



## ■ INSTRUCTIONAL REVIEW

# Bacteriophage therapy for bone and joint infections

AN INSTRUCTIONAL REVIEW

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**Antibiotic resistance represents a threat to human health. It has been suggested that by 2050, antibiotic-resistant infections could cause ten million deaths each year. In orthopaedics, many patients undergoing surgery suffer from complications resulting from implant-associated infection. In these circumstances secondary surgery is usually required and chronic and/or relapsing disease may ensue. The development of effective treatments for antibiotic-resistant infections is needed. Recent evidence shows that bacteriophage (phages; viruses that infect bacteria) therapy may represent a viable and successful solution. In this review, a brief description of bone and joint infection and the nature of bacteriophages is presented, as well as a summary of our current knowledge on the use of bacteriophages in the treatment of bacterial infections. We present contemporary published in vitro and in vivo data as well as data from clinical trials, as they relate to bone and joint infections. We discuss the potential use of bacteriophage therapy in orthopaedic infections. This area of research is beginning to reveal successful results, but mostly in nonorthopaedic fields. We believe that bacteriophage therapy has potential therapeutic value for implant-associated infections in orthopaedics.**

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### Introduction

Unfortunately for many patients undergoing orthopaedic surgery, implant-associated infections are a complication. Most commonly these infections are due to *Staphylococcus aureus* (33% to 43%), *Staphylococcus epidermidis* (18% to 40%), and *Enterococcus* species (2.5% to 15%, mainly *Enterococcus faecalis*). Less frequently, Gram-negative bacilli, including *Escherichia coli* and *Pseudomonas aeruginosa*, are implicated in implant-associated infections (4% to 7%).<sup>1</sup> These pathogenic infections contribute to the failure of the reconstruction, requiring its replacement and often causing chronic and/or relapsing disease.<sup>2</sup> As such, implant infections have a significant impact on patients' quality of life and healthcare systems worldwide. In the USA alone, there were 332,000 total hip and 719,000 total knee arthroplasties performed in 2010<sup>3</sup> and these numbers are projected to reach 572,000 and 3.48 million by 2030, respectively.<sup>4</sup> To treat such periprosthetic joint infections (PJIs) in the American healthcare system cost approximately \$566 million in 2009 and are anticipated to reach \$1.62 billion in 2020.<sup>5</sup>

Antibiotic resistance is a significant problem associated with orthopaedic implant infections.<sup>6</sup> Many strains of implant-infecting *S. aureus*

have high rates of antibiotic resistance, with similar problems reported for *S. epidermidis*.<sup>7,8</sup> Although rates of hospital-acquired methicillin-resistant *S. aureus* (MRSA) have decreased in recent years,<sup>9</sup> antibiotic resistance among Gram-negative bacteria is increasing with some species of *Enterobacter*, *Acinetobacter*, *Klebsiella*, and *Pseudomonas* showing high rates of multidrug resistance including recent panresistant *Acinetobacter baumannii* isolates.<sup>10</sup> Further, the formation of bacterial biofilm negatively impacts the effectiveness of antibiotics, often leading to an increase in the minimal inhibitory concentration up to 1,000-fold, to control the infection compared to their planktonic (free-floating) counterpart.<sup>11</sup> This is probably a result of diminished antimicrobial effects on bacteria in the biofilm environment,<sup>12</sup> which stems from an overall decrease in metabolic activity of bacterial cells or quorum sensing that leads to enhancement of the exchange of antibiotic resistance genes between bacterial cells.<sup>13</sup>

**Bacteriophages.** Bacteriophages (phages) are viruses that infect bacteria and with estimates of 10<sup>31</sup> phage types, they are the most abundant biological particles on earth and found everywhere bacteria reside.<sup>14</sup> Phage replication within an infected bacterium will kill the host by lysis, which releases the newlyformed phage particles. As such, phages

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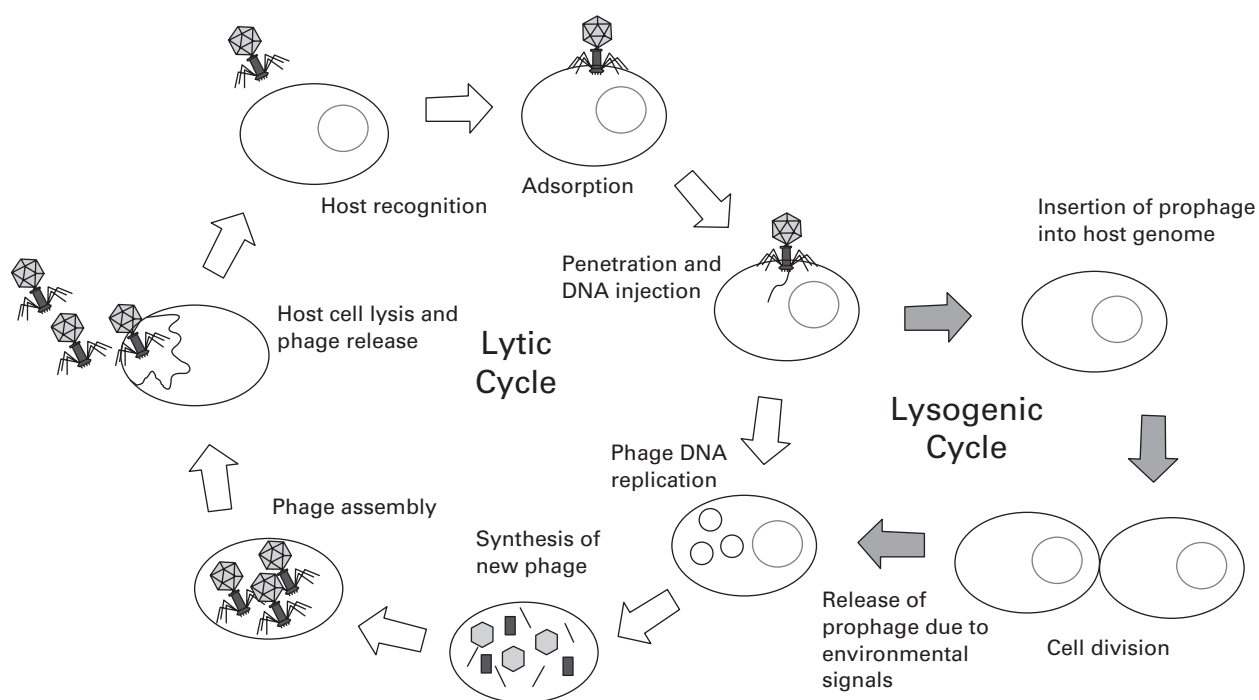


Fig. 1

The phage life cycle. Lytic phages proceed through the lytic cycle, in which the host is lysed and progeny phages are released into the environment. Temperate phages can go through the lytic or the lysogenic cycle. In the lysogenic cycle, the phage genome is incorporated into the host genome (prophage) but can be induced by environmental stressors, leading to the expression of phage DNA and the lytic cycle. Adapted from Doss et al.<sup>16</sup>

are potentially potent antibacterial agents, especially against multidrug-resistant infections.

