

Lessons Learned From the First 10 Consecutive Cases of Intravenous Bacteriophage Therapy to Treat Multidrug-Resistant Bacterial Infections at a Single Center in the United States

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Background. Due to increasing multidrug-resistant (MDR) infections, there is an interest in assessing the use of bacteriophage therapy (BT) as an antibiotic alternative. After the first successful case of intravenous BT to treat a systemic MDR infection at our institution in 2017, the Center for Innovative Phage Applications and Therapeutics (IPATH) was created at the University of California, San Diego, in June 2018.

Methods. We reviewed IPATH consult requests from June 1, 2018, to April 30, 2020, and reviewed the regulatory process of initiating BT on a compassionate basis in the United States. We also reviewed outcomes of the first 10 cases at our center treated with intravenous BT (from April 1, 2017, onwards).

Results. Among 785 BT requests to IPATH, BT was administered to 17 of 119 patients in whom it was recommended. One-third of requests were for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Mycobacterium abscessus*. Intravenous BT was safe with a successful outcome in 7/10 antibiotic-recalcitrant infections at our center (6 were before IPATH). BT may be safely self-administered by outpatients, used for infection suppression/prophylaxis, and combined successfully with antibiotics despite antibiotic resistance, and phage resistance may be overcome with new phage(s). Failure occurred in 2 cases despite in vitro phage susceptibility.

Conclusions. We demonstrate the safety and feasibility of intravenous BT for a variety of infections and discuss practical considerations that will be critical for informing future clinical trials.

Keywords. bacteriophage therapy; phage therapy; multidrug-resistant infections.

Bacteriophages (phages) are viruses that infect specific bacterial hosts to set up lytic and/or lysogenic replicative cycles; lytic phages lead to lysis of bacterial cells, with the newly released virions infecting other bacterial cells in an exponential manner. Phages were first described in the early 20th century and were used in the 1920s and 1930s for treatment of various infectious syndromes, including dysentery, furunculosis, and urinary and respiratory tract infections (though success rates are unclear). Advent of the antibiotic era led to discontinuation of bacteriophage therapy (BT) in Western medicine, though treatment centers have persisted in Eastern Europe and the former Soviet Union [1].

Increased rates of multidrug-resistant (MDR) and extensively drug-resistant bacterial infections have ushered us into the postantibiotic era. We described the first successful case of intravenous BT to treat a systemic MDR infection at our institution in 2017 and since then have pioneered the clinical use of BT in antibiotic-recalcitrant infections in the United States [2]. To assist in this endeavor, the Center for Innovative Phage Applications and Therapeutics (IPATH) was created at the University of California, San Diego, in June 2018. In this report, we describe the outcomes of consult requests made to IPATH and discuss the first 10 cases of BT treated at our center. We focus on incremental knowledge gains made from these early cases that will inform treatment decisions for future cases and phage-related clinical trials.

METHODS

We reviewed all BT consult requests made to IPATH from June 1, 2018, through April 30, 2020. This was done by retrospective review of a prospectively developed database that has de-identified data including reason for request, bacterial pathogen, and disease process. Per institutional review board

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(IRB) recommendations, review of the de-identified database did not require informed consent. We also reviewed outcomes of 10 cases of BT treated at our center under Food and Drug Administration (FDA) and IRB oversight with informed consent of the patient.

Testing of Susceptibility of Bacteria to Bacteriophages

There is currently no gold standard testing method for developing a “phagogram,” that is, assessing bacterial susceptibility to phage. Phagograms for patients treated at our center were conducted by 2 main methodologies by the phage solution manufacturer.

1. Biolog method: This consists of inoculation of standardized bacterial suspensions with bacteriophages individually and in combination in 96-well microtiter plates containing tryptic soy media in 1% (vol/vol) tetrazolium dye and incubated at 37°C in a Biolog machine for 24 hours. Bacterial respiration leads to reduction of the tetrazolium dye, leading to a color change, which is depicted as relative units of bacterial growth [3].
2. Double agar overlay plaque assay: A lawn of the bacterial isolate is grown on an agar plate, and isolates are classified as susceptible when individual plaques are observed in drop tests with serial dilutions of the phage solution [4].

Synergy testing between antibiotics and phages was not routinely performed.

