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Competing interests statement
The authors declare that they have no competing financial interests

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OPINION — ANTI-INFECTIVES

Population and evolutionary dynamics of phage therapy

Bruce R. Levin and James J. Bull

Following a sixty-year hiatus in western medicine, bacteriophages (phages) are again being advocated for treating and preventing bacterial infections. Are attempts to use phages for clinical and environmental applications more likely to succeed now than in the past? Will phage therapy and prophylaxis suffer the same fates as antibiotics — treatment failure due to acquired resistance and ever-increasing frequencies of resistant pathogens? Here, the population and evolutionary dynamics of bacterial–phage interactions that are relevant to phage therapy and prophylaxis are reviewed and illustrated with computer simulations.

The history of phage therapy — the use of bacterial viruses to treat bacterial infections — is older than most of the readers of this article (TIMELINE). Prior to the development of antibiotics, research into, and the practice of, phage therapy was a substantial enterprise in Europe, parts of Asia and North and South America, and continues to be a viable, if not thriving, industry in some countries of eastern Europe[1–3] (see phage therapy providers in the Online links). The demise of phage therapy in western medicine in the 1930s and early 1940s can, in part, be attributed to inconsistent therapeutic results and, in part, to its eclipse by effective, broader spectrum antibiotics that became available at that time[4–7]. Ironically, the epitaph of phage therapy was written more than a decade before the genetics of bacteriophage and the mechanisms of bacterial pathogenesis became important subjects of research. Passive immunization, that is, serum therapy for bacterial infections, suffered a similar fate after the advent of antibiotics, despite its demonstrated efficacy for treating *Pneumococcus* bacteraemias and pneumonias[8], diphtheria and other bacterial, as well as viral diseases[9]. The epitaph of serum therapy was written more than 30 years before we knew about T cells and B cells and even longer before the development of monoclonal antibodies.

Now, to paraphrase Victor Hugo, phage therapy is an idea whose time has come again. Fuelled by concerns about antibiotic resistance and lost ground in the antimicrobial chemotherapy 'arms race', the idea of using phages for treating and preventing bacterial infections is experiencing a rebirth. This has taken a number of forms, including the rediscovery of detailed, successful experiments, such as those of H. William Smith and M.B. Huggins[10–12], new experiments[13–19], and mathematical models to facilitate a better understanding of how phage might control bacterial infection[20–24]. Much of this renewed hope for phage therapy also comes from an improved understanding of the genetics and biology of bacteriophage[25–27] and the possibilities offered by genetically engineering bacteriophages for these applications[7,28]. In addition to their use for treating or preventing human infections, phages are being developed for agriculture, to rid environments and domestic animals of the pathogens that could contaminate food supplies[29,30], to control infections in high-density poultry production[31] and for the treatment of fish pathogens in aquaculture[18,32]. Phage have also been proposed as an alternative to antibiotic sprays to control bacterial infections in high value crops, such as citrus canker on oranges[33].

In this perspective, we consider how an understanding of population and evolutionary biology of bacteria–phage interactions will be important to the success and development of the use of phage for therapy and prophylaxis. We first review the elements of the population and evolutionary dynamics of bacteriophage that are necessary to understand how these viruses can prevent or treat bacterial infections, and when their utility for these purposes will be thwarted by resistance. We then consider three different arenas for clinical and epidemiological applications of phages: acute infections — rapidly growing infections by bacteria that, at low densities, can be cleared by the constitutive and/or inducible defences — such as invasive infections caused by *Staphylococcus* or *Pneumococcus*; chronic infections — replicating populations of bacteria that are maintained for extensive periods of time and

are not cleared by the host's defences — such as *Pseudomonas aeruginosa*, which infects cystic fibrosis patients, and *Mycobacterium tuberculosis*; and environmental prophylaxis — killing or reducing the virulence of bacteria before they cause infections — such as killing *Escherichia coli* O157:H7 in the environment, or in cattle, before it contaminates beef carcasses and reducing the density of pathogenic bacteria on fruits and vegetables.

For each of these applications, we address the conditions that are necessary for a phage to limit the proliferation of bacterial populations in treated mammals and the environment, when bacterial resistance to phages is anticipated to evolve, and its impact on the success of phage therapy and prophylaxis. The evolution of phage-resistant bacteria is, in fact, expected for many applications of phage, which leads us to further consider the extent to which phage evolution will overcome bacterial resistance; whether bacterial fitness and virulence will be compromised in bacteria that evolve resistance to phages; and the prospects for using multiphage therapy to avoid resistance. We discuss the role of mathematical models and *in vitro* and laboratory animal experiments in the development of phage therapy and prophylaxis.

