Review Article

Bacteriophage therapy for management of bacterial infections in veterinary practice: what was once old is new again

RA Squires[*§]

[*]Discipline of Veterinary Science, College of Public Health, Medical and Veterinary Sciences, James Cook University, Townsville, Queensland, Australia.
[§]Author for correspondence. Email: richard.squires@jcu.edu.au

Abstract

Bacteriophages (or phages) are naturally-occurring viruses that can infect and kill bacteria. They are remarkably diverse, numerous and widespread. Each phage has a narrow host range yet a large majority of bacteria studied so far play host to bacteriophages, hence the remarkable phage diversity. Phages were discovered just over 100 years ago and they have been used for treatment of bacterial infections in humans and other animals since the 1920s. They have also been studied intensively and this has led to, and continues to lead to, major insights in the fields of molecular biology and recombinant DNA technology, including that DNA is the genetic material, nucleotides are arranged in triplets to make codons, and messenger RNA is needed for protein synthesis. This article begins with a description of bacteriophages and explains why there has recently been a strong resurgence of interest in their clinical use for treatment of bacterial infections, particularly those caused by organisms resistant to multiple antimicrobial compounds. The history of bacteriophage therapy is briefly reviewed, followed by a review and critique of promising but very limited clinical research on the use of bacteriophages to treat bacterial infections in dogs. Other potential veterinary uses and benefits of bacteriophage therapy are also briefly discussed. There are important practical challenges that will have to be overcome before widespread implementation and commercialisation of bacteriophage therapy can be achieved, which are also considered.

KEY WORDS: *Bacteriophage, phage, phage therapy, bacteria, infection, antimicrobial resistance*

UPEC Uropathogenic *Escherichia coli*

Introduction

Bacteriophages (or phages) are viruses that can infect and kill bacteria. They are remarkably diverse, numerous and widespread in nature. Phages were discovered just over 100 years ago and

have been used for treatment of bacterial infections in humans and other animals since the 1920s. After penicillin became widely available, interest in bacteriophage therapy waned substantially in much of the western world but continued elsewhere, especially in some East European countries.

The emerging antimicrobial resistance crisis has caused a resurgence of interest in bacteriophage therapy. Bacteriophages can kill their host bacteria regardless of whether or not the host is resistant to multiple antimicrobial compounds. There has recently been a marked increase in the number and quality of research publications dealing with phage therapy. A few of these publications have dealt with clinical trials of a phage preparation for treatment of *Pseudomonas aeruginosa* otitis externa in dogs. However there are important challenges that will need to be overcome if bacteriophage therapy is to become a mainstream treatment modality that is used widely.

The purpose of this article is to provide veterinary practitioners, particularly companion animal practitioners, with basic knowledge and understanding of bacteriophage therapy, including some of the challenges to its use, so that they are in a stronger position to consider its use in their patients when future opportunities arise.

What are bacteriophages?

Bacteriophages are viruses that parasitise prokaryotes, including bacteria. They were discovered a little over 100 years ago and some of them were studied intensively from the 1940s onwards, contributing substantially to developments in the fields of molecular biology and recombinant DNA technology. Virulent (or lytic) bacteriophages can infect, lyse and kill bacteria, including pathogens of veterinary importance that are resistant to multiple antimicrobial compounds. Immediately after infection, the bacterial cellular machinery is redirected to manufacture of phage particles, which are released in large numbers when the host cell lyses. These newly-formed virus particles are then available to infect additional bacterial cells. The typical structure of a T4 phage (often used in therapy) and the T4 lytic infection cycle are presented in Figure 1. Electron micrographs of five phages isolated from a sewage treatment plant (in Palmerston North, NZ) that were able to kill a broad range of canine and feline uropathogenic *Escherichia coli* (UPEC) strains are shown in Figure 2. Bacteriophages have been used to treat bacterial infections in animals and humans since the early 1920s (Sulakvelidze *et al.* 2001). In view of the emerging and worsening global antimicrobial resistance crisis (Van Puyvelde *et al.* 2018), bacteriophage therapy as an alternative or supplement to conventional antimicrobial therapy has received renewed attention in recent years (Golkar *et al.* 2014; Grant *et al.* 2016).

Bacteriophages are extremely diverse, numerous and widespread. Their origins are very probably ancient (Hatfull and Hendrix 2011). Almost every bacterial species that has been studied in sufficient detail has been found to host at least one kind of bacteriophage, often many. Most bacteriophages are host strain-specific, explaining (together with ancient ancestry) their extraordinary diversity (Hatfull 2015). Phage particles are also hugely abundant in the external environment. For example, it has been estimated that coastal seawater contains approximately 10^7 tailed phage particles per mL (Wommack and Colwell 2000). Sewage-polluted rivers probably contain far higher concentrations. The number of phage particles on Earth at any instant is estimated at 10^{31}; an astronomically huge figure. There is also massive turnover (10^{23} phage infections per second) of this population (Hatfull and Hendrix 2011).