RESULTS AND DISCUSSION

IPATH Consultation Experience

Between June 1, 2018, and April 30, 2020, IPATH fielded a total of 785 requests for BT from patients and physicians within the United States (n = 536, 68.2%) and internationally (n = 158, 20%; missing designation n = 91, 11.6%). Of all requests, the majority were for bacterial infections (n = 644, 82%), 7 (0.9%) were for viral infections, 33 (4.2%) were for noninfectious diseases, and 64 (8.2%) were for nonmedical conditions. IPATH’s role was to assess the validity of the consult requests in order to determine if BT was clinically indicated. We then matched consult requests with commercial/academic laboratories that had experience in developing phages active against a particular bacterial species. We facilitated single-use Investigational New Drug (IND) applications to the FDA and the local IRB and related applications by providing templated material, walking treating physicians through the process, and helping formulate treatment plans. We recommended a phage hunt for BT in 119/644 (18.5%) bacterial cases, though only a minority of patients received BT under compassionate use indications, 17/119 (14.3%), as noted in Figure 1. Reasons for attrition are also noted in Figure 1 and include lack of bacterial isolates for phage

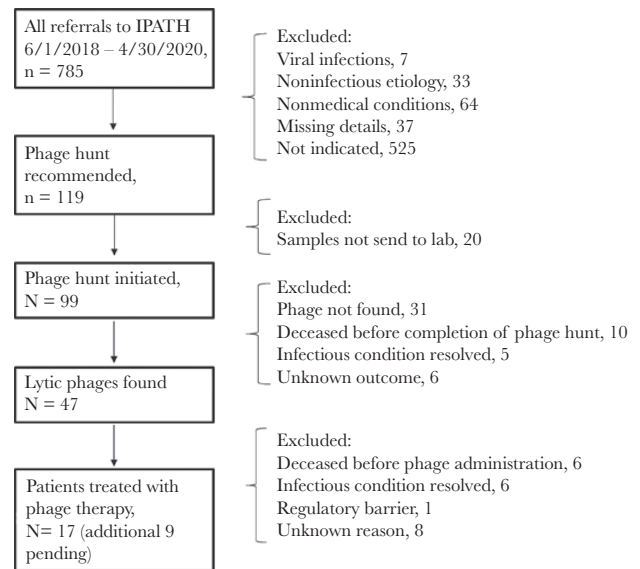


Figure 1. Flowchart depicting outcome of all bacteriophage therapy requests at the Center for Innovative Phage Applications and Therapeutics. Abbreviation: IPATH, Center for Innovative Phage Applications and Therapeutics.

susceptibility testing, resolution of the infectious condition, or death of the patient before finding matching lytic phages or before phage administration if matching phages were found.

Table 1 describes the outcomes of BT requests by bacterial pathogen. As can be noted, the majority of requests among all bacterial infections were for infections due to *Pseudomonas aeruginosa* (92/644, 14.3%), *Staphylococcus aureus* (77/644, 12%), and *Mycobacterium abscessus* (47/644, 7.3%), together accounting for a third of all requests. Other common organisms included enteric gram-negative bacilli *Escherichia coli* (39/644, 10.7%), *Klebsiella pneumoniae* (27/644, 4.2%), *Enterobacter* species (9/644, 1.4%), *Burkholderia* species (20/644, 3.1%), and *Mycobacterium avium* (23/644, 3.6%). As noted in Table 1, there was a significant delay from time to request for phage therapy to actual administration to the patient, ranging from 28 to 386 days with a median of 170.5 days.

Current Status of Compassionate Use Bacteriophage Therapy in the United States

As phage therapy remains experimental, each case required approval from the FDA under a single-use IND. Requirements for a successful application include evidence of clinical need, proof of in vitro bacterial susceptibility to the phage(s), genetic characterization of the phage(s) with particular focus on plasmids encoding for resistance mechanisms, lack of lysogenic activity, sterility of the final product based on USP 71 testing requirements, and minimal endotoxin concentration. Drug resistance genes are often carried on plasmids, which may be transferred to other bacteria through several mechanisms, including horizontal gene transfer via bacteriophages. Temperate phages integrate into the host bacterial genome (then called

Table 1. Outcomes of Request for Phage Therapy Received at IPATH From June 1, 2018, to April 30, 2020, Based on Infecting Bacterial Pathogen

