Personalized bacteriophage therapy to treat pandrug-resistant spinal \textit{P. aeruginosa} infection

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Abstract

A pandrug-resistant *P. aeruginosa* spinal abscess was treated with surgery and personalized phage therapy that was added to cefiderocol and colistin. After a 2-stage approach, despite bacterial persistence with expression of small colony variants, the patient cured with local and intravenous injections of purified bacteriophages as part of the treatment.

Full Text

Bone and joint infections (BJI) are one of the most difficult-to-treat bacterial infection, especially in the era of antimicrobial resistance. Lytic bacteriophages can rapidly and selectively target and kill bacteria whilst producing new bacteriophage particles in an exponential and self-sustained reaction. Accordingly, lytic phages are considered to have a high therapeutic potential for the treatment of bacterial infectious diseases [1]. Moreover, regarding their often-described synergistic anti-biofilm activity when combined with antibiotics, phages are considered as particularly promising adjuvant therapy for the treatment of complex BJI [2,3]. Currently, phage Active Pharmaceutical Ingredients (pAPIs) production follows requirements of quality and safety, which guarantee adequate composition and acceptable levels of residual contaminants [1,4].

A 74-year-old man with melanoma treated with anti-PD1 (pembrolizumab) experienced a catheter-related bacteremia due to multidrug-resistant *Pseudomonas aeruginosa* in January 2018. He was treated successfully with colistin and meropenem that he received during eleven days. He developed spinal pain during summer 2018 and spondylodiscitis with spinal abscess was diagnosed (Figure 1, panel A and B). Puncture revealed pan drug-resistant *P. aeruginosa* in culture, with resistance to all antibiotics including ceftazidime/avibactam (MIC 64 mg/L), ceftolozane/tazobactam (MIC >256 mg/L) and colistin (MIC 8 mg/L). He received in a French hospital colistin (colistimethate sodium; 3 MUI/8h) and rifampin 900 mg once a day, and as he developed acute kidney injury, antibiotics were rapidly stopped. The patient was admitted in our referral center for the management of complex BJI with severe pain. He was bedridden and required continuous infusion of opioid. As no active antibiotic was available for the treatment, phage therapy, that was already used in our institution, was envisaged as salvage therapy. The strain was sent to two different private companies (in France and in the USA) to test their phages in development, and also to a European military laboratory that have purified phages. Unfortunately, the strain was fully resistant to all available phages. As a consequence, we developed a unique academic collaboration between universities and hospitals located in three different European countries (Switzerland, Belgium and France) to find, produce and administer a personalized phage cocktail to the patient, in the shortest possible time (see methods, figures C-H and supplementary tables 1 and 2).

