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Use of phage therapy to treat long-standing, persistent, or chronic bacterial infections☆

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ABSTRACT

Viruses of bacteria – known as bacteriophages or phages – have been used clinically as antibacterial agents for nearly 100 years. Often this phage therapy is of long-standing, persistent, or chronic bacterial infections, and this can be particularly so given prior but insufficiently effective infection treatment using standard antibiotics. Such infections, in turn, often have a biofilm component. Phages in modern medicine thus are envisaged to serve especially as anti-biofilm/anti-persistent infection agents. Here I review the English-language literature concerning in vivo experimental and clinical phage treatment of longer-lived bacterial infections. Overall, published data appears to be supportive of a relatively high potential for phages to cure infections which are long standing and which otherwise have resisted treatment with antibieiotics.

Contents

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus* or multi-drug-resistant *Staphylococcus aureus*.

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1. Introduction

It is only relatively recently that the importance of biofilms in bacterial infections has come to be appreciated, e.g., Percival et al. [1, 2]. In part this lag reflects that it is over only the past few decades that the very existence and importance of biofilms, in microbial ecology, has been recognized [3–5]. Paralleling these trends, though generally only coincidentally, has been a rebirth within Western medicine of an awareness of the potential for bacterial viruses (bacteriophages or phages) to serve as antibacterial agents [6, 7]. As part of that rebirth, phage interactions explicitly as anti-biofilm agents have been studied now for over 20 years—particularly see Doolittle et al. [8] for an early example. Here I consider phage use as antibacterial agents especially within the context of treatment of long-standing, persistent, or chronic bacterial infections, infections which are thought to typically possess a biofilm component [4, 9–15]. Included among such infections which could be subject to phage treatment are chronic otitis, chronic wound infections, lung infections associated with cystic fibrosis, osteomyelitis, and chronic urinary tract infections, etc.

Bacterial infections which are not acute in their duration at an absolute minimum are ones which persist over long time frames. They often involve, as well, chronic inflammatory responses along with interference with tissue healing—even when infecting bacteria are difficult to culture or relatively low in number. Persistence is a consequence of a body's immune system failing, on its own, to clear an infection. The existence of persistent bacterial infections in this modern era, however, typically also represents a failure of medical treatments to fully clear infections. Thus, we usually have two general underlying bases of long-lasting bacterial infection, both having to do with inabilities of various mechanisms and approaches to effectively eradicate bacterial etiologies: (A) genetic resistance to antibacterial mechanisms or strategies, on the one hand, e.g., antibiotic resistance [16, 17], and (B) phenotypic tolerance of antibacterial mechanisms or strategies, on the other, e.g., antibiotic tolerance [18–22]. Thus, bacterial infections can persist either because of a lack of drugs or mechanisms – the latter including the body's – to which etiologies are inherently susceptible (resistance), or due to the potential, especially for biofilms, to be less than fully susceptible to antibacterial actions, despite otherwise remaining genetically susceptible as determined in the laboratory (tolerance). Dealing with these issues requires two slightly different approaches. First is the discovery of new antibacterial drugs or strategies to which resistance has not yet evolved. Second is identifying drugs or strategies which may be less impacted by mechanisms of antibacterial tolerance. Potentially addressing both issues is the use of bacteriophages as anti-biofilm/anti-bacterial infection agents.

Phage therapy is bacteriophage use as antibacterial medicaments [6, 23]. Alternatively, and more generally [24], is what can be described as a phage-mediated biocontrol of bacteria as found within environments. As I and many others have discussed elsewhere, e.g., [25–28], an important utility of phage therapy, or biocontrol, is its relative safety. As a consequence of the growing need for somewhat non-toxic antibacterials, ones which are active even against pathogens which are otherwise multiply antibiotic resistant, publications mentioning phage therapy appear to be growing exponentially, doubling in number, according to Google Scholar, approximately every 4 years since 2003 [7]. A growing literature furthermore considers the phage therapy or biocontrol of bacterial biofilms [29, 30].

Notwithstanding this increasing knowledge base, the phage therapy of long-standing, persistent, or chronic infections is still poorly understood, especially mechanistically. Towards increasing that understanding we must consider a three-component, somewhat ecological system consisting of phages (as the antibacterial), infecting bacteria (often in a biofilm form), and the patient (providing biofilm substratum, nutrients, and immune responses). A thorough discussion of phage use as anti-biofilm as well as anti-chronic/-persistent/-long-standing-bacterial-infection agents consequently could consider (i) the phage-biofilm in vitro-treatment literature, (ii) the use of phages to treat long-standing or biofilm-associated bacterial infections experimentally in vivo or in situ such as in terms of animal models, (iii) the use of phages to treat long-standing bacterial infections in the clinic, (iv) mechanisms of phage-biofilm interactions as they may occur over smaller spatial scales (i.e., as within individual biofilms), and (v) phage-biofilm ecological interactions as they may occur over larger special scales (up to whole environments). Here, emphasis is on categories (ii) and (iii), in Sections 2 and 3 (plus Section 4), respectively. Discussion of the possible impacts of patient immunodeficiencies on treatment success is provided in Section 5. Though points (iv) and (v) are not emphasized here, I have recently reviewed these various aspects of phage-biofilm interactions [29–31], while documentation of the in vitro phage therapy-of-biofilms literature, point (i), can be found in Abedon [29, 30]. A general conclusion is that there is much reason to be hopeful that phages may be used effectively against long-standing bacterial infections, including ones which have proven to be resistant to more conventional treatments, that is, particularly following treatments involving the use of antibiotics.

Note that throughout this article I use the terms 'long-standing', 'persistent', and 'chronic' to describe bacterial infections with the goals in this usage simply to contrast with acute bacterial infections. Thus, targets of phage therapy may consist of acute bacterial infections – as seen with a majority of phage therapy animal experimentation – or instead infections which are not acute, i.e., as seen with a majority of phage therapy clinical treatments [7]. The latter is particularly given treatments preformed in locations other than the former Soviet Union [32, 33], i.e., where phage therapy is not otherwise a routine aspect of clinical practice. This review in particular should thus be viewed as an overview of progress that has been made in the phage treatment of bacterial infections which would be characterized as persistent or chronic and thereby no longer acute.

2. Experimental treatment of long-standing bacterial infections

Though animal models of acute bacterial infections are routinely employed in phage therapy experiments, the use of animal models to study the phage treatment of bacterial infections which are relatively long in duration is less common. This lesser emphasis in part is likely due simply to time requirements for development of such bacterial infections, which ideally will involve infections which have progressed, prior to treatment, for weeks or even months, and must be so maintained without excessive animal morbidity or mortality. Alternatively, one can study naturally occurring infections, e.g., Hawkins et al. [34]. Naturally occurring infections, however, obviously will be less well controlled, animals will not necessarily be easily obtained for study and experimentation, and infections themselves will not necessarily respond to the same phages as would be employed clinically with humans. The in vivo literature for phage therapy treatment of long-standing bacterial infections in any case simply is not terribly robust. This relative lack of study is problematic given the likelihood that much of the current utility of phage therapy in Western medicine would be towards the treatment of long-standing bacterial infections, ones which have not been cured via standard antibiotic treatment.

In this section I review specific animal studies of phage treatment of potentially persistent, chronic, or biofilm-associated infections (plus two in vitro studies). In the following section (Section 3) clinical studies are equivalently considered. First, however, I address the question of just how, within the context of phage treatment, we may judge model infections in animals as persistent or chronic.

2.1. Judging infections as persistent or chronic

Towards emulation of long-standing bacterial infections afflicting human patients, how might we judge animal models? Elsewhere I have considered just that question [35]. There I supply a list of animal models of phage treatment of what presumptively are bacterial infections which at least approach a persistent or chronic state. Keep in mind that a list of studies which consider phage treatment of presumptive biofilm-associated infections within animals would be somewhat longer, e.g., see for example as listed in Abedon [30], but these biofilms would not necessarily be approaching full maturity and therefore would not necessarily be legitimately deemed as persistent or chronic. Thus, the primary criteria I used to generate the above-noted list [35] was an indicated delay of over 24 h between bacterial challenge and the initiation of phage treatment. Even simply taking into account likely delays in the real-world between the initiation of infections and infection presentation to physicians, however, delays in excess of 24 h are highly likely even for acute bacterial infections. Furthermore, there is no reason to believe that a 24-h biofilm (or infection) has fully matured [2]. Rather, the 24 h cutoff represents a first-approximation means of distinguishing between the treatment of experimental infections which explicitly are acute rather than having at least slightly matured in an animal model, while at the same time not reducing consideration of appropriate animal studies to too small a number. Furthermore, as model infections in animals often are initiated with bacterial challenges which are rather large relative to the initiation of naturally occurring infections, in fact there is reason to believe that such model infections are at least potentially more mature at the 24-h point than generally would be the case with naturally occurring infections. In this subsection I further elaborate upon the discussion presented in Abedon [35] concerning how to judge experimental infections as chronic for the sake of phage therapy testing.

2.1.1. Criterion 0, infection duration

The issue of delay between bacterial challenge and initial phage application I describe as Criterion 0 in Table 1, where Table 1 otherwise serves to summarize various criteria which may be used to judge an infection as persistent or chronic prior to phage therapy experimentation. I use the number zero, rather than 1, because of the obviousness of this criterion, i.e., that persistent bacterial infections require time to become established. To the extent that animal models are intended to test the phage potential to treat bacterial infections which have already been subject to substantial antibiotic treatment, especially failure to adequately respond to such treatments over weeks or months, then those models should strive to mimic such bacterial infections as they present clinically in human patients, hence the multiple additional criteria presented in this table and as discussed below. In any case, as noted in the previous paragraph, a 24-hour cutoff to distinguish "acute" infections from more persistent ones is being used entirely for pragmatic reasons, i.e., so as to allow for the consideration of more than just a small handful of studies, rather than because 24 h of infection maturation prior to the start of treatment, even given infection initiation with relatively large numbers of bacteria, in any way implies that an infection by this point has taken on all of the characteristics of a persistent bacterial infection. Further in considering Table 1, note the odd situation with phage therapy research where in many cases pre-clinical studies are in effect attempting to replicate rather than foreshadow actual clinical experience and clinical results [36].

2.1.2. Criterion 1, infection stasis

An alternative and indeed more relevant means of assessing what amount of time is adequate between bacterial challenge and the start of treatment – to signify that an infection is in fact persistent or chronic – is to consider additional infection characteristics. Acute infections tend to worsen over time or, alternatively, will spontaneously resolve, i.e., as due to the action of the immune system. Persistent or chronic bacterial infections to a large extent should do neither. Thus, in terms of monitoring an infection, long-standing, persistent, or chronic bacterial infections, at an absolute minimum, are ones that neither substantially worsen nor substantially lessen in terms of signs over reasonably long time frames, which pragmatically speaking in terms of experimental systems could be a week or longer, and as averaged over multiple animals. Importantly, however, this relative lack of especially easily observable change is not necessarily identical to the maturation of infections into what may be seen in the clinic following months or years of infection persistence prior to phage treatment, nor indeed may the underlying pathophysiological basis of infection persistence, such as seen with treatment of diabetic wound infections (below, Section 3.6.1), be easily replicated in animal models. Still, such measures should be viewed as preferable to simply declaring some arbitrary time point (e.g., 24 h) as representing the dividing line between somewhat acute and somewhat less acute.

Related to the issue of relative infection stasis is that of experimental end points. If end points generally are ones of either animal mortality or instead to the occurrence of infection clearance without treatment, particularly over relatively short time periods, then the model should *not* be viewed as that of a persistent or chronic bacterial infection. Thus, results which tend to be stated in terms of fewer animals died given treatment generally are not of such infections, nor experiments in which infections clear both with and without treatment, just faster with

Table 1
Criteria for assessing experimental bacterial infections of animals as persistent or chronic.

	Criteria[a]	Comment	Requirement[b]
0	Adequate delay between bacterial challenge and initiation of phage treatment	At a minimum, here and somewhat arbitrarily, at least a 24-hour delay is expected, but ideally much longer time delays may be necessary (weeks to months) to truly give rise to adequate models of persistent or chronic bacterial infections	Required (sufficient delays between bacterial challenge and initiation of treatment)
1	Demonstration of infection stasis	To be considered persistent or chronic at the time of treatment, an infection should have reached stages where it is neither spontaneously lessening nor worsening over time, to a substantial extent, but instead should remain more or less constant in terms of signs, including microbiology, for at least a week given mock treatment	Required (studies involving infection stasis generally are ones which do not primarily measure differences in animal mortality nor differences in the rapidity of infection resolution, but instead consider infection resolution relative to a lack of observation of change with treatment-negative controls)
2	Treatment equivalent to as performed or as anticipated being performed in the clinic	Especially if treatment, such as debridement, is performed in the clinic, than observance of efficacy absent such action in experiments but not clinically would represent evidence against the adequacy of a given long-standing bacterial-infection model	Required (use of co-treatments which are deemed necessary in the clinic should be both used and necessary for efficacy, unless studies are specifically looking at potentials to avoid such co-treatments)
3	Successful phage treatment generally should not be trivially accomplished	If experimental treatments require, for example, only a single phage application to achieve cure, then model infections may not adequately represent long-standing, persistent, or chronic bacterial infections as they present clinically, which often require multiple dosing; complicating this criterion, treatment difficulties necessitating more robust strategies instead can be a consequence of other than infection characteristics, such as poor phage choice	Two of three (not required especially to the extent that clinical treatments using phages have proven to be similarly easily accomplished; towards pre-clinical development, however, model infections lacking in challenge will offer little means for testing new treatment strategies for improving efficacy)
4	Biofilms typically should be present	Not all chronic or persistent bacterial infections necessarily involve biofilms, but if biofilms are present clinically then they should be present in animal models, and ideally should display equivalent levels of maturation; other alternative pathophysiological means of distinguishing an infection as persistent or chronic may be substituted for demonstration of biofilm presence	Two of three (to the degree that biofilms in fact are not present clinically, then explicitly biofilm presence should not be required towards evidencing the adequacy of models of chronic bacterial infections, though as noted suitable alternative markers may be substituted)
5	Demonstration of antibiotic tolerance	Antibiotic tolerance represents an important justification for phage use against long-standing, persistent, or chronic bacterial infections (with antibiotic resistance another but distinct reason); therefore, in many cases antibiotic tolerance should be present in models of such infections	Two of three (antibiotic tolerance is not necessarily easily demonstrated, and this is particularly so to extent that multiple antibiotic-resistant bacteria are being treated)

[a] Note that in Abedon [35], but not here, I had included Criterion 1 within that of adequate delays (Criterion 0). Numbering here and in the main text after Criterion 1 thus is greater by 1 relative to as presented in Abedon [35].
[b] Criteria 0, 1, and 2 should all be viewed as required while for 3, 4, and 5 ideally at least two of these three criteria will be met.

treatment than without. Instead, what one should be looking for is improvements in signs, including in terms of infection microbiology, given treatment versus a relative lack of change in signs absent treatment, with the latter especially as measured over longer time periods, e.g., more than just a few days post the start of treatment. Thus, without treatment a non-acute bacterial infection – that is, as being considered here – should once established simply not result in substantial changes in either levels of animal morbidity nor mortality over time.