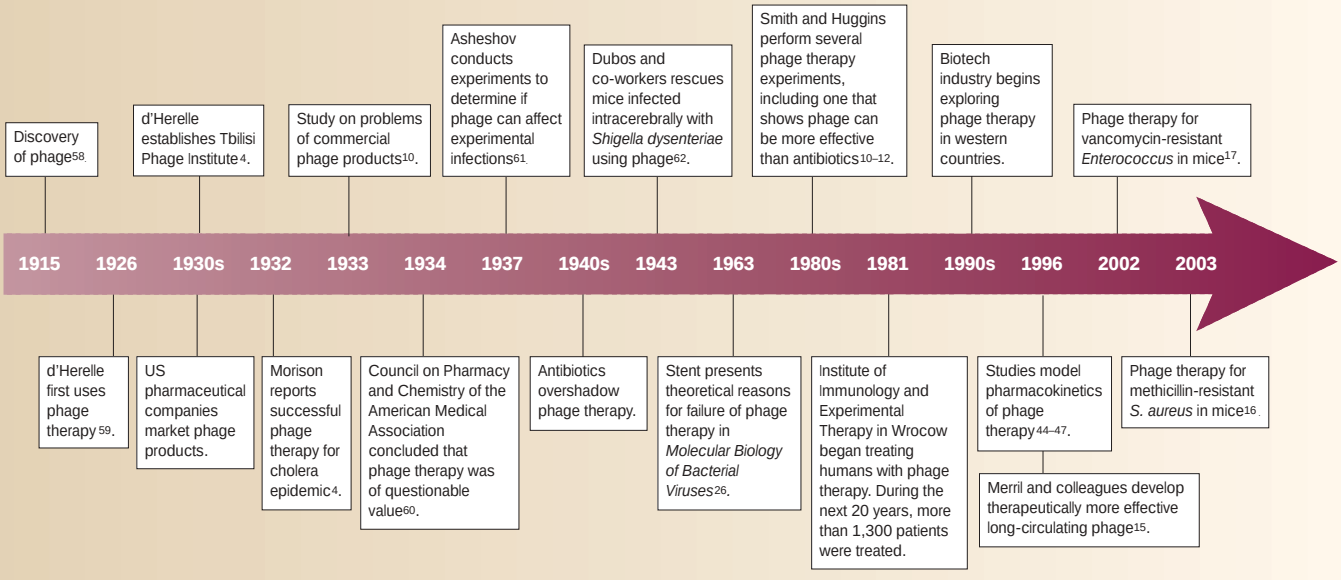
Population and evolutionary dynamics

A useful starting point for understanding how phage can control the proliferation of bacterial populations in therapeutic settings is to consider the parallels with antibiotic therapy. First, both antibiotics and phages have to be maintained at sufficient concentrations or densities, respectively, to reduce the rate of replication of the infecting population of bacteria. Second, these agents must reach the site(s) of the infection and have access to the bacteria when they are susceptible — non-replicating populations of bacteria are physiologically refractory to killing by most phages[25,26,34] as well as by antibiotics[35]. Finally, bacteria can evolve resistance to phages and to antibiotics. Resistance might arise during the course of treatment (acquired resistance) or might be transmitted (primary resistance), and the two origins of resistance have different implications for treatment.

There are also important differences between phages and antibiotics. Compared with commonly used antibiotics, a serious limitation of phages is their narrow host range. For example, not only are most coliphages that are studied in the laboratory restricted to the 'species' *E. coli*, they are able to replicate on only a few strains of this species. So, save for outbreak situations where there might be prior information about the species and strain of the infecting bacteria, the narrow host range of phages might require testing of the infecting strain for phage sensitivity before treatment. This drawback of phages could be minimized by rapid methods for identification of bacteria, or alternatively, by treatments using cocktails of phages that collectively have broad host ranges.

Phage therapy analogues of pharmacokinetics and pharmacodynamics. Whether an antibiotic will be able to kill or inhibit the growth of a susceptible target population of bacteria at a particular site depends on changes in the concentration of the antibiotic in the infected patient and at the site of infection (pharmacokinetics), and also the relationship between the concentration of the antibiotic and the growth or death rates of the bacterial population (pharmacodynamics). For phage therapy, pharmacokinetics is analogous to the change in phage densities in different tissues of the host; and pharmacodynamics is analogous to the population dynamics of the phage–bacterial interaction. There is a profound difference in the population kinetics of phage relative to the pharmacokinetics of antibiotics because phages can replicate. If the target population of bacteria is sufficiently dense and physiologically, and genetically, amenable to phage replication, treatment with just a few phage particles can result in profound increases in phage densities in tissues where the bacteria are most common. This self-amplification of the phage population will not occur when bacterial densities are too low for the phage to replicate faster than the rate at which they are lost, or if the phage does do not reproduce at all in the target bacteria (even though the phage might kill the bacteria). Under these conditions, it would be necessary to continually introduce high densities of phage in the same way that antibiotics are administered[24].

