Brief Report

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Bacteriophage therapy for empyema caused by carbapenem-resistant *Pseudomonas aeruginosa*

Peifen Chen[1,§], Ziqiang Liu[2,§], Xin Tan[2,§], Haijiang Wang[1], Yiyang Liang[1], Yingjun Kong[1], Wenxue Sun[1], Liqin Sun[1], Yingfei Ma[2,*], Hongzhou Lu[1,*]

[1] National Clinical Research Centre for Infectious Diseases, The Third People's Hospital of Shenzhen and The Second Affiliated Hospital of Southern University of Science and Technology, Shenzhen, China;
[2] Shenzhen Key Laboratory of Synthetic Genomics, Guangdong Provincial Key Laboratory of Synthetic Genomics, CAS Key Laboratory of Quantitative Engineering Biology, Shenzhen Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China.

SUMMARY *Pseudomonas aeruginosa* is a frequent causative agent of post-pneumonectomy empyema-associated broncho-pleural fistula (BPF) and it has a high mortality rate. In recent years, the therapeutic potential of bacteriophage therapy has recognized anew as antimicrobial resistance increases globally. Studies are increasingly reporting the efficacy and safety of bacteriophage therapy for the treatment of multidrug-resistant bacterial infections. However, the clinical efficacy of bacteriophage therapy in empyema has seldom been studied. The current study reports the authors' experience with bacteriophage therapy for a 68-year-old Chinese man who suffered BPF-associated empyema and pneumonia caused by carbapenem-resistant *P. aeruginosa*. A personalized lytic pathogen-specific two-phage preparation was administered to the patient continuously for 24 days in combination with conventional antibiotics. The treatment was well-tolerated, resulting in clearance of the pathogen and improvement of the clinical outcome. This experience shows that a combined conventional antibiotic treatment with bacteriophage therapy may be effective at alleviating a multidrug-resistant bacterial infection in BPF-associated empyema.

Keywords bacteriophage therapy, empyema, carbapenem-resistant *Pseudomonas aeruginosa*, pneumonia, broncho-pleural fistula

1. Introduction

Post-pneumonectomy empyema is the most severe complication of pneumonectomy (*1*). It is often associated with broncho-pleural fistula (BPF) (*2*). Stern *et al*. reported that the mortality rate of early (within 2 weeks of surgery) BPF-associated empyema was 19% and that the 1-year survival rate of early BPF-associated empyema was 47% (*1*). The organism most commonly isolated from specimens is *Pseudomonas aeruginosa* (*3*). Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) is one of the critical priority pathogens on the WHO's list. Evaluating the efficacy of new antibiotics is difficult and expensive, and especially when targeting multidrug-resistant Gram-negative bacteria (*4*). Thus, new methods for the treatment of CRPA need to be explored.

In recent years, bacteriophage therapy has been used on hard-to-treat bacterial infections, and there are several reported examples of the successful treatment of infections caused by CRPA (*5,6*). However, the clinical efficacy of bacteriophage therapy in empyema has seldom been studied. Reported here is the current authors' experience with bacteriophage therapy in a case of BPF-associated empyema and pneumonia caused by CRPA.

2. Methods

2.1. Bacteriophage preparation

Two lytic phages, PA3 and PA18, were chosen for bacteriophage therapy. The plaques of PA3 and PA18 on a lawn of *P. aeruginosa* are shown in Figure 1. The phage preparation was purified in a cesium chloride density gradient and then dialyzed using a Spectra/Por 6 membrane (MWCO 25 kDa, Sanoon Biotech, Shanghai, China) in SM Buffer (without Tris-HCl) to remove cesium chloride. Phages were then sterilized through 0.22-μm filters. Phages were titrated and evaluated

for endotoxins with an End-point Chromogenic Endotoxin Test Kit (Bioendo, Xiamen, China). The phage preparation was subsequently stored at 4°C until required.

2.2. Data collection

Clinical laboratory data including the white blood count (WBC), percentage of neutrophils (N%), sedimentation rate (ESR), procalcitonin (PCT) level, C-reactive protein (CRP) level, and liver and renal function were collected. Results of cultures of sputum, pleural effusion (PE), and bronchoalveolar lavage fluid (BALF) were also examined.

2.3. Patient surgical intervention

The patient's lung had been destroyed after tuberculosis and repeated hemoptysis for 2 years. A right upper lobe resection was performed *via* video-assisted thoracoscopic lobectomy, and the pleura was decorticated on December 10, 2021. After surgery, he suffered from empyema with BPF, which was treated with continuous negative pressure suction. A membrane-covered stent was inserted into the trunk bronchial stump *via* a bronchoscope on January 4, 2022. Due to continuous air leakage, the stent and negative pressure suction device were removed and an open-window thoracostomy was performed for the management of empyema on January 18, 2022.