	Number of Requests	Phage Hunt Initiated	Lytic Phage(s) Found	Phage Therapy Administered	Phage Therapy Pending	Median Time From Request to Administration (Range), d
<i>Pseudomonas aeruginosa</i>	92	26	18	5	1	156.5 (58–374)
<i>Escherichia coli</i>	39	6	4	2	2	260 (165–355)
<i>Klebsiella pneumoniae</i>	27	2	0	0	0	-
<i>Acinetobacter baumannii</i>	14	1	0	0	0	-
<i>Achromobacter</i> species	8	5	2	0	1	-
<i>Enterobacter cloacae</i>	6	2	2	0	1	-
<i>Enterobacter aerogenes</i>	1	1	1	1	0	252
<i>Enterobacter</i> species	2	0	0	0	0	-
<i>Serratia marcescens</i>	4	1	1	0	0	-
<i>Citrobacter</i> species	1	1	1	1	0	53
<i>Burkholderia cenocepacia</i>	8	6	0	0	0	-
<i>Burkholderia cepacia</i>	10	6	1	0	0	-
<i>Burkholderia gladioli</i>	1	1	0	0	0	-
<i>Bacillus vietnamensis</i>	1	1	0	0	0	-
<i>Pandoraea pulmonicola</i>	1	1	0	0	0	-
<i>Ralstonia mannitolilytica</i>	1	1	0	0	0	-
<i>Staphylococcus aureus</i>	77	4	1	1	0	28
<i>Staphylococcus epidermidis</i>	2	0	0	0	0	-
<i>Staphylococcus lugdunensis</i>	1	1	0	0	0	-
<i>Enterococcus faecalis</i>	10	0	0	0	0	-
<i>Enterococcus faecium</i>	2	2	2	2	0	127 (50–204)
<i>Clostridiodes difficile</i>	8	0	0	0	0	-
<i>Mycobacterium abscessus</i>	47	18	9	4	3	176 (87–386)
<i>Mycobacterium chimera</i>	7	5	4	1	0	168
<i>Mycobacterium avium</i>	23	1	0	0	0	-
<i>Mycobacterium smegatis</i>	1	0	0	0	0	-
<i>Mycobacterium chelonae</i>	2	0	0	0	0	-
<i>Mycobacterium xenopi</i>	1	0	0	0	0	-
<i>Mycobacterium bolletii</i>	1	0	0	0	0	-
<i>Mycobacterium genavense</i>	1	0	0	0	0	-
<i>Mycobacterium</i> species	7	0	0	0	0	-
<i>Borrelia burgdorferi</i>	37	0	0	0	0	-
Other organisms	45	0	0	0	0	-
Polymicrobial	86	7	1	0	1	-
Not applicable	141	0	0	0	0	-
Missing organism name	70	0	0	0	0	-
Total	785	99	47	17	9	Overall median, 170.5 (28–386)

Among 17 cases who received phage therapy during this time period, 4 were treated at IPATH. This included cases of infection due to *Pseudomonas aeruginosa* (n = 2), *Staphylococcus aureus* (n = 1), and *Escherichia coli* (n = 1).

Abbreviation: IPATH, Center for Innovative Phage Applications and Therapeutics.

prophage) and, when they switch to a lytic cycle based on certain environmental triggers, can activate antibiotic resistance genes [5]. The FDA currently requires genetic sequencing of the phages to be used for BT to demonstrate lack of known antimicrobial resistance plasmids in the genome and lack of lysogeny as a safety measure. As phage solutions are manufactured by growing the phage in the host bacterial broth, there is concern for endotoxin contamination. Per the FDA, the maximal allowable endotoxin limit for a single dose is 5-EU/kg body weight per hour of administration. The process from identification of a potential patient to actual clinical phage administration under compassionate use indications is detailed in Figure 2 and, as noted earlier, involves a significant time lag.

Lessons Learned From Clinical Use of Bacteriophage Therapy

A brief overview of the first 10 cases of BT at our center is provided in Table 2. Five of these have been published in granular detail in prior reports, and others are pending [2, 6–9]. In general, we treated MDR and antibiotic-recalcitrant cases of abdominal abscesses, pulmonary infections in patients with cystic fibrosis and lung transplant, bone and prosthetic joint infections, ventricular assist device infections, recurrent urinary tract infection, and recurrent bacteremia from an infected aortic graft. All active infections were treated with a combination of phage and systemic antibiotics. Each patient was treated after informed consent and through a single-use IND application and under close oversight by the FDA and local IRB.

