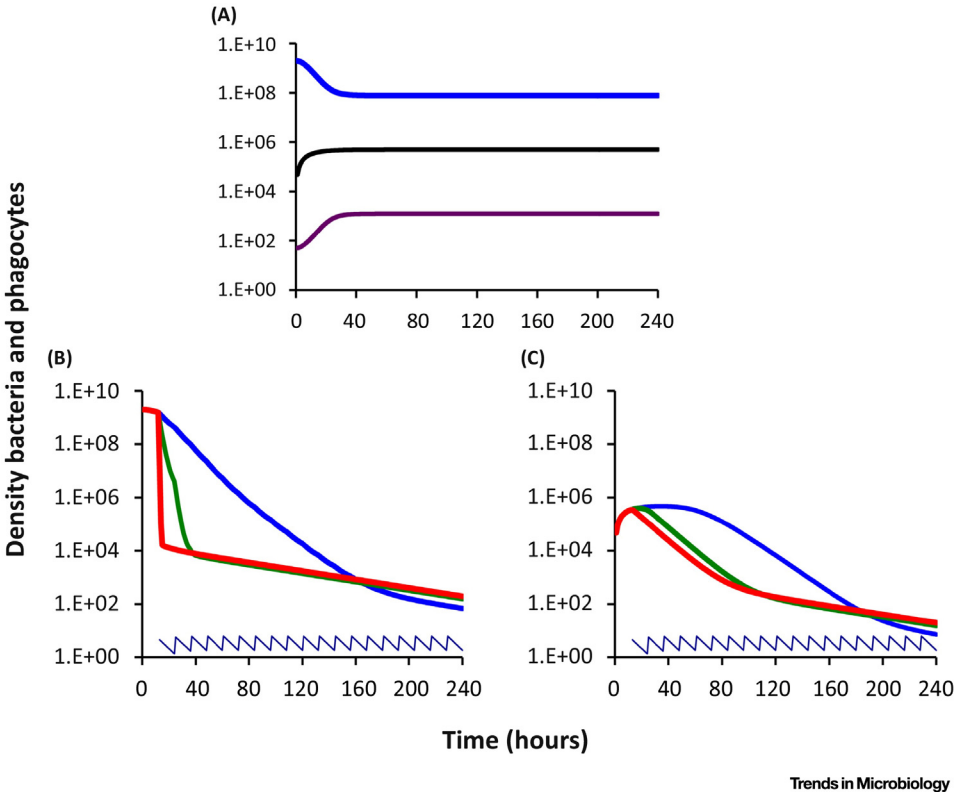

Figure 5. Simulation Results: The Population Dynamics of the Treatment of a Bacterial Population by Phagocytic Leucocytes Alone, and in Combination with Antibiotics. Changes in the densities of viable bacteria and phagocytes with a low rate of phagocytosis, $\delta = 10^{-7}$. (A) Phagocytosis alone. Blue, free bacteria, N_T; black, bacteria within phagocytes, N_{TP}; purple, free phagocytes, P. (B,C) Treatment with the different classes of antibiotics. Red, highly bactericidal; green, weakly bactericidal; blue, bacteriostatic. (B) Changes in the viable density of free bacteria. (C) Changes in the viable density of bacteria with phagocytes.

In Vitro Pharmacodynamics and the Rate of Clearance of Infections

What do the models used here tell us about the utility of *in vitro* studies on the PD of antibiotics and bacteria and experiments with neutropenic mice for the design of regimens for the treatment of acute self-limiting infections in immune-competent patients? First, they illustrate the insufficiency of PK/PD indices as predictors of the efficacy of antibiotic treatment regimens when the MIC is the unique PD parameter. Note that the strongly and weakly bactericidal and bacteriostatic (S, W, and B**)** antibiotics used in the simulations have the same PK/MIC indices; however, the rate of clearance of the infection can differ between them depending on the interaction between the PD of the antibiotics and the efficacy of phagocytosis (for additional considerations of the limitations of MICs see [56–58]). For instance, as the rate, and thereby the contribution, of phagocytosis declines, the rate of clearance of the infection become increasingly dependent on the PD of the antibiotic, such that the more bactericidal a drug is, the more rapidly the infection will be cleared.