Timeline | Highlights in the development of phage as a potential therapeutic agent for bacterial infections



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Resistance, partial resistance and immunity to phages. There are three primary, inherited, mechanisms by which sensitivity of bacteria to phage could be eliminated or reduced. The first is envelope resistance. By mutations of single genes, usually resulting in the alteration or loss of the receptor to which the phage adsorbs, a bacterium can become refractory to a phage to which its ancestors were susceptible. Envelope resistance is usually limited to the phages that use the receptor (which might include several phage species) and is typically absolute, so that resistant bacteria are no longer killed by the phage and the phage do not replicate in those bacteria. There are also partial resistance mechanisms, often reflected by mucoid colonies, which provide general protection against phages that use different receptors. Phage can adsorb to, and replicate in, partially resistant bacteria, but their rate of adsorption is low and, as a result, the phage have relatively little effect on the density of the bacterial population (modelling of the population dynamics of the phage–bacterial interaction is considered below). Finally, there is restriction-modification immunity. Bacteria, or the plasmids they carry, might encode restriction endonucleases that degrade the genomes of infecting phage and thereby block the phage lytic cycle. In part because many phages have mechanisms to avoid restriction[36], and in part because of the relatively high rate of modification, it is unlikely that restriction immunity will be as important to phage therapy as envelope resistance or partial resistance mechanisms.

Evolutionary arms races: phages fight back, host-range mutation. From one perspective, it would seem that phages have a considerable advantage over antibiotics: they can evolve and thereby overcome bacterial resistance. If resistant bacteria evolve, selection will favour mutant phages that are capable of killing and replicating in these bacteria. In turn, the bacteria can evolve again and produce mutants that are not only resistant to the host-range phages, but are also resistant to the original phage population. Limited experimental work with coliphages has shown that this arms race seems to end after a few cycles of co-evolution, resulting in a resistant bacterial population from which further host-range phage mutants cannot be isolated[37,38]. Perhaps after a transient state in which bacterial densities are low, resistant bacteria almost invariably evolve to dominance in experimental populations of *E. coli* and phage, and the community returns to nearly the same, resource-limited density as in the absence of phage[39,40]. Although there is recent evidence for continuous (or at least longer and more complex) cycles of resistance and host range mutation[41,42], it is not clear whether in this arms race the bacterial population is maintained at a lower density than would occur in the absence of phage. This problem of phage–bacterial arms races requires further basic research.

Modelling the population dynamics of bacteriophage and phage therapy. Simple, or even quite complex mathematical and computer simulation models cannot capture all of the details and complexities of the population and evolutionary dynamics of the interactions between bacteria and phage in flasks or chemostats, much less those in heterogeneous habitats of infected mammals. The utility of simple models is to identify, in a quantitative way, the dominant factors that contribute to the population dynamics and to the evolution of the interactions between bacteria and phage both *in vitro* and in treated mammals. Simple models can be used to generate hypotheses about the conditions under which phage will control or prevent bacterial infections, design phage therapy protocols *a priori* and facilitate interpretation of the results of experimental studies of phage therapy and prophylaxis.

Mathematical models have been used for studying the population and evolutionary dynamics of bacteriophage[43–46] and, more recently, have been used to consider how these dynamics apply to phage therapy[19–23]. In these models, the rate at which a bacterial population declines due to phage predation, the rate at which the phage population increases and the levels at which they are maintained depends primarily on five parameters — the infectivity of the phage, as determined by the adsorption rate, δ; the burst size, or number of phage progeny that are produced from a single cell, β; the latent period, λ, which is the time between adsorption and burst; the rate at which the phage are killed or removed from the site of the infection, ρ; the maximum rate bacterial growth, ψ — and two variables — the density of susceptible bacteria, S; and the density of phage, P. Neglecting the latent period, as long as the rate at which bacteria are attacked and killed by phage (as measured by the product $P\delta$) exceeds the rate at which the bacteria replicate, ψ, the bacterial population will decline owing to phage predation. In addition, as long as the rate of phage replication in sensitive bacteria (as measured by the product, $S\delta\beta$) exceeds the rate of which the phage are killed or removed from the habitat, ρ, the phage population will increase.