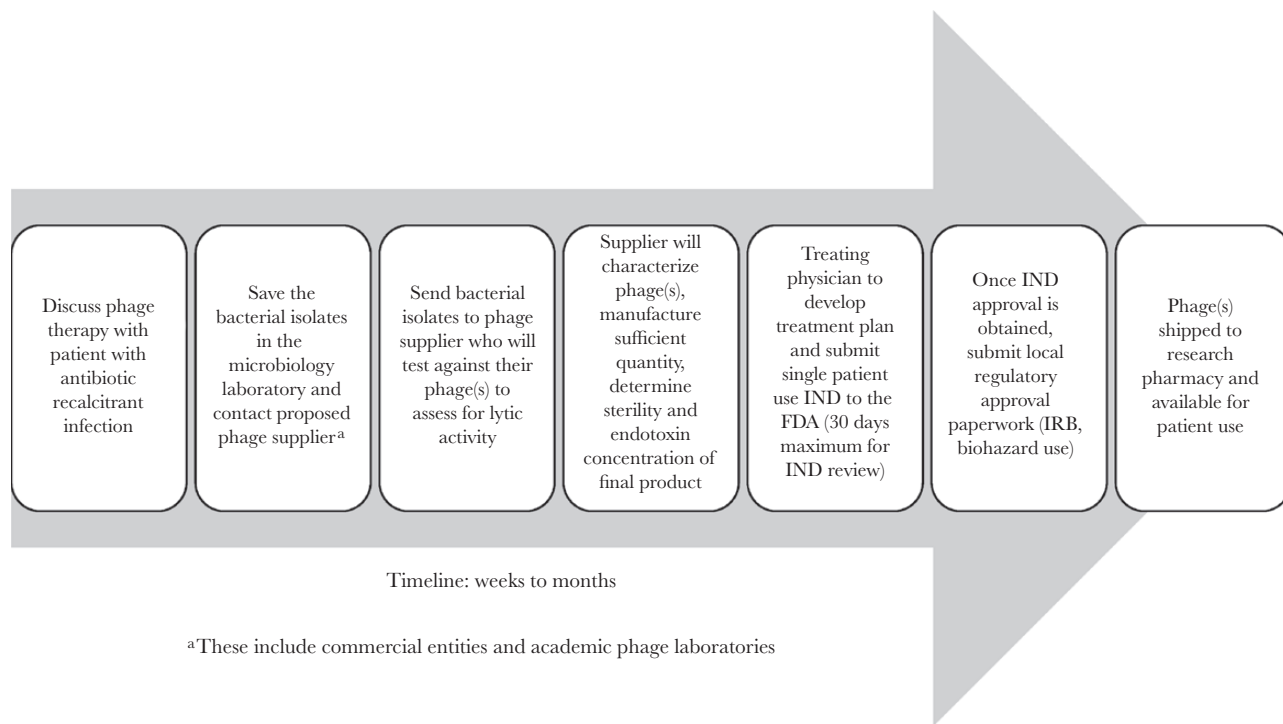


Figure 2. Timeline of the process to initiate bacteriophage therapy for a patient on a compassionate use basis in the United States. Abbreviations: FDA, Food and Drug Administration; IND, Investigational New Drug; IRB, institutional review board.

Intravenous and Nebulized Phage Administration Appears Safe

The first BT case made it clear that application of bacteriophages is a viable therapeutic option for the treatment of systemic MDR infections. This was the first time, to our knowledge, that BT was administered via the intravenous (IV) route and was well tolerated [2]. We have since treated all our patients through the IV route. Case 2 received nebulized phage in addition to IV phage, which was well tolerated, without bronchospasm. Nebulized phage administration was staggered, so there was a 1-week period of nebulized BT only and 1 week of IV BT only during episode 1, in addition to 2 weeks of combined IV and nebulized phage (with concomitant antibiotics). In this case, we determined that an equivalent amount of phage was obtained from the bronchoalveolar lavage specimens regardless of route of administration. However, as noted in Table 2, the nebulized version required 4 times the volume per dose compared with the IV route due to environmental loss, and thus we have focused on using the IV route for subsequent pulmonary infections [6].

One patient, Case 8, developed an adverse event while receiving BT. This occurred when a new phage cocktail at a concentration of 1×10^{11} phage-forming units (PFU)/mL was first administered. The patient developed fever, wheezing, and shortness of breath ~2 hours after each of 2 consecutive doses, which resolved with acetaminophen, solumedrol, albuterol nebulization, and diphenhydramine. The same phage cocktail

was subsequently well tolerated when multiple titrations were administered as a dose escalation study; the patient subsequently received a 10^{10} -PFU/mL dose without incident. Source of fever in this case was unclear; the endotoxin concentration of the original preparation was 4.3 EU per dose (well below the allowable limit of 5 mg/kg per hour per FDA requirements [298 EU in this patient]), the phage solution was sterile, and blood cultures remained negative. We hypothesize that there may have been additional pyrogens in the solution (perhaps related to solvents used during dilution or manufacture), which were then diluted with subsequent lower concentrations of the phage solution.