As with other viruses, bacteriophages recognize specific molecules on the surface of the host cell. Some examples of entry receptors used by bacteriophages include peptidoglycan, lipopolysaccharide, flagella, and surface sugars that make up capsules and slime layers.<sup>15</sup> The diversity in these molecules, even within a single bacterial species, means that most phages are specific for the target bacterial strain that they infect. During an infection, the bacteriophage genome enters the bacterial cell and immediately shuts down many host processes and begins viral reproduction (Figure 1).<sup>16</sup> Formed viral particles are released by lysis resulting in the death of the host bacterial cell, enabling infection of neighbouring cells. Many bacteriophages exhibit a purely lytic lifecycle, but some bacteriophages do not immediately kill the bacterial host. These temperate bacteriophages integrate the phage DNA genome into the bacteria chromosome and go dormant, reactivating and entering lytic growth when the host stress response (as a result of UV light, temperature, heavy metals, radiation, etc.) is activated (Figure 1). Temperate bacteriophages also provide immunity from further infection from related bacteriophages and contribute to horizontal gene transfer that can lead to the spread of antibiotic resistance and toxins. Therefore, only fully lytic bacteriophages are used in therapeutic applications.

Although bacteriophage therapy may appear as a new form of treatment, reports of their human application go back to the 1960s, especially in the former Soviet Union and Eastern Europe, particularly at the Eliava Institute of Bacteriophage,

Microbiology, and Virology (EIBMV) of the Georgian Academy of Sciences (Tbilisi, Georgia) and the Hirsfeld Institute of Immunology and Experimental Therapy (HIIET) of the Polish Academy of Sciences (Wroclaw, Poland). Published results from many of these studies were in Russian, Georgian, and Polish journals and which were not widely seen by Western scientists.<sup>17</sup> Many of these older studies have been previously extensively reviewed.<sup>17-19</sup> We will focus specifically on bone and joint infections and present significant findings from contemporary published in vitro, animal, and clinical studies.

**Search strategy.** We searched two main databases, MEDLINE (PubMed) and Google Scholar, as well as reference lists of the various articles identified. The following keywords were used in our searches: bacteriophages orthopaedic infections, bacteriophages joint infections, bacteriophages bone, bacteriophage therapy bone, phage therapy bone, phage therapy orthopaedics, and phage therapy bone joint infections. Published articles were then divided into the following categories:

1. review articles on bacteriophages, bacteriophage therapy, bone and joint infections;
2. in vitro studies that reported the effects of various bacteriophages on bacterial infection of Kirschner (K)-wires, implantable scaffolds, and cultured cells;
3. animal studies evaluating the effect of bacteriophage therapy on bone infections; and
4. clinical studies using bacteriophage treatment for bone and joint infections. We also searched on [clinicaltrials.gov](http://clinicaltrials.gov) with

the term “phage” or “bacteriophage” and results indicated 42 clinical trials on many conditions, but none related to bone infections. When the terms “phage” and “bone” were used together, results indicated one relevant trial taking place in Poland and dealing with bacteriophage therapy of patients with non-healing postoperative wounds or bone, upper respiratory tract, genital or urinary tract infections in whom extensive antibiotic therapy failed, or the use of the targeted drug is contraindicated.

### Preclinical studies

**Animal studies.** Animal studies focusing on bacteriophage therapy for bone and joint infections are lacking. Only one rabbit, one rat, and four mouse studies have been published.<sup>20–25</sup> As implant-related bacterial infections resistant to multiple antibiotics represent one of the major problems of orthopaedic surgery, Yilmaz et al<sup>20</sup> tested the hypothesis that local application of bacteriophages would be effective against biofilm-forming bacteria. To test this hypothesis, the authors inserted a 0.8 cm piece of a plastic intravenous catheter with a pre-established biofilm (either MRSA or *P. aeruginosa*) into rat tibial medullary canal (implant-related infection model). All rats were assessed for implant-related osteomyelitis by aspiration of the surgical site on the 14th day and the rats were divided into four groups for each bacterial species: control; antibiotic-treated; phage-treated; and combination of antibiotic/phage-treated. Results showed that in the *S. aureus*-infected animals, antibiotic administration significantly decreased bacterial numbers by approximately 2.9-fold, whereas treatment with antibiotic plus bacteriophages created about a ten-fold decrease in colony-forming units (CFUs). Treatment with bacteriophage alone resulted in about 1.6-fold reduction in CFUs compared to control. In the *P. aeruginosa* group, the number of CFUs was significantly lower in each treatment subgroup compared with the control subgroup; about 5.6-, 2.3-, and 8.6-fold lower with antibiotic, bacteriophage, and a combination of both treatments, respectively. These results led the authors to conclude that the addition of bacteriophage treatment to an appropriate antibiotic regimen aided in removal of the biofilm of both bacteria species, and to recommend that bacteriophage therapy should be added to the standard antibiotic regimen as it is a valuable adjunct for eradicating orthopaedic implant-related infections.<sup>20</sup>

A similar study was conducted by Kaur et al<sup>21</sup> where they tested the effects of naked wire, hydroxypropylmethylcellulose (HPMC)-coated wire, and phage and/or linezolid (synthetic antibiotic)-coated K-wire. These wires were surgically implanted into the intramedullary canal of mouse femora, followed by inoculation of *S. aureus* (MRSA). Results showed that the mouse group implanted with K-wire coated with the combination of phage and linezolid had the maximum beneficial effects: decreased inflammation of the joint; reduction in bacterial adherence in the adjoining joint tissue (Figure 2); and increased the return of locomotion and motor function of the limb. Phage and linezolid treatment alone were also equally effective to each other in all assays (both showed decreased inflammation by day ten post-implantation; same locomotion activity and motor function over 15 days; and reduction

in bacterial adherence by day ten) but slightly lower than the combination of the two in all assays.<sup>21</sup>

The possible treatment of MRSA chronic osteomyelitis was also the focus in a rabbit model.<sup>22</sup> In this study the authors established acute and chronic osteomyelitis in the distal end of rabbit femora for three weeks, followed by treatment with a cocktail of seven virulent bacteriophages (four doses; one every two days for a week). The experiment showed the presence of periosteal reaction, increased sclerosis and osteolysis, sequestrum formation, and other sequelae such as arthritis of the knee in the untreated group, while none of these were observed with the phage-treated rabbit group; rather, these treated animals recovered from osteomyelitis within two weeks of receiving phage therapy. This recovery was accompanied by improvements in the appetite and activity of the rabbits as well as diminished local oedema, erythema, and induration. The authors also observed minimal radiological changes associated with osteomyelitis and no signs of infection, and new bone formation (histologically). In another rabbit group where phage therapy was started after the sixth week of infection, representing chronic osteomyelitis, the results showed that radiological features of osteomyelitis persisted and in one rabbit, arthritis of the knee developed.<sup>22</sup> Collectively, the data support the potential of phage therapy to treat difficult infections caused by multidrug-resistant bacteria.