Bacteriophages have been used as tools and studied extensively in the development of molecular biology and recombinant DNA technology. Just some of the discoveries made through study of phages include DNA being the genetic material (except in RNA viruses); nucleotides are arranged in triplets to form codons; messenger RNA is needed for protein synthesis; and restriction endonucleases, which arose in bacteria during the course of evolution to cleave infecting bacteriophage DNA, can be used as workhorses by researchers and technologists in recombinant DNA work (Salmond and Fineran 2015). The study of bacteriophages continues to yield remarkably useful and exciting insights and new tools. Recently this includes tools derived from the bacterial immune system, or CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), which act against phages and plasmids (Uppada *et al.* 2018). These tools are now being used extensively for editing the genomes of many plants and animals, including, potentially, humans and companion animals (Lee *et al.* 2018).

The diversity of phages is as impressive as their massive abundance. Their diversity mirrors, indeed seems to exceed, that of their host bacteria and members of *Archaea*. In one small study, a single visit to a sewage treatment plant in Palmerston North, NZ, and collection of a small volume of effluent fluid, was sufficient to discover 40 distinct bacteriophages capable of killing canine and feline uropathogenic *Escherichia coli* (UPEC) strains (Freitag *et al.* 2008). The mean proportion of UPEC strains that could be killed by an individual bacteriophage was 21/53 (40 (min 17, max 72)%). Only 3/53 of the studied UPEC strains could not be killed by any of the phages that were collected during that single collection visit. The 10 most effective phages could each, individually, kill >50% of the 53 studied UPEC strains. Used in combination, i.e. in a phage cocktail, as might be used clinically, these 10 phages could be expected to kill up to 92% of the studied UPEC strains.

There is remarkable diversity in phage biology. It is very likely that collection of effluent fluid from a much larger sewage treatment facility (for example, in a much larger city), or perhaps repeated visits to a small facility, would have provided correspondingly greater phage diversity. Related to this supposition, it was reported as early as 1896 that water drawn from the Ganges and Jumna rivers in India had marked antimicrobial activity, specifically, against *Vibrio cholerae* (Hankin 1896). Hankin found that the antimicrobial factor or substance passed through fine porcelain filters and was variably heat-labile. These findings have been interpreted by many subsequent authors as early evidence of bacteriophage activity, although authors of a recent detailed analysis have disputed this interpretation (Abedon *et al.* 2011b).

Why is there resurgent interest in the use of bacteriophage therapy?

Emerging and worsening antimicrobial resistance, which has recently driven a resurgence of interest in bacteriophage therapy, represents an enormous threat to public health; one that is exacerbated by an almost empty antimicrobial pipeline (Burki 2017). The implications of this threat for New Zealand has been reviewed by the Royal Society of New Zealand – Te Apārangi (Anonymous 2017). The global cost of this worsening crisis is reportedly high and growing, although reliable estimates are difficult to obtain. A systematic literature review of reports on the economic burden caused by antimicrobial resistance was recently published (Naylor *et al.* 2018). This article reported an economic burden ranging from US$21,832 per case to a decrease in global gross domestic product (GDP) of >US$3 trillion (in 2013 dollars) by 2050.

Multiple, potential coping strategies for worsening antimicrobial resistance have been proposed. Among these bacteriophage therapy has received relatively little, but increasing, attention. A key point is that bacteriophages can infect and kill their bacterial hosts regardless of whether or not the hosts are resistant to multiple antimicrobial compounds. This is because antimicrobial resistance and phage susceptibility are, for the most part, unrelated. In general, antimicrobial resistant bacteria are no less susceptible to phage killing (Allen *et al.* 2017). Indeed, one recent paper indicates that, at least for one bacterial host species (*Acinetobacter baumannii*), antimicrobial resistant bacteria were more susceptible to phage killing than antimicrobial susceptible strains (Chen *et al.* 2017).

Although the worsening antimicrobial resistance crisis has strengthened recent interest in bacteriophage therapy, there are many other infectious disease situations, beyond the treatment of antimicrobial-resistant infections, for which phage therapy holds promise. For example, a key, potential advantage is that bacteriophages multiply at the site of a bacterial infection. Difficult-to-access infections may therefore be more amenable to bacteriophage therapy than to antimicrobial therapy, if a sufficient number of phage particles can initially be applied to, or gain access to, the

site of infection (Payne *et al.* 2000). Phage particles are much larger than antimicrobial molecules, so ensuring they gain access to the sites of infection can be a substantial challenge, as discussed in detail in one recent review (Nilsson 2014). The exquisite host specificity of bacteriophages (Freitag *et al.* 2008; Jensen *et al.* 2015) is another important potential advantage. Non-pathogenic and potentially health-promoting bacteria in the normal microbiome are likely to be spared because of this (Miller-Ensminger *et al.* 2018). Indeed, the host specificity is often so extreme as to require use of mixtures of several different phages (phage cocktails) to treat infections (Freitag *et al.* 2008; Melo *et al.* 2016). More sophisticated approaches to the development of expanded-range phage mixtures have been developed recently (Mapes *et al.* 2016).

Purified phage preparations have been safely administered to humans topically, orally, intravenously and into body cavities (Brussow 2016; Sarker *et al.* 2016; Speck and Smithyman 2016). Phages and phage subcomponents have also been sprayed onto food products (Galarce *et al.* 2014), including pet foods (Heyse *et al.* 2015), to increase food safety. This is a preventative rather than a therapeutic use of phages and therefore will not be considered further in this review.