2.1.3. Criterion 2, treatment equivalence

Even starting with sufficient delays between bacterial challenge and the start of treatment, it is reasonable also to expect that animal models of long-standing bacterial infections, to the extent possible, will be treated in a manner which anticipates treatment in the clinic. That is, this author assumes that animal studies at some level do indeed represent pre-*clinical* analyses, rather than more simplistic exercises in phage therapy proof-of-principle. These experiments thereby should be at least attempting to treat infections in a manner that would be translatable to human treatment. Thus, for example, for persistently infected wound models, debridement would be anticipated. Ideally, then, animal models for the phage treatment of biofilms will employ infections which have been maturing for at least longer than 1 day (and, ideally, for quite a bit longer), have stabilized in terms of signs (which will require somewhat more than 1 day to assess), and then which will be treated in a manner which anticipates or, instead, which recapitulates treatments as either they would be or are performed clinically. This criterion is relevant especially in terms of addressing the adequacy of an experimental infection as a model for treatment development and testing, versus simply indicating whether an infection technically may be viewed as long-standing rather than acute. That is, how well does the infection mimic what we expect to face in the clinic in terms of necessary approaches to treatment?

2.1.4. Criteria 3 to 5, further robustness

Criterion 0 can be viewed as a first approximation, Criterion 1 as actual demonstration of a basic persistent or chronic nature of an infection, and Criterion 2 as indicating a greater robustness of a model in terms of its representation of an infection as it either is or will actually be treated clinically. Building on these criteria of adequate delay, infection stasis, and translatability, in Abedon [35] I then present three more criteria, suggesting that conformity to at least two might be viewed as indicative of a reasonable model of a persistent or chronic bacterial infection. Conformity to only one, or, indeed, to none of these further criteria, however, should be viewed simply as lower levels of rigor in demonstrating the suitability of such infection models for pre-clinical testing and/or development of phage therapy protocols, that is, rather than outright rejection of the usefulness of such models. Especially the first of these (Criterion 3) and the third (Criterion 5) are drawn explicitly from reported experience treating long-standing bacterial infections with phages in the clinic, with Criterion 3 arguably the most important of these additional criteria. Indeed, all three should be viewed as further measures of the degree to which infection models are robustly mimicking what may be observed clinically.

Note that an argument can be made that Criteria 3 to 5, as well as 1 and 2, should be more relevant to describing an infection as not acute, that is, rather than adhering simply to potentially arbitrary durations of infections prior to the initiation of treatment (Criterion 0). Though I certainly agree with this point, at the same time a *lack* of adequate infection development or maturation prior to treatment initiation will be expected to contribute to an *inadequacy* of a model. For example, at an extreme would be the relatively common practice of simultaneous phage and bacterial administration to an animal. Therefore, though in principle it should be possible to precisely authenticate a model infection as adhering to Criteria 1 through 5 and thereby as adequate for experimentation despite some only *minimal* duration of development – in

so doing potentially rendering Criterion 0 as less relevant – in practice the need for providing adequate time for infection development prior to the start of phage treatment should generally be recognized as useful in phage therapy animal experimentation.

2.1.5. Criterion 3, lack of triviality

There should be an expectation that treatment of persistent or chronic bacterial infections using phages will not necessarily be easy. Instead, at a minimum, multiple phage applications typically will be necessary, and often, as noted in the next section (Section 2.1.6), modern clinical *treatment* of long-standing bacterial infections which have proven to resist antibiotic treatment often will span numerous days or, indeed, many weeks. Such infections therefore could be viewed as not trivial to treat. Thus, if model infections are cleared following only a single application of phages, thereby in fact being relatively trivial to treat using phages, and that is not something which is also observed clinically, then we might question whether the model infections themselves are adequate representations of bacterial infections, that is, as those infections typically will present for phage treatment in the clinic.

Note, of course, that evidence that an infection will or will not clear after only a single phage application requires that such single-application experiments be explicitly performed. In addition, keep in mind that poor phage performance can result from a large number of alternative issues – besides infection characteristics – including poor phage choice along with poor phage therapy technique. Elsewhere, towards eliminating these latter issues, I draw attention to how to properly appreciate as well as debug phage therapy experiments [37–39]; see also the Appendix to Abedon [40]. Furthermore, with regard to this issue of clinical precedence, it is important to recognize that the older literature considering clinical studies should be viewed as an invaluable resource for appreciating what might be expected in terms of the phage treatment of experimental infections in animals. That is, the older literature, in this case especially from the 1930s and older, should not simply be ignored for reasons of age, obscurity, or relative lack of ease of access [41].

The next two criteria (4 and 5) may be viewed as ways of generating greater confidence in the suitability of an animal model for robust experimentation. This can be important particularly should treatment approaches turn out to be highly efficacious despite minimal effort, e.g., such as infection clearance following application of only a single phage dose. In such cases it becomes especially important to justify the suitability of an animal model for the experiments performed.

2.1.6. Criterion 4, biofilm presence

Demonstration of biofilm formation within infections – see Percival et al. [1] for approaches as to how to go about doing this – represents one additional means of microbiologically characterizing an infection as potentially persistent or chronic. Not all such infections, however, necessarily possess a biofilm component, and also it should be possible to observe biofilm prior to an infection maturing to the point to being viewed as persistent or chronic. Nevertheless, the presence of a biofilm should be seen as supportive of the idea that an infection in fact is not simply acute. This helps to further verify suitability for phage therapy testing, as anti-persistent or -chronic infection agents, particularly given adherence as well to criteria 0 through 2, along with either 4 or 5.

As noted just above, biofilm presence could help lend support to arguments that an experimental treatment may be especially effective due to qualities of the treatment itself rather than because model infections happen to be inherently easy to treat. In other words, the greater extent that a model system may be demonstrated to be adequate for testing a procedure, then the more useful subsequent experimental results. More generally, indication of biofilm formation represents simply an additional characterization of the pathophysiology an infection. Thus, criteria besides biofilm presence may be used instead, and particularly so as phage treatment-independent measures of the robustness of an infection model. Such additional measure can include demonstration of anticipated tolerance to other treatments, i.e., such as to antibiotics.

2.1.7. Criterion 5, antibiotic tolerance

Substantial differences can exist in terms of antibiotic minimum inhibitory concentrations, as typically measured in vitro, versus as may be observed under more complex conditions, such as given bacteria residence within biofilms. The result is that biofilm-containing bacterial infections can be more resistant to antibiotics than may be anticipated from standard minimum inhibitory concentration determinations, a phenomenon known as antibiotic tolerance [18–22]. Antibiotic tolerance thus is equivalent to a failure to clear an infection despite otherwise genetic susceptibility of the target organism to the antibiotic being used, and is, essentially by definition, a characteristic of persistent or chronic bacterial infections which do clear in the course of antibiotic treatment despite an in vitro susceptibility of etiologies to the same antibiotics. Demonstration of antibiotic tolerance by infections caused by bacteria which otherwise are not outright antibiotic resistant therefore should lend support to the adequacy of an animal model for characterizing as well as developing the potential for phages to treat post-antibiotic treatment persistent or chronic bacterial infections as typically are observed clinically. Determination of antibiotic tolerance, unlike antibiotic resistance, however, is not necessarily a trivial process, particularly to the degree that persisting bacteria are few in number or otherwise difficult to culture [1].

Note that consideration of antibiotic tolerance is not meant to imply that experiments should be designed in which phage treatment explicitly follows, within a single experiment, a demonstration of antibiotic failure, since if nothing else such an experiment likely would be excessively complex and time consuming. In addition, demonstration of antibiotic tolerance would be both impractical as well as potentially irrelevant given use of target bacteria which are genetically antibiotic resistant (e.g., MRSA). Thus, demonstration of antibiotic tolerance should be seen simply as a further test for aspects of long-standing, persistent, or chronic bacterial infections, again as tend to be candidates for phage therapy in this modern age, but a criterion which nevertheless should be viewed as optional. Optional, that is, especially given prior demonstration of some resistance by infections to phage impact (i.e., other than single-dose success) along with other measures that an infection in fact is persistent or chronic (besides criteria 0 through 2), such as biofilm presence.

2.1.8. Experimental persistent or chronic infection criteria, conclusions

In conclusion regarding the judging of animal models, one cannot declare an infection to be persistent or chronic simply because it is not explicitly acute over relatively short time spans. Further, even if persistent or chronic, an infection still is not necessarily an adequate model for the difficulties of treating these bacterial infections as they present in the clinic. I agree that it can be important to show that pre-clinical phage treatment of model in vivo bacterial infections is at least possible before transferring techniques to the clinic, but demonstrating that possibility is not necessarily the same as studying the treatment process towards realistic testing of efficacy nor towards possible improvement in clinical approaches. To satisfy those latter needs, then an in vivo model must adequately mimic infections as they present in the clinic in terms of pathophysiology, microbiology, and difficulty of treatment. Indeed, ideally animal models would be used for the sake of *refining techniques* – under reasonably realistic, ethically more acceptable, and otherwise relatively inexpensive circumstances – prior to the initiation of clinical trials.

2.2. Phage treatment of chronic or persistent bacterial infections in animals

Below I discuss animal infections and other infection models of various types which clinically could represent non- or less-acute bacterial infections. This is not to say that the presented studies necessarily in

fact should be deemed as models of actual persistent or chronic bacterial infections. Thus, they are discussed here both in light of the criteria summarized in Table 1 (and otherwise within the previous subsection), and also in terms of simply experimental design and results. A minimum of a 24-hour delay between bacterial challenge and phage application is taken as indicative of at least a first approximation of subsequent treatment of a non- or less-acute bacterial infection.

Note as a caveat that many phage studies unfortunately do not do an adequate job of indicating exact phage doses, i.e., in terms of phage titers (in phages or plaque-forming units/ml) in combination with volumes of phage formulated product applied—see Abedon [38, 42] for further discussion. Therefore, though I strive to present as much phage dosing information as may be gleaned from individual publications, ultimately in many instances that information simply does not appear to exist, and thus phage titer or dose-volume information often can be missing. I have by contrast intentionally *not* included bacterial challenge densities. Though that information usually does exist in publications, generally initial bacterial densities are more useful for the sake of experiment repeatability than for interpretation of results, where it is interpretation of results which is the emphasis here.

2.2.1. Abscesses

Abscesses are defined by the accumulation of pus within tissues, as usually associated with bacterial infections. Though abscesses certainly can be skin associated, e.g., boils, they can be found elsewhere within the body as well. The two examples presented below, however, are of phage treatment of experimentally initiated skin abscesses.

Capparelli et al. [43] applied phages (phage M^{Sa}) to 4-day-old *Staphylococcus aureus* abscesses. A total of 10^9 phages per mouse per dose were applied subcutaneously, following subcutaneous bacterial challenge. Phage doses were either single or instead consisted of a series of four, once per day. Abscesses were assessed on day four, clock starting at what I assume is the start of phage administration (i.e., as resulting in assessment at the same time points for both single and multiple dosing). Significant reductions were seen in both abscess weight and number of bacteria associated with phage-treated abscesses, with greater reductions seen with multiple versus single dosing, and with reductions as compared with as seen with untreated controls at the same time points. Reductions in bacterial counts per abscess were approximately 2 logs and 4 logs with single or multiple dosing, respectively. Phage application at the same time as bacterial challenge resulted instead in a lack of abscess development. The latter could be due either to less infection maturity at the point of phage addition or instead because larger numbers of phages were added relative to numbers of bacteria present, i.e., as phages would have been applied prior to bacterial population growth given simultaneous phage-bacteria application. The greater effectiveness seen with multiple versus single application is, of course, suggestive of a utility to multiple phage dosing in the course of phage treatment of non-acute bacterial infections.

Wills et al. [44] treated rabbits following subcutaneous bacterial challenge (*S. aureus*) with phages applied either 5, 12, or 24 h later. Phage LS2z was used with a total of 5×10^7 phages injected into the site of bacterial challenge. No impact was seen on abscess formation by any of these treatments, as determined four days after phage application. With zero time delay between bacterial challenge and phage addition, by contrast, there was substantial impact on abscess formation, though in these latter, simultaneous experiments 5×10^9 rather than 5×10^7 phages were applied. This discrepancy in phage numbers applied possibly may be explained by a dose-response analysis in the same study in which it was found that zero-delay doses of less than 6×10^7 phages were ineffective in protecting rabbits from abscess formation while a dose of 6×10^7 phages resulted in some but otherwise not absolute protection from abscess formation. These experiments overall thus might indicate that with time infecting bacteria become more tolerant to phage application (e.g., such as due to biofilm formation), and certainly show that the use of greater phage numbers per dose can be more efficacious than the use of fewer phages. In any case, follow up experiments given delays between bacterial challenge and phage treatment using multiple phage dosing and/or higher phage titers per dose (e.g., 5×10^9) would seem to be useful.

2.2.2. Chronic otitis

Otitis refers to inflammation of the ear, and bacterial infections are one cause of otitis. Externa refers to the outer ear, that is, the visible portion, while media refers to the middle ear, i.e., as immediately internal to the ear drum. *Pseudomonas aeruginosa* is the primary etiology that has been subject to the otitis-targeted phage therapy, and all of the papers concerning this treatment, three including one human treatment as considered in Section 3, are roughly associated with a single research group. It is of interest that for all three studies the treatment, despite the chronic nature of the infections being treated, involved only a single phage dose.

Hawkins et al. [34] describe a veterinary clinical trial of dogs displaying chronic otitis (3 or more months duration), all with a presumptive *P. aeruginosa* etiology. Dogs had previously undergone 3 or more antibiotic courses. A phage cocktail consisting of six different phages was applied with a dose of 10^5 of each phage type to a single ear, with one of the phage types being that used by Marza et al. [45] (below). Reductions in clinical severity scores (ranging from about 8% to about 56% with a mean of about 30%) and *P. aeruginosa* counts were noted after 48 h, with the latter, though statistically significant, nevertheless not highly substantial (reductions ranged from about 30% to about 97% with a mean of about 67%). Bacteriophage counts increased extensively in the course of treatment, but no side effects were noted. It is conceivable that greater efficacy may have been observed if additional phage doses had been administered. Nonetheless, the authors indicate that (p. 312), "Follow up of the ten included dogs was incomplete but generally positive; in three, the chronic ear infections appear to have resolved following bacteriophage therapy. In a further three the *P. aeruginosa* component of the ear infection appears to have resolved." Of interest, treatment doses had been stored at −70 °C until immediately prior to dosing. No untreated controls are indicated other than untreated ears.

Marza et al. [45] reported on the treatment of a St. Bernard dog with phages against *P. aeruginosa*-associated chronic bilateral otitis externa. Dosing was with only 400 phages per ear, with at first only the right ear treated. After 27 h, that ear was no longer discharging and no longer appeared to be inflamed. In addition, the phage count appears to have increased by many log, "over a million" fold in the course of treatment. The left ear was otherwise not affected by the phage treatment, but was subsequently treated with similar results. Efficacy was not associated with immediate elimination of *P. aeruginosa*, however, though nine months following treatment with the phages the ears were described as "completely recovered" as well as free of *P. aeruginosa*. Though no indication is given of how long the dog may have been infected prior to phage treatment, the authors indicate that multiple antibiotic courses had been attempted prior to the commencement of treatment with phages.