A more recent mouse study investigated the capability of a bioengineered injectable hydrogel capable of encapsulating *P. aeruginosa* bacteriophage cocktail to deliver active phages to the site of bone infections.<sup>23</sup> The bacteriophage injectable hydrogel was initially successfully tested for its antimicrobial activity in vitro (for both planktonic and biofilm phenotypes) prior to its utility to treat *P. aeruginosa* in vivo. For the in vivo study, the authors removed a 2.5 mm segment of the mouse radius and applied either bacteria or bacteria/bacteriophage-encapsulating hydrogel in 4.0 mm perforated polyimide sleeves that were fitted over the ends of the radius spanning the defect. Seven days post-surgery, defects treated with control hydrogels (bacteria only) showed higher concentration of live bacteria, indicating an established infection, whereas those defects treated with the bacteriophage-encapsulating gels contained 4.7-fold fewer live bacteria compared to controls. These results further support the development of various strategies that utilize bacteriophages used locally to treat bone infections.<sup>23</sup>

It is also noteworthy that two other mouse studies reported the use of bacteriophages (*S. aureus* A5/L) to treat *S. aureus* infections in immunosuppressed mice (as a result of cyclophosphamide injections) by the same laboratory.<sup>24,25</sup> In the first study, results revealed that the high numbers of bacterial CFUs in organs (kidney, liver, and spleen) as well as elevated tumour necrosis factor (TNF) and interleukin-6 (IL-6) serum concentrations in immunocompromised and *S. aureus*-infected mice were decreased significantly (about three- and eight-fold, respectively, for the cytokines) with phage application.<sup>24</sup> Additionally, the phages significantly increased the percentage of circulating neutrophils and immature cells from the myelocytic and lymphocytic lineages as well as of myelocytes and immature neutrophils in the bone marrow in immunocompromised and *S. aureus*-infected mice. The second study used the same approach of testing the prophylactic effect of bacteriophages

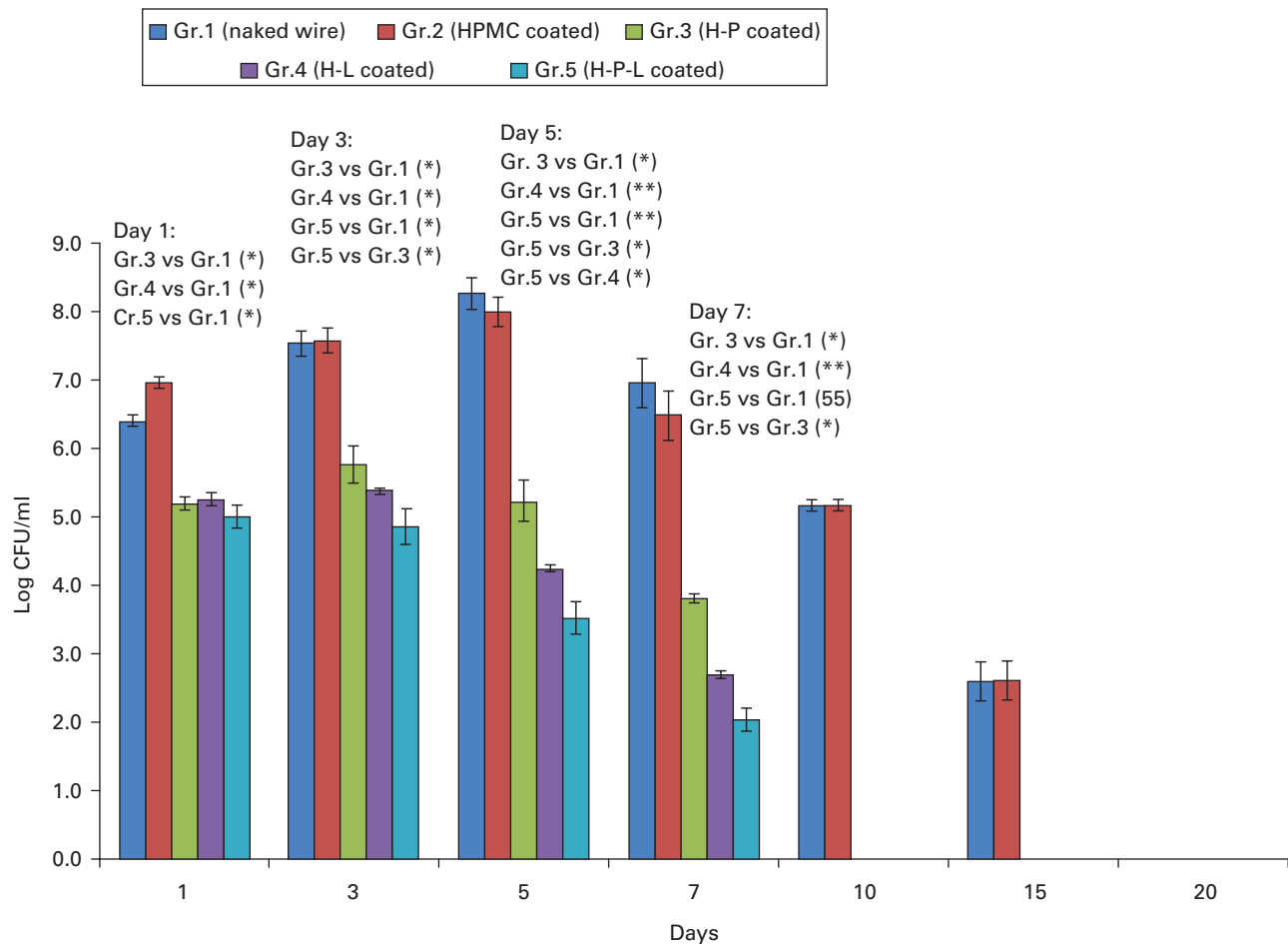


Fig. 2

Phage titre (Log colony-forming unit (CFU)/ml) in the adjoining joint tissue of mice on different days post-infection with *Staphylococcus aureus*. Each data point represents the mean  $\pm$  SD of three ( $n = 3$ ) values at each timepoint. \* $p < 0.05$ ; \*\* $p < 0.01$ . HPMC-coated, Hydroxypropylmethylcellulose-coated as biopolymer (4% w/v); H-P, Phage ( $10^9$  PFU/ml) mixed with HPMC gel; H-L, linezolid (5% w/w) mixed with HPMC gel; H-P-L, phage as well as Linezolid mixed with HPMC gel. Adapted from Kaur et al.<sup>21</sup>

in mice, but this time in chemotherapy-induced immunosuppression and bone marrow transplant upon infection with *S. aureus*.<sup>25</sup> Specifically, the authors showed that the application of bacteriophages to the immunocompromised mice with a bone marrow transplant significantly reduced by > 90% the bacterial load in spleen and liver. More importantly, these phage-treated mice exhibited a 72% long-term survival versus 8.2% survival of untreated mice (Figure 3). Further analyses of leucocyte number and blood cell type composition also showed that phage application roughly doubled the number of circulating leucocytes and neutrophils (from 15.4% to 46.4%). Lastly, phage application led to an increase in the bone marrow myelocytic cell lineage from 26.6% to 46.8%.<sup>25</sup>