We collegially proposed the following 2-stage strategy (approved by the ethic committee of our hospital and by the French national health care authority; performed after the patient gave informed consent). First stage: open surgery, L2-L3 and L3-L4 discal debridements (discectomy), local administration of the three-phage cocktail, partial stabilization bridging the infected area with osteosynthesis from T11-T12-L1
to L5-sacrum, systemic cefiderocol antibiotherapy for 6 weeks. Second stage: reconstruction with interbody fusion cages in L2-L3 and L3-L4, and spinal osteosynthesis outside the septic location, from T12-L1 to L5-S1, to finalize consolidation. For the first stage, magistral preparation of the phage cocktail was done extemporaneously by our hospital pharmacist, as described in supplementary material 3. During the first stage, the phage cocktail (dilution in 7 mL; final phage titer of $10^6$ pfu/mL) was administered locally at L2-L3 and L3-L4 after abscess evacuation, debridement, and lavage with bicarbonate solution. Additive quality controls (QCs) were performed by the French hospital pharmacy that included sterility test for bacteria and fungi and endotoxin concentration. Cefiderocol was started intravenously after the surgery, 2g during 3 h every 8 h (6g/day) for a duration of 6 weeks. Quickly, the pain was significantly reduced. During the treatment, the patient experienced *Clostridioides difficile* diarrhea, but no other serious adverse event was reported. Two weeks after the end of the first stage (two months after the initial surgical debridement), the second stage was performed. The patient had neither systemic (no fever, CRP 10 mg/L) nor clinical signs of persistent infection. The same phage cocktail with same dilution and phage titer was locally administered (same volume and concentration) before insertion of the intersomatic cages at L2-L3 and L3-L4 level. Cefiderocol was started again intravenously pending the culture results. Unfortunately, *P. aeruginosa* still grew in culture from bone biopsy with a small colony variant phenotype, but remained susceptible to the phage cocktail and cefiderocol (Figure 1, panel I-M, supplementary table 2). Whole genome sequencing (WGS) of this strain identified numerous acquired genes accounting for this high level of resistance to antibiotics (supplementary table 3). Colistin (colistimethate sodium) was added intravenously at the dose of 2 MUI/8h (6 MUI/day), despite the strain became phenotypically resistant to this antibiotic, in order to potentially have synergistic effect with cefiderocol [7]. As the cultures revealed persistence of the *P. aeruginosa*, phages were also added intravenously in three-hours infusions (30 mL, phage titers $10^6$ pfu/mL) every day for 21 days. Under the treatment, the patient experienced abdominal pain related to gall stone migration and relapsing *C. difficile* infection. No adverse event potentially related to phage therapy was noticed. Antibiotics (cefiderocol and colistin) were stopped at 3 months. The outcome was favorable during the follow-up (21 months), without implant loosening nor clinical signs of infection (Figure 1, panel Q-R), and the patient was walking without pain (supplementary video file). Unfortunately, he died from COVID-19 infection in December 2020.

Phage therapy is an emerging option for complex BJI, especially in the era of worldwide dissemination of resistance [1]. At the present time, no phages are available on the market, and some companies recently performed clinical trials in the setting of burn patients or in patients with bacteremia [8,9]. Few data have been published about phage therapy in patients with BJI. Some patients were treated using phages under development by private companies, and others were treated by academic structures, due to the lack of availability or lack of activity of phages in development [10–12]. Despite not being able to definitely determine the relative contribution of phages and antibiotics in the improvement of the patient in the present case study, we believe that personalized phage therapy is a potential adjuvant treatment of complex BJI, in particular due to pandrug-resistant *P. aeruginosa*. We are also convinced that it could well be extended to other phage-susceptible pathogenic bacterial species to prevent recurrence of chronic infection, and has to be evaluated in dedicated clinical trials. Besides, this study highlighted also again
the safety of purified phages, administered several times locally and intravenously. Regarding a highly personalized approach for phage therapy and as exemplified in this study, European academic collaboration, in addition to industrial phages under development, is crucial to develop the field.