The full dynamics of the interactions between bacteria and phage depend on the details of the models, how the populations are maintained over time, the contributions of latent periods, the distribution of variation in these parameters and the existence of refuges[44–46]. To an adequate approximation, the average levels of bacteria and phage in these communities can be seen from the equilibrium points[44], which with the above parameters are, $S = \rho/\delta\beta$ and $P^* = \psi/\delta$. So, phage with a high adsorption rate and burst size would reduce the density of an infecting population of bacteria more than a phage with a lower adsorption rate, but would be present at a lower density.

By adding terms for additional populations of bacteria, *R1*, *R2*, …, these models can be extended to account for the evolution of envelope resistance (a bacterial population with $\delta = 0$) or partial resistance (a bacterial population with $0<\delta<10^{-11}$). By adding phage populations, *P1*, *P2*, …, the models can be extended to account for the evolution of host-range phage (phage with values of $\delta>0$ for bacteria that are resistant to other phage).

In BOX 1, we use computer simulations to illustrate the principles discussed above and the contributions of some of these parameters to the efficacy of phage in controlling the density of a bacterial population and the dynamics and consequences of the evolution of resistance.

Evolution of resistance to multiple phages. By analogy with the multi-drug treatments used against tuberculosis and HIV, the simultaneous use of multiple phages might seem to provide a means of preventing the evolution of a resistant bacterial population. The logic is compelling. Different phages often use different bacterial receptors and therefore require independent mutations to effect resistance to each phage. So, on first consideration it would seem that unless mutants with generalized resistance or partial resistance mechanisms evolve, a cocktail of different phages for which there is no cross-resistance should be able to prevent multiple phage resistance and provide indefinite control of the bacterial population.

Whether this optimistic outcome for multi-phage therapy will be achieved is unclear at this time. We are aware of one theoretical and empirical investigation of this problem, in which bacteria (*E. coli*) were confronted with multiple phages (three) with independent adsorption sites and for which there was no cross resistance; the bacteria also possessed a restriction-modification system to which all three phages were sensitive[47]. The results of this study were inconsistent with the 'optimistic outcome.' The experimental populations were quickly dominated by bacteria with envelope resistance to all three

infecting phage. How general this outcome is remains to be seen. In our opinion, the question of whether resistance can be avoided by multi-phage therapy requires additional theoretical and experimental study. Limited theoretical consideration[47] and the (unpublished) simulations we have done indicate that bacterial evolution of resistance to multiple phages is far more feasible than intuition would suggest. As we discuss below, the answer to this question is particularly relevant to the use of phage to treat long-standing populations of bacteria, such as those in chronic infections and in the environment.

Phage therapy of acute infections

Most of the recent experimental work on phage therapy in humans and other animals has examined acute infections in uncompromised hosts[13,15–17]. We postulate that phages, like antibiotics, are effective in treating these types of infections because they reduce the densities and rates of dissemination of the infecting populations of bacteria to levels at which they can be controlled by the constitutive and specific immune defences of the host. This certainly seems to be the case for *E. coli* K1 infections of the mouse thigh model[10,34]. With more than 3×10^7 *E. coli* K1 inoculated into the thigh, the untreated mouse almost invariably dies of sepsis within 30–40 hours. Almost no mortality is observed when the infecting inoculum contains less than 5×10^6 bacteria (R.M. Zappala, T. DeRouin, N. Walker & B.R.L., manuscript in preparation), so the threshold density determining mortality is approximately 10^7, and treatment merely needs to reduce bacteria to a level commensurate with this threshold. The threshold phenomenon readily explains why bacterial resistance does not thwart the efficacy of treatment in preventing mortality. If the infecting population of bacteria is large enough, phage-resistant cells will be present at the start of therapy or will be generated and, as a consequence of phage-mediated selection, will increase both in density and relative frequency. However, as long as the density of the resistant bacterial population does not exceed a level that can be controlled by the host defences, their presence need not preclude the efficacy of phage therapy. In the event that resistance to single phage strains reduces the efficacy of treatment, multiple phages for which there is no cross resistance might provide a reasonable solution. In BOX 2, we use a simulation to illustrate control of an infection by the host defences and a phage, showing why the emergence of resistance need not preclude successful therapy.

The theoretical prediction described above and illustrated in BOX 2 is supported by the results of experimental studies of phage therapy to treat *E. coli* K1 infections in laboratory mice[10,34]. Although mutants resistant to treatment with K1 phage could be isolated, when phage treatment was administered early enough, the mice survived these otherwise lethal infections.