**In vitro studies.** Similar to the scant number of in vivo studies, there are also very few published in vitro studies related to bone and orthopaedic applications of bacteriophages. One particular study investigated coated orthopaedic K-wires in order to examine their effects on MRSA colonization.<sup>26</sup> Specifically, the authors coated stainless steel orthopaedic grade K-wires using a combination of HPMC mixed with phage alone, linezolid alone,

and phage and linezolid together, and examined their potential to inhibit MRSA. Analysis by scanning electron microscopy (SEM) and fluorescent staining revealed significant reduction (by about 50%) in bacterial adhesion (within 48 hours), especially on phage/linezolid wires in comparison to naked, as well as HPMC-coated wires. Coated K-wires with phage or linezolid alone also reduced the amount of bacterial adhesion, but only by about 30% in comparison to the controls. Interestingly, the authors also showed that the frequency of emergence of resistant bacterial mutants was also negligible in presence of both phages and linezolid. Based on these results the authors concluded that a local delivery system utilizing phages and linezolid is effective in destroying adhered bacteria (and reducing emergence of resistant mutants) and could be effective if used with orthopaedic implants.<sup>26</sup>

A second study investigated the antibacterial activity of a tailored bacteriophage cocktail against planktonic and biofilm-associated *S. aureus* established on 3D-printed porous titanium scaffolds.<sup>27</sup> In exposing growing *S. aureus* (two strains) cultures to various individual bacteriophages or as a cocktail, the authors

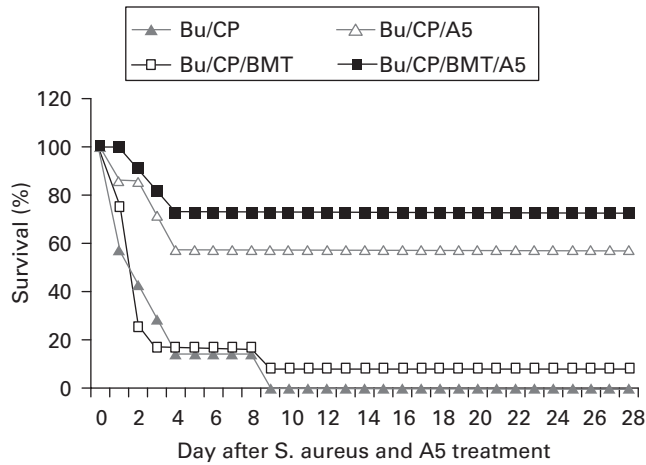


Fig. 3

Effects of A5 phages on survival of Bu/CP- and Bu/CP/BMT-treated and *Staphylococcus aureus*-infected mice. Mice were infected with a lethal dose ( $1 \times 10^9$  colony-forming units/mouse) of *S. aureus* and monitored for survival for 28 days. There were 20 mice in each group. Adapted from Zimecki et al.<sup>25</sup>

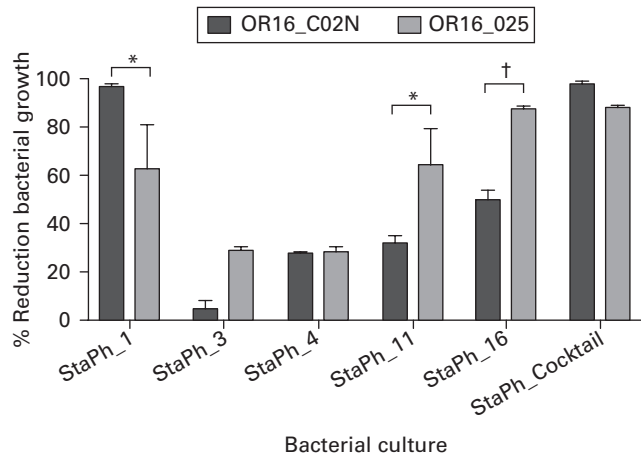


Fig. 4

Inhibition of bacterial growth by phages. Percentage reduction in the eight-hour growth of *Staphylococcus aureus* strains, ORI16\_C02N, and ORI16\_025 when exposed to the phage types StaPh\_1, StaPh\_3, StaPh\_4, StaPh\_11, and StaPh\_16 alone or combined as a StaPhage cocktail compared with untreated bacterial cultures. Adapted from Morris et al.<sup>27</sup>

reported a 5% to 100% reduction of bacterial growth with each individual phage but a 90% to 100% reduction with the phage cocktail (Figure 4). Similarly, when *S. aureus*-established biofilm containing scaffolds were exposed to either cefazolin or the phage cocktail, results indicated sensitivity of both *S. aureus* strains to cefazolin, although viable bacterial numbers recovered from untreated and cefazolin treated biofilm-coated titanium implants were comparable for both strains. Further, the antibiofilm activity of the phage cocktail on the two *S. aureus* strains showed some differences; bacterial numbers with one of the two strains were similar as those of untreated, while for the second strain there was a statistically significant decrease

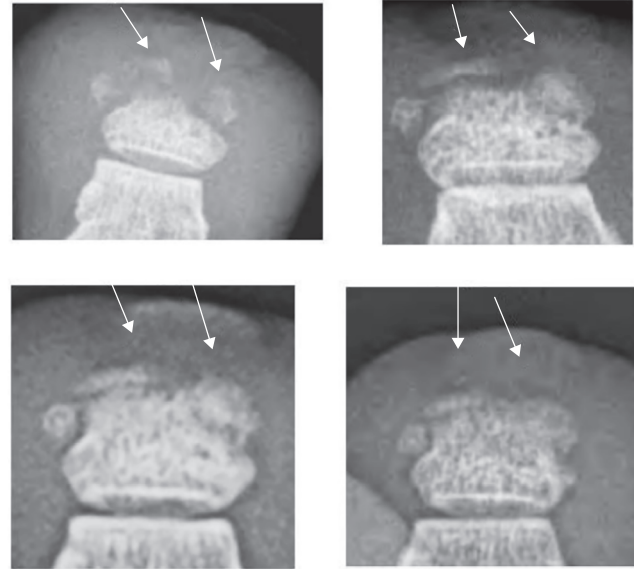


Fig. 5

Radiographs of ulcerated toe over the course of treatment showing reossification of the toe. Radiographs taken on a) April 2015; b) July 2015; c) June 2015; and d) September 2015. Arrows indicate areas of reossification from photo (a) to photos (b-d). Adapted from Fish et al.<sup>34</sup>

(about 3.3-fold) in bacterial numbers after exposure to the phage cocktail compared with the untreated implants. This led the authors to conclude these in vitro data provide support for in vivo studies that would further investigate the potential use of custom phage cocktails for management of orthopaedic implant-related infections caused by *S. aureus*.<sup>27</sup>