The results of these simulations also provide an explanation for the observation that antibiotics with very different *in vitro* PD properties, like those designated bactericidal and bacteriostatic, can be similarly effective for treating uncompromised patients even with 'serious bacterial infections' [51]. If we consider the time before the clearance of free bacteria, N_T, as a measure of the efficacy of treatment, this model suggests that if the rate of phagocytosis is great enough, the course of therapy would be relatively insensitive to the PD-based antibiotic categories, and bacteriostatic antibiotics can be as effective as bactericidal ones (Figure 3C).

Resisting Resistance

There is no question that, if the dominant population of the target bacteria is genetically resistant to the treating antibiotic, primary treatment will be ineffective – be the infection acute or chronic [59–63]. There is also no doubt that, for chronic infections – like tuberculosis, or those caused by *Pseudomonas* in cystic fibrosis patients – resistance evolving during the course of therapy, acquired resistance, can thwart effective therapy [64]. This is also the case for patients under treatment for endocarditis or metastatic infections, like those with *Staphylococcus* [65,66].

As suggested by this model, if the rate of phagocytosis is low, if minority populations of bacteria resistant to the treating drug are present or generated during the course of therapy, they will ascend to dominance (Figures 2 D and 4 D). Consistent with this prediction is the rapid evolution of mutational antibiotic resistance in neutropenic patients [67–71] or patients with inherited disorders of the phagocytic system [72,73]. If, however, the rate of phagocytosis is great enough, the model predicts that, even if they exist before the onset of therapy, resistant minority populations will not ascend to dominance during the course of treatment.

Implications for Treatment: Sometimes Less Can Be Better

In recent years, the orthodox 'hit them hard', high-dose approach to antimicrobial chemotherapy that has prevailed for more than a century [74] has been challenged by Troy Day, Andrew Read, and their colleagues [28,75–78]. The basis of their proposition about the failings of these 'orthodox' treatment protocols are mathematical models and experiments with a mouse malaria model. In their models and interpretations of their data, treatment failure can be attributed to the ascent of resistance during the course of therapy. By reducing the direct or apparent [28,78,79] competition with dominant populations of susceptible cells, these drugs promote the ascent of minority populations of resistant cells at a rate that increases with the dose of the selecting antibiotic.

We believe that Paul Ehrlich's 'hit them hard', high-dose chemotherapy mandate remains appropriate for life-threatening infections in immune-impaired hosts, where toxicity,

disturbances of the microbiota, and the risks of acquired resistance are secondary to the benefits of rapid clearance of the infecting microbe [80,81] (also see [82]). For acute, normally self-limiting infections in immune-competent hosts, the results of this study support the proposition that there are conditions where low-dosage chemotherapy can be as effective as higher doses, albeit for reasons different from those proposed by Read, Day, and colleagues. In accord with our heuristic model, if the rate of phagocytosis is great enough, low doses of antibiotics can be as effective as greater doses, whether there is a resistant minority population or not.

There is indeed evidence that low doses of antibiotics can be as effective as higher doses (e.g., [83,84]). There is indeed evidence that low doses of antibiotics can be as effective as higher doses (e.g., [83,84]), via mechanisms such as reductions in the virulence of the target bacteria [85], or by antibiotics augmenting the efficacy of phagocytosis [86,87]. Our own observations and models complement these views, supporting the potential advantage of lower dosages.

To be sure, theory and evidence suggest that low doses of antibiotics will select for resistance [88–91]. We are, however, unaware of studies demonstrating this selection for resistance with low doses of antibiotics in situations where phagocytic leucocytes play the dominant role in clearing the infection, as considered in our models. Consistent with this view, selection for resistance occurs more frequently in bacteria colonizing mucosal surfaces than in those involved in invasive infections, probably confronting a stronger phagocytic response. Finally, in modest support for the clinical predictions of this model are the observations for some infections, like yaws and uncomplicated urinary-tract infections, that therapeutic success can be achieved with short-course dosages, including single-day antibiotic therapy [92–95].

