

Phage therapy for diabetic foot infection

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The treatment of diabetic foot infections (DFIs) represents a costly and growing challenge to the NHS. DFIs can be difficult to treat for a variety of reasons, including late presentation of advanced infection, and antibiotic tolerance or resistance. Bacteriophage (phage) are ubiquitous viruses that infect and kill bacteria in a species-, sometimes even strain-, specific manner. Phages have been used to treat bacterial infection since 1919, but their use in the geopolitical West ceased in the 1930s due to a variety of factors, including the mass production of antibiotics. The modern antibiotic resistance crisis has driven renewed interest in phage therapy and 2,241 patients with mostly with antibiotic refractory infections have been treated since 2000, 79% of whom improved. This includes at least 310 patients with chronic wound infections, among whom 86.1% achieved clinical resolution or improvement of infection. Reassuringly, the available evidence suggests that phage therapy is safe and without notable side effects. Some phages also possess enzymes capable of degrading the biofilms that afford antibiotic tolerance to bacteria and underpin many chronic infections. Phages also act independent of antibiotic resistance, allowing the treatment of even pan-resistant bacteria, and topical or local application to DFIs means antimicrobial activity is independent of a patient's peripheral perfusion. Presently only an option when antibiotics are not meeting a patient's clinical needs, future integration of phage therapy at all levels of DFI care will radically transform the outlook for DFIs in the UK. Reducing the number of serious infections and amputations will not only benefit patients but will deliver vast savings to the NHS and reduce the amount of antibiotics used, making phage therapy a tangible response to the antibiotic resistance crisis.

Over 4.9 million people in the UK have diabetes mellitus, herein diabetes, and this could rise to 5.5 million by 2030 (Diabetes UK, 2020a). Around one third of patients with diabetes will develop a diabetic foot ulcer at some point (Armstrong et al, 2017). The aetiology of diabetic foot ulcers (DFUs) generally reflects trauma superimposed upon peripheral neuropathy and peripheral arterial disease. In the UK, approximately 2–2.5% of the diabetic population have a foot ulcer

at any one time, representing a significant health burden and costing the NHS approximately £1 billion annually (Kerr et al, 2019). Approximately half of all DFUs become infected, with wound care and antibiotics the mainstay of treatment (Prompers et al, 2007; Lipsky et al, 2012). Amputation may be considered when clinical resolution cannot be achieved by antibiotics. A recent study in the UK found that one year after diagnosis, 55% of DFI patients were still infected and almost 15% had

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undergone amputation (Ndosi et al, 2018). It has also been reported that there are 169 diabetic foot amputations performed each week in England alone, although the proportion of those due to ischaemia in the absence of infection is not known (Diabetes UK, 2018). It is often the clinical and microbiological complexity of an infection rather than antibiotic resistance that necessitates amputation (Dörr et al, 2021). Amputation is a costly outcome for both the patient and NHS, with each major amputation alone estimated to cost £8,213, excluding additional associated inpatient, outpatient or postoperative costs (Kerr et al, 2019). The treatment of DFIs therefore represents a significant and sizeable clinical challenge for the NHS.

Diabetic foot infections (DFIs) can be difficult to treat for several reasons, including late presentation of complex acute infections, complicating osteomyelitis or resistance to antibiotics. Antibiotic resistant bacteria encode genetic resistance mechanisms. Although DFIs often contain bacteria resistant to one or more antibiotics, complete resistance to antibiotics is in fact rarely encountered (Dörr et al, 2021). Antibiotic tolerance is more common and is likely responsible for many chronic or recurrent infections (Sulaiman and Lam, 2021). Unlike resistance, antibiotic tolerance means that bacteria can survive, but not grow, in the presence of antibiotics. Tolerance can occur because of phenotypic, not genetic, changes in the state of the bacteria. This strategy allows a small population of bacterial cells to 'shut down' and attempt to survive unfavourable environmental conditions (i.e. antibiotics) (Yann and Bassler, 2019).

However, antibiotic tolerance can also be afforded to bacteria by extracellular polysaccharide matrices known as biofilms, which are thought to underlie many chronic infections (Sharma et al, 2019; Yann and Bassler, 2019). It is therefore unsurprising that for some DFI patients the prospects of clinical resolution of infection can remain poor despite multiple rounds of appropriate antibiotics, which itself risks selecting for antimicrobial resistance. Moreover, nephrotoxic antibiotics can be harmful for patients who already have significant renal impairment. Novel antimicrobial strategies, which mitigate tolerance or resistance to antibiotics, ideally with fewer adverse effects, are therefore urgently needed.