2.2.3. Chronic wound infection

An infected wound is not necessarily a chronic wound infection. Bacterial infections of burn wounds, for example, can be quite acute. In any case, here it is animal wound-infection models with delays of 24-h or greater between bacterial challenge and phage application which are considered, with the ongoing caveat that 24-h delays between bacterial challenge and the start of phage treatment in most cases is not a reasonable criterion that the infection being treated is not acute, nor static or mature. Osteomyelitis, as often wound associated, is considered in a separate subsection. For general reviews of wound treatment using phages, see Loc-Carrillo et al. [46] and Abedon [30].

Shivaswamy et al. [47] treated *Acinetobacter baumannii* excisional wounds of diabetic rats. Phages (phage 38) were applied once by topical

spraying with 3×10^9 phages. This was following wound debridement and took place 48 h after bacterial challenge. Bacterial load consequently was reduced to zero by day 8 following bacterial challenge. By contrast, without treatment all rats showed both increases in bacterial load and died by day 8, thus indicating that this was an acute rather than chronic or persistent infection model. The study nevertheless is noteworthy with regard to treatment of non- or less-acute bacterial infections given the 48-hour delay before the start of phage treatment. Intramuscular injection with colistin also resulted in clearance of infections, though not until day 16 post bacterial challenge. Wound healing also occurred sooner with phage- versus colistin-treatment, though we can speculate that this was a reflection simply of phage treatment bringing infections under control faster than with colistin treatment.

Mendes et al. [48] separately treated *A. baumannii*-, *P. aeruginosa*-, or *S. aureus*-challenged excisional (pigs) or punch (rats) wounds of chemically induced diabetic animals. Cesium chloride-purified phage cocktails were applied in 100 μl aliquots consisting of 10^8 to 10^9 phages and thereby titers of 10^9 to 10^{10} per ml, starting 4 days later. At this time, phages were applied every 4 h for 24 h. Subsequently, between the 5th and 8th day – which is post bacterial challenge, though results are shown in the article in post-phage-application units – phages were applied equivalently but every 12 h instead of every 4. Debridement of wounds was performed on days 4, 5, and 8, and debridement in all cases was followed by phage application. Reductions in bacterial counts relative to phage-untreated controls were seen with *P. aeruginosa* and *S. aureus* (2 to 4 log reductions) but not with *A. baumannii* infections in the pig model. Equivalent results were seen with rats, but though *A. baumannii* counts were reduced and overall bacterial counts in those trials were reduced as well, only the latter occurred with statistical significance. It is also worth noting that only the *S. aureus* infections along with *A. baumannii* in rats showed a *lack* of decline in bacterial densities *without* phage treatment, thus arguing against the *P. aeruginosa* infection as chronic or persistent. Wound healing was also faster with phage treatment than without for all but *A. baumannii* phage-treated infections. Thus, in this model the *S. aureus* infection most consistently approximated what could be a persistent or chronic infection, and with debridement and multiple phage application over a four day period *S. aureus* was reduced in number by about 3 logs in the rat model and about 4 logs in the pig model. These latter results may be viewed as a good start towards successful eradication of infections, perhaps especially keeping in mind that clinical treatment of long-standing bacterial infections often will take place over multiple weeks (Section 3).

Seth et al. [49] treated *S. aureus*-infected punch wounds of rabbits. Six days following bacterial challenge, and then occurring every other day until animals were euthanized on day 12, wounds were treated with phages, were treated via debridement, or were treated with a combination of both. In separate experiments, infections with bacteria that were deficient in biofilm production were also treated with phages. Phages were applied seemingly in amounts of 10^6 per dose (or perhaps 10^7 as there appears to be a discrepancy in numbers or units in the article). Of the infections with biofilm-competent bacteria, phage treatment alone had little impact on bacterial counts. Debridement alone appears to have had a slightly greater effect. In both cases, however, changes were not statistically significant relative to no treatment. By contrast, bacterial counts were reduced by approximately 2 log units given treatment with a *combination* of debridement and phage application. Even greater reductions in bacterial numbers (3 log units) were seen, using phage treatment alone, of wounds infected with biofilm-deficient bacteria. Wound healing also was better with combination treatment (debridement plus phages) as well as given treatment of infections caused by the biofilm-deficient bacteria. Though these are exciting results, nevertheless follow up experiments perhaps employing more phages per dose would have been useful before concluding that debridement is absolutely essential to treatment success using the phage employed. Nonetheless, certainly these results are supportive of a utility to debridement in the treatment of chronic wound infections. Overall, the experimental model used by Seth et al. would appear to be a reasonably good one from the perspective that treatment effectiveness was not trivially accomplished, but nevertheless was demonstrably achievable.

2.2.4. Cystic fibrosis

Biofilm formation is implicated in chronic bacterial infections of the respiratory tracts of cystic fibrosis patients [50], particularly as associated with *P. aeruginosa*, though *Burkholderia* spp. can also serve there as important chronically infecting pathogens [51]. Cystic fibrosis infections consequently have been considered in terms of various animal models. Not all animal models actually involve biofilm formation, or chronic or persistent infection, however. What follows are various approaches to phage treatment of models of lung infections, in vivo but also in vitro, which in principle *could* serve as bases for treatment of non-acute infection studies. See Saussereau and Debarbieux [52] and Soothill [53] for consideration of phage treatment of *P. aeruginosa* infections more broadly. See also Abedon [36] and Hraiech et al. [54] for additional review of treatment of lung-associated infections both clinically and in animals. Note that a number of studies have involved delays between bacterial challenge and phage application of less than 24 h, i.e., instead only 2 h, and as a consequence are not reviewed here [55–59].

Darch et al. [60] present an in vitro rather than in vivo model of *P. aeruginosa* lung infection, utilizing synthetic sputum as the growth and treatment environment. Within this environment *P. aeruginosa* forms what the authors describe as "aggregates" but might alternatively be interpreted as microcolonies, presumably mimicking the status of this bacterium within the lungs of cystic fibrosis patients. These structures, after only 4 h of growth, were found to be somewhat resistant to phage treatment as tested (using phages NP3 and PA2). Unfortunately, how many phages were applied is not easily appreciated, nor were the number of phages present over the course of experiments monitored. The approach employed, nonetheless, likely could be useful towards developing anti-*P. aeruginosa* lung therapies in the context of cystic fibrosis. Of further interest, exopolysaccharide-deficient bacterial mutants still formed aggregates in the synthetic sputum medium but those aggregates were substantially more susceptible to phage treatment, after 18 h, than the isogenic wild-type *P. aeruginosa*.

Waters et al. [61], in a research letter, describes both an in vitro and an in-mouse *P. aeruginosa* model. The in vitro model is also in an artificial sputum medium. The authors indicate that phages (PELP20), at a titer of 10^8/ml (presumably final, in situ concentration), were "administered 72 hours after establishment of *P. aeruginosa* mature biofilm" (p. 666), with numbers of bacteria assayed 24-hours later. The results were approximately 3-log reductions in bacterial counts relative to those seen at the same time point with untreated controls. In the mouse model, phages at a dose of 2×10^7 were applied intranasally 24 and 36 h post infection, or 48 and 60 h post infection. With both treatments, at 72 h, complete bacterial clearance was observed, which compares with roughly 10^4 bacteria per lung without treatment, though in a minority of cases negative-treatment controls also showed a lack of bacteria present. The authors also note that no reduction in bacterial counts were observed using ultraviolet-treated phages, which they indicate as lacking in lytic activity. Using an adapted *P. aeruginosa* strain, which the authors describe as "readily established chronic infection in the murine lung" (p. 666), phages were administered at 6 and 6.5 days post infection, with counts determined at 7 days. Here bacteria were reduced to zero in 70% of the treated infections while all of the untreated infections possessed in excess of 10^4 bacteria/lung. While 30% of treated infections still possessed bacteria, they were reduced in number to less than 10^4 bacteria/lung. This study provides good indication of the potential for phages to clear relatively long-term *P. aeruginosa* infections in mouse lungs. It also would appear to be a good model for exploring possible causes of less than 100% treatment efficacy. In particular, could dosing with higher phage titers or instead with yet additional phage doses have resulted in more extensive bacterial clearance using

their infection model, and also would longer delays between bacterial challenge and phage application have negatively impacted results?

Pabary et al. [62] treated mouse lungs with a phage cocktail intranasally 24-h post challenge with *P. aeruginosa*. Dosing was with 1.2×10^9 phages per mouse, or a titer of 6×10^{10} phages/ml suspended in 20 μl. The result was elimination of bacteria from six of seven mice as determined via bronchoalveolar lavage 24-hours post phage application, whereas all of the untreated mice, except one, retained substantial numbers of bacteria. That one untreated mouse with lower bacterial counts, by contrast, was found to have fewer bacteria than the one treated mouse which retained bacteria, though both counts were roughly 5-fold to over one-thousand-fold lower than with all of the other untreated mice (which had a median of 6×10^3 bacteria per ml of lavage). The authors indicate from a different experiment that the mice "became terminally unwell in less than 24 h" (p. 746), given a lack of treatment. Thus, these experiments represent an impressive success against what would seem to be fairly well established but nevertheless acute bacterial infections, and perhaps are indicative of a utility of employing relatively high-titer phage dosing. That suggestion, however, is not definitively supported by this study as only a single dosing phage titer was characterized, and comparisons between studies in terms of dosed phage titers, particularly where different phage strains are employed, are unlikely to be valid.

Semler et al. [63] exposed mice to cyclophosphamide before establishing *Burkholderia cenocepacia* lung infections, as generated using a nebulizer and nose-only inhalation. Individual endotoxin-depleted stocks of phages, of five different types, were applied either as aerosols or instead intraperitoneally 24 h post bacterial challenge, with bacterial numbers in lungs determined at 72 h post bacterial challenge. Only aerosol treatment resulted in significant reductions in bacterial densities relative to the no-treatment control and this is despite intraperitoneal treatment with no bacterial challenge initially (1 day post phage application) showing higher phage densities in association with lungs than aerosol phage delivery. Average differences in bacterial densities with aerosol phage treatment (phage KS12) were over 2 logs after both 1 and 2 days post-treatment (48 and 72 h post bacterial challenge) in comparison with mock-treated controls. Less reduction was seen with a second phage (phage KS4-M) with a little over 1 log differences on average after 1 day and about half a log after 2 days (post-treatment). Note that higher differences were reported by these authors than indicated above, but using median bacterial densities rather than means, thus implying that some individual phage treatments per trial were somewhat more successful than others. It is of interest that bacterial densities with mock phage treatments were higher in the phage KS12 trials by as much as 1 log relative to the phage KS4-M trial. Keeping track of dosing quantitively given aerosol delivery is difficult, especially in terms of phage numbers in lung lumens on a per ml intraluminal basis, though titers of applied phages were in excess of 10^9/ml (and with suggestion that titers as high as 10^{11}/ml may have been used). The authors indicate that ratios of phages to bacteria were higher with phage KS12 (131 phages per bacterium) than for phage KS4-M (11 phages per bacterium), thereby potentially also contributing to the greater killing seen with phage KS12. Indeed, the authors report similar levels of killing using phage KS12 versus KS4-M when lower numbers of phages were added of the former as well as substantially higher killing with a third phage, KS5, given a higher ratio of applied phages to bacteria of 32 versus a lower ratio of 2. In any case, this study indicates that reductions in *B. cenocepacia* densities can be accomplished using aerosolized phages in the lungs of immunosuppressed (cyclophosphamide-treated) mice. In addition, they explicitly note (p. 4011) that "generally, the higher the [ratio of applied phages to bacteria], the better the therapeutic effect."

Carmody et al. [64] treated *B. cenocepacia* infections intranasally or intraperitoneally with phages (BcepIL02) 24 h after bacterial challenge. Phage doses are described in terms of ratios of phages to bacteria. If the latter were initial bacterial concentrations, then phage numbers used were either 10^9 or 10^{10} depending on that initial bacterial concentration. Reductions in bacterial densities relative to various mock-treatment controls 48 h after phage application were lower—not statistically significantly so given intranasal phage administration, but statistically significantly lower by greater than 1 log given intraperitoneal administration. This result is despite observations that delivery of phages to lungs was somewhat more effective given intranasal administration, but may be explained by the specific location of bacteria, which over time appears to have been other than within the lung's lumen. Unfortunately, it is difficult to tell what number of phages were applied in these experiments, 10^9 versus 10^{10}. These findings nevertheless are useful in that they would seem to imply that not all lung-infecting bacteria may be easily reached via topical phage application to within the lungs. Notable, for Semler et al. [63] intraperitoneal phage delivery to treat a *B. cenocepacia* lung infection in mice was ineffective in reducing bacterial densities (above).

2.2.5. Mastitis

Mastitis is an inflammation of mammary glands. It is a problem not only with human mothers but also in the dairy industry.

Gill et al. [65] tested the potential for a phage (bacteriophage K) to treat *S. aureus*-associated 'subclinical' mastitis in cows. As the cows were selected on the basis of pre-existing disease, the infections can be viewed as presumptively persistent or chronic. Susceptibility of *S. aureus* isolates from different cows was determined in vitro prior to the start of trials, though it should be noted that this assay employed spot testing rather than plaque formation, where spot testing is not necessarily truly indicative of phage host range [66]. Approximately 10^{10} phages/ml were applied via intra-mammary infusions within 10 ml volumes, and this was done daily over five-day periods. Though slightly greater efficacy was seen with phage treatment than with saline treatment (16.7% bacteriological cure rate vs. zero), the difference was not found to be statistically significant. Possible exacerbation of mastitis, at a rate lower than cure rate, was also noted with phage treatment but not in phage-less controls. A potential for phage inactivation as well as interference with phage adsorption within the mammary gland was noted in this study, though it is uncertain to what extent this may have impacted results. In addition, the authors noted that cures were obtained especially in association with cows "experiencing extremely mild forms of… mastitis" (p. 2917). As such, it is possible that with less mild infection bacteria are more difficult to kill using phages or, at least, with the phage type used. It is also possible, given the noted apparently hostile environment for phage survival or adsorption within the mammary gland, that application of greater phage numbers than were used might have resulted in greater cure rates, though it is important to emphasize that phage numbers used in these experiments already were rather high and that possible negative consequences of phage application were already observed at the doses used.

2.2.6. Osteomyelitis

Osteomyelitis is a bone infection. Though a chronic, antibiotic tolerant form of these infections is envisaged as a target for modern phage therapy, in the older, pre-antibiotics literature phages appear to be have been employed generally to treat these infections (Section 3.4). Thus, initial treatment of infections with phages given infection with antibiotic-resistant bacteria could be a reasonable strategy given such a diagnosis. A typical etiology is *S. aureus* and the two studies reviewed here are both of treatments of experimental MRSA infections.

Ibrahim et al. [67] treated, with phages, rabbits that had been experimentally infected with *S. aureus* (methicillin resistant, i.e., MRSA). Bacterial challenge was to 1 mm holes that had been drilled into rabbit tibias following incision. Infections appear to have been allowed to develop for at least 21 days prior to treatment. Treatment was with an unknown quantity of phages (3×10^8 phages/ml) which were applied intramuscularly, once per day for 14 days. This resulted in recovery as determined via x-ray one week "post treatment". Not well indicated,

however, is whether this is post the end of treatment or instead post the start of treatment. Equivalent treatment with ceftaroline did not result in equivalent recovery even 14 days "post treatment" and description of untreated infections is not provided. This study nevertheless is suggestive of a phage potential to cure this experimental osteomyelitis infection, presumably even given three-weeks of infection development prior to phage treatment, though better description of experiments would have been useful.