Concluding Remarks and Implications for Research

We have focused on the treatment of acute, normally self-limiting infections in immune-competent hosts because this is by far the most common use of antibiotics in the developed world. It is possible that this use of antibiotics as life-style-preserving rather than life-saving drugs is the dominant source of selection for resistant bacteria in the community, and from there into hospitals. In addition to direct selection for resistance in the target bacteria, there is collateral selection for resistance in the commensal microbiota, with the potential for these resistant populations to become opportunistic pathogens [96].

Models Are More Useful When They Do Not Fit the Data than When They Do

Although, general, mechanistic and, we believe, realistic, the model used here is heuristic. Its role is to illustrate principles, generate a hypothesis, and guide and facilitate the interpretation of empirical studies, not to provide numerically precise analogs of the dynamics of the joint action of phagocytic leucocytes, antibiotics, and bacteria in specific situations (e.g., [29]). Arguably, not to us, simple heuristic models of the sort considered here are most useful when they fail in a qualitative way to account for the results of experiments. That way they demonstrate that one or more of the biological assumptions made in the construction of the models are in error. By modifying the models to improve their predictive ability, one can develop and refine a hypothesis about the processes responsible for deviation from fit, and potentially elucidate the mechanisms responsible for the phenomena under consideration.

Although the PD and PK parameters used in our simulations are in realistic ranges for antibiotics and bacteria such as *S. aureus* and *Escherichi coli* [46,97,98], the phagocytosis parameters are chosen to generate dynamics that mimic that of a self-limited infection, and thus ensure bacterial clearance over a clinically realistic term. More precise data and estimates of the different parameters of this model can be obtained *in vitro* by following the changes in the

Outstanding Questions

What are the relative contributions of the patient's innate and adaptive antibacterial defenses and antibiotics to the rates at which these infections are cleared?

How well do antibiotic-dosing regimens based on the pharmacokinetics of these drugs in serum, *in vitro* estimates of MICs, and neutropenic animal model experiments predict the rates at which these infections are cleared?

What is the effect of phenotypic and inherited resistance emerging during the course of therapy on the rates at which these infections are cleared?

densities of viable free and engulfed bacteria in different types of leucocyte phagocytes, with and without antibiotics [99–102]. It should be noted, however, that, in our model, we are not considering the synergistic effects of antibiotics promoting phagocytosis and promoting bacterial killing [87]. The functional synergy between phagocytosis and antibiotics is able to produce a complete eradication of the bacterial population from the infection site, a desired goal in antibiotic chemotherapy [103].

We also see the simple heuristic model of the type used here as a first step in developing a comprehensive theory of the joint action of antibiotics and the innate immune system in clearing bacterial infection (see Outstanding Questions). A more comprehensive model should include the effects of vaccination in enhancing phagocytosis efficiency (opsonophagocytosis) [104–108]. Of particular interest in expanding these models is to develop the theory needed to guide and evaluate programs for the use of antibiotics in vaccinated or nonvaccinated populations.

Hypotheses Are for Testing, Not Championing, and Certainly Not Implementing

Most importantly, the hypotheses derived from the analysis of the properties of these models can be tested (and rejected) with experimental infections in laboratory animals, in particular knockout mice with different inherited defects in the efficacy and densities of phagocytic leucocytes [109,110], and/or by chemically inhibiting or stimulating the production of granulocytes [111]. The idea would be to establish infections in these mice and follow the changes in the densities of bacteria at the site of the infection and in blood with different rates/efficacy of phagocytosis and when treated with antibiotics with different PD properties. In accord with the predictions of these models, at a sufficiently high rate of phagocytosis, antibiotics with different PD properties will be similarly effective, lower doses of antibiotics will be as effective as higher doses, phenotypic resistance (persistence) will have little effect on the rate of clearance, and minority populations of genetically resistant bacteria will not ascend. As the rate of phagocytosis declines, bactericidal drugs will be increasingly more effective than bacteriostatic ones, higher doses of antibiotics will be more effective than lower doses, persistence will play an increasingly important role in the course of therapy, and minority populations of resistant bacteria can ascend and become the dominant population during therapy.