A brief history of bacteriophage therapy

The history of phage therapy has been previously reviewed (Summers 2001; Chanishvili 2012; Wittebole *et al.* 2014). Bacteriophages were discovered about 100 years ago, independently, by Twort in London in 1915 and d'Herelle in Paris, in 1917 (Summers 2011). Twort described "glassy transformation" of his micrococcal colonies. Today, when phages are grown on a lawn of bacteria on agar, the clear spots caused by focal bacterial lysis are called plaques as shown in Figure 3. Soon after he made his discovery, d'Herelle began to investigate the use of bacteriophages for treatment of infectious diseases of animals. He studied pasteurellosis in water buffaloes, salmonellosis in chickens and shigellosis in rabbits (Summers 2001). Having established to his own satisfaction their safety and promising efficacy, he went on to carry out human trials. D'Herelle's first research report on phage therapy in humans, published in 1921, described success in the treatment of bacillary dysentery in children. He later reported apparent success in the treatment of bubonic plague, using direct injection of a bacteriophage preparation into the buboes (infected lymph nodes). Remarkably, bacteriophages from d'Herelle's original, start-up company remained commercially available to French physicians until 1978. Bacteriophage therapy continued to be available and used clinically in France until the early 1990s (Abedon *et al.* 2011a).

Early progress in developing efficacious bacteriophage preparations was hampered by a deep lack of understanding of phage biology. Early on, many researchers believed that bacteriophages were enzymes rather than viruses (Summers 2001). Some commercially-available preparations contained

preservatives that would undoubtedly have inactivated, and therefore rendered ineffective, the phages. Later on, the fact that much of the work on bacteriophage therapy was being done in the Union of Soviet Socialist Republics (USSR) gave bacteriophage therapy a so-called Soviet Taint in western Europe and North America (Summers 2001).

In the 1940s, the increasing clinical use of penicillin, and appreciation of its dazzling efficacy, led to a substantial decline of interest in bacteriophage therapy, particularly in the United States of America and United Kingdom. Phage therapy continued to be studied and used extensively in France, Germany, Russia and the rest of Eastern Europe (Summers 2011).

Throughout the 1980s, excellent veterinary research on phage therapy was carried out by Smith and his colleagues at the Houghton Institute for Animal Disease Research (UK). He studied the therapeutic use of bacteriophages in young farm animals, mostly directed against bacterial diarrhoeal diseases (Smith and Huggins 1982, 1983; Smith *et al.* 1987a). This work addressed many of the weaknesses of earlier experimental studies of bacteriophage therapy. In a set of landmark studies, these researchers investigated the use of phage therapy in calves, piglets and lambs. They also carried out rigorous experimental studies in mice. They focussed upon treatment of diarrhoea caused by a particular, well characterised strain of *E. coli*. In 1987 they showed that severe, experimental diarrhoea in calves could be cured by a single dose of 10^5 phage particles and could be prevented by doses as low as 100 particles (Smith *et al.* 1987b). They obtained their phages from ordinary sewage.

Little was published about bacteriophage therapy in the 1990s in journals indexed by Medline (https://www.ncbi.nlm.nih.gov/pubmed/). However from 2000–2010 numerous publications considered a range of topics including use of bacteriophage therapy in the management of antimicrobial resistant infections (Biswas *et al.* 2002); use of bacteriophages to “cleanse” farm animals of potentially zoonotic infections in the immediate pre-slaughter period, i.e. before transportation of the animals to the abattoir (Loc Carrillo *et al.* 2005); and the use of bacteriophages or bacteriophage components to control foodborne bacteria in packaged food products (Greer 2005). Since 2009 a much larger number of articles, covering a remarkably broad range of bacteriophage therapy-related topics, has been published each year in journals indexed by Medline and Web of Science (https://clarivate.com/products/web-of-science/).

Recent studies of bacteriophage therapy in companion animals

There is an excellent and growing literature on the use of bacteriophages in livestock, particularly intensively-reared livestock, to improve food safety and diminish the use of antimicrobials (e.g.

Endersen *et al.* 2014 and Kazi and Annapure 2016). In comparison to livestock, very little research has so far been published on the use of bacteriophages in companion animals. The author is aware that bacteriophage therapy has occasionally been used by companion animal and equine practitioners on an exceptional basis but, so far, very few research or clinical publications are to be found.

There have, however, been a few promising, recent developments and publications on phage treatment of *P. aeruginosa* otitis externa, including in dogs (Wright *et al.* 2009; Hawkins *et al.* 2010). Indeed, a commercial veterinary product for treatment of *Pseudomonas* otitis (containing six bacteriophages) became available in Europe as a consequence of some of this research, although it is not currently available. The following paragraphs describe this small body of research on phage therapy in dogs.