From studies of acute infections, the fact that a phage seems to replicate well on the bacterium does not assure its efficacy for therapy against that bacterium[10,15,20,34]. Although the therapeutic failure of a phage is readily shown empirically, understanding why a phage that replicates in the target bacteria fails when it is used for treatment is more difficult and requires a quantitative appreciation of the dynamics of the phage infection process[34]. One purely empirical approach towards improving the success of phage therapy has been to search the environment for many phages and to identify those that are most effective in preventing mortality from a normally lethal infection[10]. Alternatively, phages can be selected to perform better in the mammalian host[15]. The population biology perspective indicates that a simple way of screening a pool of naturally occurring phages for those that are most effective therapeutically is to treat the infection with a mixture of phages and to isolate those phages that show the greatest increase in frequency *in vivo*. These would then be used in pure culture to evaluate their therapeutic efficacy. More generally, an understanding of the phage–bacterial dynamics will be necessary if engineering approaches to phage therapy are to be developed.

Phage therapy of chronic infections

In chronic infections, bacterial populations are maintained at substantial densities for extensive periods — years or even decades. Although there might be a measurable, specific immune response to antigens that are expressed by the infecting bacteria, by themselves the specific and constitutive defences of the host mammal are unable to clear the infection, or can only do so slowly. For some chronic infections, such as tuberculosis, single antibiotics are able to control the proliferation or dissemination of the bacteria sufficiently to lead to clinical remission for an extensive period of time before resistance develops and the patient relapses. Multi-drug therapy can also result in indefinite remission. Antibiotics can also reduce the morbidity and the rate of mortality owing to chronic *P. aeruginosa* infections in cystic fibrosis patients. Most commonly, however, antibiotic treatment leads to the emergence of mutant bacteria that are resistant to the treating antibiotics, and which increase in density — thereby replacing the susceptible population of bacteria and ultimately leading to treatment failure.

Can phage do any better than antibiotics in treating chronic infections? Would the use of multiple phages avoid the evolution of resistance that commonly thwarts multi-drug antibiotic therapy of these infections? At this point, it is unclear whether phage can be effective in treating tuberculosis or the *P. aeruginosa* infections of cystic fibrosis, although there have been reports of phage being effective in treating the *Pseudomonas* infections that are responsible for the destructions of skin grafts[15] and infections of burns[49]. On first consideration, it might seem unlikely that a phage would be able to kill *M. tuberculosis*, or other bacteria that survive and proliferate in macrophages or other somatic cells. There is, however, at least one study that indicates that mycobacteriophage kills *M. tuberculosis* and *Mycobacterium avian* in macrophages that are maintained *in vitro*[49]. As noted earlier, even if phages were initially able to sufficiently reduce the densities of bacteria in chronic infections to prevent symptoms, at this time it is unclear whether they will be any better than antibiotics in preventing acquired resistance — even when multiple phages with independent receptor sites are used.

By analogy with chemostat studies, the treatment of chronic infections might present results that are not easily understood without the use of models. The main paradox from chemostat studies is that high densities of (partially) susceptible bacteria can coexist with high densities of phages. Determining which phage provides the best prospect for control of a chronic infection is therefore a fundamentally different matter (and requires different methods) than determining which phage is best for treating an acute infection.

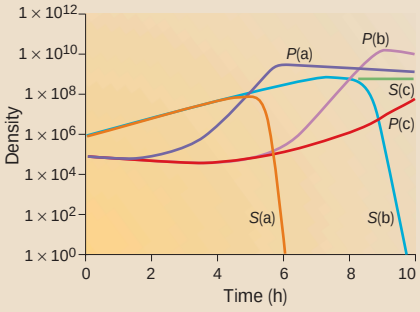
Environmental prophylaxis

In some settings, an environmental population of bacteria is the primary source of colonization and symptomatic infections. Feedlots and cows harbour *E. coli* O157 and other enteropathogenic bacteria that contaminate carcasses; *Staphylococcus* and other bacteria responsible for nosocomial (hospital-acquired) infections are often transmitted by biotic and abiotic surfaces, which are obvious targets for decontamination. Environmental prophylaxis is the use of phage to control populations of bacteria before they cause infections. On first consideration, this application of phage is appealing for several reasons.