Kishor et al. [68] also used phages to treat rabbits which had been experimentally infected with *S. aureus*, also methicillin resistant, in a chronic osteomyelitis model. I subsequently published a commentary on this article [35]. In the Kishor et al. study, rabbits were treated separately starting after either three or six weeks of infection, were treated every two days for one week, and were treated with a cocktail of seven phages. (Perhaps confusingly, treatment groups may not have been consistently named throughout the article.) Phages were injected into soft tissues associated with the infections ("intralesionally") at doses of perhaps roughly 10^{10} phages. Results were compared with untreated controls for the three-week infection treatments but not for the six-week treatments. Efficacy was determined by a variety of means (microbiologically, radiopathologically, and histopathologically), with three-week infections cured given phage treatment but not cured without treatment. After six weeks of infection, curing was also seen upon phage treatment, though not without sequelae, and also these latter experiments were done without associated untreated controls. Though in Abedon [35] I express concern that the outcomes of this study are almost too efficacious, nevertheless this study represents perhaps the strongest evidence to date that phage treatment can be effective against an experimental chronic infection.

2.2.7. Other

Trigo et al. [69] treated *Mycobacterium ulcerans* subcutaneous infections of mice. As the name of the organism suggests, *M. ulcerans* is a cause of skin ulcers. Although susceptible to antibiotics, surgical removal of the infected area is used to address more advanced cases. This study employed a 33-day delay between bacterial challenge and phage (D29) administration. Though that would appear to be a long delay, nevertheless this is an ulceration-prevention study rather than one involving ulcer treatment, as ulcers absent treatment did not emerge until day 68, at which point untreated mice were sacrificed for ethical reasons. Thus, we can question whether technically this model may be considered to be one of a persistent or chronic infection, even given the long delay between bacterial challenge and phage application. Phages were applied as a single dose subcutaneously consisting of 10^8 phages. Though this application was in the same footpad as the initial bacterial inoculation, there is no indication of how close, spatially, to the bacterial inoculation the phage inoculation was (e.g., into the growing lesion versus next to it, or indeed whether such a distinction is even possible). Swelling of lesions continued past the point of phage application for another 5 weeks, again indicating that, despite the long delay prior to treatment, this study does not obviously involve the treatment of an infection which had reached an approximation of a persistent steady state (i.e., infection stasis; Criterion 1, Section 2.1.2). By roughly 16 weeks post phage application swelling had decreased to that seen at the time of phage application, though the experiment was stopped prior to the observance of decreases to pre-bacterial challenge footpad thickness. Numbers of bacteria present per footpad on day 35 post treatment declined by roughly 2 logs with phage treatment, relative to the point of phage application, and showed slightly greater declines relative to the untreated control at the same 35-day time point. No side effects of phage treatment were detected.

Capparelli et al. [43] considered a number of different *S. aureus* infection models including treatment of 10-day-old systemic infections using phage M^{Sa}. A total of 10^9 phages/mouse were applied, following intravenous bacterial challenge ten days earlier with an otherwise non-lethal dose that the mice were unable to clear on their own. Assessment ten days post phage administration indicated persistence of bacteria in untreated mice, but complete clearance in treated mice was seen in those organs which were assessed.

3. Treatment of chronic or persistent bacterial infections in the clinic

Contrasting experimental animal models for testing phage treatments – the vast majority of which have *not* been reviewed in the previous section – modern clinical phage treatment of bacterial infections in fact typically are indeed of what can be reasonably characterized as persistent or chronic. This generally is the case given substantial efforts to treat infections using antibiotics prior to the initiation of phage treatment, and particularly so where antibiotic treatment has not been abandoned prematurely given evidence of outright etiology resistance to the antibiotics being used. For circumstances in which prior treatment with antibiotics was not attempted, and especially given a lack of information as to the duration of treatments before the initiation of phage treatment, the question of whether infections truly are long-standing can be more difficult to assess. Nevertheless, we can consider three criteria for *not* considering clinical treatments to be of non-acute bacterial infections: (i) the treatment was preventative, (ii) the treatment was of a demonstrably acute infection, or (iii) the treatment was of an infection that would not typically be recognized as persistent or chronic (e.g., cholera). Thus, especially with this last point in mind, and as with animal models (above), in this section phage treatment is distinguished by the presumptively persistent or chronic infections being treated. Unlike animal models, however, the literature on phage treatment of likely non-acute bacterial infections in the clinic is more substantial, and particularly so in terms of the older literature, which dates back to 1923 and consists of numerous English- as well as non-English-language publications. Here I limit myself to English-language studies, and otherwise make no promise of comprehensiveness. For a more complete bibliography of the English-language phage therapy literature, see http://publications.phage-therapy.org [70] along with Alves and Abedon [41]. See also Chanishvili [71] for review of a large sampling of the non-English-language phage therapy literature.

Elsewhere I review a number of examples of treatment of bacteria-infected wounds [30]; see also Loc-Carrillo et al. [46] as well as Eaton and Bayne-Jones [72]. Not all of these examples are of *chronic* wound infection, and are mostly not considered in this section (skin ulcers are the exception). Indeed, and as suggested above, for a number of the studies presented here it is uncertain whether or to what degree treated infections in fact are chronic or persistent. In the more modern literature especially, however, and as repeatedly noted, it is typical for patients to be treated with antibiotics prior to *resorting* to phage therapy, so in these cases there very much is a tendency for infections to be no longer acute at the point of the initiation of phage treatment. Consistently, Międzybrodzki et al. [73] reports that the phage-treated infections described in their study were present for a median of 43 months before the start of phage therapy. Such long-duration infections are not necessarily trivial to treat using phages, even if ultimately successfully, with Międzybrodzki et al. [73] reporting as well that individual patients were treated over spans with a median of 55 days.

3.1. Eaton and Bayne-Jones' caveat (and suggestion)

For an important as well as a somewhat critical review of the early phage therapy literature, see Eaton and Bayne-Jones as published in 1934 [72, 74, 75]. In assessing the literature reviewed in this section (below), it is important to not overlook their concerns [72], which are well expressed in this quote (p. 1848):

> It is impossible to read these many reports of the successful bacteriophage treatment of suppurative conditions without feeling that a great many of the authors are extremely uncritical. It is difficult to believe that success in from 95 to 99 per cent of cases has been

> due to a therapeutic agent that has proved so disappointing in the more critically conducted laboratory experiments. A consideration of many of the detailed case reports of what are stated by the investigators to be dramatic cures reveals that the process of recovery has not run an unusually swift course. Many of the conditions treated are self limiting, and, in those which are not, the usual methods of surgical intervention have been practiced. The fact that the patient recovered has, therefore, little significance. The speed of recovery as compared with other similar conditions not treated by bacteriophage may have some significance, but it is well known that many suppurative conditions may show prompt improvement in from twenty-four to forty-eight hours, especially after drainage has been instituted. The prevention of recurrence in such conditions as furunculosis and the healing of chronic suppurative conditions of long standing under bacteriophage therapy are perhaps the most convincing effects observed. These effects could well be due to the action of bacteriophage filtrates as vaccines.

With "critically conducted laboratory experiments", what is being referred to are early animal experiments [74]. The early phage therapy literature (roughly pre-1940) thus may or may not lend support to the idea that phages in fact are capable of effectively treating chronic or persistent bacterial infections. Nevertheless, as Eaton and Bayne-Jones note, there does appear to be ample suggestion that phage application can be effective in the treatment of bacterial infections in the clinic (though the last sentence in the quotation referring to the possible importance of immunological contributions to efficacy, whether associated with components of relatively unpurified phage lysates or instead products of in situ phage-induced lysis of targeted bacteria, remains in my opinion an intriguing possibility). Furthermore, these results appear to be reflected to a reasonably large degree in the more recent literature (i.e., roughly post-1980). Indeed, you will note a substantial gap in the dates of the literature reviewed. This gap clearly is real, though not necessarily as broad as the 50 year, early-1930s to early 1980s, lack of English-language examples as presented here would suggest, i.e., see Alves and Abedon [41] for access to a more complete listing of the phage therapy literature. For example, see in particular the English-language writing on the treatment of staphylococcal infections by MacNeal and colleagues [76–78].

Below I review published accounts of clinical treatments of presumptively persistent or chronic or at least 'less acute' bacterial infections, as summarized by disease and etiology in Table 2. See the previous section on animal experiments for missing general descriptions of different disease categories. Overall, I believe that these examples are suggestive of a potential for phages to treat chronic or persistent bacterial infections, including ones which have not otherwise responded sufficiently to antibiotic treatment. It is important to keep in mind, however, that most of these studies, though not all, do not represent properly controlled experiments, and also that reporting bias, including by myself, could result in an impression of greater efficacy than might otherwise have been the case. It is also my opinion that success using phages against bacterial infections, acute or otherwise, is more likely in the hands of more experienced phage therapist, or indeed can differ when using different phages, which could explain at least some discrepancies in the results obtained by different groups. In short, there is reason to be optimistic about the potential of phage therapy, including against chronic or persistent bacterial infections, though absolute proof of a consistent, not-placebo-related efficacy associated with phage treatments is not yet robust.

3.2. Abscesses

Barrow and Soothill [79] suggest that phages may find it difficult to "multiply rapidly" in abscesses due to "overwhelming numbers of other bacteria" (p. 271). A more modern interpretation of this concern may specifically refer to a reduced potential for phages to impact persistent or chronic bacterial infections due to a presence of biofilms. Notwithstanding that issue, a fairly large number of reports in fact do describe successful phage treatment of abscesses in the clinic. To the extent that the duration of these infections prior to the commencement of phage therapy is not reported, then treatments in many cases cannot be explicitly considered to be chronic or persistent. Nevertheless, what follows are reports of the phage therapy of abscesses, including especially the skin abscesses, furuncles and carbuncles (i.e., boils). Eaton and Bayne-Jones [72] note that (p. 1848), "Of the various suppurative conditions treated by bacteriophage, the results with furunculosis and staphylococcic abscesses seem most convincing."

Fadlallah et al. [80], in 2015, presented a case study of the treatment of a corneal abscess using anti-*S. aureus* phages. The infection was nosocomial, MRSA was identified, and it initially responded to vancomycin. Eventually, however, vancomycin-resistant *S. aureus* emerged. It is unclear from the publication what specifically the patient's condition was at the point of initiation of phage treatment (i.e., see "this condition lasted…", p. 167), though presumably this was reoccurrence of corneal abscess as well as interstitial keratitis, and these had been persisting for roughly a dozen years prior to phage treatment. Phages were applied topically as well as intravenously using the commercially available phage (in Georgia), SATA-8505, and took place over a total of 4 weeks. Topical treatments involved both eye drops and nasal spray. At three as well as six months following treatment, signs had stabilized and cultures were negative.

Cisło et al. [81], in 1987, treated 2 cases of furunculosis both orally and locally with phages. In both cases "outstanding improvement" was observed. More from this article is found under the heading of "Venous leg ulcers".

Ślopek et al. [82] is a milestone English-language human phage therapy report also published in 1987, which was at or near the transition to what can be described as the modern or "revival" era of phage therapy [7, 83]. This report documents phage therapy as used in clinical practice in Poland in the mid-1980s and is based on six previous reports [84–89]. Summarized are 550 cases involving the treatment of *Escherichia*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Staphylococcus* etiologies. Antibiotics were applied along with phages in 152 of these cases, and antibiotic failure prior to the start of phage treatment was seen in 518 cases. In treatment of furunculosis, good results are reported in all 55 cases. A more recent study by the same group [90] indicates that the treatment of abscesses was at least in part topical. Furunculosis is indicated there, again, as 100% cured. These latter infections were associated with *S. aureus*, with 90 cases treated. For these treatments, phages were supplied to practitioners who treated patients in various clinics and hospitals (additional general descriptions of this latter study can be found below under the sub-heading, "Other", i.e., Section 3.8). Weber-Dąbrowska et al. [90] also provides before and after phage-treatment photos of a nasal-area abscess found on the face of an infant. Subsequently, treatments were performed in-house, in the Phage Therapy Unit associated since 2005 with the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław, Poland [73]. Though in-house results appear to be substantially less effective (e.g., roughly half as many successes), it is difficult to tell from that publication just how much lower this is for abscesses (furuncles or carbuncles) specifically.

Cipollaro and Sheplar [91], from 1932, present 108 cases of phage treatment of pyoderma. One patient possessed multiple abscesses on her hands which potentially were chronically present. Phage treatment of *S. aureus* appears to have consisted of subcutaneous (2 ml) as well as intra-lesional (0.25 ml) injection, at 2-day intervals for two weeks. Abscesses were nearly gone after 4 days of treatment and the patient remained abscess free for at least over four months. Another patient with multiple furuncles on the back of the neck received multiple injections, every 1 or 2 days, directly into the furuncles, of 0.25 ml of the phage, as well as 1 ml subcutaneously into the arm. The result was five months lesion free compared with no more than one month over a period of 15 years prior to this treatment. Similar treatment of an

Table 2
Human treatment studies discussed in Section 3, as distinguished by etiologies.