The first, preliminary paper, published in 2006, provided two very brief case reports: a human with skin burns and *P. aeruginosa*-infected, failing skin grafts and a dog with chronic otitis externa (Marza *et al.* 2006). The dog was a 5-year-old St Bernard, reported to have atopy and chronic, bilateral otitis externa, associated with *P. aeruginosa* infection. The otitis had failed to resolve despite repeated courses of topical and systemic antimicrobials. A bacteriophage preparation was applied topically to both patients. Substantial multiplication of the applied *Pseudomonas*-targeting bacteriophage (as surrogate evidence of bacterial killing) was noted in both patients shortly after topical treatment, much more impressive in the dog. Little clinical detail and no information about the phages used are provided in this first paper. The dog's right ear was treated, and was reported to be dramatically improved 27 hours after the bacteriophage application with no adverse consequences. Then the other ear was treated. The bacteriophage used in this dog multiplied at least a million-fold in the right ear shortly after it was administered. *P. aeruginosa* was subsequently isolated from the ears of this dog in the weeks after bacteriophage treatment and there were cycles of clinical improvement and deterioration. However the ears were reported to be consistently better than before the bacteriophage treatment and no further antimicrobial treatment was used. Nine months after phage application, both ears were described as having completely recovered and *P. aeruginosa* was not subsequently cultured. It is not stated how many samples were collected for culture and sensitivity.

A second study of bacteriophage therapy for chronic, *P. aeruginosa*-associated otitis externa in dogs was published 4 years later by members of the same British research group (Hawkins *et al.* 2010). Ten dogs with *P. aeruginosa*-associated otitis externa each received, into one of their external auditory canals, a 0.2 mL cocktail of six bacteriophages, 100,000 plaque-forming units of

each phage in the mixture. The chosen combination of six bacteriophages had previously been shown to have *in vitro* activity against 90% of *P. aeruginosa* strains derived from canine otitis externa cases. The total number of strains examined to obtain this percentage figure is not stated in the paper. Dogs were re-examined 48 hours after a single bacteriophage treatment. A clinical score, quantitative bacterial culture and bacteriophage quantitation were done before and after the treatment. There was a 30% mean reduction in clinical score at 48 hours, a 67% reduction in bacterial count, and a 99.1-fold mean increase in bacteriophage count. Limited information is provided about which particular bacteriophages (of the six different types in the cocktail) had increased in number in each dog and whether the effective phage or phages differed from dog to dog, as one might expect. It is noteworthy that the phage cocktails used in each dog were not optimised for each particular dog. A standard product was used, presumably with a view to rapid commercialisation. Indeed, a commercial veterinary product was successfully developed out of this research. Much better results might have been anticipated if phage cocktails tailored to the particular *P. aeruginosa* strains infecting each dog had been used. However, as will be discussed below, that would have made registration of a commercial product much more challenging, if not impossible. It would be interesting to know whether *in vitro* susceptibility of bacteria to the phage cocktail was tested in these dogs and whether or not *in vitro* results correlated with *in vivo* clinical improvement of the dogs.

Although there were limitations in the design and execution of these two veterinary studies, the results were sufficient to support the licensing of a commercial product in Europe, and these findings are very promising. It would be interesting to compare such a fixed ingredient product with personalised phage therapy, i.e. choosing and using the best phages for a particular infection on the basis of *in vitro* susceptibility of bacteria.

This research group also carried out a randomised, double-blind, placebo-controlled, phase I/II clinical trial of a therapeutic bacteriophage preparation for use in humans, similar to the cocktail used in the previously described study of canine otitis (Wright *et al.* 2009). This clinical trial was for treatment of chronic otitis due to antimicrobial-resistant *P. aeruginosa* in humans. This was the first clinical trial of its kind.

What challenges are impeding the widespread adoption and implementation of bacteriophage therapy?

Many challenges will need to be overcome if bacteriophage therapy is to become a mainstream, commercially-viable therapeutic option for humans and companion animals with difficult-to-manage bacterial infections rather than remaining a therapeutic modality used only piecemeal or in

the worst of clinical circumstances. Some of these challenges are unfortunately formidable. As a consequence, many researchers and companies have turned away and focussed their attention on non-therapeutic uses of bacteriophages such as in food safety, nutraceutical development and clinical diagnostics (Lu and Koeris 2011). However the growing antimicrobial resistance crisis is also formidable, therefore some researchers and companies are continuing their efforts to address challenges in several distinct areas.

The challenge of insufficient high-quality evidence

There is a pressing need for further well-controlled clinical trials of bacteriophage therapy to be completed in humans and companion animals and the results published. Data from experimental animal studies (Biswas *et al.* 2002; Shivaswamy *et al.* 2015), and a considerable volume of published clinical experiences, mostly from Eastern Europe, support the view that bacteriophage therapy can be highly efficacious under a variety of circumstances, including when treating serious bacterial infections caused by organisms resistant to multiple antimicrobial compounds (Verstappen *et al.* 2016). On the other hand, *in vitro* susceptibility to phage is not always a reliable predictor of *in vivo* efficacy (Tsonos *et al.* 2014). Considerably more robust evidence is required.

The challenge of meeting regulatory requirements

Existing medicinal regulatory frameworks are designed to deal with ready-to-use pharmaceutical and biological products. Fixed-ingredient phage cocktails containing, for example, equal numbers of six different phages against a particular strain or species of pathogenic bacteria, although quite different from conventional pharmaceutical compounds, are somewhat reminiscent of modified live vaccines or medical products that contain more than one active ingredient. Every animal treated with such a phage remedy would receive the same product. As described above, one phage-containing medicinal product has already been successfully licensed for veterinary use in Europe (Hawkins *et al.* 2010).