Outpatient Self-Administered BT Is Feasible

Most of the patients we treated with BT had persistent infections before BT and were well versed with outpatient self-administered parenteral antibiotic therapy. With this in mind and with the object of preventing a long hospitalization (and associated costs) in otherwise ambulatory patients, we have now treated 6 patients (Cases 5–10 in Table 2) with outpatient self-administered BT via an indwelling peripherally inserted central catheter. The first dose of BT was administered in a dedicated research clinic space by S.A. with frequent vital sign monitoring every 15 minutes for the first 2–3 hours following the first dose. An anaphylaxis kit containing antihistamine, epinephrine, and steroid was available at the bedside. Each patient underwent

Table 2. Bacteriophage Therapy Cases Treated at the University of California, San Diego; of Note, All Cases Had Previously Failed Multiple Attempts of Antibiotic Treatment and/or Were Due to Highly Drug-Resistant Organisms

Age, Gender	Type of Infection	Organism	Bacteriophage	Phage Concentration, Dosing, and Duration	Key Clinical Points and Outcome
1 68-year-old male [2]	Infected pancreatic pseudocyst, abdominal abscesses, and septic shock	<i>Acinetobacter baumannii</i>	ϕIV (4 phages), ϕIVB (2 phages), ϕPC (4 phages) ^a	5 × 10 ⁹ PFU/mL q2h (16 wk) followed by 5 × 10 ⁹ PFU/mL IV q6h (2 wk); ϕPC was instilled percutaneously into abdominal drains q8–12h (18 wk)	Patient had an episode of necrotizing pancreatitis complicated by XDR <i>A. baumannii</i> infected pseudocyst, abdominal abscesses, and critically ill on 3 vasopressors (ongoing infection for 4 mo before BT). Received BT + antibiotics. Patient recovered fully from the infection. Duration from phage request to administration: 21 d AE: No phage-related adverse event Outcome: Success
2 67-year-old male [3]	Pneumonia in lung transplant recipient	<i>Pseudomonas aeruginosa</i>	Episode 1: AB-PA01 (4 phages) ^b Episode 2: AB-PA 01 m1 (5 phages) ^b and Navy phage cocktail 1 (3 phages) ^a Suppression: Navy phage cocktail 2 (2 phages) ^a	Episode 1: 4 × 10 ⁹ PFU/mL IV q6h, nebulized q12h (4 wk) Episode 2: 5 × 10 ⁹ PFU/mL IV q6h, nebulized q12h and 1 × 10 ⁹ PFU/mL IV q2h, nebulized q4h (4 wk) Suppression: 5 × 10 ⁷ PFU/mL IV q4h (8 wk)	Post-transplant course complicated by recurrent MDR <i>P. aeruginosa</i> pneumonia. Had 2 distinct pneumonia episodes resolved with BT + antibiotics followed by phage alone as suppressive therapy. No infection recurrence while on suppressive phage. Duration from phage request to administration: 22 d AE: No phage-related adverse events Outcome: Success
3 77-year-old male [6]	Subdural and epidural empyema and cranial osteomyelitis	<i>Acinetobacter baumannii</i>	Single phage ^a	2.14 × 10 ⁷ PFU/mL IV q2h (8 d)	Patient underwent a craniotomy for trauma complicated by MDR <i>A. baumannii</i> subdural and epidural empyema and cranial osteomyelitis status post debridement and flap repair. Flap healed while on BT but patient was transitioned to hospice due to lack of neurologic improvement. Duration from phage request to administration: 12 d AE: No phage-related adverse event Outcome: Uninterpretable
4 26-year-old female [5]	Pneumonia in cystic fibrosis	<i>Pseudomonas aeruginosa</i>	AB-PA 01 (4 phages) ^b	4 × 10 ⁹ PFU/mL IV q8h (8 wk)	Acute on chronic respiratory failure due to MDR <i>P. aeruginosa</i> pneumonia unresponsive to antibiotics. Resolved with BT + antibiotics and no further CF flare for the next 3 mo; underwent lung transplant 9 mo later. Colistin was stopped while on BT and acute kidney injury resolved. Duration from phage request to administration: 23 d AE: No phage-related adverse event Outcome: Success
5. 60-year-old male	Ventricular assist device infection	<i>Pseudomonas aeruginosa</i>	GD-1 (3 phages) ^a	1.9 × 10 ⁷ PFU/mL IV q8h (6 wk)	Patient developed MDR <i>P. aeruginosa</i> infection of the driveline complicated by sternal osteomyelitis and recurrent bacteremia for ~9 mo before BT with multiple surgical debridements and prolonged antibiotic courses. Developed bacteremia 1 wk after starting BT, resolved with change in antibiotics. Developed recurrent DL drainage following end of BT. Duration from phage request to administration: 37 d AE: No phage-related adverse event Outcome: Failure