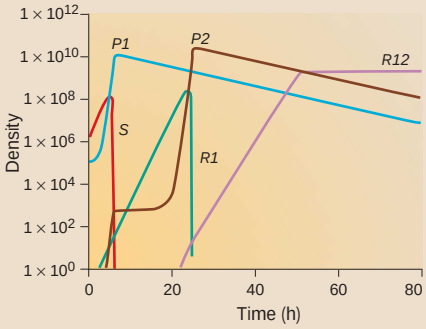
Box 1 | Population and evolutionary dynamics in batch culture

From a population dynamic perspective, the interactions between phage and bacteria are analogous to those of a predator and its prey. When the density of a susceptible population of bacteria (*S*) is too low, the phage (*P*) do not make contact with the bacteria sufficiently frequently for the rate of phage replication to overcome the rate of phage loss (rate, ρ). As a result, the phage population declines, whereas the bacterial population increases. When the density of the bacteria reaches a sufficient level, phage growth becomes positive. As the phage density increases, the density of the susceptible bacteria, *S*, declines owing to phage-mediated mortality (figure part **a**). Quantitatively, the rate at which the density of phage increases is proportional to how well these viruses can capture bacteria — as measured by the adsorption rate, δ — and also to how rapidly and efficiently they convert these captured bacteria into phage — as measured by the latent period, λ, and burst size, β; see figure part **a**. In this figure, β = 100 particles, λ = 0.25 hours and ρ = 0.10 hour^{-1}. δ = 10^{-8}, 10^{-9} and 10^{-10} for curves (a), (b) and (c), respectively.

a **The contribution of the adsorption rate, δ, to the control of a susceptible bacterial population**



b **Semi-stochastic simulation of the evolution of bacterial resistance and host-range phage**



If bacteria that are resistant to the dominant phage, *P1*, arise by mutation from the population of *S*, the density of resistant bacteria, *R1*, increases because they are not killed by phage, despite a growth rate disadvantage relative to their phage-sensitive ancestors; see figure part **b**, which shows the changes in density over time of *S*, *P1*, *R1*, *P2* and *R12*. The selection coefficients for *R1* and *R12* are 0.1 and 0.3, respectively. The rates of mutation to resistance in the bacteria and host-range changes in the phage are both μ = 10^{-8}, and the total volume of the habitat is assumed to be *V* = 10 ml. As the resistant bacteria (*R1*) become more common, selection increasingly favours a host-range phage, *P2*, that can replicate in both the resistant and the susceptible bacteria. These host-range phages arise by mutation in the original *P1* population, increase in density, reduce the *R1* bacterial population, and become the dominant population of phage. Owing to the increase in the *P2* phage population, bacteria that are resistant to *P1* and *P2* (*R12*) have an advantage, and when they are generated by mutation from the *R1* population, they increase in density and become the dominant bacteria. In the short-term, this 'evolutionary arms race' stops when there are no more host-range phage mutants that can replicate in this second-order resistant population (figure part **b**). The equations for the simulations depicted here and in BOX 2 are presented in online BOX S1.

In simulations of the population and evolutionary dynamics of the phage–bacteria interactions shown in the figure, the phage can eliminate all of the sensitive bacteria in the culture. In reality, there are at least two reasons not to anticipate complete extinction of the bacterial population. First, there may be physical refuges, such as surfaces or biofilms, where the phage are unable to adsorb to the bacteria[47]. Second, the bacterial population might reach stationary phase and therefore might be physiologically refractory to the phage.

In these simulations, several assumptions are made — the maximum rate of growth of the susceptible bacteria (*S*) is assumed to be ψ = 1 hour, the concentration of resource where the growth rate is half its maximum value, k, is equal to 0.25 μg ml^{-1}, the conversion efficiency, e, is 5 × 10^{12} μg, the maximum amount of resource, *R*, is 1,000 μg ml^{-1} and the rate of adsorption is constant (see online BOX S1 for simulation equations).

First, the narrow host range of phages might not be problematic because there are often one or only a few pathogenic strains of bacteria that are responsible for contamination of a particular environmental setting. Second, preventing infections is more attractive and cost-effective than treating them. Third, as long as the phage can adequately replicate in the target populations of bacteria, environmental prophylaxis would not require continuous applications of phages. But will phage prophylaxis work, either in the short or the long term? Smith and Huggins[11,12] obtained short-term success with phage prophylaxis for the treatment of calf diarrhoea, and the study was so thorough and convincing that its potential use in other settings seemed assured. At this time, however, owing to the problems of resistance, it is unclear how long a phage prophylaxis protocol will work.