Personalised phage therapy is radically different. Each animal would potentially receive a unique cocktail, based on the demonstrated phage susceptibility of the infecting bacterial pathogen(s) causing illness. This is already happening on a very small scale in the worst of clinical circumstances in humans and companion animals (Fish *et al.* 2018). How will such phage-based medicinal products be defined and regulated? Fauconnier (2017) has described them as being "...somewhere between magistral formulas and industrially made medicinal products". Considerable challenges will need to be overcome before widespread implementation is achieved. Indeed, a new paradigm for management of bacterial infectious diseases will need to be developed, beginning

when a bacterial pathogen is isolated from a diseased individual, identified and determined to be resistant to multiple antimicrobial compounds (Chan *et al.* 2013).

Expert working groups in Europe and the United States of America have already done some of the work of scrutinising pharmaceutical regulatory frameworks with a view to identifying and tackling bottlenecks and potential tangles and finding ways forward to facilitate widespread implementation of personalised phage therapy (Huys *et al.* 2013; Pelfrene *et al.* 2016; Fauconnier 2017).

The challenge of generally narrow phage host ranges

Narrow host range of most phages can be viewed as both a challenge and a benefit, as previously discussed. The traditional approach to narrow phage host ranges has been to use phage cocktails rather than individual phages (Chan *et al.* 2013). Newer approaches include genetic modification of phages to broaden their host range and co-incubation of phages with multiple bacterial strains, including their cognate host, and selection of phage mutants that develop broadened host range (Mapes *et al.* 2016).

An interesting question is “How many different phages should be in a cocktail”? The vast majority of reports describe cocktails that contain four to eight different phages, but this author was unable to find reasons, other than the following of historical precedent, why the number should not be much greater.

The challenge of emerging bacterial resistance

Bacteria have co-evolved with phages for hundreds of millions of years, so it is unsurprising that they have evolved multiple resistance mechanisms. Conversely, phages have evolved great diversity and a multiplicity of attack mechanisms. It has been reported that the use of phage cocktails tends to slow the emergence of bacterial resistance as compared with the use of individual phages (Fischer *et al.* 2013; Yu *et al.* 2017). Phages act synergistically when used in cocktails (Schmerer *et al.* 2014). The enormity of phage diversity would usually permit different individual phages, or even different phage cocktails, to be used sequentially in personalised treatment, should this prove necessary because of lack of effectiveness or emergence of resistance in particular patients.

Challenges related to adverse clinical effects of phage therapy

Concerns have been expressed that massive and rapid lysis of bacterial cells during phage therapy could occasionally be associated with rapid release of endotoxins, including superantigens, causing significant morbidity (Matsuda *et al.* 2005). However clinical descriptions of this potential adverse effect of phage therapy are not readily available. Still, animal experiments have shown that lysis-deficient phages can be used in serious infections, such as severe septic peritonitis, and their use

was associated with lower mortality than was seen when using a conventional, lytic phage for treatment (Matsuda *et al.* 2005). Lysis-deficient phages kill bacteria without inducing endotoxin release.

Poorly-manufactured phage preparations can also be contaminated with endotoxin, with the potential to cause morbidity, especially when delivered by nebuliser. Modern manufacturing techniques are available to prevent this (Cooper *et al.* 2014).

Challenges associated with rapid removal of phage by the immune system, especially from the bloodstream

Therapeutically applied bacteriophages are exposed to, and stimulate, the immune system of the animal under treatment (Krut and Bekeredjian-Ding 2018). This can produce a synergistic beneficial effect, with the immune system (particularly phagocytes) enhancing the ability of phage to resolve the infection (Roach *et al.* 2017). When phages are administered intravenously, many are removed rapidly by the reticuloendothelial system and phage-neutralising antibodies. Serial passage of phages through animals, and consequent selection of progressively longer-circulating mutants, has been achieved by researchers (Merril *et al.* 1996).

Challenges associated with the potential for harmful gene transfers

Some phages are capable of transmitting potentially harmful genes to their bacterial hosts. This matters if the bacterial cell does not die (i.e. is not lysed) immediately after phage infection. Some phages (described as temperate) can integrate into the host bacterial chromosome after infecting the cell and may bring toxin-encoding genes with them. The process is termed lysogeny and the phage is said to have entered the lysogenic lifecycle (Feiner *et al.* 2015). The process is reminiscent of retroviral integration into a vertebrate cell, in that the integrated phage genome is transmitted to subsequent generations of bacteria without being transcribed or translated and it does not cause bacterial cell lysis. At a later time the integrated phage genome ceases to be repressed, exits the lysogenic lifestyle, is expressed to make numerous viral particles and causes cell lysis. It is said to have entered the lytic cycle.

The use of only virulent (rapidly lytic) phages for therapeutic purposes, not those prone to undergo lysogeny, reduces the risk of harmful horizontal gene transfer substantially (Hobbs and Abedon 2016). Currently, bacteriophages intended for inclusion in any new commercial phage therapy product for use in humans, either in isolation, or as part of a cocktail, would need to be thoroughly characterised and safety tested to satisfy regulatory authorities. This would typically require full genome sequencing of each phage with a search for potentially deleterious genetic components, especially genes that encode toxins or bacterial virulence factors or predispose towards lysogeny.