Another study sought to test commonly used materials such as hydroxyapatite (HA) and beta-tricalcium phosphate ( $\beta$ -TCP), which are commonly used in bone repair.<sup>28</sup> Specifically, the authors evaluated the possibility of loading HA and  $\beta$ -TCP ceramics (used as bone substitutes) with phages targeting *E. coli* K12. Examining the lytic kinetics (antibacterial activity) of phage-loaded ceramics placed in growing cultures of *E. coli* K12, revealed that phage-loaded HA and  $\beta$ -TCP materials induce fast bacterial lysis between two and three hours after their introduction, leading to a roughly ten-fold reduction with both phage-loaded materials. In addition, it was shown that most phages were retained in dense and microporous HA and  $\beta$ -TCP samples for at least six days, which suggests strong interaction between phages and ceramics; and yet, it did not prevent bacterial attachment and lysis. These results led the authors to conclude that phage-loaded ceramics could be used as prophylactic treatments for bone repair.<sup>28</sup>

Finally, Kolenda et al<sup>29</sup> evaluated the activity of a combination of three bacteriophages alone or in association with vancomycin or rifampicin against *S. aureus* biofilm and an osteoblast (MG63) infection model in vitro. Exposure of growing bacteria in liquid culture to the combination of the three phages resulted in complete bactericidal activity within three hours. Similarly, 24-hour exposure of *S. aureus* biofilms to different phage concentrations (approximately  $10^5$  to  $10^8$  plaque-forming unit (PFU)/ml) and with antibiotics (vancomycin or rifampicin)

**Table I.** Summary of recent clinical studies utilizing bacteriophage therapy.

Study	Clinical diagnosis, n	Bacteria species	Treatment approach	Clinical outcome
Fish et al <sup>33</sup>	Diabetic toe ulcers with <i>S. aureus</i> infected bone and soft tissue (n = 6)	<i>S. aureus</i>	Topical application of phage solution to ulcer	Ulcers healed generally in seven weeks; one patient required 18 weeks of treatment
Fish et al <sup>34</sup>	Distal phalangeal osteomyelitis (n = 1)	<i>S. aureus</i> (MRSA)	Phage solution injection into the soft tissue surrounding the distal phalanx	Reossification of the distal phalanx within 3 mths; 3 yr follow-up patient still free of osteomyelitis
Ferry et al <sup>35</sup>	Osteomyelitis adjacent to the cement located in the right sacroiliac joint (n = 1)	<i>P. aeruginosa</i>	Phage solution injection into the cavity in contact with bone every 3 days, totalling 4 administrations; also antibiotic therapy	Rapid healing within 14 days with no presence of bacteria
Ferry et al <sup>36</sup>	PJI of the right hip (n = 1)	<i>S. aureus</i>	Phage solution injection into the joint; also antibiotic therapy	Favourable outcome at 18 mths post-treatment without any clinical signs of persistent infection
Onsea et al <sup>37</sup>	Severe musculoskeletal (pelvis/femur) infections, osteomyelitis (n = 4)	<i>P. aeruginosa</i> ; <i>S. epidermidis</i> ; <i>S. agalactiae</i> ; <i>S. aureus</i> ; <i>E. faecalis</i>	Phage solution delivered through draining system in close contact with infected bone; collagen sponge soaked in phage solution was placed on the infected bone prior to wound closure; phage solution three times per day for 7 to 10 days; also antibiotic therapy	With single course of phage therapy, no recurrence of infection in periods ranging from 8 to 16 mths
LaVergne et al <sup>38</sup>	Traumatic brain injury and craniectomy complicated by postoperative infection (n = 1)	<i>A. baumannii</i>	Phage solution administered intravenously every 2 hrs for 8 days	No further signs of infection at the craniotomy site
Patey et al <sup>39</sup>	Pelvic bone infection (n = 1); Complex fracture of right foot (n = 1); mandibular fracture, osteosynthesis, and fistulized infection (n = 1); Femoral fracture under hip prosthesis (n = 1); Left knee prosthesis infection (n = 1); Osteomyelitis of the left tibia (n = 1); Left tibia fracture, followed by reopened bone infection (n = 1)	<i>S. aureus</i> (n = 3); <i>P. aeruginosa</i> ; <i>S. aureus</i> (MRSA) (n = 3); <i>Staphylococcus sp.</i>	Phage solution administered preoperatively and via catheter in days following operation (n = 3); Phage solution administered peroperatively (n = 3); Surgery, phage therapy with commercial phage suspension; also antibiotic therapy	Complete cure (n = 5); Initial partial disinfection; Disappearance of <i>S. aureus</i>
Nir-Paz et al <sup>40</sup>	Left bicondylar tibial plateau fracture (n = 1)	<i>A. baumannii</i> ; <i>K. pneumoniae</i>	Phage solution delivered intravenously over 35 mins and over 5 days; second treatment course was given for an additional 6 days, one week later	Graft healing; elimination of subtle chronic bone pain; 8 mth post-treatment follow-up indicated no positive cultures for either bacterial strain
Tkhilaishvili et al <sup>41</sup>	Right knee peri prosthetic infection and chronic osteomyelitis of the femur (n = 1)	<i>P. aeruginosa</i>	Phage solution applied locally during surgery followed by additional phage solution every 8 hrs through each of the four drains as a local delivery system for five days; also antibiotic therapy	Eradication of infection, and no side effects: 10 mth follow-up visit patient reported no pain in the right knee

*A. baumannii*, *Acinetobacter baumannii*; *E. faecalis*, *Enterococcus faecalis*; *K. pneumoniae*, *Klebsiella pneumoniae*; MRSA, methicillin-resistant *Staphylococcus aureus*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. agalactiae*, *Streptococcus agalactiae*; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*

showed that antibacterial activity of the phages was dose-dependent, with the highest phage concentration showing the highest antimicrobial activity. The presence of the antibiotics provided a synergistic effect at the lowest concentrations. Further, the intracellular bacterial count of infected osteoblasts treated with phages, as well as with vancomycin, was significantly higher than in cells treated with lysostaphin (control). Collectively, the data suggest that the combination of phages tested was highly active against *S. aureus* biofilm but inactive against intracellular bacteria in osteoblasts.<sup>29</sup>

**Clinical studies.** The earliest study related to skeletal infections treated by bacteriophages was published by Sakandelidze and Meipariani.<sup>30</sup> The authors treated 236 patients with *Staphylococcus*, *Streptococcus*, and *Proteus* infections that included osteomyelitis, peritonitis, lung abscesses, and

postsurgical wound infections with bacteriophages that were administered subcutaneously or through surgical drains and eliminated antibiotic-resistant infections in 92% of the patients. A second human study, published in French in 1979, also reported bacteriophage treatment of seven cases of infection (two after insertion of a hip prosthesis; two from knee arthritis; one tibial osteomyelitis; one from a nonunion of the femur; and one following Harrington spinal stabilization).<sup>31</sup> Specifically, the authors reported that all seven cases were long-term infections with resistant microorganisms and that results were positive with five patients, fair in one, and failure with another. They concluded that bacteriophage therapy may be helpful in the treatment of long-term infections.<sup>31</sup>