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Resources

[i]https://data.mendeley.com/datasets/cwfsmxt74d/draft/files/5f581be7-a26d-463a-8d0e-13dcdd268e03/FigureS1-1.tif?dl=1

References

1. Gould, I.M. (2009) Antibiotic resistance: the perfect storm. *Int. J. Antimicrob. Agents* 34, S2–S5
2. Harris, P.N. *et al.* (2015) β-Lactam and β-lactamase inhibitor combinations in the treatment of extended-spectrum β-lactamase-producing Enterobacteriaceae: time for a reappraisal in the era of few antibiotic options? *Lancet Infect. Dis.* 15, 475–485
3. Cerqueira, G.C. *et al.* (2017) Multi-institute analysis of carbapenem resistance reveals remarkable diversity, unexplained mechanisms, and limited clonal outbreaks. *Proc. Natl. Acad. Sci. U. S. A.* 114, 1135–1140
4. Levin, B.R. and Antia, R. (2001) Why we don't get sick: the within-host population dynamics of bacterial infections. *Science* 292, 1112–1115
5. Arason, V.A. and Sigurdsson, J.A. (2010) The problems of antibiotic overuse. *Scand. J. Prim. Health Care* 28, 65–66
6. Gulliford, M. *et al.* (2009) Selective decrease in consultations and antibiotic prescribing for acute respiratory tract infections in UK primary care up to 2006. *J. Public Health (Oxf.)* 31, 512–520
7. Quie, P.G. (1984) Phagocytes vs. bacteria, biological detente. *Zentralbl. Bakteriol. Mikrobiol. Hyg. A* 256, 401–407
8. Chung, Y.S. and Kocks, C. (2012) Phagocytosis of bacterial pathogens. *Fly (Austin)* 6, 21–25
9. Sansonetti, P. (2001) Phagocytosis of bacterial pathogens: implications in the host response. *Semin. Immunol.* 13, 381–390
10. Ernst, J.D. and Stendahl, O.,eds (2006)In *Phagocytosis of bacteria and bacterial pathogenicity* (vol. 12), Cambridge University Press
11. Malech, H.L. *et al.* (2014) The role of neutrophils in the immune system: an overview. *Methods Mol. Biol.* 1124, 3–10