Conclusion

Bacteriophage therapy may, in future, prove to be a valuable component in a multi-pronged approach to dealing with the worsening challenge of increasing antimicrobial resistance in bacterial pathogens. However more evidence from high-quality clinical trials will be needed to increase confidence in the breadth of their effectiveness, and regulatory frameworks for licensing biological therapeutic agents will probably need to be reconsidered and adjusted if personalised phage therapy is to be approved for use beyond the worst clinical circumstances.

Acknowledgements

The author is most grateful to Dr Thurid Johnstone, his former PhD student, who co-authored with him an earlier, non-peer-reviewed article on bacteriophage therapy, upon which some parts of this new article are based (Squires and Johnstone 2017).

References

Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM. Phage treatment of human infections. *Bacteriophage* 1, 66–85, 2011a

Abedon ST, Thomas-Abedon C, Thomas A, Mazure H. Bacteriophage prehistory. *Bacteriophage* 1, 174–8, 2011b

Allen RC, Pfrunder-Cardozo KR, Meinel D, Egli A, Hall AR. Associations among antibiotic and phage resistance phenotypes in natural and clinical *Escherichia coli* isolates. *MBio* 8, e01341-17, 2017

***Anonymous.** *Antimicrobial Resistance – Implications for New Zealanders.* https://royalsociety.org.nz/assets/documents/Antimicrobial-resistance-factsheet-May-2017.pdf (accessed 14 June 2018). Royal Society – Te Apārangi, Wellington, NZ, 2017

Biswas B, Adhya S, Washart P, Paul B, Trostel AN, Powell B, Carlton R, Merril CR. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. *Infection and Immunity* 70, 204–10, 2002

Brussow H. Targeting the gut to protect the bladder: oral phage therapy approaches against urinary *Escherichia coli* infections? *Environmental Microbiology* 18, 2084–8, 2016

Burki T. Antibiotic development pipeline slows to a trickle. *Lancet Infectious Diseases* 17, 1128–9, 2017

Chan BK, Abedon ST, Loc-Carrillo C. Phage cocktails and the future of phage therapy. *Future Microbiology* 8, 769–83, 2013

Chanishvili N. Phage therapy--history from Twort and d'Herelle through Soviet experience to current approaches. *Advances in Virus Research* 83, 3–40, 2012

Chen L-K, Kuo S-C, Chang K-C, Cheng C-C, Yu P-Y, Chang C-H, Chen T-Y, Tseng C-C. Clinical antibiotic-resistant *Acinetobacter baumannii* strains with higher susceptibility to environmental phages than antibiotic-sensitive strains. *Scientific Reports* 7, 6319, 2017

Cooper CJ, Denyer SP, Maillard JY. Stability and purity of a bacteriophage cocktail preparation for nebulizer delivery. *Letters in Applied Microbiology* 58, 118–22, 2014

Endersen L, O'Mahony J, Hill C, Ross RP, McAuliffe O, Coffey A. Phage therapy in the food industry. *Annual Review of Food Science and Technology* 5, 327–49, 2014

Fauconnier A. Regulating phage therapy: the biological master file concept could help to overcome regulatory challenge of personalized medicines. *EMBO reports* 18, 198–200, 2017

Fauconnier A. Guidelines for bacteriophage product certification. *Methods in Molecular Biology* 1693, 253–68, 2018

Feiner R, Argov T, Rabinovich L, Sigal N, Borovok I, Herskovits AA. A new perspective on lysogeny: prophages as active regulatory switches of bacteria. *Nature Reviews: Microbiology* 13, 641-50, 2015

Fischer S, Kittler S, Klein G, Glunder G. Impact of a single phage and a phage cocktail application in broilers on reduction of *Campylobacter jejuni* and development of resistance. *PloS One* 8, e78543, 2013

Fish R, Kutter E, Wheat G, Blasdel B, Kutateladze M, Kuhl S. Compassionate use of Bacteriophage therapy for foot clcer treatment as an effective step for moving toward clinical trials. *Methods in Molecular Biology* 1693, 159–70, 2018

Freitag T, Squires RA, Schmid J. Naturally occurring bacteriophages lyse a large proportion of canine and feline uropathogenic *Escherichia col*i isolates *in vitro*. *Research in Veterinary Science* 85, 1–7, 2008

Galarce NE, Bravo JL, Robeson JP, Borie CF. Bacteriophage cocktail reduces *Salmonella enterica* serovar Enteritidis counts in raw and smoked salmon tissues. *Revista Argentina de Microbiologia* 46, 333–7, 2014

Golkar Z, Bagasra O, Pace DG. Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. *Journal of Infection in Developing Countries* 8, 129–36, 2014

Grant A, Hashem F, Parveen S. *Salmonella* and *Campylobacter*: antimicrobial resistance and bacteriophage control in poultry. *Food Microbiology* 53, 104–9, 2016

Greer GG. Bacteriophage control of foodborne bacteria. *Journal of Food Protection* 68, 1102–11, 2005

Hankin EH. The bactericidal action of the waters of the Jumna and Ganges rivers on *Cholera* microbes. *Annales de l'Institut Pasteur* 10, 511–23, 1896

Hatfull GF, Hendrix RW. Bacteriophages and their genomes. *Current Opinion in Virology* 1, 298–303, 2011