Phage therapy

What is phage therapy?

Phage therapy is an exciting antimicrobial strategy that has the potential to transform

the care of a wide range of bacterial infections, including DFI. Bacteriophage (phage) are viruses that infect and kill bacteria in a species-, sometimes even strain-, specific manner. Globally, there are an estimated 1,031 phage, representing an enormous, ever-changing, pool of genetic diversity that is in an inexorable evolutionary wrestling match with its bacterial hosts (Comeau et al, 2008). Collectively, phage are the most abundant biological entity on the planet and are found wherever bacteria are found, including as part of human commensal flora (Townsend et al, 2021). We have evolved, and continue to exist, in permanent contact with phages; for example, there are more phages on/in you than cells in your body (Liang and Bushman, 2021).

Discovered in the UK in 1915, naturally occurring phages have been successfully used to treat bacterial infections for over 100 years, known as phage therapy (Twort, 1915; Chanishvili, 2012). Globally, the 1920s and '30s were the 'golden age' for phage therapy. However, this enthusiasm declined with the mass production of antibiotics which, at the time, were easier to make, market and use (Summers, 2012). Ironically, enthusiasm in phage therapy also helped accelerate its demise, as clinicians injudiciously applied phages without first checking if the phage could kill the person's bacteria, yielding poor results. Nonetheless, the use of phage therapy persisted in the geopolitical East, particularly in Russia, Georgia and Poland, where phage therapy is still used today (Miedzybrodzki et al, 2018). The antibiotic resistance crisis is driving a modern global renaissance in phage therapy, and over 2,200 patients, most with antibiotic resistant infections, have been treated with phages since 2000 (Uyttendaele et al, 2022).

How does phage therapy work?

Phage therapy can be used in two formats: pre-formulated phage cocktails targeting one or more bacterial species or a personalised phage preparation. Both approaches are important and their value can be illustrated by a hypothetical scenario. Suppose a person with DFI presented with an infection not resolving with antibiotics. The individual could be started on a pre-formulated phage cocktail that was known to cover the main bacterial species likely to be causing the infection, analogous to empirical antibiotics. Such cocktails can be broad, as shown by metagenomic analysis of Georgian and Russian phage cocktails (McCallinn et al, 2018). However, such empirical use would

be short-lived. In the same way that most antibiotic therapy is now guided by laboratory analyses, because of the specificity of phages it is paramount that a patient's clinical isolate is tested for susceptibility to phages. First, the individual's isolate would be tested for susceptibility to the pre-formulated cocktail, with laboratory analyses typically taking 18–24 hours. If susceptible, then the person with diabetes could continue to receive the cocktail. However, if the infection relapsed during treatment, indicating potential phage resistance, or if the patient's bacteria was not covered by the initial cocktail, a personalised formulation would be required. In this case, a sample of the bacteria causing the person with diabetes' infection would be sent to a national specialist centre holding a phage library. The individual's bacteria would be tested against the different phages in the library and a bespoke formulation prepared and sent back to the hospital. If the phage library did not hold a suitable phage, then academic partners could be contacted regarding alternative phages, perhaps isolated on-demand from the environment, or to 'train' weakly acting phages to kill the bacteria. Because of the vast environmental diversity of phages, such a phage therapy infrastructure would be able to effectively deliver the UK an inexhaustible supply of phages. This scenario illustrates that pre-formulated off-the-shelf phage cocktails should cover most individuals with diabetes; however, these will always need to be backed up by access to personalised phage therapy.

Phage therapy in chronic wound infections

Phage therapy has long been used to treat wound infections in Russia and Eastern Europe. Although most of the Eastern literature is not accessible to Western audiences, the few available sources paint a fascinating picture of widespread phage use (Chanishvili, 2012). For example, during the 1938–39 Russo-Finnish War three Red Army mobile sanitary brigades gave over 6,000 wounded soldiers prophylactic phages against gas gangrene, reportedly causing a 30% decrease in the incidence of gas gangrene relative to soldiers that did not receive phages. Later, during World War II, phage preparations were supplied to soldiers for prophylactic use in case of injury, reportedly again reducing the frequency of gas gangrene and subsequent amputations. Phages were also widely used to treat wounded soldiers. Phages were given topically with dressing changes and by subcutaneous injection at the infection

site. Cocktails of phages covering bacterial species commonly found in wounds were used. One such cocktail, known as 'pyophage' is still manufactured by the Georgian Eliava Institute today and available to buy in pharmacies. Individuals with antibiotic resistant chronic wound infections have also been treated with phages in Poland. One report shows that in 1990s, among the total 1,307 patients treated, 68 of 77 (88.2%) cases of individuals with varicose ulcers showed marked improvement or a full recovery and 13 of 16 (81%) of those with decubitus ulcers also made a full recovery (Weber-Dabrowska et al, 2000).