12. Culpepper, L. and Froom, J. (1997) Routine antimicrobial treatment of acute otitis media: is it necessary? *JAMA* 278, 1643–1645
13. Froom, J. *et al.* (1997) Antimicrobials for acute otitis media? A review from the International Primary Care Network. *BMJ* 315, 98–102
14. Hoberman, A. *et al.* (2011) Treatment of acute otitis media in children under 2 years of age. *N. Engl. J. Med.* 364, 105–115
15. Rovers, M.M. *et al.* (2006) Antibiotics for acute otitis media: a meta-analysis with individual patient data. *Lancet* 368, 1429–1435
16. Spiro, D.M. *et al.* (2006) Wait-and-see prescription for the treatment of acute otitis media: a randomized controlled trial. *JAMA* 296, 1235–1241
17. Tahtinen, P.A. *et al.* (2011) A placebo-controlled trial of antimicrobial treatment for acute otitis media. *N. Engl. J. Med.* 364, 116–126
18. van Buchem, F.L. *et al.* (1981) Therapy of acute otitis media: myringotomy, antibiotics, or neither? A double-blind study in children. *Lancet* 2, 883–887
19. Venekamp, R.P. *et al.* (2015) Antibiotics for acute otitis media in children. *Cochrane Database Syst. Rev.* CD000219
20. Dykewicz, M.S. and Hamilos, D.L. (2010) Rhinitis and sinusitis. *J. Allergy Clin. Immunol.* 125, S103–S115
21. Rautakorpi, U. (2006) *Common Infections in Finnish Primary Health Care,* Tampere University
22. Howe, R.W. *et al.* (1997) A randomized controlled trial of antibiotics on symptom resolution in patients presenting to their general practitioner with a sore throat. *Br. J. Gen. Pract.* 47, 280–284
23. Sheikh, A. *et al.* (2000) Antibiotics for acute bacterial conjunctivitis. *Cochrane Database Syst. Rev.* CD001211
24. Ankomah, P. and Levin, B.R. (2014) Exploring the collaboration between antibiotics and the immune response in the treatment of acute, self-limiting infections. *Proc. Natl. Acad. Sci. U. S. A.* 111, 8331–8338
25. Drusano, G.L. *et al.* (2011) Impact of granulocytes on the antimicrobial effect of tedizolid in a mouse thigh infection model. *Antimicrob. Agents Chemother.* 55, 5300–5305
26. Gjini, E. and Brito, P.H. (2016) Integrating antimicrobial therapy with host immunity to fight drug-resistant infections: classical vs. adaptive treatment. *PLoS Comput. Biol.* 12, e1004857
27. Handel, A. *et al.* (2009) Exploring the role of the immune response in preventing antibiotic resistance. *J. Theor. Biol.* 256, 655–662
28. Day, T. and Read, A.F. (2016) Does high-dose antimicrobial chemotherapy prevent the evolution of resistance? *PLoS Comput. Biol.* 12, e1004689
29. Drusano, G.L. *et al.* (2014) Interaction of drug- and granulocyte-mediated killing of *Pseudomonas aeruginosa* in a murine pneumonia model. *J. Infect. Dis.* 210, 1319–1324
30. Toutain, P.L. *et al.* (2002) The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. *Res. Vet. Sci.* 73, 105–114
31. Craig, W.A. (1998) Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin. Infect. Dis.* 26, 1–10 quiz 11–12
32. Craig, W.A. (2001) The hidden impact of antibacterial resistance in respiratory tract infection. Re-evaluating current antibiotic therapy. *Respir. Med.* 95, S12–S19 discussion S26–S27
33. Craig, W.A. (2001) Does the dose matter? *Clin. Infect. Dis.* 33, S233–S237
34. Frimodt-Moller, N. (2002) How predictive is PK/PD for antibacterial agents? *Int. J. Antimicrob. Agents* 19, 333–339
35. Sy, S.K. *et al.* (2016) Pharmacokinetics and pharmacodynamics in antibiotic dose optimization. *Expert Opin. Drug Metab. Toxicol.* 12, 93–114
36. MacGowan, A. (2011) Revisiting beta-lactams – PK/PD improves dosing of old antibiotics. *Curr. Opin. Pharmacol.* 11, 470–476
37. Mouton, J.W. *et al.* (2005) Standardization of pharmacokinetic/pharmacodynamic (PK/PD) terminology for anti-infective drugs: an update. *J. Antimicrob. Chemother.* 55, 601–607
38. Roberts, J.A. (2011) Using PK/PD to optimize antibiotic dosing for critically ill patients. *Curr. Pharm. Biotechnol.* 12, 2070–2079
39. Andes, D. *et al.* (2009) *In vivo* pharmacodynamic characterization of a novel plectasin antibiotic, NZ2114, in a murine infection model. *Antimicrob. Agents Chemother.* 53, 3003–3009
40. Andes, D. and Craig, W.A. (1998) *In vivo* activities of amoxicillin and amoxicillin-clavulanate against Streptococcus pneumoniae: application to breakpoint determinations. *Antimicrob. Agents Chemother.* 42, 2375–2379
41. Van Bambeke, F. *et al.* (2006) Cellular pharmacodynamics and pharmacokinetics of antibiotics: current views and perspectives. *Curr. Opin. Drug Discov. Dev.* 9, 218–230
42. Ambrose, P.G. *et al.* (2007) Pharmacokinetics-pharmacodynamics of antimicrobial therapy: it's not just for mice anymore. *Clin. Infect. Dis.* 44, 79–86
43. Mouton, J.W. *et al.* (2011) Conserving antibiotics for the future: new ways to use old and new drugs from a pharmacokinetic and pharmacodynamic perspective. *Drug Resist. Updat.* 14, 107–117
44. Blaser, M.J. (2016) Antibiotic use and its consequences for the normal microbiome. *Science* 352, 544–545
45. Baquero, F. *et al.* (2013) Antibiotic resistance shaping multi-level population biology of bacteria. *Front. Microbiol* 4, 15
46. Regoes, R.R. *et al.* (2004) Pharmacodynamic functions: a multiparameter approach to the design of antibiotic treatment regimens. *Antimicrob. Agents Chemother.* 48, 3670–3676
47. Wiuff, C. *et al.* (2005) Phenotypic tolerance: antibiotic enrichment of noninherited resistance in bacterial populations. *Antimicrob. Agents Chemother.* 49, 1483–1494
48. Levin, B.R. and Udekwu, K.I. (2010) Population dynamics of antibiotic treatment: a mathematical model and hypotheses for time-kill and continuous-culture experiments. *Antimicrob. Agents Chemother.* 54, 3414–3426
49. Finberg, R.W. *et al.* (2004) The importance of bactericidal drugs: future directions in infectious disease. *Clin. Infect. Dis.* 39, 1314–1320
50. Pankey, G.A. and Sabath, L.D. (2004) Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clin. Infect. Dis.* 38, 864–870
51. Nemeth, J. *et al.* (2015) Bacteriostatic versus bactericidal antibiotics for patients with serious bacterial infections: systematic review and meta-analysis. *J. Antimicrob. Chemother.* 70, 382–395
52. Bigger, J.W. (1944) Treatment of staphylococcal infections with penicillin. *Lancet* 244, 497–500
53. Balaban, N.Q. *et al.* (2013) A problem of persistence: still more questions than answers? *Nat. Rev. Microbiol.* 11, 587–591
54. Balaban, N.Q. *et al.* (2004) Bacterial persistence as a phenotypic switch. *Science* 305, 1622–1625
55. Levin, B.R. *et al.* (2014) Persistence: a copacetic and parsimonious hypothesis for the existence of non-inherited resistance to antibiotics. *Curr. Opin. Microbiol.* 21, 18–21
56. Gehring, R. and Riviere, J.E. (2013) Limitations of MIC as the sole criterion in antimicrobial drug dosage regimen design: the need for full characterization of antimicrobial pharmacodynamic profile especially for drug-resistant organisms. *Vet. J.* 198, 15–18
57. Mueller, M. *et al.* (2004) Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: kill curves versus MIC. *Antimicrob. Agents Chemother.* 48, 369–377
58. Udekwu, K.I. *et al.* (2009) Functional relationship between bacterial cell density and the efficacy of antibiotics. *J. Antimicrob. Chemother.* 63, 745–757
59. Cosgrove, S.E. (2006) The relationship between antimicrobial resistance and patient outcomes: mortality, length of hospital stay, and health care costs. *Clin. Infect. Dis.* 42, S82–S89