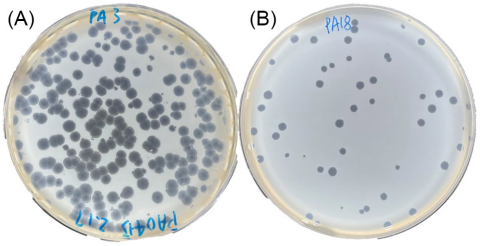

Figure 1. Plaques of PA3 (A) and PA18 (B) on a lawn of P. aeruginosa.

3. Results and Discussion

3.1. Clinical history before phage therapy

A 68-year-old Chinese male was admitted due to an intermittent cough, sputum for 7 years, and hemoptysis for 2 years. He was diagnosed with pulmonary tuberculosis in 2014 and treated with an anti-tuberculosis drug for 1 year. In 2016, he had a second episode of pulmonary tuberculosis and received anti-tuberculosis treatment for 6 months. He had recurrent hemoptysis starting in 2020 and was treated again with the anti-tuberculosis drug for 3 months. After admission, he was diagnosed with a destroyed right lung, bronchiectasis with a *P. aeruginosa* infection, and obsolete pulmonary tuberculosis. After the right upper lobectomy, he developed empyema with BPF and pneumonia. *P. aeruginosa* was isolated from cultures of sputum, BALF, PE, and lung tissues obtained during surgery. He was treated with a variety of antibiotics including amikacin, azithromycin, imipenem, and ceftazidime-avibactam (Figure 2). A PE culture was positive for CRPA on January 12, 2022. After obtaining consent from the Ethics Committee of the Third People's Hospital of Shenzhen (ethics approval no. 2021-068) and the patient's family for experimental treatment, phage therapy was initiated.

3.2. Bacteriophage therapy and clinical outcome

The bacteriophage was nebulized twice daily and injected intrapleurally once daily between January 14 and 25, 2022. Following the intrapleural injection, negative pressure drainage was stopped for 4 hours. Amikacin, ceftazidime-avibactam, and fosfomycin were concomitantly administered intravenously. Since *P. aeruginosa* was still present in PE on January 19 (reported on January 22), the dose of the phage was increased on January 25 (11 days after phage therapy). The phage that was intrapleurally injected daily remained in the right pleural space until the next day. The bacteriophage was nebulized three times on January 25 and 26 and then nebulized twice daily.

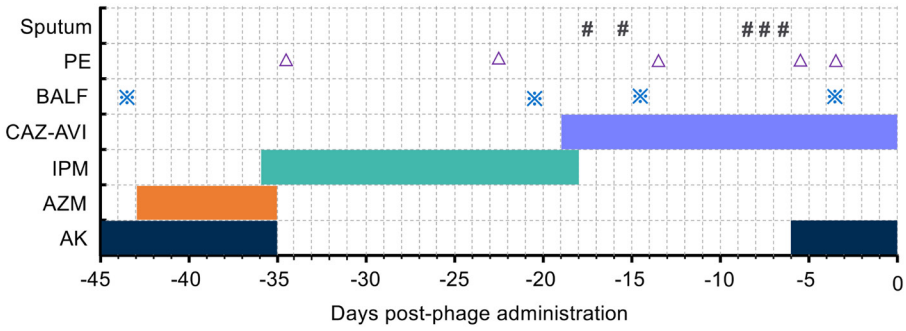

Figure 2. Bacterial culture and antibiotics administered before bacteriophage therapy. CRPA-positive cultures from sputum (#), PE (Δ) and BALF (※). PE, pleural effusion; BALF, bronchoalveolar lavage fluid; CAZ-AVI, ceftazidime-avibactam; IPM, imipenem; AZM, azithromycin; AK, amikacin.

Table 1. Details of the bacteriophage administration

Bacteriophage cocktail	Component bacteriophages	Titer (PFU/mL)	Endotoxin concentration (EU/mL)	Route of administration and frequency
1	PA3	1.25×10^{10}	190	0.4 mL of phage was added to 4.6 mL normal saline nebulized twice or three times daily or intrapleural injected once daily
	PA18	1.25×10^{10}		
2	PA3	3.0×10^{10}	2,200	
	PA18	1.5×10^{11}		

EU, endotoxin units; PFU, plaque forming units.