Hatfull GF. Dark matter of the biosphere: the amazing world of bacteriophage diversity. *Journal of Virology* 89, 8107–10, 2015

Hawkins C, Harper D, Burch D, Anggard E, Soothill J. Topical treatment of *Pseudomonas aeruginosa* otitis of dogs with a bacteriophage mixture: a before/after clinical trial. *Veterinary Microbiology* 146, 309–13, 2010

Heyse S, Hanna LF, Woolston J, Sulakvelidze A, Charbonneau D. Bacteriophage cocktail for biocontrol of *Salmonella* in dried pet food. *Journal of Food Protection* 78, 97–103, 2015

Hobbs Z, Abedon ST. Diversity of phage infection types and associated terminology: the problem with 'Lytic or lysogenic'. *FEMS Microbiology Letters* 363, doi: 10.1093/femsle/fnw047 2016

Huys I, Pirnay J-P, Lavigne R, Jennes S, De Vos D, Casteels M, Verbeken G. Paving a regulatory pathway for phage therapy. Europe should muster the resources to financially, technically and legally support the introduction of phage therapy. *EMBO Reports* 14, 951–4, 2013

Jensen KC, Hair BB, Wienclaw TM, Murdock MH, Hatch JB, Trent AT, White TD, Haskell KJ, Berges BK. Isolation and host range of bacteriophage with lytic activity against

methicillin-resistant *Staphylococcus aureu*s and potential use as a fomite decontaminant. *PloS One* 10, e0131714, 2015

Kazi M, Annapure US. Bacteriophage biocontrol of foodborne pathogens. *Journal of Food Science and Technology* 53, 1355–62, 2016

Krut O, Bekeredjian-Ding I. Contribution of the immune response to phage therapy. *Journal of Immunology* 200, 3037–44, 2018

Lee JG, Sung YH, Baek IJ. Generation of genetically-engineered animals using engineered endonucleases. *Archives of Pharmacal Research*, doi 10.1007/s12272-018-1037-z, 2018

Loc Carrillo C, Atterbury RJ, el-Shibiny A, Connerton PL, Dillon E, Scott A, Connerton IF. Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Applied and Environmental Microbiology* 71, 6554–63, 2005

Lu TK, Koeris MS. The next generation of bacteriophage therapy. *Current Opinion in Microbiology* 14, 524–31, 2011

Mapes AC, Trautner BW, Liao KS, Ramig RF. Development of expanded host range phage active on biofilms of multi-drug resistant *Pseudomonas aeruginosa*. *Bacteriophage* 6, e1096995, 2016

Marza JA, Soothill JS, Boydell P, Collyns TA. Multiplication of therapeutically administered bacteriophages in *Pseudomonas aeruginosa* infected patients. *Burns* 32, 644–6, 2006

Matsuda T, Freeman TA, Hilbert DW, Duff M, Fuortes M, Stapleton PP, Daly JM. Lysis-deficient bacteriophage therapy decreases endotoxin and inflammatory mediator release and improves survival in a murine peritonitis model. *Surgery* 137, 639–46, 2005

Melo LDR, Veiga P, Cerca N, Kropinski AM, Almeida C, Azeredo J, Sillankorva S. Development of a phage cocktail to control *Proteus mirabilis* catheter-associated urinary tract infections. *Frontiers in Microbiology* 7, 1024, 2016

Merril CR, Biswas B, Carlton R, Jensen NC, Creed GJ, Zullo S, Adhya S. Long-circulating bacteriophage as antibacterial agents. *Proceedings of the National Academy of Sciences* 93, 3188–92, 1996

Miller-Ensminger T, Garretto A, Brenner J, Thomas-White K, Zambom A, Wolfe AJ, Putonti C. Bacteriophages of the urinary microbiome. *Journal of Bacteriology* 200, e00738-17, 2018

Naylor NR, Atun R, Zhu N, Kulasabanathan K, Silva S, Chatterjee A, Knight GM, Robotham JV. Estimating the burden of antimicrobial resistance: a systematic literature review. *Antimicrobial Resistance and Infection Control* 7, 58, 2018

Nilsson AS. Phage therapy--constraints and possibilities. *Upsala Journal of Medical Sciences* 119, 192–8, 2014

Payne RJ, Phil D, Jansen VA. Phage therapy: the peculiar kinetics of self-replicating pharmaceuticals. *Clinical Pharmacology and Therapeutics* 68, 225–30, 2000

Pelfrene E, Willebrand E, Cavaleiro Sanches A, Sebris Z, Cavaleri M. Bacteriophage therapy: a regulatory perspective. *Journal of Antimicrobial Chemotherapy* 71, 2071–4, 2016

Roach DR, Leung CY, Henry M, Morello E, Singh D, Di Santo JP, Weitz JS, Debarbieux L. Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. *Cell Host and Microbe* 22, 38–47, 2017

Salmond GP, Fineran PC. A century of the phage: past, present and future. *Nature Reviews: Microbiology* 13, 777–86, 2015

Sarker SA, Sultana S, Reuteler G, Moine D, Descombes P, Charton F, Bourdin G, McCallin S, Ngom-Bru C, Neville T, *et al.* Oral phage therapy of acute bacterial diarrhea with two coliphage preparations: a randomized trial in children rrom Bangladesh. *EBioMedicine* 4, 124–37, 2016