60. French, G.L. (2005) Clinical impact and relevance of antibiotic resistance. *Adv. Drug Deliv. Rev.* 57, 1514–1527
61. Klugman, K. (2007) Clinical impact of antibiotic resistance in respiratory tract infections. *Med. Mal. Infect.* 37, 6–10
62. Klugman, K.P. (2007) Clinical impact of antibiotic resistance in respiratory tract infections. *Int. J. Antimicrob. Agents* 29, S6–S10
63. MacGowan, A.P. (2008) Surveillance, clinical implications of antimicrobial resistance for therapy. *J. Antimicrob. Chemother.* 62, ii105–ii114
64. Lechtzin, N. *et al.* (2006) Outcomes of adults with cystic fibrosis infected with antibiotic-resistant *Pseudomonas aeruginosa*. *Respiration* 73, 27–33
65. Lash, D.B. *et al.* (2015) Differences in method-specific vancomycin MICs and induced daptomycin resistance in an infective endocarditis patient. *Case Rep. Infect. Dis.* 2015, 175810
66. Sotillo, A. *et al.* (2016) Development of daptomycin resistance during therapy in a patient with methicillin-resistant *Staphylococcus aureus* endocarditis: a case report. *Enferm. Infecc. Microbiol. Clin.* 34, 534–535
67. Sanchez-Diaz, A.M. *et al.* (2014) Emergence and long-lasting persistence of linezolid-resistant *Enterococcus faecium*-ST117 in an oncohematologic patient after a nine-day course of linezolid. *Microb. Drug Resist.* 20, 17–21
68. Mehta, S. *et al.* (2006) Emergence of linezolid-resistant enterococci in a neutropenic patient. *J. Hosp. Infect.* 62, 125–127
69. Prabhu, R.M. *et al.* (2005) Emergence of quinolone resistance among viridans group streptococci isolated from the oropharynx of neutropenic peripheral blood stem cell transplant patients receiving quinolone antimicrobial prophylaxis. *Eur. J. Clin. Microbiol. Infect. Dis.* 24, 832–838
70. Santini, C. *et al.* (1988) Emergence of penicillin-resistant viridans streptococci causing septicemia in granulocytopenic patients. *Eur. J. Epidemiol.* 4, 391–392
71. Carratala, J. *et al.* (1995) Emergence of quinolone-resistant *Escherichia coli* bacteremia in neutropenic patients with cancer who have received prophylactic norfloxacin. *Clin. Infect. Dis.* 20, 557–560 discussion 561–563
72. Kaplan, J. *et al.* (2008) Chediak–Higashi syndrome. *Curr. Opin. Hematol.* 15, 22–29
73. McNeil, J.C. (2014) *Staphylococcus aureus* – antimicrobial resistance and the immunocompromised child. *Infect. Drug Resist* 7, 117–127
74. Ehrlich, P. (1913) Address in pathology, on chemiotherapy: delivered before the Seventeenth International Congress of Medicine. *Br. Med. J.* 2, 353–359
75. Day, T. *et al.* (2015) Is selection relevant in the evolutionary emergence of drug resistance? *Trends Microbiol.* 23, 126–133
76. Huijben, S. *et al.* (2013) Aggressive chemotherapy and the selection of drug resistant pathogens. *PLoS Pathog.* 9, e1003578
77. Kouyos, R.D. *et al.* (2014) The path of least resistance: aggressive or moderate treatment? *Proc. Biol. Sci.* 281, 20140566
78. Read, A.F. *et al.* (2011) The evolution of drug resistance and the curious orthodoxy of aggressive chemotherapy. *Proc. Natl. Acad. Sci. U. S. A.* 108, 10871–10877
79. Read, A.F. and Taylor, L.H. (2001) The ecology of genetically diverse infections. *Science* 292, 1099–1102
80. Woods, R.J. and Read, A.F. (2015) Clinical management of resistance evolution in a bacterial infection: a case study. *Evol. Med. Public Health* 2015, 281–288
81. Rolain, J.M. and Baquero, F. (2016) The refusal of the society to accept antibiotic toxicity: missing opportunities for therapy of severe infections. *Clin. Microbiol. Infect.* 22, 423–427
82. Hansen, E. *et al.* (2017) How to use a chemotherapeutic agent when resistance to it threatens the patient. *PLoS Biol.* 15, e2001110
83. Giamarellou, H. (1993) Low-dosage cefepime as treatment for serious bacterial infections. *J. Antimicrob. Chemother.* 32, 123–132