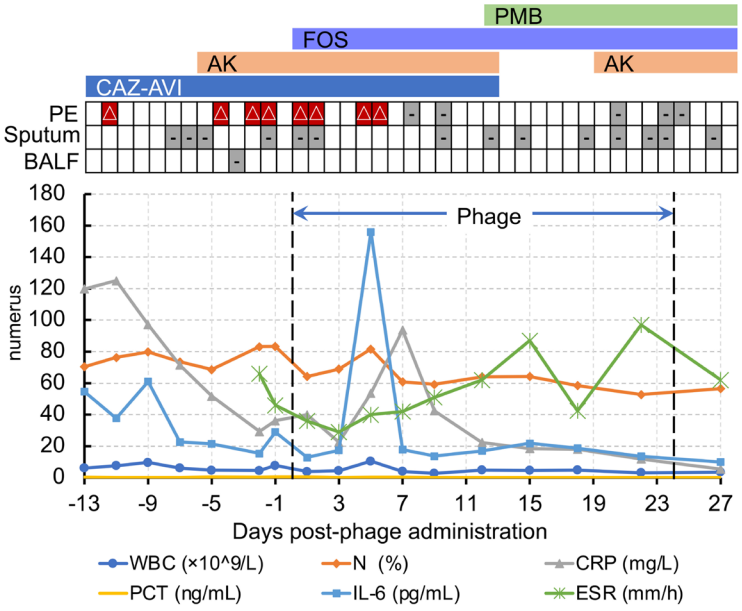

Figure 3. Patient clinical data during phage therapy. (A). Graph of bacterial cultures, inflammatory markers, and the duration of antibiotic and bacteriophage administration. CRPA-positive cultures from PE (Δ), CRPA-negative cultures from PE and sputum (-).

Fosfomycin and polymyxin were also administered then. The details of phage administration are shown in Table 1. All antibiotics were stopped 28 days after phage therapy.

P. aeruginosa was isolated from PE samples on day 0, 1, 4, and 5 after phage therapy. Carbapenem-sensitive *P. aeruginosa* was detected on February 27, 2022, from day 7 of phage therapy to discharge, but cultures of PE did not yield any CRPA (Figure 3 A). Carbapenem-sensitive *P. aeruginosa* was considered to be a colonizer since the patient did not have any symptoms and the volume of PE did not increase.

Inflammatory markers including WBC, N%, PCT, ESR, CRP, and IL-6 tended to gradually decrease during the period of bacteriophage therapy, although a peak in IL-6 was observed on day 5 after therapy and a peak in CRP was observed on day 7. Changes in the levels of IL-6 and CRP were presumably caused by the open-window thoracostomy. In addition, there were no serious adverse reactions to the therapy in terms of the patient's liver and renal function except for a slight increase in liver function that was observed on day 18 after phage therapy, when polymyxin B was administered. Liver function quickly returned to normal after polymyxin was stopped (Figures 3 A and 3 B). Moreover, the volume of PE decreased and the consolidations evident on pre-treatment chest X-rays and CT scans gradually improved (Figures 3 C and 3 D). The patient did not have a cough, sputum, or shortness of breath when he was discharged from the hospital on March 4, 2022.

Empyema has been described as a possible sanctuary for drug-resistant bacteria since antibiotics have been proven to reach the site of infection at subtherapeutic concentrations, thus increasing the risk of treatment failure (*7*). The delivery of phages to the desired site remains a major challenge for bacteriophage therapy. In the current case, the phage preparation was administered locally *via* an intrapleural injection to eradicate empyema. Due to safety concerns, negative pressure drainage was stopped only for 4 hours initially following the intrapleural injection. However, PE cultures were still positive for CRPA after 5 days of treatment (Figure 3A). The phages did not seem to have reached the site of injection. After open-window thoracostomy, the phages that were injected intrapleurally remained in the right pleural space for a longer period. No CRPA was detected in PE cultures from then on, and the patient was discharged with no signs of infection.

