Supplemental Digital Content

Progress and pitfalls of bacteriophage therapy in critical care: a concise definitive review

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Table S1. Studies assessing the efficacy of phage therapy for the treatment of infections relevant to critical care.

Bacteria	AMR[a]	Host	Setting[b]	Treatment mode[b]	Adjunct antibiotic[c]	Clinical improve	Survival or Primary endpoint[d]	Bacterial loads	Phage resistance	Ref.
Respiratory infections										
P. aeruginosa		Mouse		Monophage IN single dose	No		↑	↓		(1)
		Mouse		Monophage IN single prophylactic dose	No		↑	↓		(1)
	MDR	Human, case study	VAP	Cocktail [4], Nebulized and IV, twice daily	GEN +CIP, CFZ-TAZ	Yes	Yes	↓		(2)
	MDR	Human, case study	Lower respiratory tract infection	Cocktail [4/5], IV (6hr) + nebulized (12hr)	PIP-TAZ, COL	Yes	Yes	↓	Yes	(3)
	MDR	Human, case study	Lower respiratory tract infection	Cocktail [4] IV (12hr)	COL	Modest	Yes	↓		(3)
K. pneumoniae		Mouse		Monophage, IP single dose	No			-		(4)
		Mouse		Monophage, IP single prophylactic dose	No			↓		(4)
	MDR	Human, case study	Lung infection	Cocktail [2], Inhaled + nasogastric tube	CZA, LIN, AVI, COL,		Yes	↓		(5)

					MER, TOB, SXT					
A. baumannii	CARB	Neutropenic mouse		Monophage, IN single dose	No		↑	↓		(6)
	CARB	Neutropenic mouse		Monophage, IN single dose	No		↑	↓		(7)
E. coli		Mouse		Monophage, IN single dose	No		↑	↓		(8)
	MDR, ESBL	Mouse		Monophage, single dose IP or IN	No		↑	↓		(9)
BCC	MDR	Neutropenic mouse		Monophage, IP single dose	No			-		(10)
	MDR	Neutropenic mouse		Monophage, nebulized single dose	No			↓		(10)
	MDR	Human, case study	Lower respiratory tract infection	Monophage, 1-2 times daily, 6wks	CAZ-AVI, PIP-TAZ	No	No			(3)
S. aureus	MRSA	Rat	Experimental VAP	Cocktail [4], IV five doses	TEC		↑, =TEC	↓, =TEC	No	(11)
	MRSA	Rat	Experimental VAP	Cocktail [4], nebulized single prophylactic dose	No		↑	↓	No	(12)
	MRSA	Neutropenic mouse		Cocktail [3], IN 2hr and 6hr	No			↓, =VAN	No	(13)
	MRSA	Human, Case study	Pneumonia, sepsis	Cocktail [3], IV five doses	VAN, CLI, MEM, AZI	No	No		Yes	(14)
Abdominal infections										
E. faecalis	VRE	Mouse	VRE + SRFE	Cocktail [2], IP single dose	AMP	Yes	↑, >AMP	↓, <AMP	No	(15)

P. aeruginosa		Mouse		Monophage, IP single dose	No	No	↑	↓		(16)
E. coli (ExPEC)	MDR	Neutropenic Mouse	Oral, chemotherapy induced translocation	Monophage, IP single dose	No	Yes		↓		(17)
A. baumannii	MDR	Human, case study	Necrotizing pancreatitis	Cocktails [2/4], IC (6 to12hr intervals), then IV	AZM, MIN, COL, AZI, RIF	Yes	Yes		Yes	(18)
E. coli		Phase I/II human trial	Diarrhea in hospitalized children	T4-like coliphages [11] Or Microgen coliproteus cocktail [~17]		No	Yes (safety)	-	Yes, impact unclear	(19)
Bloodstream infections										
P. aeruginosa	CARB	Mouse		Monophage, IP single dose	No		↑	↓		(20)
		Rat	Experimental endocarditis	Cocktail [12], IV bolus or continuous infusion	CIP			↓, =CIP	No	(21)
	MDR	Human, case study	Aortic graft infection	Monophage, single injection into fistula	CAZ	Yes	Yes	↓		(22)
	MDR	Human, case study	Bacteremia	Cocktail [3] , IV (6hr), Topical (8hr)	No	Yes	Yes	↓		(23)
	MDR	Human, case study	Bacteremia	Cocktail [2], IV every 6 hrs	MER, TOB, PMB	Unclear	No	↓	No	(24)
E. coli		Mouse		Monophage, IP single dose	No		↑	↓	No	(25)
		Mouse		Monophage, IV single dose	No			↓		(26)
	MDR, ESBL	Mouse		Monophage,	No			↓		(9)