84. Yoo, S.E. *et al.* (2005) The effect of low-dose doxycycline therapy in chronic meibomian gland dysfunction. *Korean J. Ophthalmol.* 19, 258–263
85. Lorian, V. (1985) Low concentrations of antibiotics. *J. Antimicrob. Chemother.* 15, 15–26
86. Mandell, L.A. and Afnan, M. (1991) Mechanisms of interaction among subinhibitory concentrations of antibiotics, human polymorphonuclear neutrophils, and gram-negative bacilli. *Antimicrob. Agents Chemother.* 35, 1291–1297
87. Root, R.K. *et al.* (1981) Interactions between antibiotics and human neutrophils in the killing of staphylococci. *J. Clin. Invest.* 67, 247–259
88. Andersson, D.I. and Hughes, D. (2014) Microbiological effects of sublethal levels of antibiotics. *Nat. Rev. Microbiol.* 12, 465–478
89. Gullberg, E. *et al.* (2011) Selection of resistant bacteria at very low antibiotic concentrations. *PLoS Pathog.* 7, e1002158
90. Baquero, F. *et al.* (2002) Antibiotic consumption and resistance selection in *Streptococcus pneumoniae*. *J. Antimicrob. Chemother.* 50, 27–37
91. Levin, B.R. *et al.* (1999) Population biology, evolution, and infectious disease: convergence and synthesis. *Science* 283, 806–809
92. Tepper, M. *et al.* (2016) Mass treatment with single-dose azithromycin for yaws. *N. Engl. J. Med.* 375, 1093–1094
93. Pitout, J.D. *et al.* (2016) Tackling antimicrobial resistance in lower urinary tract infections: treatment options. *Expert Rev. Anti Infect. Ther.* 14, 621–632
94. Michael, M. *et al.* (2003) Short versus standard duration oral antibiotic therapy for acute urinary tract infection in children. *Cochrane Database Syst. Rev.* CD003966
95. Rubinstein, E. (2007) Short antibiotic treatment courses or how short is short? *Int. J. Antimicrob. Agents* 30, S76–S79
96. Baquero, F. *et al.* (1990) Capnophilic and anaerobic bacteremia in neutropenic patients: an oral source. *Rev. Infect. Dis.* 12, S157–S160
97. Johnson, P.J. and Levin, B.R. (2013) Pharmacodynamics, population dynamics, and the evolution of persistence in *Staphylococcus aureus*. *PLoS Genet.* 9, e1003123
98. Udekwu, K.I. and Levin, B.R. (2012) *Staphylococcus aureus* in continuous culture: a tool for the rational design of antibiotic treatment protocols. *PLoS One* 7, e38866
99. Miles, A.A. *et al.* (1938) The estimation of the bactericidal power of the blood. *J. Hyg. (Lond.)* 38, 732–749
100. Leijh, P.C. *et al.* (1981) Kinetics of phagocytosis and intracellular killing of *Staphylococcus aureus* and *Escherichia coli* by human monocytes. *Scand. J. Immunol.* 13, 159–174
101. Hampton, M.B. *et al.* (1994) A single assay for measuring the rates of phagocytosis and bacterial killing by neutrophils. *J. Leukoc. Biol.* 55, 147–152
102. Swamydas, M. *et al.* (2015) Isolation of mouse neutrophils. *Curr. Protoc. Immunol.* 110, 3.20. 1-3.20.15
103. Dagan, R. *et al.* (2001) Evidence to support the rationale that bacterial eradication in respiratory tract infection is an important aim of antimicrobial therapy. *J. Antimicrob. Chemother.* 47, 129–140
104. Huang, S.S. *et al.* (2009) Continued impact of pneumococcal conjugate vaccine on carriage in young children. *Pediatrics* 124, e1–e11
105. Song, J.Y. *et al.* (2013) Pneumococcal vaccine and opsonic pneumococcal antibody. *J. Infect. Chemother.* 19, 412–425
106. Whitney, C.G. *et al.* (2003) Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *N. Engl. J. Med.* 348, 1737–1746
107. Wilson, R. *et al.* (2017) Correction: naturally acquired human immunity to pneumococcus is dependent on antibody to protein antigens. *PLoS Pathog.* 13, e1006259
108. Wilson, R. *et al.* (2017) Naturally acquired human immunity to pneumococcus is dependent on antibody to protein Antigens. *PLoS Pathog.* 13, e1006137