In the broadest sense, phage prophylaxis encompasses a heterogeneous set of applications that defies any single approach. The target population of bacteria might be replicating and physiologically susceptible to phage infection, or be in stationary phase and refractory to phage infection. The environment might be homogenous so that all the phage have access to their target bacteria and the bacteria are dense enough for the phage to replicate. Or the population of bacteria could be physically structured, with only pockets of sensitive bacteria that are sufficiently dense for phage replication. The population dynamic perspective indicates that each of these settings presents different challenges. If the target population is replicating, phage prophylaxis has direct parallels to phage therapy for chronic infections and could be thwarted by phage-resistant bacteria; moreover, the widespread application of phage in this way might promote the evolution of resistant strains that preclude the efficacy of phages for treating infections. If the population is not growing, or growing only slightly, phages might not be able to replicate in or kill the bacteria. Structured bacterial populations (for example, biofilms and bacterial populations that are growing on other surfaces) might afford limited opportunities for phages to access the bacteria. Each application for prophylaxis might have its own idiosyncrasies and therefore require specific models and methods to evaluate and effect success.

Primary (transmissible) resistance

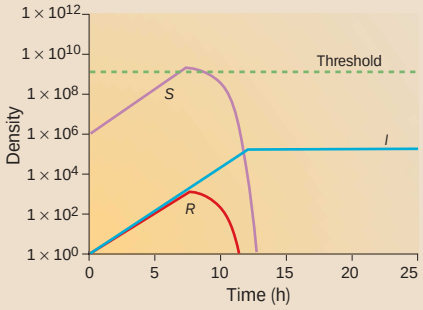
Acquired resistance might not preclude the effective phage treatment of acute, or possibly even chronic, infections of susceptible pathogens. Phage, however, would not be effective in treating infections with virulent

bacteria that are already resistant to their action — primary resistance. At this time, it is not clear whether the widespread use of phages to treat or prevent infections with specific pathogens will lead to ever-increasing frequencies of pathogens that are resistant to their action — in the same way that has been seen for antibiotics. Clearly, bacterial resistance has not lead to the extinction of phage at large, and although naturally occurring strains of bacteria might be resistant to the phage that attack other strains of their species, that resistance could be coincidental — a property of that bacterial strain — rather than a property that has evolved in response to confrontation between that strain of bacteria and that phage.

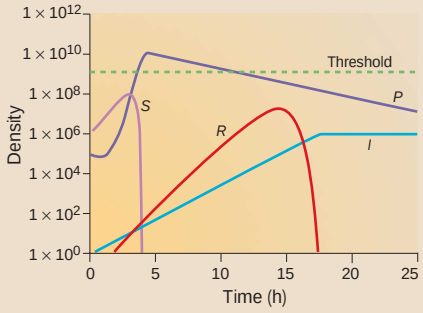
Box 2 | Acute infection with host defences supplemented by phage therapy

When a mammalian host is infected by bacteria, a series of signal transduction events activate several constitutive host defence mechanisms, including the release of cytotoxic chemicals and the migration of phagocytic cells to the site of infection. The net effect of these responses is a decline in the density of the bacterial population, possibly followed by clearance of the bacteria. This first line of defence is relatively rapid and nonspecific, in contrast to the specific immune response, which might take days or weeks to become activated[55,56]. To model the control of bacteria by this early mechanism, we follow others in assuming that this nonspecific host response starts at an initial level and increases in proportion to the density of the bacteria at the site of the infection[20,57]. In the absence of intervention, the density of the bacterial population (*S*) rises rapidly until the magnitude of the host defences (*I*) is sufficient for the rate of bacterial mortality to exceed the bacterial growth rate (figure part **a**). We assume there is a threshold — 10^9 bacteria ml^{-1} — beyond which the host dies. With the parameters of this simulation, the defences of the host alone are insufficient to keep the bacterial density below this threshold level. The addition of phage (*P*) tips the balance in favour of the host (figure part **b**). Although the density of the bacteria increases, owing to the combination of phage and the host defences, the bacterial growth rate becomes negative before the density reaches the lethal threshold. Although phage-resistant bacteria increase in density (*R*), their population is also reduced by the host defences before they reach the lethal threshold.

a Host control of a phage-sensitive and phage-resistant population of bacterial infection in the absence of phage



b Host and phage control of a phage-sensitive and phage-resistant bacterial infection in the presence of phage



In simulations of an acute infection with host defences supplemented by phage therapy, shown in the figure, the resource concentration and bacterial growth parameters are identical to those in the simulations in BOX 1 — the maximum rate of growth of the susceptible bacteria (*S*) is assumed to be $\psi = 1$ hour and the concentration of resource where the growth rate is half its maximum value, k, is equal to 0.25 μg ml^{-1}. In these simulations, the phage parameters are $\beta = 100$ particles, $\lambda = 0.5$ hours, $\rho = 0.10$ $hour^{-1}$ and $\delta = 10^{-8}$. The host defence function used in this model is shown in equation 1.

$$\frac{dI}{dt} = \alpha I \left(\frac{N}{N + k_N} \right) \qquad (1)$$

where *N* is the total density of bacteria. In these simulations, the maximum rate at which the host defences increase was assumed to be $\alpha = 1$ $hour^{-1}$, the density of bacteria at half the maximum rate of increase in the host defenses was assumed to be $k_N = 10^4$ and the host killing parameter was assumed to be $\nu = 10^{-4}$ (see online BOX S1). The horizontal broken line (10^9 bacteria ml^{-1}) is the lethal threshold above which the infection cannot be controlled by the host defences alone.