There are also multiple modern reports of the successful use of phage therapy to treat chronic wound infections. In the US, a 2006 document from the Southwest Regional Wound Care Centre revealed that phage had been used to treat 17 individuals with a variety of chronic wound infections, at least one of which was caused by *Pseudomonas aeruginosa*, with improvements in all patients (Southwest Regional Wound Care Centre, 2005). Three years later, in 2009, a phase I safety trial of phages for chronic venous ulcers was published, although infection was not one of the inclusion criteria, which prevented any conclusions about efficacy (Rhoads et al, 2009). In the trial, individuals had phage instilled into their wounds weekly for 12 weeks. Phage was found to be safe and without adverse effects, which the authors noted was not unexpected given the ubiquity of phages in the environment. In 2016, a case series of nine individuals with DFIs caused by *Staphylococcus aureus* and refractory to antibiotic therapy was published (Fish et al, 2016).

The individuals, who also had vascular insufficiency, received a once weekly topical application and the wound was then packed with phage-soaked gauze. All the infections resolved with phage therapy and there were no adverse effects. In 2018, phage was applied topically or by subcutaneous injection to two individuals with *S. aureus* osteomyelitis, both of whose infections resolved without adverse effects (Fish et al, 2018). There are also two reports in English available from Russia, describing the treatment of 25 people with DFI whose infections were caused by a range of pathogens, including *E. coli*, *Klebsiella spp.*, *P. aeruginosa*, *Proteus spp.* and *Staphylococcus*. Among these 25 individuals, all 13 with monomicrobial infections had elimination of, or a significant decrease in, their bacterial load, as was the case with 4/10 with polymicrobial infections (Morozova et al, 2018a; 2018b).

Recently, two reports from India have described the treatment of 66 chronic wound patients whose infections were refractory to standard treatments, including systemic antibiotics. The patients received topical phages, isolated from local environmental water sources, against a range of pathogens including *S. aureus*, *E. coli*, *P. aeruginosa*, *Morganella*, *Citrobacter*, *Proteus*, *Klebsiella* and *Acinetobacter*. Between the two studies 69.7% of infections were resolved, 27.3% had improved significantly and only two individuals (3.0%) did not respond (Gupta et al, 2019; Patel et al, 2021).

Most recently, we used topical anti-staphylococcal phage therapy as part of the clinical care of 10 individuals with DFI at high risk of amputation despite antibiotic therapy. This represents the largest application of phage therapy in the UK to date and the first application of phage therapy for DFI in the UK. We anticipate publication of these cases in due course, but at this stage can share that the experience was in line with previously published reports of phage therapy.

The safety and efficacy of phage therapy

Both the available trial and observational evidence suggest that phage therapy is safe. All 13 modern clinical or safety trials, representing the application of phages by various routes of administration among 302 individuals, found that phages were safe and no phage-related adverse events were reported (Bruttin and Brussow, 2005; Rhoads et al, 2009; Wright et al, 2009; Sarker et al, 2012; 2016; Rose et al, 2014; McCallin et al, 2018, Febvre et al, 2019; Gindin et al, 2019; Jault et al, 2019; Ooi et al, 2019; Leitner et al, 2021).

A recent systematic review of observational data, covering 2,241 cases, found that phage therapy was well tolerated with any adverse events mild (Uyttebroek et al, 2022). This is consistent with a systematic review which found no evidence of adverse effects from modern phage therapy used to treat a range of superficial infections (burn-wound infection, chronic wounds and dermatological infection) (Steele et al, 2020). As mentioned above, the lack of adverse effects is perhaps unsurprising given the ubiquity of phages in the environment, meaning that we are constantly exposed to, and have co-evolved with, phages.

Regarding efficacy there is an, albeit explainable, discrepancy between trial and observational data (Stacey et al, 2022). A recent systematic review of observational clinical data, covering 2,241 cases, suggested that 79% of

phage individuals saw clinical improvement and 87% achieved bacterial eradication (Uyttebroek et al, 2022). These data are compelling, even more so given that most of these individuals had infections refractory to antibiotics but resolvable with phage. While there is always a risk of reporting bias with observational data, this concern is mitigated because such a wide variety of sources have independently published reports of antibiotic refractory infections resolved by phage therapy.