Table 2. Continued

Age, Gender	Type of Infection	Organism	Bacteriophage	Phage Concentration, Dosing, and Duration	Key Clinical Points and Outcome
65-year-old male [4]	VAD infection	<i>Staphylococcus aureus</i>	AB-SA 01 (3 phages) ^b	3 × 10 ⁹ PFU/mL q12h (4 wk)	Patient had a VAD infection, sternal osteomyelitis, and recurrent bacteremia for >2 y before BT with multiple surgeries and prolonged courses of IV antibiotics and an open chest with visible device. Infection resolved with BT + antibiotics, and he underwent successful heart transplant. Duration from phage request to administration: 28 d AE: No phage-related adverse event Outcome: Success
61-year-old female	Prosthetic joint infection	<i>Staphylococcus aureus</i>	Episode 1: AB-SA01 (3 phages) ^b Episode 2: SaGR51øK (single phage) ^a	Episode 1: 3 × 10 ⁹ PFU/mL q12h (2 wk) + 1-time intra-articular injection Episode 2: 2.89 × 10 ¹⁰ PFU/mL 1-time intra-operative dose + IV q12h (6 wk)	Patient had persistently infected right total knee arthroplasty for >2 y before BT with several surgeries, prosthesis exchange, and prolonged IV antibiotics. Patient's pain, swelling, and purulent drainage significantly improved at the end of 2 wk of treatment in episode 1 but then recurred 5 d later. She was then re-treated almost 6 months later (episode 2) with surgical revision, systemic antibiotics, and BT with resolution of <i>S. aureus</i> infection. Duration from phage request to administration: 28 d AE: No phage-related adverse event Outcome: Success
82-year-old male	VAD infection	<i>Pseudomonas aeruginosa</i>	Episode 1: SDSU1 (2 phages: PAK_P1, E217) and SDSU2 (2 phages: PAK_P1, PAK_P5) ^b Episode 2: PPM3 (4 phages) ^b	Episode 1: 2 × 10 ⁹ PFU/mL IV q8h (6 wk) + 1-time intra-operative dose followed by PAK_P1 7.58 × 10 ⁵ PFU/mL alone for 10 d followed by SDSU2 4 × 10 ¹⁰ IV q12h for 3 wk Episode 2: 1 × 10 ⁹ PFU/mL q12h (4 wk)	Patient had a persistently infected VAD complicated by multiple hospitalizations, surgical debridements, and recurrent bacteremia for >2 y before BT. He underwent 2 rounds of BT, developed recurrent bacteremia within 1 wk of ending BT in episode 1. Retreated with BT 3.5 mo later but had recurrent bacteremia 4 wk into episode 2 while still on BT. Duration from phage request to administration: 58 d AE: Developed fever, wheezing, and shortness of breath after 2 infusions of SDSU2 1 × 10 ¹¹ PFU/mL concentration; same phage was well tolerated at the 10 ¹⁰ PFU/mL concentration. Other phage formulations well tolerated. Outcome: Failure
56-year-old male	Recurrent urinary tract infection in liver transplant recipient	<i>Escherichia coli</i>	UCS1 (4 phages) ^b	1.0 × 10 ⁹ PFU/mL IV q12h (2 wk)	Recurrent extended-spectrum beta-lactamase-producing <i>E. coli</i> UTI and prostatitis in a patient with chronic kidney disease, kidney stones, and immunosuppression. Pre-BT, patient was intermittent on systemic antibiotics for 1 y with recurrence of symptoms 1–3 wk after antibiotics discontinuation. After end of BT + erlapenem, there was a lack of recurrent symptomatic UTI symptoms with 12 wk of follow-up though urine culture was positive. Duration from phage request to administration: 355 d AE: No phage-related adverse event Outcome: Success