Etiology	Disease[a]	Year	Authors	Reference
Enterobacter	Urinary tract infections	2010	Letkiewicz et al.	[106]
Enterococcus	Bacterial prostatitis	2009	Letklewicz et al.	[105]
	Diabetic skin ulcers	2018	Morozova et al.	[110]
Escherichia	Abscesses	1987	Ślopek et al.	[82]
	Bed sores	2000	Weber-Dąbrowska et al.	[90]
	Osteomyelitis	2000	Weber-Dąbrowska et al.	[90]
	Urinary tract infections	2010	Letkiewicz et al.	[106]
		2000	Weber-Dąbrowska et al.	[90]
		1987	Ślopek et al.	[82]
		1930	Rice	[102]
	Venous leg ulcers	2009	Rhoads et al.	[113]
		2002	Markoishvili et al.	[114]
		2000	Weber-Dąbrowska et al.	[90]
		1987	Cisło et al.	[81]
		1987	Ślopek et al.	[82]
	Other (fistulas)	2000	Weber-Dąbrowska et al.	[90]
Klebsiella	Abscesses	1987	Ślopek et al.	[82]
	Bed sores	2000	Weber-Dąbrowska et al.	[90]
	Osteomyelitis	2000	Weber-Dąbrowska et al.	[90]
	Otitis	2000	Weber-Dąbrowska et al.	[90]
		1987	Ślopek et al.	[82]
	Urinary tract infections	2010	Letkiewicz et al.	[106]
		2000	Weber-Dąbrowska et al.	[90]
		1987	Ślopek et al.	[82]
	Venous leg ulcers	2000	Weber-Dąbrowska et al.	[90]
		1987	Cisło et al.	[81]
		1987	Ślopek et al.	[82]
	Other (fistulas)	2000	Weber-Dąbrowska et al.	[90]
Proteus	Abscesses	1987	Ślopek et al.	[82]
	Bed sores	2000	Weber-Dąbrowska et al.	[90]
	Osteomyelitis	2000	Weber-Dąbrowska et al.	[90]
	Otitis	1987	Ślopek et al.	[82]
	Urinary tract infections	2010	Letkiewicz et al.	[106]
		2000	Weber-Dąbrowska et al.	[90]
		1987	Ślopek et al.	[82]
	Venous leg ulcers	2002	Markoishvili et al.	[114]
		2000	Weber-Dąbrowska et al.	[90]
		1987	Cisło et al.	[81]
	Other (Fistulas)	2000	Weber-Dąbrowska et al.	[90]
Pseudomonas	Abscesses	1987	Ślopek et al.	[82]
	Bed sores	2000	Weber-Dąbrowska et al.	[90]
	Diabetic skin ulcers	2018	Morozova et al.	[110]
	Osteomyelitis	2000	Weber-Dąbrowska et al.	[90]
	Otitis	2009	Wright et al.	[108]
		2000	Weber-Dąbrowska et al.	[90]
		1987	Ślopek et al.	[82]
	Urinary tract infections	2011	Khawaldeh et al.	[117]
		2000	Weber-Dąbrowska et al.	[90]
		1987	Ślopek et al.	[82]
	Venous leg ulcers	2009	Rhoads et al.	[113]
		2002	Markoishvili et al.	[114]
		2000	Weber-Dąbrowska et al.	[90]
		1987	Cisło et al.	[81]
		1987	Ślopek et al.	[82]
	Other (fistulas)	2000	Weber-Dąbrowska et al.	[90]
	Other (cystic fibrosis)	2011	Kvachadze et al.	[120]
Staphylococcus	Abscesses	2015	Fadlallah et al.	[80]
		1987	Ślopek et al.	[82]
		1932	Cipollaro and Sheplar	[91]
		1931	Kahn	[101]
		1930	Alderson	[97]
		1930	Crutchfield and Stout	[98]
		1929	Larkum	[95]
	Bed sores	2000	Weber-Dąbrowska et al.	[90]
	Diabetic skin ulcers	2018	Morozova et al.	[110]
		2018	Fish et al.	[111]
		2016	Fish et al.	[112]
	Osteomyelitis	2000	Weber-Dąbrowska et al.	[90]
		1932	Schultz	[92]
		1923	McKinley	[107]
	Otitis	2000	Weber-Dąbrowska et al.	[90]
		1987	Ślopek et al.	[82]
	Skin ulceration (facial)	2013	Lecion et al.	[109]
	Urinary tract infections	2010	Letkiewicz et al.	[106]
		2000	Weber-Dąbrowska et al.	[90]
		1987	Ślopek et al.	[82]
	Venous leg ulcers	2009	Rhoads et al.	[113]
		2002	Markoishvili et al.	[114]
		2000	Weber-Dąbrowska et al.	[90]
		1987	Cisło et al.	[81]
		1987	Ślopek et al.	[82]
	Other (fistulas)	2013	Lecion et al.	[109]
		2000	Weber-Dąbrowska et al.	[90]
	Other (cystic fibrosis)	2011	Kvachadze et al.	[120]
Streptococcus	Venous leg ulcers	2002	Markoishvili et al.	[114]
Unspecified[b]	Abscesses	1987	Cisło et al.	[81]
		1932	Schultz	[92]
		1930	Rice	[102]
	Bacterial prostatitis	2012	Międzybrodzki et al.	[73]
	Bed sores	1930	Rice	[102]
	Osteomyelitis	1930	Rice	[102]
	Otitis	1930	Rice	[102]
	Venous leg ulcers	1930	Rice	[102]

[a] Disease names correspond to various subsections in the main text. Parentheticals, however, do not correspond to subsections.
[b] For Rice [102] generally these are presumably either *Escherichia* or *Staphylococcus*.

approximately 5 by 10 cm skin abscess is described, also with success. A total of 62 furunculosis and 5 carbunculosis cases were equivalently treated, with 100% cure rate for those patients who completed treatment. Of interest, the authors suggest that phage treatment reduces scar formation, toxemia, and pain in comparison (presumably) to what at the time were standard treatments. Discussed in terms of side effects are local reactions at the site of injection (if not the lesion itself), local reactions at lesions, and, more rare, systemic reactions, the latter involving fever, malaise, and vomiting. Of interest, the authors note (p. 289) that, "All lesions showed marked improvement after the reaction."

Schultz [92], also from 1932, indicates that (p. 303), "Bruynoghe and Maisin, as early as 1921 [93], injected 'phage' near the base of furuncles and carbuncles of six patients, and noted in all of them marked improvement within 48 hours. Gratia soon afterward (1922) reported excellent results in about forty cases of staphylococcus infection, including furunculosis, carbuncles, and subcutaneous abscesses. Good results have also been reported in the treatment of furunculosis and carbuncles by Larkum, 1928 [94], 1929 [95]; Raiga, 1929 [96]; Alderson, 1930 [97]; Crutchfield and Staut, 1930 [98]; and others." Note that two Gratia publications are cited by Schultz, both of which are in French, but not distinguished from one another in the quoted passage [99, 100]. Schultz provides further (p. 305) – in terms of the "Results reported to the Bacteriophage Research Laboratory of Stanford University" (p. 304) – that "The reports on skin and wound infections reveal a similar trend of results. Of 63 cases of furunculosis, 44 (70 per cent) were decidedly helped by 'phage.' The very pronounced shift in the clinical course of a goodly number of these cases seems to leave little doubt as to the therapeutic relationship of the 'phage.' Of 16 cases diagnosed as carbuncles, 14 responded promptly to the administration of 'phage.' I have personally observed the rapidity with which severe lesions in several cases regressed." And from p. 307, in terms of administration, "…in the case of furunculosis, carbuncles, etc., the most satisfactory way is to inject subcutaneously with a small needle, fractions of 1 cc. near the base of the lesion. The total amount injected in this way at one time probably should not exceed 2–3 cc."

Kahn [101], in 1931, treated a number of abscesses and other pyodermic infections successfully, using anti-staphylococcal phages obtained from the Swan-Myers Company of Indianapolis, Indiana (U.S.A.). As was also the case for Cipollaro and Sheplar [91], more systemic treatment (injection into the arm, subcutaneously perhaps in most or all cases) along with more localized treatment tended to be employed. Five cases of furunculosis were treated. In all five cases this resulted in resolution of the furuncles, though in two cases non-phage ointment was employed as well.

Alderson [97], in 1930, provides a number of case studies. Case 3 is treatment of furuncles of greater than one-week duration. Phages were subcutaneously injected, 1 ml on the first day and otherwise 2 ml, per day every third day, for four treatments. Before the fourth treatment the patient was described as "almost well". In addition to phages, an ammoniated mercury ointment had been locally applied. Case 4 consisted of recurring furuncles which had lasted several weeks. A similar treatment, consisting of two rather than three 2-ml injections was performed to similarly favorable results. Cases 4, 5, 6, and 7 were similar in terms of both procedures and results. The treatment of Case 10, also of furuncles, is described as follows (p. 204): "Three subcutaneous injections of 3 cc. each were given at twenty-four hour intervals with only slight local reaction at the site of injection. About twelve hours after the first injection, the two unincised furuncles on the neck became more turgid and painful and opened spontaneously, draining considerable fluid pus. On the day after the second subcutaneous injection, the lesion on the cheek underwent rapid regression, while the three draining furuncles on the neck were practically dry. At the time the patient presented himself for the third injection the dressing was dry, and healing had progressed in all the infected areas. No further visits were required. There have been no recurrences since the foregoing episode (ten months)."

Crutchfield and Stout [98], in 1930, reported (p. 1012) on "cases of deep-seated furuncles. It was in this group that we obtained our most spectacular results. Liquefaction, cessation of pain, reduction of the induration and resolution occurred within from three to six days." Acne varioliformis treatment "were not less spectacular than the preceding one" where "in all but one case recovery was obtained", but with slow improvement seen with the exception. Treatment of "a pustular type of acne" (p. 1015), by contrast, did not yield satisfactory results. Two tables listing details on 57 cases are presented. Two cases involving abscesses are described in further detail: "Case 46.—D. P., a man, aged 40, had generalized furunculosis of six months' duration. The lesions were resistent [*sic*] to manganese butyrate, vaccines and dietary treatment. Six injections of polyvirulent [perhaps meaning polyvalent] bacteriophage were administered at two day intervals. The lesions became softer two days after the first injection, and there was a progressive resolution. The patient was discharged after the sixth injection, and there has been no evidence of a recurrence after six months." "Case 7.—S. B., a salesman, white, aged 40, presented recurring small, hard, painful furuncles, of eight years' duration, on the face, neck and scalp. The case was diagnosed as acne varioliformis. On Aug. 13, 1929, 0.25 cc. of bacteriophage was administered. On August 17, the lesion was less painful and there was a central liquefaction; 1 cc. of phage was administered. On August 21, the lesion was healing. When the patient was observed on August 23, 27 and 29, there were no new lesions. By September 3, he had recovered, and there were no recurrences." In terms of delivery, they note that (p. 1011), "When injections were supplemented by local application or by irrigations, the amelioration of symptoms was more rapid than when injections were used alone. Local applications in very superficial small areas seemed quite efficacious." In terms of when phage therapy was indicated as well as the idea of what might be serving experimental as negative controls, the following passage is quite informative (p. 1012): "Most of our patients presenting chronic conditions had been treated by the usual methods of drainage, vaccines, protein therapy, diet and supportive treatment. Our comparison of results is based on the duration of severity and the usual sequelae of similar groups of patients treated by the usual methods." Explicitly, the authors indicate that (p. 1020), "The clinical results are shown to be much better than in similar cases in which other means of treatment was used."

Rice [102], from 1930, treated various *S. aureus* and "Bacillus coli" infections mostly using phages obtained from the Swan-Myers Company of Indianapolis. There were 66 cases of boils and carbuncles which were treated. Indicated results are excellent (55, or 83%), intermediate (5), and failures (4) as well as 1 in which no report was provided. For the failures it is suggested that part of the problem was one of etiology susceptibility to available phages. An additional issue mentioned is the depth of the infection and that injecting phages in these instances apparently was not permitted. On p. 347 the author notes, "We have had 10 cases of generalized furunculosis of extreme grade—fifty to three hundred and fifty boils present simultaneously in each case—in children from five months to ten years of age. All of these have shown spectacular improvement immediately after the application of the bacteriophage. Most of these children were in bad condition when we began treatment—emaciated, badly nourished, running considerable temperature, extremely uncomfortable, and in several instances considered to be critically ill. The bacteriophage was applied directly as a wet dressing, or in several instances injected directly into the lesions with a fine needle. In every case except one the child was markedly improved the following day, the temperature was lower, the pain and soreness less, the early boils tending to abort, the older ones moving rapidly toward liquefaction, and the child apparently turned toward recovery." A number of additional details associated with treatments are discussed.

For abscesses, also from Rice [102], the corresponding results of 27 treated were 24 (or 89%) excellent, 1 intermediate, 1 without improvement, and 1 for which a report was lacking. Some of the treated abscesses had been present for months or years. One abscess was 15 cm deep and 100 ml in volume. From pp. 348–349 for that case: "The patient's color was bad, he had considerable temperature, was badly emaciated, and otherwise in an unsatisfactory condition. After the injection of bacteriophage into the cavity he showed prompt improvement, the wound closed completely, and he gained 22 pounds in an interval of five weeks." Abscesses generally had been opened prior to treatment with phages instilled or injected into the resulting cavity. Good results, however, were seen especially when abscesses had not first been opened. When particularly deep, then injection into an abscess was via a catheter. Additional abscess treatment details and suggestions are provided.

Larkum [95], in 1929, provided a tabular review of phage treatment of staphylococcal infections, with many of those references described not being in English—see Alderson [97] for review of many of these papers in greater detail. In the Bruynogie and Masin [93] study, 6 patients were treated for furunculosis (note that the citation to the study indicated by Larkum I believe is not correct and I have indicated the likely alternative, though see also Bruynoghe and Maisin [103] and Bruynoghe and Maisin [104]). They were given 0.5 to 2 ml phages which were injected either into or near to the lesion. Results are described as, "Rapid diminution in induration and complete disappearance 24 to 48 hours". Side effects are described as "Slight temperature rise; local edema and soreness". For Gratia [100], 50 patients were treated, though not all treatments were of abscesses. Phage application was of 0.5 to 3 ml injected, at least at first, into the lesion. Results are described as, "Injections influence evolution of folliculitis, furunculosis, subcutaneous abscesses, giving unquestionable favorable results. One recurrence during 3 months". Side effects are described as, "Slight temperature; local edema, pruritus and pain general following intravenous injection" (the latter for treating septicemias). Larkum [94], from 1928, describes the treatment of 66 patients with furunculosis. 4 ml of phage preparations were applied via, "Two subcutaneous inoculations 24 hour interval". Results are indicated as, "Improvement in all except one[.] Termination of furunculosis for 6 weeks or more in most cases." Side effects are described as "None or slight local" (all preceding quotes in this paragraph are from p. 36).

Other studies reviewed in this same table may also involve abscess treatment but are not explicitly indicated as such.

A second table is provided on p. 38 by Larkum [95] in which more recent treatments of furunculoses are summarized. Overall, 208 patients were treated, with 88 experiencing no recurrence after 6 or more months later (42%), and a total of 78% experiencing no recurrences over that duration or shorter. Only 3% experienced no improvement. Results are further subdivided in terms of furuncle duration prior to treatment. For cases of 1-year or more duration, the equivalent numbers are similar (15 out of 25 patients, or 60%, for which data was available showed no recurrence and only 1 out of 32 patients showed no improvement). Summarizing, Larkum [95] notes, "The lesions present regress, the exudate changes from purulent to serous, the pain is promptly relieved, and the lesions start healing within 24 hours from the time of inoculation. There are exceptions to be sure, but they are exceedingly rare. The statement that a patient has been free of boils for 6 months or more does not give a true picture. Six months is the minimum time in such instances. Many of these patients have had no boils during two years. Finally the statement that boils recurred should be amplified. Most of these recurrences were mild, few and small boils in marked contrast to the previous condition in the same patient." Overall for the study (which include more than just treatment of furuncles), 42% of patients saw no side effects, 47% showed mild side effects, 10% general side effects ("temperature and malaise"), and for 1% side effects are described as "Severe".

3.3. Bacterial prostatitis

Bacterial prostatitis is an inflammation of the prostate gland. Chronic bacterial prostatitis, as considered here, is associated with bacterial urinary tract infections.

Międzybrodzki et al. [73], from 2012, report a rate of pathogen eradication using phage therapy in six cases of chronic bacterial prostatitis of 50%. Phage administration involved, in some cases, application of wet compresses to the glans of the penis. The authors make a point of including these infections when indicating cases that are "especially difficult-to-treat" (p. 114).

Letklewicz et al. [105], as published in 2009, describe the treatment of three *Enterococcus faecalis*-infected cases of chronic bacterial prostatitis, a report provided by the same group as that of Międzybrodzki et al. [73]. Phages in this case were applied rectally against pathogens which antibiotic or alternative treatments had previously failed to eradicate over multiple months. Rectal treatment was chosen due to the connection of veins unidirectionally from the rectum to the prostate in combination with evidence that phages can enter the blood following rectal delivery. Phages in 10 ml volumes, with titers of 10^7 to 10^9 phages/ml, were applied twice daily for 28, 33, or 30 days. The result in all three cases was *E. faecalis* culture-negative prostatic fluids and prostate return to normal condition upon digital rectal examination. No changes in bone marrow, kidney, liver or pancreas function were noted during treatment.