However, only two of the seven modern efficacy trials have demonstrated evidence of efficacy (Wright et al, 2009; Ooi et al, 2019). For efficacy to be observed a therapeutic amount of the right phage(s) must be delivered to the right place to treat infections containing enough susceptible bacterial cells. This 'Goldilock's constellation' is easier to achieve on an individual basis and has been harder to consistently achieve in a trial setting. Trials that have demonstrated efficacy have got this right and trials that have not have simply not fulfilled one or more elements of this constellation. There haven't been any trials where this constellation has been achieved and efficacy has not been shown (Stacey et al, 2022). While it is important to consider the safety and efficacy of phage therapy in general, as the findings are applicable across infection types, a recent systematic review found that 86.1% of 310 individuals with chronic wounds achieved clinical resolution or improvement with phage therapy (Steele et al, 2020).

Advantages of phage therapy for diabetic foot infection (DFI) care

There are several features of phage therapy that make it a particularly attractive therapeutic option for the care of DFIs. Most individuals with difficult-to-treat DFIs are inpatients on intravenous antibiotics which come with significant side effects, including nephrotoxicity, which is unhelpful as many people with DFI may already have impaired renal function. In contrast, as described above, phage therapy has a promising safety profile. Moreover, although excretion in urine is possible, phages are readily destroyed by the immune system and there is no evidence that nephrotoxicity is associated with phage therapy. Another advantage of phage therapy is that because the phages are so specific about the bacteria they kill, few, if any, other species will be affected (Mu et al, 2021). This means that unlike broad spectrum antibiotics, whose decimation of commensal flora can cause diarrhoea or even pave the way

for opportunistic infections such as *Clostridium difficile*, phage therapy leaves commensal flora intact. There are also no reports of allergic responses to phages, making them suitable alternatives for individuals with antibiotic hypersensitivity. Indeed, some DFI individuals have suggested that, given the choice, they would prefer to try phage therapy alone before intravenous antibiotics (Macdonald et al, 2020).

Phages, being biological agents, are not pharmacologically equivalent to antibiotics. For example, some phages possess enzymes that are able to degrade the biofilms which help enable bacteria to survive antibiotics (Ferriol-Gonzalez and Domingo-Calap, 2020). Moreover, although phages cannot replicate in the dormant bacterial persister cells found within a biofilm, phages can bind and enter those cells ready to replicate when the bacterial cells return to a replicating state (Harper et al, 2014). This anti-biofilm ability of some phages will be invaluable to the care of DFIs. Another unique pharmacological feature of phages is that, as biological agents, phages do not show a classical dose-response curve but are 'auto-dosing'.

If bacterial hosts are plentiful there is substantial phage replication. But when no hosts remain phage replication cannot continue, and any remaining phages are readily destroyed by the immune system. This naturally concentrates phages at sites of infection. Notably, there is strong evidence that some combinations of phages and antibiotics can synergise, with phages and antibiotics exerting different selection pressures on bacteria (Segall et al, 2019). Therefore, as the bacteria evolves to avoid one it may render itself more susceptible to the other.

Phage therapy is also straightforward to administer. Topical phage therapy (simple suspensions of phage, for example in sterile saline) may simply be dripped onto the wound and/or used to soak the dressings applied (Fish et al, 2016; Patel et al, 2021). To be effective phages need to physically encounter their bacterial host, so allowing phages to soak into the wound before dressing may permit deeper penetration of phages into the wound. Topical phage therapy is sufficiently straightforward that it could be applied in a community setting or even by an individual at home. However, where DFIs are complicated by osteomyelitis topical phages may need to be supplemented by subcutaneous administration to the site of infection (Fish et al, 2016). That phage therapy for DFIs is administered locally is also

advantageous as it means that antimicrobial activity is not impeded by poor perfusion.

A long-term future for phage therapy for DFI in the NHS

Phage therapy has the potential to transform the care of diabetic foot infections. Presently, in the UK phage therapy may only be used where licensed alternatives (i.e. antibiotics) are not meeting a person's clinical needs. This means that in the first instance only individuals at high risk of amputation despite antibiotic therapy or with antibiotic refractory chronic infections may be suitable for phage therapy.

In the long-term phage therapy will be used to prevent individuals developing serious DFIs. There will be two aspects to prevention. First, and of particular commercial interest, will be the use of prophylactic phage-containing medical products, such as dressings or socks. There is a significant commercial incentive to develop such products as, although naturally-occurring phages themselves are not protectable, such products are. The second aspect to prevention of serious DFIs is the treatment of mild DFIs with phage therapy. This will be undertaken, as described above, using off-the-shelf phage cocktails and it is anticipated that this will play a key role in reducing the number of serious DFIs.