Schmerer M, Molineux IJ, Bull JJ. Synergy as a rationale for phage therapy using phage cocktails. *PeerJ* 2, e590, 2014

Shivaswamy VC, Kalasuramath SB, Sadanand CK, Basavaraju AK, Ginnavaram V, Bille S, Ukken SS, Pushparaj UN. Ability of bacteriophage in resolving wound infection caused by multidrug-resistant *Acinetobacter baumannii* in uncontrolled diabetic rats. *Microbial Drug Resistance* 21, 171–7, 2015

Smith HW, Huggins MB. Successful treatment of experimental *Escherichia coli* infections in mice using phage: its general superiority over antibiotics. *Journal of General Microbiology* 128, 307–18, 1982

Smith HW, Huggins MB. Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. *Journal of General Microbiology* 129, 2659–75, 1983

Smith HW, Huggins MB, Shaw KM. Factors influencing the survival and multiplication of bacteriophages in calves and in their environment. *Journal of General Microbiology* 133, 1127–35, 1987a

Smith HW, Huggins MB, Shaw KM. The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. *Journal of General Microbiology* 133, 1111–26, 1987b

Speck P, Smithyman A. Safety and efficacy of phage therapy via the intravenous route. *FEMS Microbiology Letters* 363, fnv242, 2016

***Squires RA, Johnstone T.** Bacteriophage therapy: a ray of hope in the war against antimicrobial-resistant bacteria. *Australian and New Zealand College of Veterinary Scientists, Dermatology Chapter, Science Week Proceedings.* Pp 40–5, 2017

Sulakvelidze A, Alavidze Z, Morris JG, Jr. Bacteriophage therapy. *Antimicrobial Agents and Chemotherapy* 45, 649–59, 2001

Summers WC. Bacteriophage therapy. *Annual Review of Microbiology* 55, 437–51, 2001

Summers WC. In the beginning. *Bacteriophage* 1, 50–1, 2011

Tsonos J, Oosterik LH, Tuntufye HN, Klumpp J, Butaye P, De Greve H, Hernalsteens JP, Lavigne R, Goddeeris BM. A cocktail of *in vitro* efficient phages is not a guarantee for *in vivo* therapeutic results against avian colibacillosis. *Veterinary Microbiology* 171, 470–9, 2014

Uppada V, Gokara M, Rasineni GK. Diagnosis and therapy with CRISPR advanced CRISPR based tools for point of care diagnostics and early therapies. *Gene* 656, 22–9, 2018

Van Puyvelde S, Deborggraeve S, Jacobs J. Why the antibiotic resistance crisis requires a One Health approach. *Lancet Infectious Diseases* 18, 132–4, 2018

Verstappen KM, Tulinski P, Duim B, Fluit AC, Carney J, van Nes A, Wagenaar JA. The effectiveness of bacteriophages against methicillin-resistant *Staphylococcus aureus* ST398 nasal colonization in pigs. *PloS One* 11, e0160242, 2016

Wittebole X, De Roock S, Opal SM. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence* 5, 226–35, 2014

Wommack KE, Colwell RR. Virioplankton: viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews* 64, 69–114, 2000

Wright A, Hawkins CH, Anggard EE, Harper DR. A controlled clinical trial of a therapeutic bacteriophage preparation in chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa*; a preliminary report of efficacy. *Clinical Otolaryngology* 34, 349–57, 2009

Yu L, Wang S, Guo Z, Liu H, Sun D, Yan G, Hu D, Du C, Feng X, Han W, *et al.* A guard-killer phage cocktail effectively lyses the host and inhibits the development of phage-resistant strains of *Escherichia coli*. *Applied Microbiology and Biotechnology* 102, 971–83, 2017

Submitted 4 January 2018

Accepted for publication 14 June 2018

Figure 1. Anatomy and lytic infection cycle of phage T4. The phage DNA genome is initially within the icosahedral head. After attachment of the phage to the bacterial cell via its fibres (1), the tail shortens and phage DNA is injected into the bacterium (2). There, it is rapidly transcribed and translated to produce numerous new phage components (3). These autoassemble (4) and the cell lyses, releasing phage into the extracellular space (5). Image by Guido4 (https://commons.wikimedia.org/wiki/File:11_Hegasy_Phage_T4_Wiki_E_CCBYSA.png) licensed under CC by SA 4.0 (accessed 3 June 2018).

Figure 2. Electron micrographs of five phages collected in Palmerston North, New Zealand, that each had a relatively broad host range against uropathogenic *Escherichia coli* (Uranyl acetate staining, bar=100nm). The four on the left appear morphologically similar to lytic phage T4, widely used in phage therapy. The morphological findings were verified by DNA sequence analysis (Freitag *et al.* 2008). Image courtesy of Dr Thurid Johnstone, University of Melbourne, Australia.

Figure 3. Photograph of phage plaques growing in soft agar on a lawn of their host bacteria. They appear clear, glassy, dark and circular. These are ΦM12 phages, but phages used for therapy appear similar. Image by Ninjatacoshell (https://upload.wikimedia.org/wikipedia/commons/0/08/%CE%A6M12_Plaques_in_Sinorhizobium_meliloti.JPG) licensed under CC by SA 3.0 (accessed 3 June 2018).