Table 2. Continued

Age, Gender	Type of Infection	Organism	Bacteriophage	Phage Concentration, Dosing, and Duration	Key Clinical Points and Outcome
10 64-year-old male	Recurrent bacteremia and probable aortic graft infection	<i>Pseudomonas aeruginosa</i>	PPM2 (3 phages) ^b	2.6 × 10 ⁶ PFU/mL IV q12h (6 wk)	Recurrent bacteremia for the past 1.5 y with prolonged antibiotic courses and breakthrough infection. Negative blood cultures while on BT + ciprofloxacin. Weekly surveillance blood cultures remained negative ×4 with no recurrence for 12 wk after end of therapy (previous bacteremia recurrences occurred 7–10 d off antibiotics). Duration from phage request to administration: 374 d AE: No phage-related adverse event Outcome: Success

All patients received BT along with concomitant systemic antibiotics except for suppressive phage therapy in Patient 2. The first 6 patients received BT before the formation of IPATH. Phage manufacturers and bacterial susceptibility testing methods are noted in the footnote [3, 4].

Phage manufacturers: qIV, qIVB, qPC and single phage for Patient 3—Naval Medical Research Center, Fort Detrick, MD, USA; Navy Phage Cocktail 1, Navy Phage Cocktail 2, GD-1, and SaGR51eK—Adaptive Phage Therapeutics, Gaithersburg, MD, USA; ABPA-01, ABPA-01m1, and ABPA-01—Armata Pharmaceuticals (formerly AmpliPhi Corp), Marina del Rey, CA, USA; SDSU1 and SDSU2—Roach Laboratory, San Diego State University, San Diego, CA, USA; USC1—Tailored Antimicrobials and Innovative Laboratories for Phage q Research, Baylor College of Medicine, Houston, TX, USA; PPM2 and PPM3 manufactured by Walter Reed Army Institute of Research, Silver Spring, MD, USA.

Abbreviations: AE, adverse event; BT, bacteriophage therapy; CF, cystic fibrosis; DL, driveline; IV, intravenous; MDR, multidrug-resistant; PFU, phage-forming units; UTI, urinary tract infection; VAD, ventricular assist device; XDR, extensively drug resistant. *Bacterial susceptibility testing (phageogram) methods:*

^aBiolog method.

^bDouble agar overlay plaque assay.

teaching and was given clear instructions on phage storage and administration at home. We generally provided enough phage doses for self-administration for a 1-week period (with some overage), the patient had weekly clinic visits for an in-person evaluation and blood draws and a pharmacy visit for an additional 1-week phage supply. Patients completed a daily symptom diary to assess for adverse events and documented date and timing of phage administration. Case 10 was treated during the COVID-19 pandemic, and S.A. used weekly telemedicine video visits to assess the patient after the initial in-person visit for the first dose of phage administration.

None of the patients treated with BT as an outpatient had a phage-related adverse event while administering phage at home, and all patients reported feeling comfortable with this process. Outpatient administration and avoidance of hospitalization are very important from the patient perspective, and all those treated were appreciative of the convenience while receiving experimental therapy. This was a cost-saving approach, reduced the risk of patients acquiring nosocomial infections, and allowed patients to continue with work or other regular routines. We believe that outpatient administration will be important as clinical trials are developed, in particular if prolonged therapy for a particular clinical indication is needed.

BT May Be Used Alone Safely as Suppressive Therapy

Case 2 received IV BT alone for almost 8 weeks as suppressive therapy in an attempt to prevent recurrence of MDR *P. aeruginosa* pneumonia [6]. During this time period, he was taken off systemic antibiotics and had no recurrence of *P. aeruginosa* infection while on BT and for the subsequent 3 months. We demonstrated the safety of phage alone as well as potential efficacy as a preventive approach.

Bacterial Resistance to Phage Can Develop During Therapy but May Be Overcome With New Phage(s)

We noted development of phage resistance during BT in 3 patients (Cases 1, 2, and 8); in each case, this was overcome clinically by matching resistant bacterial isolates to additional new phages in a personalized treatment strategy. Development of new phages occurred in real time, and we were able to treat patients effectively with this approach. Most patients were treated with a combination of several phages with different bacterial receptors to reduce the risk of developing bacterial resistance to the phage(s), though conversely development of resistance may come at a fitness cost to the bacteria as well. One patient, Case 7 with *S. aureus* prosthetic joint infection, was treated successfully with a single lytic phage without development of resistant organisms. Case 3 was also treated with a single phage, as only 1 was found, though treatment duration was too short to interpret any outcome. In Cases 1, 2, 5, and 8, new bacterial isolates emerged either during the course of treatment or thereafter

with different antibiotic susceptibility profiles than the original infecting strain that were more amenable to antibiotic therapy.