Above all, bacteriophage therapy was well-tolerated

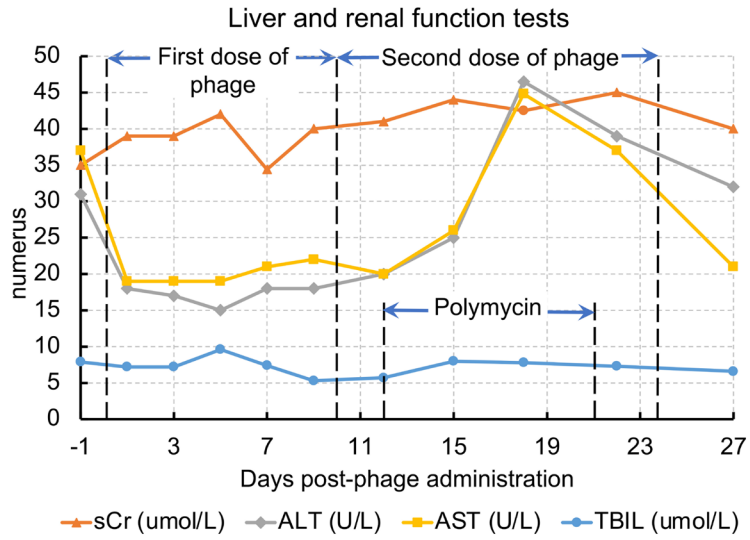

Figure 3. Patient clinical data during phage therapy. (B). Graph of liver and renal function test results over time during phage therapy.

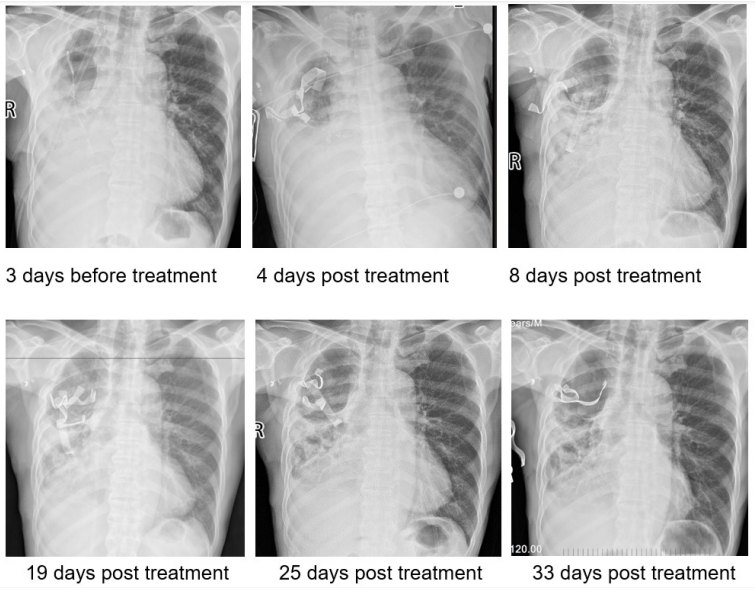

Figure 3. Patient clinical data during phage therapy. (C). The patient's chest X-rays 3 days before phage therapy and on day 4, 8, 19, 25, and 33 after phage therapy.

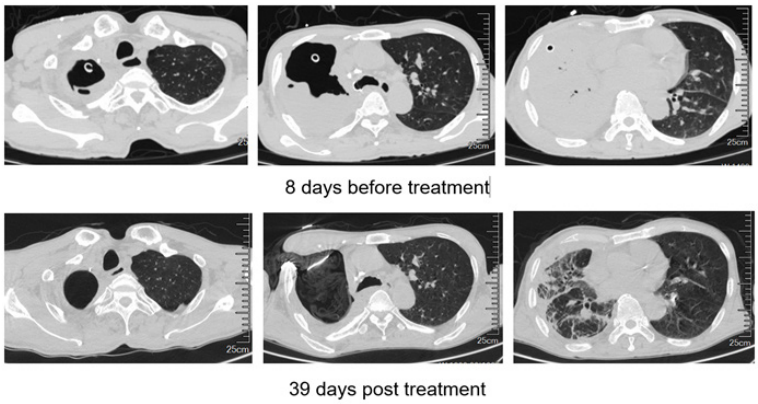

Figure 3. Patient clinical data during phage therapy. (D). The patient's chest CT scans 8 days before phage therapy and on day 39 after phage therapy.

in the current patient, with no obvious phage-associated adverse events. Adverse reactions associated with the host's defense mechanisms against a *P. aeruginosa* phage require further evaluation (*8*). The current experience shows that conventional antibiotic treatment in combination with bacteriophage therapy may be effective at alleviating a multi-drug resistant bacterial infection. However, measures such as local drug delivery systems, surgical interventions, and repeated courses of a phage are vital to clinical success in cases of surgical site infections. In a critical era of increasing antimicrobial resistance, bacteriophage therapy warrants further evaluation in well-designed clinical trials for larger-scale use.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

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[§]These authors contributed equally to this work.
**Address correspondence to:*
Yingfei Ma, Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, 1068 Xueyuan Boulevard, Shenzhen, China.
E-mail: yingfei.ma@siat.ac.cn

Hongzhou Lu, The Third People's Hospital of Shenzhen and The Second Affiliated Hospital of Southern University of Science and Technology, Shenzhen 518112, China.
E-mail: luhongzhou@fudan.edu.cn

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