In a review article outlining their experience in treating patients with bacteriophage therapy for various infections

at the Institute of Immunology and Experimental Therapy (Wrocław, Poland), Weber-Dabrowska et al<sup>32</sup> specifically reported that with 40 human cases of osteomyelitis (as a result of *S. aureus*, *E. coli*, *Klebsiella*, *Proteus*, and *Pseudomonas*) of long bones they observed 95% full recovery, defined as complete elimination of bacteria. Subsequently, many other studies have been published examining bacteriophage therapy for a multitude of infections but none for bone until very recently. To this date, only limited studies appear in recent literature that describe the application of bacteriophage therapy for osteomyelitis,<sup>33–37</sup> craniectomy site infection,<sup>38</sup> various bone infections,<sup>39</sup> and trauma-related tibial/femoral infection.<sup>40,41</sup> All of these studies are summarized in Table I.

Fish et al<sup>33</sup> reported the treatment of nine patients (who had responded poorly to recommended antibiotic therapy, two with confirmed osteomyelitis) with diabetic toe ulcers containing *S. aureus*-infected bone and soft tissue, with a commercially available bacteriophage solution (Staphylococcal phage Sb-1). The progression to closure of non-healing ulcers with chronic osteomyelitis was rapid, at an average of 5.6 weeks after initiation of phage therapy. Topical application of a single Staphylococcal phage solution appeared to be safe and efficacious when compared to previous conventional treatment controls, despite the often polymicrobial nature of these wounds. In patients with unresolved osteomyelitis, the signs of osteomyelitis and/or cellulitis rapidly improved and the wounds healed without relapse. In one patient when the bacteriophage solution was applied weekly to a high-risk large gangrenous toe ulcer, the wound healed quickly and steadily without development of infection or other complications. These results led the authors to conclude that topical application of a staph monophage preparation can be used successfully to treat infected toe ulcerations with bone infection.<sup>33</sup>

A second case study by some of the same authors involved a 63-year-old Caucasian female who developed a diabetic ulcer of the distal right second toe and distal phalangeal osteomyelitis as a result of a MSSA infection.<sup>34</sup> The authors' treatment consisted of injecting bacteriophages (once) into the soft tissue surrounding the distal phalanx with no antibiotics. The authors reported that after a week of treatment, the erythema increased, suggesting worsening of the infection. Following application of levofloxacin (500 mg) there was no change in the amount or intensity of the erythema or reduction of oedema, and thus the antibiotic was discontinued. Application of bacteriophages continued once weekly for another six weeks and results showed progressive reossification of the distal phalanx (as determined radiologically) coupled with decreased erythema and oedema, which also continued to improve after the injection treatment was discontinued. The same patient was seen for another three years and on the third year she exhibited an ulcer of the same treated toe, in the same location, although smaller and more superficial and without any sign of recurrent osteomyelitis (Figure 5). The authors concluded that bacteriophage therapy can be successfully applied to the standard care of diabetic foot ulcers with osteomyelitis.<sup>34</sup>

Ferry et al<sup>35</sup> reported that a man in his early 60s developed a fistula with clinical evidence of infection of the cement located

in the right sacroiliac joint two months following a cementoplasty for bone metastases located at that site. Following surgery to remove the cement and to debride an abscess located in the psoas muscle, and antibiotic treatment, the patient developed a catheter-related bacteremia due to ceftazidime-resistant *P. aeruginosa* as well as persistent osteomyelitis seen on a CT scan. With local application of a selected cocktail of bacteriophages (previously shown to be effective in killing *P. aeruginosa*) every three days, totalling four administrations, the authors reported successful treatment; healing was rapid with no bacteria growing in culture (following biopsy). Lastly, the authors suggested that bacteriophage therapy is promising with patients harbouring an extensively drug-resistant bone and joint infection because: 1) bacteriophages and antibiotics are synergistic; 2) there is no cross-resistance between antibiotic resistance and bacteriophage resistance; and 3) some in vitro and animal models have demonstrated that bacteriophages could have antibiofilm activity.<sup>35</sup> The same group also treated an 80-year-old patient with acute postoperative methicillin-susceptible *S. aureus* (MSSA) infection with debridement and antibiotics without success.<sup>36</sup> Finally, an implant retention (debridement, antibiotics, and implant retention (DAIR)) procedure for a relapsing *S. aureus* chronic PJI was performed followed by application of a selected cocktail of *P. aeruginosa* and *S. aureus* bacteriophages, which was locally injected in the joint cavity at the end of the procedure. At 18 months, there were no longer any clinical signs of persistent infection.<sup>36</sup>

In a recent study, *P. aeruginosa*, *S. epidermidis*, *Streptococcus agalactiae*, *S. aureus*, and *E. faecalis* were identified in four patients (each patient had a different combination of these bacterial species) with musculoskeletal infections (chronic osteomyelitis of the ileum; nonunion of the distal femur following open segmental fractures of the right femur; postoperative wound problems following crush lesions of the right thigh; complex femur fractures; condylar fracture of the knee; and infection of the surgical site with abscess formation with osteomyelitis of a fractured femur).<sup>37</sup> When these patients were treated with a combination of antibiotics and phages (for a duration between seven and ten days), the authors reported that one month from the start of phage therapy, CRP and white blood cell (WBC) levels returned to normal in all patients. In addition, at eight to 16 months follow-up after phage therapy, three of the four patients' clinical (i.e. inspection of the wound or scar, blood tests, and general health status) and radiological investigations showed no signs of recurrence, and these patients were declared infection-free. The fourth patient had to undergo additional surgical revision procedures for management of complex bone fractures and, eight months after the initial treatment, was infected by a new *S. epidermidis* strain, which was treated with an antibiotic regimen; the patient became infection-free. Taken together, this study showed the successful application of a single course of phage therapy combined with antibiotics in the treatment of severe musculoskeletal infections.<sup>37</sup>

LaVergne et al<sup>38</sup> described a 77-year-old patient with multidrug-resistant *A. baumannii* infection as a result of traumatic brain injury and craniectomy complicated by postoperative infection. Bacteriophages were administered intravenously every two hours for eight days with a total of 98 doses. Results