Letkiewicz et al. [106] is a review on the subject of phage treatment of chronic bacterial prostatitis. There, summary of an additional 22 cases of treatment of chronic bacterial prostatitis is provided, having *E. coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *P. aeruginosa*, or *Streptococcus haemoliticus* etiologies. Phages were delivered rectally, orally, or to the glans of the penis. Treatments lasted 22 to 99 days (for a mean of 47). Pathogens were eliminated in (at least) half of the cases, with no side effects observed.

3.4. Osteomyelitis

Weber-Dąbrowska et al. [90], a study published in 2000, summarize the treatment of "osteomyelitis of the long bones" along with "suppurative osteitis after bone fractures". In both cases etiologies consisted of *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, or *S. aureus*. Respectively, full recovery was seen in 38 of 40 (95%) and 37 of 41 (90%). Marked improvement without elimination of the target organism, again respectively, is seen with 2 (5%) and 4 (10%) cases. Ślopek et al. [82] presents identical numbers, though the Weber-Dąbrowska et al. [90] are results from 1987 to 1999 while those of Ślopek et al. are from 1981 to 1986.

Schultz [92], from 1932, provides limited description of the treatment of osteomyelitis. A total of 22 cases were phage treated, 5 of which benefitted from the treatment, and perhaps 2 of those were chronic. The following caveat was then provided, however (p. 305): "It must be admitted that interpretation of results in infections of this type are not easily made, especially when they relate to the more chronic cases."

Rice [102] in 1930 summarized 11 cases of osteomyelitis for which results are described as excellent (4), fair (3), and no effect (4). Further, the author notes (p. 355) that, "Our general impression is that in osteomyelitis the value of phage is less than would be indicated by these figures. If there is dead bone in the lesion it [phage therapy] has apparently little or no value. When all necrotic bone has been removed, we have obtained good results, but under such circumstances the result may have been good without phage."

McKinley [107], in 1923, describes a case of *Staphylococcus* osteomyelitis (case 3). A sequestrum was curetted and this appears to have led to an infection that was observed 9 days later. A phage preparation was injected into the wound and by the next day the amount of pus was reduced from substantial to little. On the following day the infection had improved further, with further dosing and improvement following. By one week following the start of the treatment the wound was nearly healed, with subsequent complete healing. A caveat, though, is that an approximately 9-day-old infection is obviously not chronic.

3.5. Otitis

Wright et al. [108], published in 2009, is a phase I/II (safety and initial efficacy) randomized, placebo-controlled double-blinded clinical trial using phages to treat *P. aeruginosa* associated with chronic otitis (12 phage treated and 12 placebo treated). Phages consisted of a cocktail of 6 types (of 10^5 phages/ml), and were equivalent to those used by Hawkins et al. [34]. These were applied as a single dose in 0.2 ml volumes directly into ears. A variety of measures were used to gauge success and phage-treated patients were judged to have improved to a statistically significant degree relative to placebo-treated controls, as determined on day 7. In addition, phages increased in number at least 200-fold in treated patients, and such increases when observed were seen for an average of 23 days post treatment. In addition (p. 354), "clearance of bacteriophages was observed after resolution of infection in all cases where this occurred." Furthermore, *P. aeruginosa* counts were statistically significantly lower in the phage-treated versus placebo controls on days 7, 21, and 42 following the initiation of treatment (a mean of 24% of starting number with phage treatment versus 108% for the placebo control on day 42, for example). In terms of side effects, the same number of "treatment-emergent adverse events" were reported in the phage treated group as in the placebo control group. These "events" furthermore were characterized by the authors as "mild to moderate". Overall, this study represents perhaps the most important to date supporting the phage potential to effectively treat persistent or chronic bacterial infections.

Weber-Dąbrowska et al. [90], from 2000, indicate results of 33 individuals treated with phages for purulent otitis media, as caused by *Klebsiella*, *Pseudomonas*, or *S. aureus*. Full recovery was seen with 28 patients (88%) whereas marked improvement, but not elimination of the pathogen, was seen with another 3 (9%).

Ślopek et al. [82], as published in 1987, combines data for blepharitis (4), conjunctivitis (7), and otitis media (5). Etiologies included *Klebsiella*, *Proteus, Pseudomonas*, or *Staphylococcus*. Treatments were local and appear to have been 100% effective.

Rice [102] in 1930, under a heading of "Mastoidectomy wounds and running ears" states that of 9 cases, 6 gave excellent results, 2 good results, and 1 was a treatment failure. Noted is that (p. 355), "Several of these cases had been running for years, and were dry and odorless for the first time in months within a week or ten days after phage was used."

3.6. Skin ulcers

A skin ulcer is a break in the skin which is distinct especially from a rapidly imposed injury, i.e., a laceration is also a break in the skin but not an ulcer (though a wound which fails to heal can lead to an ulcer as too can externally imposed non-laceration injuries, the latter as is the case with bed sores). Ulcers can become infected and infections can persist. Here emphasis is on diabetic foot ulcers, venous leg ulcers, and decubitus ulcers (bed sores), as presented in that order based on how recent the most recent phage-treatment studies were published. In addition is the following:

Lecion et al. [109], from 2013, describe the treatment of a single patient with facial ulcerations infected with *S. aureus*. Phage-containing wet compresses were applied (containing roughly between 10^7 and 10^9 phages/ml, with 2 to 6 ml applied per dose). These were replaced 3 to 5 times per day. Treatment was for 32 days, followed by a break of 20 days, and then for 55 additional days. Numbers of bacteria present essentially were *not* reduced over the course of treatment. Nevertheless, clinical improvement was observed (p. 261): "significant reduction of ulceration dimension had been observed and it was followed by signs of epithelialization." To what degree this success might be due specifically to phage treatment is difficult to say.

3.6.1. Diabetic foot ulcers

Combinations of poor circulation, high glucose content of blood, and neuropathies in diabetics can result in both wounds of the feet and poor wound healing. Wounds can become infected, fail to heal, and subsequent amputation represents a common response to treatment failures.

Morozova et al. [110], in 2018, describes diabetic foot ulcer treatment using phages. The presentation is primarily from a methods perspective. Phage treatment generally is of patients who respond poorly to standard treatments, and, as a consequence of these prior treatment failures, is initiated particularly when amputation has otherwise been recommended. The authors employ phages which are available to them commercially (in Russia) along with phages obtained from other sources. Etiologies consist of *Enterococcus, P. aeruginosa*, or *Staphylococcus*. Recommended is the use of highly antibacterially virulent phages, high phage titers, and also, particularly in the case of treatment of *P. aeruginosa* infections, phage cocktails. In the course of treatments, wounds generally are debrided, rinsed with phages, and covered with gauze which has been soaked with phages. These treatments are then repeated as many as four times daily. Two case studies are presented consisting of the treatment large ulcers infected with MRSA. The duration of these successful treatments is approximately 3–4 weeks.

Fish et al. [111], from the same volume as Morozova et al. [110], presents two case studies of phage treatment of poorly vascularized diabetic toe ulcers infected with *S. aureus*, cases which differ from those provided by Fish et al. [112] (below). These are cases 2 and 6, where for both cases treatments were stopped sooner than would have been typical and for reasons which appear to have been otherwise unrelated to the treatment process. As also described by Morozova et al. [110], in these studies phage treatments generally were not initiated until infections had failed to respond to standard treatments (antibiotics), with amputation thereby otherwise indicated (I present additional treatment details below, while discussing the Fish et al., 2016, publication). With case 2, a total of three phage doses (one per week) were provided. The apparent result was no toe amputation along with complete ulcer healing. With case 6, the patient was injected with phages in the vicinity of ulcers, rather than treatments consisting of purely topical application. As with patient 2, patient 6 was treated only 3 times (weekly) with phages. The outcome, also, was both complete ulcer healing and an avoidance of amputation.

Fish et al. [112] from 2016, as with Fish et al. [111], presents case studies of phage treatment of poorly vascularized, *S. aureus*-infected diabetic toe ulcers, in this earlier article of five patients with infected ulcers which had not responded to antibiotics and for which toe amputation was indicated. The severity of these ulcers ranged over what is described as moderate or mild. The treated *S. aureus* was determined to be methicillin sensitive in all but one case. As with Fish et al. [111], phage application was weekly, but in all cases topical (direct application of phages as well as within dressings, with dressings changed after 48 h). Titers of the phage (Sb-1) preparation – obtained commercially from the former Soviet republic of Georgia – consisted of between 10^7 and 10^8 phages/ml, and is indicated as purified, presumably away from lysis products, using a column-purification process [111]. In addition to skin ulcers, three patients displayed clinically infected bone, with osteomyelitis confirmed in another two. Bone removal – as necessary (and not undertaken in all cases) – along with wound debridement was carried out. Resolution of inflammation tended to be rapid, resulting in closure, with treatments spanning an average 5.6 weeks (range of 4 to 19). Per Fish et al. [111], above, however, we can question whether all of the provided dosings in fact were absolutely necessary for resulting treatment success.

3.6.2. Venous leg ulcers

Venous leg ulcers are a consequence of improper functioning of veins. The result is a decrease in potential to carry blood from the legs back to the heart, and this can lead to localized tissue damage which in turn can result in ulceration of overlying skin, and subsequent infection.

Rhoads et al. [113] in 2009 presented a phase I safety trial of venous leg ulcer treatment. Ulcers must have been present for at least 30 days for inclusion in the study. Phages were applied topically once per week for 12 weeks, in combination with debridement using ultrasound. Etiologies targeted were *E. coli*, *P. aeruginosa*, and *S. aureus*, though the eight-phage cocktail employed was not confirmed to be highly efficacious prior to the initiation of treatment, but rather was optimized for safety particularly in terms of phage molecular characterization. Phages were applied with titers of approximately 10^8/ml. No efficacy was observed but also no toxicity was seen with phage treatment, both as in comparison with saline treated controls.

Markoishvili et al. [114], from 2002, is a PhagoBioDerm study of treatment of skin ulcers, including venous stasis ulcers, in Tbilisi, Georgia. PhagoBioDerm is a phage-impregnated artificial skin product which also contains the analgesic benzocaine and the antibiotic, ciprofloxacin, as well as the enzyme, α-chymotrypsin. Phages employed consisted of a cocktail ("PyoPhage") targeting *E. coli*, *Proteus*, *P. aeruginosa*, *S. aureus*, and *Streptococcus*. In this study the treatments of a total of 107 patients are summarized, all of whom failed to respond to conventional therapies prior to the initiation of PhagoBioDerm treatment, with targeted etiologies at least in some cases demonstrably resistant to antibiotics. Complete healing is indicated for 70% (of 96 so-monitored) of PhagoBioDerm-treated patients and improvement along with elimination of suppuration was seen in another 25%. Time until recovery ranged from approximately 1 to over 60 weeks. No phage-minus nor ciprofloxacin-minus controls were included in the study. Jikia et al. [115], as presented below, is also a PhagoBioDerm study.

Weber-Dąbrowska et al. [90] in their 2000 study considered the treatment of 77 patients with varicose ulcers of the lower extremities. Bacteria consisted of *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, or *S. aureus*. A total of 47 showed full recovery (61%) whereas 21 (or 27%) showed recovery but without elimination of target bacteria. Another 9 patients were not affected by the phage therapy. Their Fig. 2 provides before and after shots of a phage-treated patient.

Cisło et al. [81], from 1987, treated 31 patients with suppurative skin infections which had not responded to antibiotic treatment. Etiologies

included *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *Staphylococcus*. Phage treatments were both local and non-local (oral) and did not overlap temporally with antibiotic treatment, nor local disinfection. A total of 20 cases of "Trophic ulcers of legs in venous and arterial vessels insufficiency" were treated of which 10 showed "outstanding improvement" and another 5 "marked improvement". Side effects included painfulness in 2 cases and eczema (2 cases). From p. 177, "The treatment was terminated only when the skin lesions disappeared completely or three subsequent cultures gave negative bacteriologic result." In considering all 31 cases, treatment durations ranged from 1 to 16 weeks, on average taking 10 to 20 days.

Ślopek et al. [82], also from 1987, summarized under the heading of "Varicose veins of legs" the treatment of 36 patients. Etiologies are as for Weber-Dąbrowska et al. [90] but minus *Proteus*. Full eradication of the infection was seen in 27 cases, or 75%. For another 9 "marked improvement with tendency towards healing" is indicated.

Rice [102], published in 1930, described 3 cases of what are referred to simply as "Leg ulcers". Two of these treatments are indicated as having given rise to excellent results and one to good results. There is some speculation that bed rest may have given rise to two of these results rather than phages, though alternatively it is pointed out that previous bed rest, prior to phage treatment, did not result in similar outcomes.

3.6.3. Bed sores (a.k.a., decubitus or pressure ulcer)

Bed sores are a consequence of long-term application of pressure to specific locations on the body which results in an impeding of blood flow and/or associated mechanical damage to the skin. Resulting breaks in the skin can then persist, i.e., as ulcers, and become bacterially infected.

Weber-Dąbrowska et al. [90], in 2000, summarized treatments of infected decubitus ulcers. Of 16 patients treated with phages, 13 showed full recovery (81%) while for 3 there was no impact of phage treatment. Etiologies consisted of *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *S. aureus*. As for osteomyelitis (above), the numbers presented by Ślopek et al. [82] for phage treatment of decubitus ulcers are identical to those of Weber-Dąbrowska et al. [90]. Ślopek et al. [82], however, do indicate explicitly that the treated ulcers were "long-term", and that all treated infections were antibiotic resistant.

Rice [102], from 1930, reported on 21 cases of treatment of bed sores. Results are described as excellent (15), good (2), and poor (1), along with one patient who died before completion of phage treatment due to the primary disease (i.e., as not specifically due to the bed sores nor, presumably, the treatment). Ignoring the latter, then these are 83% excellent and 11% good outcomes. That conclusion, however, is qualified by the following statement (p. 353): "In reporting that 15 cases gave excellent results we do not mean that all were entirely healed. If the lesion became clean, was granulating, and starting to close in a badly debilitated patient, we have considered the result excellent." The author then goes on to discuss various cases in additional detail. Overall, it appears that phage treatment was associated with the healing or at least partial healing of relatively large bed sores, i.e., up to roughly 10 cm in diameter. Etiologies consisted of *Staphylococcus* or (presumably) *E. coli* as based on the specificity of the phages used.

3.7. Urinary tract infections

The urinary system, going from outside inward, consists of the urethra, bladder, ureters, and kidneys, with bacterial infections more likely with or towards the urethra and less so towards the kidneys. In addition to the urinary system are the genitals, together making up the urogenital system. The latter includes the prostate, the treatment of which is addressed above (Section 3.3). The current subsection, in turn, is limited to consideration of infections specifically of the urinary system. Of interest, Schultz [92] indicates that of early phage studies, a substantial number, as listed by the author, involved treatment of (p. 303) "colon infections of the urinary tract." The author further indicates, however, that treatments tend to be more effective against more acute rather than less acute infections, and further, though without explanation, that phage treatment of acute cases may be successful for reasons other than explicitly phage "action". Critical review of early years of phage treatment of urinary tract infections can be found in Eaton and Bayne-Jones [72]. Given the large number of early reports published on the phage treatment of urinary tract infections, the question of whether the infections treated were necessarily chronic, the inconsistent results observed, and that a good if rather old review of this literature exist in Eaton and Bayne-Jones [72], only a sampling of especially the early literature is reviewed below. It is important to note, however, that among phage treatments that Eaton and Bayne-Jones [75] report "perhaps" may be effective is against cystitis as caused by either "colon bacilli" or *Staphylococcus* (this is in addition to local infections by *Staphylococcus*, the potential against which greater certainty is expressed by these authors). A current clinical trial addressing the potential for phage use against urinary tract infections is being planned as reported by Leitner et al. [116].