Phage and Antibiotics Combination Can Lead to Successful Outcome Despite Presence of In Vitro Antibiotic Resistance

This was assessed in depth for Case 1, whose *A. baumannii* isolate demonstrated in vitro resistance to minocycline, but the combination of phage and minocycline showed synergistic activity against the organism [2]. We have not assessed phage-antibiotic synergy systematically to date, though there is great interest in developing phage-antibiotic synergy testing assays that will help determine optimal treatment choices for a patient [10]. Phage and antibiotic combinations were tested before BT in Case 9 only and did not show antagonism between the phages and ertapenem, though there was lack of synergy.

Treatment Failure Can Occur Despite In Vitro Phage Susceptibility

Both cases of *P. aeruginosa* VAD infection were associated with therapeutic failure of adjunctive BT. Both of these were chronic (>1-year) biofilm-based infections. We hypothesize that treatment failure may be related to poor biofilm penetration of the phages and/or presence of multiple biofilm-based pseudomonal strains at baseline that were not all susceptible to the phage(s) administered. Both also developed bacteremia within the first week of BT from pseudomonal strains with different antimicrobial susceptibility patterns that cleared with a change in systemic antibiotic administration. These new bacterial isolates remained susceptible to administered phages in vitro. Case 5 was noted to have phage-specific neutralizing serum at the time of recurrent bacteremia, but we did not have baseline samples for comparison. Additionally, filamentous lysogenized phages are known to occur in *P. aeruginosa* biofilms as a biofilm-promoting virulence factor [11]; these prophages may be associated with inhibition of adsorption of extraneous phage administered as therapy. Lastly, loss of potency of stored phage is possible, and thus stability studies will be helpful for future cases.

CONCLUSIONS

Our experience with BT highlights the burgeoning interest and need for alternative antimicrobial therapeutics for effective management of infections that are recalcitrant to traditional antibiotics mainly due to multidrug resistance and biofilm characteristics; in just 2 years, we received 785 requests. Based on our referral pattern, the top 3 organisms of interest for BT are *P. aeruginosa*, *S. aureus*, and *M. abscessus*, together comprising ~30% of all referrals, though we noted a wide spectrum of organisms. Identification of the main organisms of interest for BT is helpful for researchers in order to focus their efforts based on clinical need. The safety profile and favorable outcomes of our locally treated cases are highly encouraging for increasing use of BT both on a compassionate use basis and in particular

for upcoming clinical trials. Since the creation of IPATH as the first BT center in the United States, other sites in the United States have developed clinical BT centers as well, including Baylor College of Medicine, Mayo Clinic, and Johns Hopkins University. There are also well-established international centers including the Eliava Institute (Tbilisi, Georgia), Queen Astrid Military Hospital (Brussels, Belgium), Centre Hospitalier Universitaire de Lyon (Lyon, France), Westmead Institute for Medical Research (Sydney, Australia), and HUJI-HMC Phage Therapy Institute (Jerusalem, Israel).

The National Institutes of Health and Cystic Fibrosis Foundation have funded phage-related clinical trials and development of bacteriophage libraries focusing on *P. aeruginosa* and *Burkholderia* species, and a number of commercial entities are in the process of initiating clinical trials; multiple other funding opportunities are available for basic science, translational, and clinical phage-related work. Factors we believe will be important for successful trials include development of a broadened host range for phage(s) used for BT using genetic engineering techniques, stability testing of the final phage product to ensure that the concentration delivered to the patient at bedside is at goal, pharmacokinetic/dynamic studies to evaluate for optimal dosing concentration, interval, and duration of BT, impact of immune response to the phage on clinical outcome, and rapid phage susceptibility platforms and assessment of phage-antibiotic synergy to identify optimal treatment [12, 13].

In conclusion, our experience with BT highlights the promise of BT for multiple clinical indications. Significant work is needed to identify predictors of success and for design of clinical trials that will lead to more widespread use.

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Patient consent. Each patient in the case series was treated with written informed consent approved by the University of California San Diego Human Research Protections Program (UCSD HRPP) and a specific IND from the Food and Drug Administration. The retrospective review of the case series was approved by the UCSD HRPP protocol

number 200163. The review of the de-identified consult request data set did not require institutional review board oversight as determined by the UCSD HRPP.

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