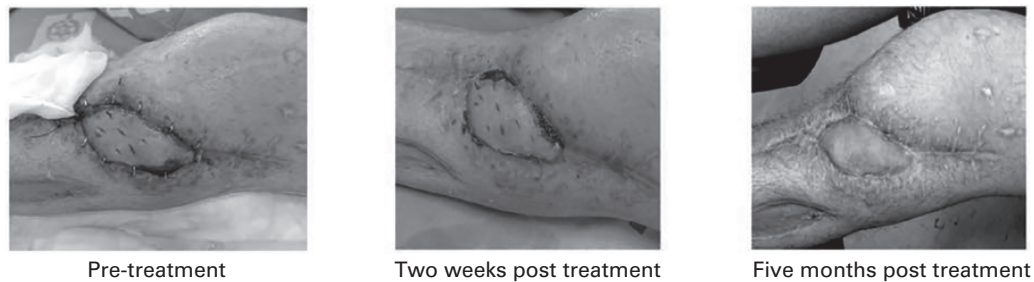


Fig. 6

Wound healing of a patient as induced by phage treatment. Before treatment, the flap edges did not heal well, with dehiscence and evisceration. By two weeks after treatment, the wound had completely healed and there was no dehiscence and evisceration of flap. By five months after treatment, complete healing of the wound was observed. Adapted from Nir-Paz et al.<sup>40</sup>

from this treatment showed that the craniotomy site and skin flap healed well, and there were no further signs of infection at the site nor in urine and blood. Unfortunately, before receiving the second phage cocktail, the patient's family decided to withdraw care including extubation and the patient died.<sup>38</sup>

Patey et al<sup>39</sup> have described several cases of bone infection managed with bacteriophage therapy between 2006 to 2018. They reported a range of bone-related conditions, including pelvic bone infection (*S. aureus* and *P. aeruginosa*); complex fracture of the foot (*S. aureus*); jaw fracture, osteosynthesis and fistulized infection (*S. aureus*); femoral fracture under hip prosthesis (*S. aureus*); knee prosthesis infection (*Staphylococcus*); osteomyelitis of the tibia (*S. aureus*); and operated tibial fracture, followed by reoperated bone infection (*S. aureus*). For most of these conditions, bacteriophage therapy led to complete cure. In addition, the authors point out that the local application of bacteriophages was completely safe.<sup>39</sup>

Nir-Paz et al<sup>40</sup> reported the treatment of a 42-year-old male patient with trauma-related (bilateral Grade IIIA open left bicondylar tibial plateau fracture) bacterial osteomyelitis associated with extensively drug-resistant *A. baumannii* and multidrug-resistant *Klebsiella pneumoniae*. Despite months-long multiple irrigations, debridement, and flap coverage, bacterial infection resistant to carbapenems and colistin was still present and could not be eradicated with antibiotics. The patient received a combination of bacteriophages  $\phi$ AbKT21phi3 and  $\phi$ KpKT21phi1, targeting both bacterial strains, along with intravenous antibiotics. Specifically, the patient received three doses every day for five days and a second treatment course was given for an additional six days one week later. Results showed that by two weeks following treatment, the wound had completely healed with no dehiscence or evisceration of flap, and by five months after treatment, complete healing of the wound was observed (Figure 6). By eight months post-treatment, no positive cultures for either bacterial species were obtained from any site and the patient's wound had closed with no secretions detected. Most importantly amputation which had previously been considered, was avoided.<sup>40</sup>

A final study reported on the use of bacteriophage therapy to treat an 80-year-old female with chronic relapsing PJI of the knee and chronic osteomyelitis of the femur caused by multidrug-resistant *P. aeruginosa*.<sup>41</sup> During removal of the knee implant the surgeons placed four drainage tubes: one

into the femoral and one into tibial canal via drill holes, and two tubes into the former prosthesis area. A single 100 ml loading dose of purified bacteriophage was applied locally during surgery, followed by administration of 5 ml of bacteriophage solution containing  $10^8$  PFU/ml every eight hours through each of the four drains as a local delivery system for five days. After surgery, antibiotic therapy was also administered with colistin (150 mg every 24 hours), meropenem (1 g every 12 hours), and ceftazidime (2 g every 12 hours). Reimplantation of the prosthesis was performed four weeks following this treatment and at the time all intraoperatively collected periprosthetic tissue samples remained negative for bacteria. Ten months after reimplantation, the patient reported no pain in the right knee, the soft tissue at the surgical site was unremarkable, and the mobility satisfactory.<sup>41</sup>

Despite these successfully treated human bone infections summarized in Table I, and as mentioned in the "Search Strategy", there are currently no clinical trials reported in the clinicaltrials.gov database, with the exception of one conducted in Poland that is peripherally related to bone. It is therefore clear that, given the success of the studies we have identified, clinical trials specifically designed to treat bone and joint infections with bacteriophages are lacking.

**Bacteriophage therapy as a viable treatment for orthopaedic-related infections.** The lack of extensive preclinical and clinical studies reveal a need for further research. The identification and testing of individual bacteriophages or cocktails to induce lysis of bacterial strains commonly found with bone and joint infections may offer another form of treatment for skeletal infections. A recent study by Barros et al<sup>42</sup> reported the identification and isolation of lytic bacteriophages against multidrug-resistant *S. aureus*, *E. faecalis*, and *E. coli* obtained from orthopaedic implant-associated osteoarticular infections. These bacteriophages showed low latent periods, high burst sizes, broad host ranges, and tolerance to several environmental conditions. More importantly, they also showed high efficiency and specificity to infect and reduce clinically important bacteria, including MRSA and vancomycin-resistant *Enterococci*. Their results suggest that these bacteriophages represent a promising approach to control orthopaedic implant-associated bacterial infections.<sup>42</sup>

Given the antimicrobial effectiveness of bacteriophages and reports of successful use in treating orthopaedic-related