Khawaldeh et al. [117] in 2011 presented a case report of an antibiotic-resistant *P. aeruginosa* urinary tract infection associated with a patient having received ureteric stents and possessing bladder ulceration. Antibiotics were applied over a two-year period prior to the start of phage treatment. A cocktail consisting of six phages at titer of 10^6/ml was instilled into the bladder in 20 ml volumes twice daily for 10 days. The (presumably) instilling urinary catheter was clamped for 30 min following instillation. Starting day 6, co-treatment with the antibiotics meropenem and colistin (the latter, a.k.a., polymixin E) began. *P. aeruginosa* viable counts declined by 1 log prior to day 6, and by about another 5 fold on day 6 (one time point) prior to the start of antibiotic treatment. Counts then progressed to zero (post day 7) following the start of antibiotic treatment. Phage numbers in urine declined, despite ongoing phage instillation, starting day 8, which is after numbers of target bacteria had declined to zero, suggesting ongoing phage replication within the urinary tract had occurred but only so long as host bacteria were present in substantial numbers. This study is suggestive of some utility to phage treatment in impacting chronic bacterial infections (i.e., 50-fold reduction in bacterial numbers) and that phage treatment can increase the susceptibility of these infections to antibiotics. This latter conclusion, however, is complicated due to a *lack* of reported colistin treatment prior to in association with phage treatment.

Letkiewicz et al. [106], in 2010, summarized the in-Polish publication by Boratyńska et al. [118] which described the treatment of 15 patients experiencing recurrent urinary tract infections. Etiologies consisted of *E. coli*, *Enterobacter aerogenes*, *K. pneumoniae*, *P. vulgaris*, and *S. aureus*, which are described as being "resistant to the available chemotherapies" (p. 106). Treatments consisted of oral phage delivery, following gastric neutralization, with three patients also treated simultaneously with antibiotics. Treatments lasted 3–11 months (mean of 5.4) with 12–36 month lessening of symptoms in combination with elimination of the targeted pathogen seen for 5 patients, and remission ("after 3–6 months") seen with another 3, suggesting a success rate of roughly 50%. Letkiewicz et al. [106] also report on a Russian study in which 46 patients with chronic or acute urogenital inflammation were treated via phage administration directly into the urinary bladder, resulting in 92% clinical improvement among treated patients [119].

Weber-Dąbrowska et al. [90] reported in 2000 on 78 urinary tract infections which were phage treated. These were caused by *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, or *S. aureus*. Full recovery was seen with 59 (76%) of treated patients. Another 9 (11.5%) recovered but without full elimination of the etiology. No effect of phage treatment was seen with another 10.

Ślopek et al. [82] from 1987 considered urinary tract infections under the heading of "Suppurative inflammations of the genitourinary tract". Of 42 cases, antibiotic resistance is described for 37. Phage therapy was fully successful for 39 cases (93%) whereas for one more case

marked improvement is indicated. Etiologies are the same as for Weber-Dąbrowska et al. [90]. Ślopek et al. provides as well this detail (p. 578), "In 2 cases of suppurative vaginitis due to infection with pyogenic *Staphylococci,* the washings with the specific bacteriophages totally eliminated the inflammation."

Schultz [92], as published in 1932, reported on 191 cases of "colon infections of the urinary tract" which were treated with phages. Of these, 151 are indicated as having been chronic. While 87% of the acute cases "responded promptly to treatments" (p. 304), "The results for the *chronic* cases were less impressive." These latter results the author attributes at least in part to urinary tract "anatomic disturbances". Notwithstanding this pessimism, still 47% (72) of the cases are reported as having either been cured or clinically improved following phage treatment, though in the case of 15 (p. 305) "infections recurred within ten days."

Rice [102], from 1930, described the treatment of cystitis, that is, a bladder infection, along with urinary fistulae, which are abnormal connections between the urinary system and other, non-urinary system organs (e.g., the colon). For the three cases of cystitis, results are described as excellent, with the target organism probably *E. coli*. Phages are described (p. 352) as "instilled into the bladder and retained as long as possible." A number of articles are listed also involving phage treatment of urinary tract infections. Results from treatment of urinary fistulae are described as excellent (2), good (2), and poor (1). Note (p. 352) that, "In all of the cases the characteristic pus discharge was checked and the lesion became clean or relatively so."

3.8. Other

Lecion et al. [109], as published in 2013, describe the phage treatment of chronically infected suppurating fistulae of various types, all infected with *S. aureus* (5 cases). Phages were applied in concentrations of roughly between 10^7 and 10^9 per ml via various combinations of orally (10 ml), via irrigation, or within wet compresses (2 to 6 ml), 1 to 5 times daily depending on route. Treatments were of duration of minimally four weeks. Wound healing was observed for one patient, clinical improvement for another 2, and no change for a further 2. Wound healing was associated with substantial reductions in bacterial counts, with clinical improvement there were roughly 1-log reductions in bacterial counts, and little or no non-transient reductions in bacterial counts were observed with no change in patient clinical status. The transient reductions in bacterial counts noted above appear to have been associated with the appearance of resistance to the treatment phages. The study, however, is primarily concerned with quantification of infecting bacteria. The authors indicate that using phages present at high titers can be useful towards phage therapy success.

Weber-Dąbrowska et al. [90] summarized, in 2000, the treatment of chronic suppurative fistulas. Etiologies included *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, and *S. aureus*. Based on 180 cases, 168 or 93% showed full recovery. Another 12 (7%) showed marked improvement, meaning that recovery was not associated with complete elimination of infecting bacteria. Equivalent numbers by Ślopek et al. [82] are also 180 cases, also 168 with infections eliminated, and also marked improvement in 12 cases, with equivalent etiologies.

Kvachadze et al. [120], from 2011, briefly reported on the phage treatment of both *Pseudomonas* and *Staphylococcus* in a seven-year-old cystic fibrosis patient who otherwise had undergone long-term antibiotic treatment. Phages were delivered via nebulization every 4 to 6 weeks, for 9 treatments in total. Results included substantial reductions in bacterial counts, general improvement in the patient's condition, and a subsequent 50% reduction in antibiotic use by the patient.

Other infections treated, as summarized by Weber-Dąbrowska et al. [90], included mucopurulent chronic bronchitis, laryngitis, and rhinitis; purulent mastitis; pyogenic post-operative infection; and suppurative sinusitis, etc. Full recovery for those listed, respectively, was 83% (224/271), 93% (41/44), 82% (29/35), and 83% (38/46). See also the results of presented by Ślopek et al. [82], of 92% (67/74) "suppurative process was fully eliminated" for "Suppurative inflammation of the respiratory tract".

Though not all of these treatments were necessarily of truly persistent or chronic infections, Weber-Dąbrowska et al. [90] note (p. 548) that phage treatment was of "1307 patients with different suppurative infections caused by multi-drug-resistant bacteria. The majority of cases were long, persisting infections in which antibiotic therapy had failed." Ślopek et al. [82] similarly states (p. 570) that "in 518 cases, i.e. in 94.2% the treatment preceding phage therapy failed, among others, due to resistance of bacteria to antibiotics and chemotherapeutics used. … Only in 31 cases (5.6%) bacteriophages were used in patients not pretreated with either antibiotics or chemotherapeutics." Ślopek et al. [82] notes as well, however, that in part (p. 582), "Unfavorable treatment results may be accounted, to a great extend [*sic*], to too late initiation of the treatment…"

Weber-Dąbrowska et al. [90] further indicate that treatments lasted from 1 to 12 weeks, for an average of 32 days. General description of these treatments is as follows (p. 548):

> Our studies included isolation and identification of bacterial strains from patient specimens, determination of the sensitivity of the isolated strains to specific [phages], and preparation of crude sterile [phage] lysates for therapy… In each case, [phages] were administered orally 3 times daily in the amount of 10 ml (children 5 ml) 30 min before eating, after neutralization of the gastric juice. Local administration depended upon a localization of the suppurative process. [Phages] were applied directly to the wounds, as ear and nose drops, as infusions to the fistulas, washing of the nasal cavity[,] suppurative lesions of pleura and peritoneum, decubitus and fistulas, intraperitoneally during the washing of the peritoneal cavity and topically in the cases of multiple skin abscesses.
>
> A more or less equivalent description is provided as well by Ślopek et al. [82].

4. Recent treatment of a persistent *Acinetobacter baumanni* infection

Recently, in 2016, a well-publicized clinical treatment of a long-standing, persistent bacterial infection took place in California, U.S.A. This was of a 68-year-old diabetic man for an antibiotic-resistant *A. baumanni* infection, an infection which, at the point of initiation of phage treatment, had been present for over 100 days [121]. This infection originally was identified with a pseudocyst associated with a necrotizing pancreatitis which had been induced by a gallstone. Antibacterial treatment involved azithromycin, colistin, meropenem, rifampin, tigecycline, and vancomycin, including in combination. Overall the *A. baumanni* isolated from the patient was found to be resistant to a total of 8 antibiotics. Over the course of 3-plus months the patient's condition continued to deteriorate by numerous criteria (p. 4): "The patient's prognosis was grave when bacteriophage therapy was first initiated… the patient was unresponsive to commands and had developed renal failure… Over the next 36 h, his clinical condition was stable, but he remained comatose… with worsening renal and hepatic function." Day 109 of the illness is indicated as the start of phage treatment. In this section I take a detailed look at that phage treatment towards ascertaining the possible role or roles of phage therapy in eventual patient recovery.

4.1. Initial phage dosing

From a Naval Research Medical Center collection of 200 anti-*A. baumanni* phages, a total of 98 initially environmentally sourced phages were tested against an *A. baumanni* strain that had been isolated from the patient, dubbed bacterial strain 'TP1'. From these phages a cocktail was devised, named 'ΦIV', which consisted of four phages. These phages individually were able to inhibit the growth of an

in vitro culture of TP1 for over 20 h (their Fig. 3), thus suggesting a low potential for evolution to phage resistance by this strain to each of the specific phages making up this cocktail. These phages had also been characterized previously as possessing distinct host ranges (from p. 2: "each phage was different from the others"). The phages were supplied for an indicated (their Table 3) total of 16 weeks intravenously, starting on day 111 of the illness.

The dosage used is described as 5×10^9 phages, which given a blood volume of about 5 l implies a maximum blood titer upon mixing of about 10^6 phages/ml, with a measured titer of 10^4 per ml five minutes following phage application, and substantial further declines in blood titers noted over the subsequent hour of phage exposure to patient plasma. These latter assays, however, did not specifically employ the ΦIV cocktail but rather were based on a subsequently used cocktail (ΦIVB; Section 3.4) which had an only one-phage overlap with ΦIV. Nevertheless, such titers, at any of these levels, are not very high and this suggests that phage population growth in association with target bacteria likely was necessary to reach sufficient phage numbers to ultimately achieve bacterial eradication, i.e., as via an active treatment [25]. Since neither such phage replication nor phage titers in direct association with the targeted bacteria in situ were determined, nor indeed bacterial counts following phage application, we at best can only conjecture that phage predation pressure against targeted bacteria during phage treatments may have been strong enough to result, on its own, in substantial as well as relatively rapid reductions in bacterial numbers (Section 4.2). Potential phage bactericidal impact on target bacteria in this study is, in other words, largely speculative (see also further issues raised in Sections 4.2 and 4.3). Dosing took place up to every 2 h, though was temporarily suspended early on while antibiotic treatment was continued, though the latter was as a different antibiotic combination than as applied prior to phage application (Section 4.2). Later phage dosing was every 6 to 8 h, and this continued, as stated by the authors, through until day 167.

Another four phages were also included in an additional cocktail. These phages were derived from other sources (one from the AmpliPhi Corporation and three as isolated from the environment, with inclusion of all four phages stemming from efforts which had taken place at Texas A&M University). This second cocktail, dubbed 'ΦPC', was supplied via an intracavitary method, which the authors clarify as (p. 6), "Through percutaneous catheters draining the pseudocyst cavity, the gallbladder, and a third intra-abdominal cavity." By this they mean that these phages were used to wash the above, and this was done every 6 to 12 h. Dosage explicitly is not indicated for these ΦPC phages, but these were the first phages applied, i.e., starting on day 109. These phages individually, rather than collectively as a cocktail, appear to have inhibited strain TP1 growth for only about 10 h in vitro, that is, until presumably phage-resistant mutants arose to prominence in these cultures.

4.2. Patient condition following initial phage dosing

Following the intracavitary and then intravenous phage administration – I infer that this was *soon after*, though this isn't explicitly stated – the patient's condition substantially improved. Especially, the patient awoke and was conversant. This observation is suggestive that phage application was causal in this improvement, though of course this is not definitive proof of causality. Particularly, no indication of infection microbiology, i.e., numbers of *A. baumannii* present in association with the patient, is indicated. Ideally, that is, dramatic improvements in patient condition as due to phage treatment would have been mirrored by reasonably substantial reductions in infection bacterial loads, but this was not reported (and presumably not determined).

In addition, the authors indicate that the patient's condition actually took a turn for the worse 2 days after the start of intravenous phage treatment, and at the same time meropenem dosing was increased while phage treatment was temporarily discontinued. Indeed, the exact timing of the dramatic improvement in the patient's condition following the initiation of phage treatment – in my opinion the most relevant phage-treatment result in the study [121] – is not indicated. Also around this same time the antibiotic minocycline was introduced, though the exact timing that the latter took place relative to the start and suspension of phage treatment as well as to the timing of the worsening of and improvement in the patient's condition is difficult to precisely ascertain.

The actual role of the phage treatments in the initial improvement in patient condition thus would appear to be murky at best, at least as can be garnered from the published account. Continued patient improvement over a large number of weeks nevertheless appears to have occurred during the course of ongoing treatment using these same phages. During that time however, so far as seems to be indicated, target bacteria might have been resistant to the phages being applied (Section 4.3).

4.3. Phage resistance of two A. baumannii isolates

A second *A. baumanni* strain was isolated, in this case after initiation of phage treatment. This was dubbed bacterial strain 'TP2', and was found to be completely resistant to all four ΦPC phages (the first phage cocktail applied). Experimentally, in vitro (their Fig. 3), resistance subsequently and independently arose in strain TP2 cultures also against all four of the ΦIV phages (the second cocktail applied), and this resistance arose in strain TP2 much faster than as seen experimentally with bacterial strain TP1. As strain TP2 initially displayed sensitivity to these ΦIV phages, these data appear to suggest that application of cocktail ΦPC and then application of cocktail ΦIV, each for only a few days, could have resulted in selection for a bacterial strain, TP2, which relative to strain TP1 was able to more rapidly evolve resistance to the phages found in cocktail ΦIV. It is important to keep in mind, however, that the prevalence of strain TP2 in the infection at the time of its isolation is not reported and that its isolation occurred while phages to some degree presumably were present in situ, as this was post the start of phage treatment. Phage presence in particular could have biased isolations *during plating* against phage-sensitive bacteria [122, 123], i.e., against bacteria which were more TP1-like in their phage resistance. It therefore is possible that strain TP2 was simply present in these infections and subsequently selectable at the point of its isolation rather than necessarily being highly prevalent at the *time* of its isolation.