Resistance might not be all bad

There are circumstances in which the evolution of phage-resistant bacteria could be positive. Resistance can reduce the fitness of the bacteria[37,38] and could thereby impair the ability of the bacteria to compete with its phage-sensitive ancestors and colonize mammalian hosts. Moreover, the receptors that are used by some phage to attack bacteria might be capsules or other virulence determinants, which implies that the development of phage resistance would immediately impact virulence[10–12,28]. In this case, bacterial evolution of resistance to phage would have the benefit of creating mutant bacteria that were no longer capable of causing disease, and as long as phage are present as a selective agent, the resistant, avirulent mutants might replace virulent forms. Phages could even be chosen specifically for this property. A possible complication to this hypothesis is that subsequent evolution might restore the fitness or virulence of the resistant bacteria, either by selecting susceptibility or by compensatory evolution through second site mutations, as has been observed with antibiotic resistance[50–53]. At this time, we lack sufficient data to make a general statement about whether resistance to single and multiple phages will commonly engender a cost in the fitness, or reduce the virulence of, pathogenic bacteria, or how those costs will change through subsequent evolution. These data can certainly be obtained and will be crucial both to the development of phage therapy and for predictions about the problems of acquired and primary (transmissible) resistance to therapeutic phage.

Conclusions and recommendations

It would seem that increasing problems of antibiotic resistance and environmental costs of using antibiotics for these purposes provides sufficient motivation for the development of phages and other alternatives to prevent and treat bacterial infections in domestic animals, aquaculture, some crops, and perhaps in decontaminating food supplies. Phage success in these agricultural endeavours will be a stepping stone for their development for human medicine. Less clear at present is whether the financial, as well as the medical, veterinary and public health incentives, are great enough to promote substantial programmes for the research and

development of protocols for phage therapy and prophylaxis. Also remaining to be seen is how the regulatory agencies will respond to the use of replicating and evolving viruses for treatment and environmental prophylaxis. Success in even limited applications of the use of phage for therapy and prophylaxis, and carefully controlled studies of the evolutionary, as well as environmental effects of these applications, will be crucial to these financial and regulatory considerations.

We, like many others, believe there is sufficient scientific justification to study and develop the use of bacteriophage for therapy and prophylaxis. Our knowledge of the genetics, physiology, molecular biology and population and evolutionary biology of bacteriophage and the mechanisms of bacterial pathogenesis is much greater than 60 years ago. This knowledge is being applied for the rational, as opposed to purely empirical, development of phages and protocols for their use in the treatment and prevention of bacterial infections. On the basis of the results of studies so far (many of which are cited above), there is good reason to believe that the development of phage for treating and preventing bacterial diseases will be successful, at least in limited settings.

Mathematical and computer simulation models can be important in the development of phage for therapy and prophylaxis. They can be used to understand, in the necessarily quantitative way, the conditions under which phage can control bacterial populations in infected mammals and the environment; to generate hypotheses, interpret the results and explain the successes and failures of experiments using phage to control populations of bacteria; and to design and evaluate protocols for the use of phages toward these ends. However, unless the assumptions behind the construction of these models are well justified empirically and the values of the parameters used in the analysis of their properties are in realistic ranges, their utility will be restricted to the career opportunities they provide for the modellers.

Given the time scale and the magnitude of the motivation, it is understandable that the development and widespread use of antibiotics to treat bacterial infections long preceded the serious concerns about the evolution and spread of resistance, much less the development and use of measures to prevent or limit this evolution. Although it is easy to make postulates about resistance (such as those presented here), we do not yet know if acquired or primary resistance will become problematic for phage prophylaxis and therapy. These empirical questions have to be, and can be, readily addressed. Relative to the euphoric, miracle-drug phase of the antibiotic era, the development of phage therapy and prophylaxis will almost certainly remain a modest enterprise, both in size and pace. There be will be plenty of time to address the problem of resistance to phage and to develop procedures to minimize the effects of this resistance.

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Competing interests statement

The authors have declared that they have no competing financial interests.

Online links

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