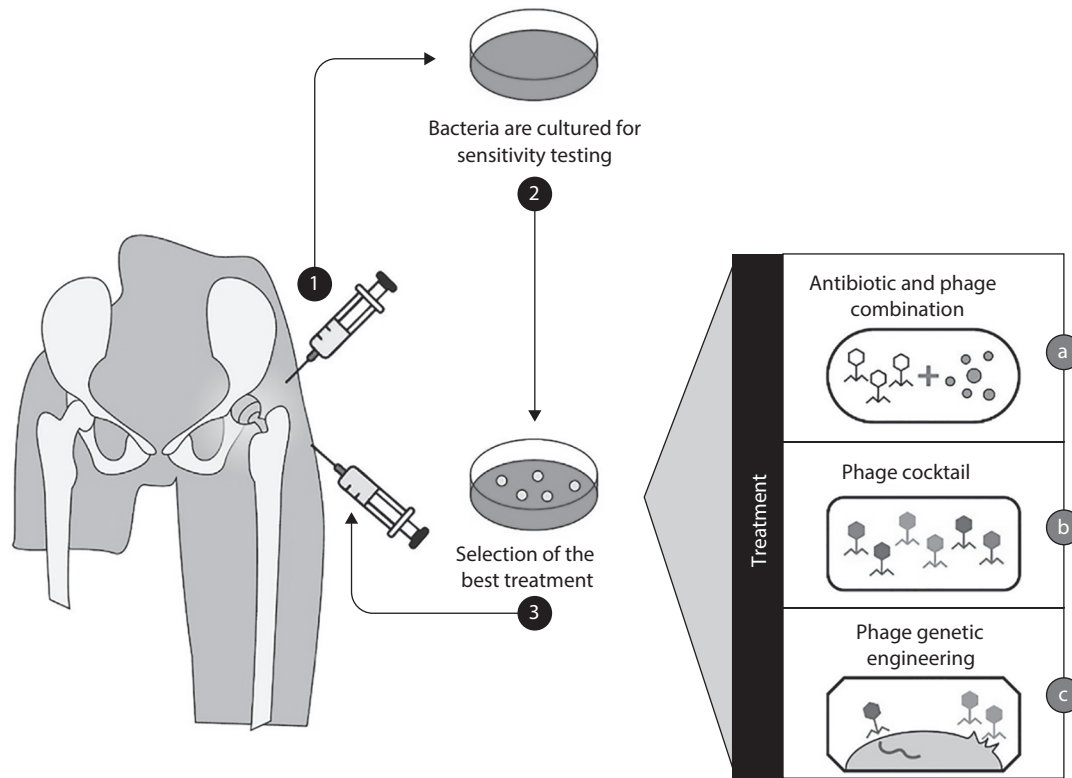


Fig. 7

Personalized combinatorial phage therapy. Various treatments for periprosthetic joint infection can be developed using a combination of either phages and antibiotics, phage cocktail, or engineered phages in order to create personalized therapy. Adopted from Romero-Calle et al.<sup>45</sup>

infections, the potential use for establishing bacteriophages as antibacterial therapeutics seems promising (Figure 7). Bacteriophages exhibit high specificity for the bacteria they infect, making them a narrow spectrum agent. Therefore, phage therapy does not disrupt the normal microbial flora of the host microbiome as can happen with broad-spectrum antibiotics, which can lead to other problems such as emergence of *Clostridioides difficile*. Although narrow spectrum treatments typically require accurate diagnosis, cocktails of multiple phages (a phenomenon called synergy) provide a broader spectrum of activity against known pathogens.<sup>13</sup> Such phage cocktails may be active against various strains of the same bacterial species, and killing the target bacteria may be more effective than the lytic activities of single cocktail phages.<sup>43</sup> It was previously shown that synergy can be obtained when one phage is able to facilitate the infection of the same bacterium by another phage.<sup>44</sup> This approach of synergistic phages may significantly improve production of phage preparations for therapeutic application, since it enhances their clinical efficacy.<sup>43</sup>

Ideally phage therapy of individual patients requires selection of phages for their specificity, also known as affinity.<sup>46</sup> It is important that the isolated/identified bacterium is sensitive to the chosen phage. If this is not the case, then the therapeutic application of the phage will be ineffective. As such, a better approach would be the use of phage cocktails that will improve the phages' lytic spectrum.<sup>47</sup> It would be more practical to select

phages with broad spectrum-strain lytic activity. Further, once the therapeutic phage(s) is selected, it is important to investigate the concept of multiplicity of infection (MOI) - the ratio of phage infections per bacteria - and MOI input, the number of phages administered per cell.<sup>45</sup> Another factor is the killing titre, the number of effective bactericidal phage particles delivered (the number of plaque-based phage counts), which can also be used to streamline the therapeutic application of the phage.<sup>48</sup> Determining the aforementioned aspects of the phage will ensure successful pharmacodynamics and therapeutic efficacy.<sup>49</sup>

Two other important advantages of bacteriophages are their abundance in the environment and adaptability. Bacteria can become resistant to bacteriophages but, unlike traditional antibiotics, it is often simple to isolate new bacteriophages from environmental sources. Bacteriophages can also adapt to resistance, either through natural selective means or with a directed engineering approach.<sup>13</sup> Although many favour the use of natural bacteriophages, genetically engineered bacteriophages can be less immunogenic, have broader host range, or carry specialized payloads, such as clustered regularly interspaced short palindromic repeats (CRISPR), to more effectively kill host bacteria. Other advantages of phages include their ability to replicate at the site of infection, thus continuing the treatment where it is most needed.<sup>50</sup> This increased phage density must be sufficiently robust so that their numbers can

be sustained in the vicinity of target bacteria to achieve desired levels of bacterial eradication.<sup>18</sup>

Several decades of studies on bacteriophage therapy, including recent clinical trials, have demonstrated that there are no serious side effects in humans.<sup>45</sup> Further, bacteriophages are also extremely stable and can be stored for several months at room temperature.<sup>19</sup> Phages can also be stored in colder temperatures or in the presence of reagents that can enhance phage stability in a water suspension.<sup>43</sup> Additionally, phages can be preserved by freeze-drying, spray drying, or encapsulation. Lastly, phage stability is achieved when the titre does not significantly decrease for several days, while others preserve their stability for years.<sup>43</sup> Pirnay et al<sup>51</sup> discuss in greater detail the storage, quality, and safety requirements that need to be validated and monitored.<sup>52</sup>

Delivery of phages is yet another advantage. Simple injections (intraperitoneally, intramuscularly, and intravenously) have all been used successfully to deliver bacteriophages in both animal and humans. These types of injections represent efficient means of phage delivery to virtually all organs and tissues and are significantly better than oral delivery.<sup>53</sup> Bacteriophages considered for therapeutic purposes must be fully lytic, so candidate phages must be screened for genes associated with lysogeny, antibiotic resistance, or toxins.<sup>43</sup> Lastly, phage-mediated bacterial lysis may result in the release of bacterial endotoxins caused by Gram-negative bacteria infections (as is the case with many antibiotics), but this issue is less of a concern with local phage treatment of infections.

As phages are not classified as living or as chemicals, their regulation is complicated. According to Romero-Calle et al,<sup>45</sup> in Belgium the status of therapeutic phage preparations is defined as industrially prepared medicinal products or as magistral (compounded) preparations prepared in pharmacies. As such, natural phages and their products can be processed by a pharmacist as raw materials (active ingredients), providing compliance is observed with certain provisions of the European Directive requirements for medicinal products for human use. Other countries permit the use of phage therapy on compassionate grounds when all other therapies have failed or if the condition is immediately life-threatening.<sup>45</sup> Elsewhere it has been suggested that there is a need to create a regulatory framework to allow quick supply of bacteriophage cocktails for personalized therapy with the framework based on the Quality by Design (QbD) concept, which is already applied to the development and production of biopharmaceuticals, and incorporates process and product quality, in a risk-based manner.<sup>54</sup> Therefore, understanding patients' needs coupled with the specific science and quality characteristics of the phage product will be linked to safety and efficacy, both critical components of QbD. In the USA, the Food and Drug Administration has approved the Centre for Innovative Phage Applications and Therapeutics (IPATH) to utilize phage therapy via the Emergency Investigational New Drug scheme.<sup>52</sup>

In conclusion, orthopaedic-related bacterial infections represent a major challenge with growing antibiotic resistance. Effective alternative therapeutic strategies are required.<sup>3</sup> The limited data reviewed here suggest that bacteriophage therapy may offer a solution. This is a rich area for ongoing research.



### Take home message

- Antibiotic resistance is a worldwide health problem.
- Complications from orthopaedic surgery include implant-associated infections.
- Bacteriophage therapy is a promising approach to bone and joint infections.

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