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Perspective

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Pathways to Phage Therapy Enlightenment, or Why I Have Become a Scientific Curmudgeon

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Abstract

Over the past decade I, with collaborators, have authored a number of publications outlining what in the first of these I described as ''Phage therapy best practices''—phage therapy being the use of bacterial viruses (bacteriophages) to treat bacterial infections, such as clinically. More generally, this is phage-mediated biocontrol of bacteria, including of bacteria that can contaminate foods. For the sake of increasing accessibility, here I gather some of these suggestions, along with some frustrations, into a single place, while first providing by way of explanation where they, and I, come from scientifically. Although in my opinion phage therapy and phage-mediated biocontrol are both sound approaches toward combating unwanted bacteria, I feel at the same time that the practice of especially phage therapy research could be improved. I supply also, as supplemental material, a list of ~100 English language 2000-and-later publications providing primary descriptions of phage application to humans.

Keywords: antibacterial, antibiotic, bacteriophage therapy, biological control

Introduction

Curmudgeon (*noun*)—old sourpuss (as in honor of my 60th birthday)

Bacteriophages (phages) are the viruses of bacteria. For nearly as long as phages have been known to science—known at least with some certainty[1]—they have been used clinically as antibacterial agents, and we have reached or are about to reach 100 English language 21st Century primary publications describing phage application to humans (Supplemental Data).[2] This phage *therapy* can be described more generally as phage-mediated biological control of bacteria, such as of *Listeria* contaminating foods.[3,4] Though of much less consequence, we are also approaching the 20th anniversary of the first phage therapy or at least the first phage-mediated antibacterial biocontrol-emphasizing publications that I have personally contributed to.[5,6]

Throughout my career, my interests nonetheless have focused chiefly on bacteriophage evolutionary ecology and related issues of phage organismal ecology, starting >30 years past; for example, Refs.[7] and [8]. It is from those interests that I was able to develop an appreciation of phage therapy pharmacology, and particularly of phage therapy pharmacodynamics.[9–12]

I have since taken it upon myself, given these interests and other personal proclivities, to point out situations where a better understanding of pharmacology could be helpful to improve the effectiveness of phage therapy, particularly toward phage therapy's translation from the laboratory to real-word applications; see Refs.[2,13–18] plus the Appendix of Ref.[19] as well as a companion review found also in the current issue (pp. 98–111). Here I summarize some of my suggestions, some hints as to where my sometimes exasperation with the phage therapy literature has come from, and indications of where I have discussed individual issues more thoroughly.

Toward Phage Therapy Enlightenment

The following are general suggestions toward improving phage therapy research, or at least improving the resulting literature:

1. Provide details of phage sources.[15] Included among these details should be comprehensive referencing to the previous antibacterial use of the specific phages being studied, as well as to other relevant biological properties.
2. There should be good reasons for choosing a phage or phages for phage therapy, and those reasons should be provided.[13,15] Of course, do consider excluding

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from use phages with particularly undesirable properties, including temperate phages and phages encoding bacterial virulence factors.[12]