My guess as to what is going on in terms of the observed experimental in vitro evolution of phage resistance (again, their Fig. 3) is that a mutation was present in strain TP2, but not strain TP1, which resulted in a collective as well as complete blocking of the action of the phages found in cocktail ΦPC (the first phages applied to the patient), but an only partial but still collective blocking of the action of the phages found in cocktail ΦIV (the second phages applied to the patient). The latter only partial blocking of phage action – perhaps delaying phages from reaching the surfaces of these bacteria for irreversible adsorption or otherwise delaying phage amplification in numbers in these cultures during in vitro assessment – could thereby have allowed sufficient bacterial growth in liquid culture in the presence of the ΦIV phages that further mutations to resistance to each of these phages were able to relatively rapidly appear. That is, in otherwise equivalent experiments, TP2 numbers might not have been reduced as rapidly as TP1 numbers despite phage presence during these in vitro experiments, resulting in more mutation events per TP2 versus TP1 culture and thereby faster mutation-limited bacterial evolution to phage resistance. The latter would then be seen as the sooner observed initiation of culture growth in these experiments.

Note that initial phage titers for these assays are not specified, just multiplicity of infection. Nor in addition are phage adsorption rate constants indicated. Consequently, the degree of phage population growth – once these in vitro experiments had been started – that was required to bring bacterial cultures under control is not known. Indeed, initial bacterial densities, i.e., the denominator of multiplicity of infection, are

low as experimentally indicated, and are not indicated in absolute terms. As multiplicity of infection is the ratio of phages to bacteria, low starting bacterial densities therefore could be consistent with relatively low initial phage densities, and thereby consistent with phage densities which were not necessarily sufficiently high to rapidly eliminate all still phage-sensitive bacteria found in these cultures, and particularly so if rates of phage adsorption to strain TP2 were slow. In other words, there is substantial uncertainty associated with the bacterial-resistance experiments performed, and perhaps greater uncertainty than there would have been had, for example, bacteria simply been plated for resistance in the presence of high, e.g., 10^9/ml, phage densities.

An alternative though probably less likely explanation for the pattern of development of phage resistance in these experiments could be that the TP1 strain, but not TP2, required two independent resistance mutations to develop full resistance to each of the ΦIV phage strains, i.e., as had been noted by Lenski with the evolution of *E. coli* B resistance to phage T2 [124]. In any case, apparently a bacterial strain was present in the patient's infection, TP2, which either possessed or was predisposed to evolving resistance to all eight of the initially applied phage types.

A third bacterial strain, TP3, was isolated only two days after the isolation of phage TP2, with TP3 shown in vitro to be fully resistant to all eight phages. Unlike bacterial strains TP1 and TP2, this third strain was unencapsulated, and thereby potentially less virulent against the patient. Given the timing of TP3's isolation, 8 days after the start of phage treatment, my guess is that it either arose from TP2 or instead was present in the infection perhaps even prior to the start of phage application. Furthermore, as TP3 prevalence in infections also is not indicated, so too caveats applying to the isolation of strain TP2 should apply as well to the isolation of strain TP3. Thus, it seems clear that among the bacteria found within the patient's infection were strains which either were resistant or were predisposed, perhaps by more than one mechanism, to becoming resistant to the treatment phages employed. The actual prevalence of these strains in the patient, particularly relative to phage-sensitive bacteria, however is not indicated.

4.4. Late-stage phage dosing

The response to the appearance of strain TP3, though not applied until over 100 days following the initial start of phage treatment, was the intravenous application of a third phage combination, consisting of a new phage plus one phage derived from the ΦIV cocktail. This new phage combination was dubbed ΦIVB and appeared to delay TP3 evolution of phage resistance, in vitro, equally as well as cocktail ΦIV was able to delay strain TP1 evolution of phage resistance also in vitro (both their Fig. 3). Application of these ΦIVB phages was at the same relatively low titers as ΦIV, and treatment with ΦIVB took place over only the final two weeks defining the end of treatments. A contribution of these ΦIVB phages to treatment success during those two weeks, however, is not well substantiated in the article.

4.5. Evidence of phage therapy efficacy?

Treatments with various antibiotics, as noted, took place over the course of phage treatment, and evidence is presented that phage resistance might have been associated with increased pathogen sensitivity to antibiotics. We therefore may speculate that one of these various treatments, one or more of the phages and one or more of the antibiotics – with the latter perhaps especially as modified after the start of phage treatments as this coincided with dramatic improvements in the patient's condition – resulted in reductions in bacterial loads within the patient. These efforts, possibly in combination with the action of the patient's own immune system (Section 5), potentially as against less virulent phage-resistant bacteria, presumably contributed to patient recovery.

That this outcome was substantially a consequence of phage action nevertheless does not in my opinion appear to be robustly supported by the data provided [121]. Such a conclusion, in particular, is complicated by a varying of multiple parameters during the crucial first few days of phage treatment, would have greatly benefitted from a precise timeline regarding treatment modifications along with changes in patient condition, and lacks quantitative assessments of infection microbiology. Indeed, as the authors note (p. 9), and I agree: "…we cannot exclude the possibility that reversal of his clinical deterioration was unrelated to the phage therapy." Certainly, though, the possibility of considerable phage therapy efficacy against this persistent bacterial infection also cannot be excluded.

5. Persistent infection, immunodeficiency, and phage therapy

In order for clinically apparent bacterial infections to persist within a body, whether that body is being treated with antibacterial agents or not, then the body must in some manner be immunocompromised. That is, the body must be unable to clear the infections on its own. Underlying causes of these inabilities can include especially efforts by the infecting bacteria to thwart immune system actions, but in addition are various deficiencies in the body's ability to successfully mount an immune response against pathogens more generally. For the latter, these immunodeficiencies can include poor vascularization such as seen with diabetics, insufficient nutrition to support fully functional immune responses, various diseases other than the bacterial infection in question which can interfere with immune system functioning, primary immunodeficiencies (i.e., body genetic predispositions), immunosuppression due to the action of chemotherapeutics, mechanical damage to tissues including by the infection itself that also can interfere with immune action, or insertion of various foreign materials into the body, e.g., implant-related infections for the latter. In addition, immune responses typically will tend [125] to be (p. 1026) "compromised by the needs of the host to prevent immune-mediated damage." The result can be persistent bacterial infections even by bacteria which typically are not well equipped to establish long-term bacterial infections within otherwise healthy individuals, e.g., persistent *Escherichia*, *Pseudomonas*, or *Staphylococcus* infections (Table 2), and this is in contrast to various infections which can become chronic even within relatively healthy individuals, e.g., such as those caused by mycobacteria, various sexually transmitted bacterial infections, Lyme disease, etc. Thus, a certain fraction of persistent bacterial infections, including many which have for various reasons proven to be resistant to prior antibiotic treatment, will have an associated immunodeficiency of some sort. In this section I consider the potential interplay between immunodeficienices, persistence of bacterial infections, and phage therapy efficacy.

5.1. Immunity and phage treatment

Górski et al. [126] provide an overview of various aspects of the interactions between phage therapy, immunity, and immunodeficient states, including suggesting a utility to phage therapy despite an absence of full immune system functioning. Arguments however have also been made and evidence presented, most recently by Leung and Weitz [127] and Roach et al. [59], of a utility for immune system functioning as an aid to phage therapy success. In those two studies it is explicitly innate immunity against acute bacterial infections which is considered, though in the latter, experimental study using mice, this therapeutic utility associated with proper immune system functioning was explicitly a consequence of prevention of emergence of phage-resistant bacterial populations rather than necessarily due to an inability to substantially reduce in number bacteria that were sensitive to treatment phages. Nevertheless, should we have an expectation that immunodeficiencies could result in reduced phage therapy effectiveness against chronic or persistent bacterial infections?

Perhaps consistent with a validity to this latter suggestion, note especially the long durations required to treat long-standing infections using phages, i.e., as discussed in Section 3. D'Hérelle, as translated by Smith [128], in fact noted nearly 90 years ago a distinction between newer ("acute infections or in infectious conditions that have not become fully chronic"; p. 176) and older infections ("chronic conditions"; p. 176) in terms of durations of phage treatments, indicating a need for greater numbers of phage applications in the latter case to achieve treatment success. Thus, immunodeficiencies are postulated to interfere with phage treatment [127], in at least narrow circumstances can be shown to interfere with phage treatment [59], and long-standing bacterial infections which to some degree can be responses to immunodeficiences tend to take more effort to treat using phages than more acute bacterial infections.

5.2. Immunodeficiency before versus during phage treatment

An important question to ponder is whether challenges associated with the phage treatment of long-standing bacterial infections are direct consequences of immunodeficiencies rather than indirect consequences, if indeed delays in the impact of phage treatment of persistent bacterial infections (Section 5.1) are a consequence of immunodeficiencies at all. That is, is this an issue of immune functioning not being entirely maximal *during* phage treatment, thereby resulting in less antibacterial activity during treatment (which essentially is the perspective of Leung and Weitz [127] and represents a direct impact on phage therapy success) or instead, and indirectly, due to immune systems not being fully effective in the course of infection persistence *prior to* phage treatment?

Might then equivalently treatment-refractory states be present – were such chronic or persistent infections able to become established at all – either with or without maximal immune system functioning prior to treatment? Specifically, do infections become more resistant over time to future phage action precisely because they, for example, have become overgrown due to failure of immune functions to better control bacterial growth or, in addition or alternatively, because immunity has failed to better control bacterial formation of phage-refractory biofilms?

5.3. Experimental exploration could be difficult

The above questions are not simple to address. This is because to the degree that immunodeficiencies are required for infections to persist at all over long durations, then older infections for many pathogens in many systems would not be expected to exist without such immune system deficiencies. Nevertheless, it is a relevant question, at least academically, of to what degree levels of immune system dysfunction prior to versus during phage treatment might affect the efficacy of phage treatment of chronic or persistent bacterial infections.

Ideally, for testing, immunodeficiencies in animals would be induced after persistent infection development but immediately prior to the initiation of phage treatment, though such a strategy (again) would require that persistent infections can be established at all in animals which are not immunodeficient. If immunodeficiencies are required for infection persistence, then alternatively one would need to terminate immunodeficiencies at the point of phage addition to study the impact of the presence or absence of more effective immune responses during rather than prior to phage treatment. In both cases it instead is more likely that *degrees* of immunosuppression may be compared, rather than immunosuppression versus complete lack of immunosuppression, and together these would have to vary over the course of experiments, that is, to achieve such comparisons (i.e., less immunosuppression followed by more, or more followed by less). Testing such hypotheses as to the impact of immunosuppression on efficacy either before or during phage treatments of especially long-standing bacterial infections thus is not likely be facilely achieved using animal models, but nevertheless should not be inherently impossible. It is further important that restoration of immune system functioning, even if only partial such as by adding back relevant cell types, not by itself be sufficient to fully clear infections in animal models.

5.4. Of what practical utility?

At this point in time we really don't have much appreciation of the importance of host immunity as an aid to phage treatment of chronic or persistent bacterial infections, though certainly it seems reasonable to speculate that host actions against bacterial invaders in many cases should be helpful. For example, such immune system actions could follow phage-mediated bacterial reductions to somewhat lower population densities, though not necessarily to zero bacteria (perhaps leaving behind only phage-resistant bacteria which the immune system could then eliminate, i.e., as seen in the Roach et al. [59] experiment). Alternatively, this could be following phage-mediated disruptions of immune-system inhibiting infection properties (i.e., such as following biofilm disruption), or given phage-mediated selection for resistance mutations in target bacteria which pleiotropically result in greater levels of bacteria susceptibility to immune system action (and thereby leaving phage-resistant bacteria more susceptible to immune system action than wild-type bacteria). In all of these examples the idea is that existing immune system processes might be able to function better following phage treatment, thereby potentially aiding phages towards bacterial elimination, though only of course if those immune system functions are not otherwise compromised.

Notwithstanding such issues, it remains an open question to what degree research into the role of immune systems in the aiding of phage therapy of chronic or persistent bacterial infections might have practical utility. Thus, clinically, tackling such questions of how the immune system can be helpful to phage therapy success might only be relevant to the extent, ideally unlikely, that addressing issues of patient health, in addition to direct treatment of infections, in some manner could be detrimental to phage treatment outcomes. In other words, to the extent treatments specifically designed to boost patient immune functioning are not harmful to the patient, e.g., such as might be achieved in the course of improved nutrition, or instead via tissue debridement of wounds, then it is difficult to see why such treatments would not be undertaken for the sake of potentially also positively impacting phage therapy success.

On the other hand, immunodeficient animal models – perhaps particularly to the extent that relatively mild human immunodeficient conditions may be reasonably well mimicked – likely would be useful towards testing new phage therapy strategies against long-standing bacterial infections. That is, especially to the degree that such infections come to require relatively long durations of phage application to treat, as is often the case for clinical phage treatments of more long-standing bacterial infections (Section 3), then immunodeficient infection models possibly could serve to better approximate those infections, and their treatment. Such model systems could be useful towards improved pre-clinical phage therapy development.

6. Conclusions

It is important to keep in mind that chronic or persistent infections, among bacterial infections, can be difficult to successfully treat. Of those infections which fail to resolve given antibiotic treatment, whether due to antibiotic resistance by etiologies or instead due to antibiotic tolerance by infections, the treated infections will tend to end up being relatively long in duration if indeed the patient (or limb) survives. The tendency for phage therapy to serve in many instances as a treatment of last resort therefore has a consequence of reserving phage therapy for the treatment of what generally will be difficult infection types to treat. It is encouraging, therefore, that a fairly large number of examples of seemingly successful – though nonetheless in nearly all cases not

placebo controlled – clinical phage treatments of these seemingly persistent or chronic bacterial infections exist, though it certainly is reasonable to question how effectively such successes may be transferred to less experienced practitioners.

Especially the older literature, as well as that of Ślopek et al. [82] and Weber-Dąbrowska et al. [90], and though less well documented at least in English the extensive use of commercially available phages in the former Soviet Union, is helpful with this latter concern. That is, though it would be foolish to imagine that these early phage therapists were any less experienced than more modern-day practitioners, nevertheless the breadth individuals who have been reported to have successfully treated chronic, persistent, or at least potentially non-acute bacterial infections is encouraging. Indeed, the impression one receives is that given application of an effective phage or phage cocktail which is well matched to targeted bacteria, a means of delivering these phages to those bacteria, delivering phages in sufficient numbers, and also patience in waiting for results (potentially weeks or even months of repeated dosing), together may go a long way towards relatively consistent phage therapy success against persistent or chronic bacterial infections. Nevertheless, it likely will be important for physicians who are otherwise unfamiliar with phage therapy to be formally trained in the use of phages if we are to hope to expect such consistent success given expansion of phage use as a therapeutic. Therefore, while animal experimentation will continue to be important towards phage therapy development (Section 2), it will be similarly important to both support and learn from those individuals, such as in Poland, Georgia, and also Russia [6, 23], who, with substantial experience, still routinely practice phage therapy clinically. Phage therapy, in other words, represents a potential means of curing otherwise incurable, in many cases debilitating, chronic or persistent bacterial infections, and it therefore behooves us to nurture as well as invest in this existing practitioner experience.

Competing interests

The author has advised and holds equity stake in companies with phage therapy interests, and maintains the websites phage.org and phage-therapy.org, but received no support in the writing of this manuscript.

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