**Methods**

The phage library of a fundamental microbiology laboratory located in Switzerland was screened to find active phages on the patient’s strain. Among >100 different phages active against *P. aeruginosa*, five were able to efficiently lyse the patient’s strain. The genome of three of them (named vB_Pae_4029, vB_Pae_4032 and vB_Pae_4034) were fully sequenced and annotated, which allowed checking for their truly lytic nature (absence of integrase and recombinase genes) and the absence of undesired genes, such as virulence determinants or toxins (data not shown). The phages looked as podovirus on the Electron Microscopy (EM) micrographs and belonged to the family *Schitoviridae*, genus *Litunavirus* based on the genome sequences (Figure 1, panel C-E). The phagogram and the Efficiency of Plating (EOP) tests performed as previously described [5] on the patient strain for the three unpurified phages revealed lytic activity (Figure 1, panel F-H and supplementary table 1). Production of the APIs, in compliance with a Belgian monograph describing the production process and QC system for incorporation in magistral preparations was done in the laboratory of a military hospital, under the supervision of the French national health care authority (*Agence Nationale de Sécurité du Médicament et des produits de santé*, ANSM), in collaboration with French hospital pharmacists [4, 5]. Of note, this monograph received on 10 January 2018 a formal positive advice from the Belgian Minister of Public Health asked the Federal Agency for Medicines and Health Products (FAMHP). It was conceived by representatives of the Queen Astrid Military Hospital located in Brussels, the FAMHP and Sciensano, formerly known as the Belgian Scientific Institute of Public Health. Here the three *P. aeruginosa* bacteriophages were produced using bacterial *P. aeruginosa* host strains PAO1 and ATCC® 15442™, according to the monograph for bacteriophage APIs. Bacteriophages were propagated using the double-agar overlay method in in animal-free growth medium (APS-LB broth, BD). The subsequent purification and concentration process consisted of two centrifugation steps at 6,000 and 40,000 x g for 20 and 90 min, respectively, separated by filtration through 0.45 and 0.22 µm polyethersulfone (PES) filters. The resulting bacteriophage pellet was resuspended in Dulbecco’s Phosphate Buffered Saline (DPBS) buffer to obtain high titer (10^{12} pfu/ml) bacteriophage stocks. Next, bacteriophage stocks were transferred to the clean room facility of the military hospital and conditioned as APIs by diluting to a final concentration of 10^9 pfu/ml, followed by filtration through 0.22 µm PES filters, endotoxin purification using Endotrap columns (Lionex, Germany) and final filtration through 0.22 µm medical filters. Samples of each of the three bacteriophage APIs were sent to Sciensano for QC testing, including determination of pH, endotoxin level (EU/ml), and microbial burden. The pH values for the bacteriophage APIs ranged from 7.29 to 7.35, the endotoxin content endotoxin concentration ranged from 134 to 3400 UI/mL and no bacterial growth was observed in any of the samples. Based on the above results the three bacteriophage APIs were approved by Sciensano and deemed safe for application in humans, including intravenous use [4, 5]. In addition, we obtained from the ANSM a Temporary Authorization for Use (ATU) for cefiderocol, a new cephalosporin.
evaluated in clinical trials [6], that showed activity against the patient’s *P. aeruginosa* strain (MIC = 1 mg/L, broth microdilution).

**Declarations**

**Potential conflict of interest**

All authors report no conflict of interest.

**References**


Figures
Panel A: Gadolinium T1-weighed MRI of the spine showing L2-L3 discal abscess and L3-L4 spondylodiscitis; Panel B: CT-scan showing mirror-like bone destruction close to the L2-L3 abscess; Panel C: EM micrographs of bacteriophage vB_PaeP_4029; Panel D: EM micrographs of bacteriophage vB_PaeP_4032; Panel E: EM micrographs of bacteriophage vB_PaeP_4034; Panel F: Phagogram (spot test) of bacteriophages vB_PaeP_4034 and vB_PaeP_4029 on production strain PA01; Panel G: Phagogram (spot test) of bacteriophage vB_PaeP_4032 on production strain ATCC-15442-MINI; Panel H: Phagogram (spot test) of bacteriophages vB_PaeP_4034, vB_PaeP_4032, and vB_PaeP_4029 on the patient strain; Panel I: One of the two *P. aeruginosa* morphotypes cultured from a bone sample taken from the second-stage surgery procedure, that was close to the initial strain; Panel J: One of the two *P. aeruginosa* corresponds to a stable Small Colony Variant (SCV) morphotype; Panel K: Visualization of Plaque Forming Units (PFU) of the phage vB_PaeP_4029 on the patient’s strains isolated before phage therapy or during the second-stage surgery procedure; Panel L: Visualization of Plaque Forming Units (PFU) of the phage vB_PaeP_4032 on the patient’s strains isolated before phage therapy or during the second-stage surgery procedure; Panel M: Visualization of Plaque Forming Units (PFU) of the phage vB_PaeP_4034 on the patient’s strains isolated before phage therapy or during the second-stage surgery procedure; Panel N: X-ray performed at the end of the follow-up showing no loosening of the spinal osteosynthesis and the adequate position of the intersomatic cages at L2-L3 and L3-L4 level with anterior bone fusion; Panel O: Local aspect of the lombar scar at the end of the follow-up, showing no inflammation nor discharge.

**Supplementary Files**

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