				double dose IP or IN						
A. baumannii	MDR	Mouse		Monophage or cocktail [2], IP single dose	No		↑			(27)
S. aureus	MRSA	Mouse		Monophage, IV single dose	No		↑	↓	No	(28)
	No	Mouse	Lung derived bacteremia	Monophage, IP single dose	No		↑	↓	No	(29)
	No	Rabbit	Experimental CVC	Monophage, catheter lock	No			↓		(30)
	MRSA	Human, case series (8)	Sepsis, IE, PVE	Cocktail [3], IV twice daily for 14 days	FLU, CEF, VAN	Yes	4/8	↓	Yes (n=1)	(14)
	MDR	Human, case series (2)	Bacteremia, related to Cardiothoracic surgery	Monophage as local application via drainage, every 12hr (4 doses) or local intraoperative embedded in fibrin glue.	RIF, FLU, DAP, AMP-SUL	Yes	1/2	↓	No	(5)
S. aureus, *E. faecium,* *P. aeruginosa*	MDR	Human, case study	Prosthetic infection after aortic arch replacement	Cocktail [4], local application through drainage, intraoperative local administration and per oral, each as a single dose	CEF, DAP, LIN, TOB, GEN	Yes	No, died due to new infection	↓		(5)
E. faecium	VRE	Mouse		Monophage, IP single dose	No	Yes	↑	↓		(31)
E. faecalis	VRE	Mouse		Monophage, IP single dose	No	Yes	↑	↓	No	(32-34)

Skin infections										
P. aeruginosa		Burned mouse		Monophage, IP, IM, or SC, single dose	No		↑	↓		(35)
K. pneumoniae		Burned mouse		Monophage, topical, single dose	No		↑, >SOC			(36)
P. aeruginosa, *S. aureus*	MDR	Case series (n=9)	Burn wound infection, ~30% TBSA	Cocktail [3]	No			-		(37)
P. aeruginosa		Phase I/II human trial	Burn wound infection, ~20% TBSA, n=12	Cocktail [12], topical, once daily for 7 days	At the discretion of treating physician	<SOC	=SOC	<SOC	Yes	(38)
Urinary tract infections										
E. coli (UPEC)		Mouse		Monophage, IP single dose	No		↑			(39)
E. coli	Yes	Mouse		Monophage, IP single dose	No			↓		(9)
Various		Phase I/II human trial	UTI after TURP n=28	Cocktail [>5], intravesical instillation every 12 hr for 7 days	No		5/28 =placebo	=placebo		(40)

a) AMR, antimicrobial resistance; CARB, carbapenem; ESBL, extended spectrum beta lactamase; MDR, multidrug resistant; MRSA, methicillin-resistant *S. aureus*; VRE, vancomycin-resistant *Enterococcus*;

b) Values in [ ] indicate the number of unique phages in the cocktail. CVC, central venous catheter; IC, intracavitary; IN, intranasal; IP, intraperitoneal; IV, intravenous; SC, subcutaneous; SRFE, sterile rat fecal extract; TBSA, total body surface area; TURP, transurethral resection of prostate; UTI, urinary tract infection; VAP, ventilator-associated pneumonia;

c) antibiotics; AMP, ampicillin; AVI, avibactam; AZI, azithromycin; CEF, cefazolin; CFZ, ceftolozane; CIP, ciprofloxacin; CLI, clindamycin; COL; colistin; CZA, ceftazidime; DAP, daptomycin; FLU, flucloxacillin; GEN, gentamicin; LIN, linezolid; MEM, meropenem; MIN, minocylcine; PIP, piperacillin; PMB, polymyxin B; SXT, trimethoprim-sulphamethoxazole; TAZ, tazobactam; TEC, teicoplanin; TOB, tobramycin; RIF, rifampicin; SUL, sulbactam; VAN, vancomycin.

d) ↑↓ for animal trials represents a comparison with untreated controls. For human case studies, it represents a change in the patient over the course of therapy. ><= represent comparisons with additional treatment groups, i.e. antibiotics.

Other abbreviations: BCC, Burkholderia cepacia complex; ExPEC, extra-intestinal pathogenic *E. coli*; SOC, standard of care; UPEC, uropathogenic *E. coli*.