Notwithstanding these interventions, individuals presenting with serious DFIs will also be able to access off-the-shelf phage cocktails and, where needed, personalised phage therapy. It is anticipated that phage therapy will typically be used alongside antibiotics. This will exploit the synergy that can occur between both strategies as two independent selection pressures are applied to the target pathogen. However, there will be a small group of individuals for whom the side effects of antibiotics would be so undesirable or intolerable that phage therapy alone may be considered. For example, phage alone may be appropriate for individuals with significant renal impairment. However, considering the side effects of antibiotics, individuals themselves may also prefer phage therapy alone (Macdonald et al, 2020). Taken together, these approaches should help dramatically reduce the amputation rate secondary to infection in the UK.

The integration of phage therapy into DFI care will be transformative for individuals and the wider NHS. For example, the one-off cost of a major amputation and initial provision of prosthesis, physiotherapy and wheelchair support, has been estimated to be £13,972 per person (Kerr et al, 2019). This excludes the

costs of associated outpatient care, estimated at £266 per week per person, and admission to hospital, estimated at around £250 per night (University Hospitals Birmingham, 2019). In comparison, the cost of phage therapy is anticipated to be comparable with, or cheaper than, the cost of existing antibiotics. Given the high prevalence of diabetes, it is therefore unsurprising that diabetic foot care as a whole has been estimated to cost the NHS almost £1 billion per year, or around 1% of the entire NHS budget, and almost 1 million bed days per year (Insight Health Economics, 2017; NHS Digital, 2019). Reducing the number of amputations secondary to infection will, therefore, directly benefit individuals and substantially reduce NHS costs. Moreover, when phage is used to prevent the development of serious infections this will also help reduce demand on outpatient care. Phage therapy will also help reduce the amount of antibiotics taken for DFIs by shortening the duration of DFIs in general and by reducing the number of serious DFIs. Consequently, phage therapy is not just an alternative antimicrobial but a tangible answer to the antibiotic resistant crisis.

Barriers to success

Shrewd readers will rightly be wondering why, if phage therapy is so promising, it hasn't been done before. Aside from the usual commercial disincentives associated with antimicrobial development, the main barrier to successfully delivering phage therapy is money. The preparation of phage products takes a fraction of the time and money required to develop a new antibiotic.

However, as discoveries, naturally occurring phages are not protectable and methods to characterise and produce phages have been widely published (Luong et al, 2020). Simple suspensions of phages in clinically appropriate solutions, such as sterile saline, have no associated intellectual property. Unfortunately, it is these simple suspensions of phages that are extremely useful clinically as, in the context of DFI, they can readily be used for wound washing, subcutaneous injection or even, with systemic infection, intravenously. Aside from the nature of the phage preparation itself, it is unlikely that rare or resistant infections, the sort that would require a personalised rather than off-the-shelf approach, would be financially attractive (Ferry et al, 2022). Instead, phage therapy lends itself well to a more patient-centred model, which prioritises clinical needs.

This is the objective of UK Phage Therapy which, by taking a non-profit approach, will deliver sustainable access to phages manufactured according to Good Manufacturing Practice for the NHS in the near future.

Conclusion

Phage therapy has the potential to transform the care of DFI. The use of phage therapy is not new and all available evidence strongly suggests that phage therapy is safe and, when used appropriately, highly effective. Phage therapy is particularly well suited to the care of DFIs because it can be administered topically, and antimicrobial activity is therefore independent of peripheral perfusion. Moreover, the apparent lack of adverse effects is attractive, particularly when compared to antibiotics which may be nephrotoxic and damaging to the commensal microbiota. The integration of phage therapy into all levels of DFI care, from prevention to the treatment of mild and severe DFIs, will help reduce the progression of DFIs and ultimately the amputation rate. This will deliver substantial savings to the NHS, which currently spends almost £1 in every £140 on diabetic foot care as a whole, with approximately 169 amputations per week undertaken in England alone (Diabetes UK, 2018; 2020b).

Moreover, because phages act independent of antibiotic resistance, phage therapy offers the opportunity to treat almost any antibiotic resistant bacteria, while also helping reduce the volume of antibiotics used to treat DFIs. Phage therapy is, therefore, a tangible answer to the antibiotic resistance crisis. While phage therapy has previously struggled to gain traction in the Western pharmaceutical system, the challenge for the UK is not whether it can deliver phage therapy but, as one individual put it, whether it can 'think outside the box', radically embrace phage therapy and truly put the welfare of its patients first (Macdonald et al, 2020). WINT

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