109. Sogawa, Y. *et al.* (2011) Inhibition of neutrophil migration in mice by mouse formyl peptide receptors 1 and 2 dual agonist: indication of cross-desensitization *in vivo*. *Immunology* 132, 441–450

110. Rongvaux, A. *et al.* (2014) Development and function of human innate immune cells in a humanized mouse model. *Nat. Biotechnol.* 32, 364–372

111. Zuluaga, A.F. *et al.* (2006) Neutropenia induced in outbred mice by a simplified low-dose cyclophosphamide regimen: characterization and applicability to diverse experimental models of infectious diseases. *BMC Infect. Dis.* 6, 55

112. Bleidorn, J. *et al.* (2010) Symptomatic treatment (ibuprofen) or antibiotics (ciprofloxacin) for uncomplicated urinary tract infection? – results of a randomized controlled pilot trial. *BMC Med.* 8, 30

113. Baquero, F. *et al.* (2015) Public health evolutionary biology of antimicrobial resistance: priorities for intervention. *Evol. Appl.* 8, 223–239

114. Harbarth, S. *et al.* (2002) Outpatient antibiotic use and prevalence of antibiotic-resistant pneumococci in France and Germany: a sociocultural perspective. *Emerg. Infect. Dis.* 8, 1460–1467

115. Grossman, Z. *et al.* (2016) Severe acute mastoiditis admission is not related to delayed antibiotic treatment for antecedent acute otitis media. *Pediatr. Infect. Dis. J.* 35, 162–165