References

1. Debarbieux L, Leduc D, Maura D, et al. Bacteriophages can treat and prevent *Pseudomonas aeruginosa* lung infections. *J Infect Dis* 2010;201(7):1096-1104.
2. Maddocks S, Fabijan AP, Ho J, et al. Bacteriophage Therapy of Ventilator-associated Pneumonia and Empyema Caused by *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 2019;200(9):1179-1181.
3. Aslam S, Courtwright AM, Koval C, et al. Early clinical experience of bacteriophage therapy in 3 lung transplant recipients. *Am J Transplant* 2019;19(9):2631-2639.
4. Chhibber S, Kaur S, Kumari S. Therapeutic potential of bacteriophage in treating *Klebsiella pneumoniae* B5055-mediated lobar pneumonia in mice. *J Med Microbiol* 2008;57(Pt 12):1508-1513.
5. Rubalskii E, Ruemke S, Salmoukas C, et al. Bacteriophage Therapy for Critical Infections Related to Cardiothoracic Surgery. *Antibiotics (Basel)* 2020;9(5).
6. Hua Y, Luo T, Yang Y, et al. Phage Therapy as a Promising New Treatment for Lung Infection Caused by Carbapenem-Resistant *Acinetobacter baumannii* in Mice. *Front Microbiol* 2017;8:2659.
7. Jeon J, Park JH, Yong D. Efficacy of bacteriophage treatment against carbapenem-resistant *Acinetobacter baumannii* in *Galleria mellonella* larvae and a mouse model of acute pneumonia. *BMC Microbiol* 2019;19(1):70.
8. Dufour N, Debarbieux L, Fromentin M, et al. Treatment of Highly Virulent Extraintestinal Pathogenic *Escherichia coli* Pneumonia With Bacteriophages. *Crit Care Med* 2015;43(6):e190-198.
9. Dufour N, Clermont O, La Combe B, et al. Bacteriophage LM33_P1, a fast-acting weapon against the pandemic ST131-O25b:H4 *Escherichia coli* clonal complex. *J Antimicrob Chemother* 2016;71(11):3072-3080.
10. Semler DD, Goudie AD, Finlay WH, et al. Aerosol phage therapy efficacy in *Burkholderia cepacia* complex respiratory infections. *Antimicrob Agents Chemother* 2014;58(7):4005-4013.
11. Prazak J, Iten M, Cameron DR, et al. Bacteriophages Improve Outcomes in Experimental *Staphylococcus aureus* Ventilator-associated Pneumonia. *Am J Respir Crit Care Med* 2019;200(9):1126-1133.
12. Prazak J, Valente L, Iten M, et al. Nebulized Bacteriophages for Prophylaxis of Experimental Ventilator-Associated Pneumonia Due to Methicillin-Resistant Staphylococcus aureus. *Crit Care Med* 2020;48(7):1042-1046.
13. Lehman SM, Mearns G, Rankin D, et al. Design and Preclinical Development of a Phage Product for the Treatment of Antibiotic-Resistant *Staphylococcus aureus* Infections. *Viruses* 2019;11(1).
14. Petrovic Fabijan A, Lin RCY, Ho J, et al. Safety of bacteriophage therapy in severe *Staphylococcus aureus* infection. *Nat Microbiol* 2020;5(3):465-472.
15. Gelman D, Beyth S, Lerer V, et al. Combined bacteriophages and antibiotics as an efficient therapy against VRE *Enterococcus faecalis* in a mouse model. *Res Microbiol* 2018;169(9):531-539.
16. Watanabe R, Matsumoto T, Sano G, et al. Efficacy of bacteriophage therapy against gut-derived sepsis caused by *Pseudomonas aeruginosa* in mice. *Antimicrob Agents Chemother* 2007;51(2):446-452.