3. Provide goals, techniques, and results of phage purification as this is important when treating animals and especially for clinical phage use.[15] After their purification, it is also a good idea to describe how phages are stored along with their stability during that storage.[15]
4. Consider testing phages using *in vitro* conditions that reasonably mimic those anticipated *in situ* during treatments.[13,15,17] For example, if a treatment is to be in blood or in urine, then consider studying *in vitro* phage properties within media that at least approximates those environments.
5. Provide sufficient details for replication of experiments, including phage production, processing, and characterization.[2,15,19] Crucial also will be phage titers, volumes, and, often, numbers applied during dosing as well. Be sure also to separately describe the titers of individual phages making up phage cocktails, as in many cases it can be difficult to distinguish these in publications from the titer of a cocktail as a whole.[2,15]
6. If describing dosing in terms of multiplicity of infection (MOI), it can be helpful to specify whether this is MOI_{input} (number of phages added relative to number of bacteria added to) or instead MOI_{actual} (number of phages that actually adsorb relative to number of bacteria added to). The latter in fact is the historical meaning of MOI.[14,15,20] But please do not describe phage dosing *just* in terms of MOIs as phage titer and/or phage number information can be crucial to experiment replication.
7. If basing results on bacterial numbers or densities, it is important to document what quantities of bacteria were present *in situ* just before phage application.[17] This is rather than numbers only at the point of initial bacterial addition and particularly not just numbers found at the end of experiments for no-treatment controls.
8. What is the timing of phage application relative to the timing of bacterial challenge or, clinically, relative to the point of detection of infection?[13,15] What is the route and other details of this phage application?[2,15] If possible, determine phage titers *in situ* as found especially immediately after phage application, or at least try to provide approximations of what those titers might be.[15]
9. Consider whether your therapy approach depends upon active versus passive treatments.[9–12,16,19,20] That is, will treatment success likely be dependent on phage population growth *in situ* (active treatment) or instead will phage population growth not necessarily be needed (passive treatment)? Are bacteria expected to be present in sufficient numbers to even support active treatment if that is required?[19]
10. Consider dosing with more phages or dosing more often should original treatment efforts prove insufficiently efficacious.[13,16,19]
11. It is important to not be ambiguous about the criteria used for inclusion of bacteria in a phage's host range.[15,21] For example, simply stating that a phage displays ''lytic activity'' against a given bacterial host often is not very useful if we do not know how that lytic activity was measured. A concern also is the inclusion of a bacterium in a phage's host range if plaque formation is observed but confluent lysis was expected during spot testing. This is because formation of a few plaques when many more phages have been applied can imply low efficiencies of plating and/or plaque formation by only phage host range mutants.
12. When treating with phages in combination with inhibiting concentrations of antibiotics, consider the potential for antibiotic-associated antagonism of phage infection activities.[22,23] In particular, in the presence of the concentrations of antibiotics you are using, are your phages still capable of displaying productive infections? Also, are antibiotic minimum inhibitory concentrations being determined in the same medium/under the same conditions that experiments are carried out in?
13. Describe what ingredients phages are formulated with as well as any purposes or drawbacks.[15] That is, why is what being used? It also can be useful, for example, to avoid animal-derived products for phage propagation given subsequent phage use *in vivo*.[24] Also problematic are absences of carbon and energy sources when applying phages *in vitro* such as to biofilms.[17]
14. Important as well can be details associated with the bacteria used for phage propagation, such as their possible carriage of prophages or virulence-factor genes.
15. Provide details of the individuals being treated along with their nonphage-related care.[15] How well do improvements in the condition of treated individuals coincide temporally with phage application versus other aspects of treatments such as dosing with antibiotics?[2] What other studies have explored the treatment of similar infections and how do results compare?
16. What are the specifics of the bacteria being treated and why were they chosen or targeted?[15] What are the details of preparation of bacteria for preclinical studies; for example, overnights or log phase cultures?[15] What other studies have explored preclinical or clinical application of phage therapy to this or these bacterial species?
17. Though more complex to determine than bacterial starting conditions, if it is possible to determine, then what is the bacterial state at the point of phage addition. For example, are they present as biofilms?[15,19] Indeed, and given that phages typically are not employed clinically to treat acute bacterial infections, can an experimental bacterial infection of an animal be legitimately described as chronic, that is, as often are associated with bacterial biofilms?[25]
18. Under almost all circumstances, the only reasonable indication of phage therapy efficacy should be *improvement* in the state of the treated patient, or environment, relative to *before* the point of phage addition, such as in terms of reductions in bacterial numbers.[17] Claiming as phage-treatment success just *less* of an *increase* in bacterial numbers relative to untreated controls should, by contrast, often be viewed with skepticism.
19. Be unambiguous in describing how treatment efficacy has been determined.[15,19] Ideally, this will include relevant caveats such as if number of bacteria

remaining have not been determined,[15,17] particularly as relative to starting bacterial numbers. Or, if those numbers have been determined, whether an effort to prevent phage adsorption of bacteria during enumeration was used.[13,17,19] It also can be useful to provide quantitative determinations of phage resistance rather than just statements that phage resistance was observed.[25]

20. Lastly, what might be the real-world adequacy of efficacy if observed.[2,15,19] For example, is a one log reduction in bacterial concentrations really meaningful? Were any toxicities observed?[13,15] Especially clinically, can we really distinguish the contribution of nonphage treatments to bacteria killing, for example, antibiotic cotreatments from phage-mediated killing?[2]

As a budding curmudgeon, what I would love to see is a phage therapy literature that works better for *me*. Ideally the suggestions provided here, however, might help to bolster the progress of the field of phage therapy for everyone. ¡Viva la phage therapy!

Author Disclosure Statement

S.T.A., a faculty member of the Ohio State University for >25 years, has consulted for and served on advisory boards for companies with phage therapy interests, holds equity stakes in a number of these companies, and maintains the websites phage.org and phage-therapy.org. No additional competing financial interests exist. The text presented represents the perspectives of S.T.A. alone, and no outside help was received in its writing.

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Supplementary Material

Supplementary Data

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