17. Green SI, Kaelber JT, Ma L, et al. Bacteriophages from ExPEC Reservoirs Kill Pandemic Multidrug-Resistant Strains of Clonal Group ST131 in Animal Models of Bacteremia. *Sci Rep* 2017;7:46151.
18. Schooley RT, Biswas B, Gill JJ, et al. Development and Use of Personalized Bacteriophage-Based Therapeutic Cocktails To Treat a Patient with a Disseminated Resistant *Acinetobacter baumannii* Infection. *Antimicrob Agents Chemother* 2017;61(10).
19. Sarker SA, Sultana S, Reuteler G, et al. Oral Phage Therapy of Acute Bacterial Diarrhea With Two Coliphage Preparations: A Randomized Trial in Children From Bangladesh. *EBioMedicine* 2016;4:124-137.
20. Wang J, Hu B, Xu M, et al. Use of bacteriophage in the treatment of experimental animal bacteremia from imipenem-resistant *Pseudomonas aeruginosa*. *Int J Mol Med* 2006;17(2):309-317.
21. Oechslin F, Piccardi P, Mancini S, et al. Synergistic Interaction Between Phage Therapy and Antibiotics Clears *Pseudomonas aeruginosa* Infection in Endocarditis and Reduces Virulence. *J Infect Dis* 2017;215(5):703-712.
22. Chan BK, Turner PE, Kim S, et al. Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*. *Evol Med Public Health* 2018;2018(1):60-66.
23. Jennes S, Merabishvili M, Soentjens P, et al. Use of bacteriophages in the treatment of colistin-only-sensitive Pseudomonas aeruginosa septicaemia in a patient with acute kidney injury-a case report. *Crit Care* 2017;21(1):129.
24. Duplessis C, Biswas B, Hanisch B, et al. Refractory *Pseudomonas* Bacteremia in a 2-Year-Old Sterilized by Bacteriophage Therapy. *J Pediatric Infect Dis Soc* 2018;7(3):253-256.
25. Merril CR, Biswas B, Carlton R, et al. Long-circulating bacteriophage as antibacterial agents. *Proc Natl Acad Sci U S A* 1996;93(8):3188-3192.
26. Schneider G, Szentes N, Horvath M, et al. Kinetics of Targeted Phage Rescue in a Mouse Model of Systemic *Escherichia coli* K1. *Biomed Res Int* 2018;2018:7569645.
27. Leshkasheli L, Kutateladze M, Balarjishvili N, et al. Efficacy of newly isolated and highly potent bacteriophages in a mouse model of extensively drug-resistant *Acinetobacter baumannii* bacteraemia. *J Glob Antimicrob Resist* 2019;19:255-261.
28. Capparelli R, Parlato M, Borriello G, et al. Experimental phage therapy against *Staphylococcus aureus* in mice. *Antimicrob Agents Chemother* 2007;51(8):2765-2773.
29. Takemura-Uchiyama I, Uchiyama J, Osanai M, et al. Experimental phage therapy against lethal lung-derived septicemia caused by *Staphylococcus aureus* in mice. *Microbes Infect* 2014;16(6):512-517.
30. Lungren MP, Donlan RM, Kankotia R, et al. Bacteriophage K antimicrobial-lock technique for treatment of *Staphylococcus aureus* central venous catheter-related infection: a leporine model efficacy analysis. *J Vasc Interv Radiol* 2014;25(10):1627-1632.
31. Biswas B, Adhya S, Washart P, et al. Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. *Infect Immun* 2002;70(1):204-210.
32. Cheng M, Liang J, Zhang Y, et al. The Bacteriophage EF-P29 Efficiently Protects against Lethal Vancomycin-Resistant *Enterococcus faecalis* and Alleviates Gut Microbiota Imbalance in a Murine Bacteremia Model. *Front Microbiol* 2017;8:837.
33. Uchiyama J, Rashel M, Takemura I, et al. In silico and in vivo evaluation of bacteriophage phiEF24C, a candidate for treatment of *Enterococcus faecalis* infections. *Appl Environ Microbiol* 2008;74(13):4149-4163.
34. Zhang W, Mi Z, Yin X, et al. Characterization of *Enterococcus faecalis* phage IME-EF1 and its endolysin. *PLoS One* 2013;8(11):e80435.

35. McVay CS, Velasquez M, Fralick JA. Phage therapy of *Pseudomonas aeruginosa* infection in a mouse burn wound model. *Antimicrob Agents Chemother* 2007;51(6):1934-1938.
36. Kumari S, Harjai K, Chhibber S. Bacteriophage versus antimicrobial agents for the treatment of murine burn wound infection caused by *Klebsiella pneumoniae* B5055. *J Med Microbiol* 2011;60(Pt 2):205-210.
37. Rose T, Verbeken G, Vos DD, et al. Experimental phage therapy of burn wound infection: difficult first steps. *Int J Burns Trauma* 2014;4(2):66-73.
38. Jault P, Leclerc T, Jennes S, et al. Efficacy and tolerability of a cocktail of bacteriophages to treat burn wounds infected by *Pseudomonas aeruginosa* (PhagoBurn): a randomised, controlled, double-blind phase 1/2 trial. *Lancet Infect Dis* 2018;19(1):35-45.
39. Nishikawa H, Yasuda M, Uchiyama J, et al. T-even-related bacteriophages as candidates for treatment of *Escherichia coli* urinary tract infections. *Arch Virol* 2008;153(3):507-515.
40. Leitner L, Ujmajuridze A, Chanishvili N, et al. Intravesical bacteriophages for treating urinary tract infections: A randomised, placebo-controlled, double-blind clinical trial. *Lancet Infect Dis* 2020;in press