Efficacy of phage therapy in preclinical models of bacterial infection: a systematic review and meta-analysis

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Summary

Background Antimicrobial resistance of bacterial pathogens is an increasing clinical problem and alternative approaches to antibiotic chemotherapy are needed. One of these approaches is the use of lytic bacterial viruses known as phage therapy. We aimed to assess the efficacy of phage therapy in preclinical animal models of bacterial infection.

Methods In this systematic review and meta-analysis, MEDLINE/Ovid, Embase/Ovid, CINAHL/EbscoHOST, Web of Science/Wiley, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and Google Scholar were searched from inception to Sept 30, 2021. Studies assessing phage efficacy in animal models were included. Only studies that assessed the efficacy of phage therapy in treating established bacterial infections in terms of survival and bacterial abundance or density were included. Studies reporting only in-vitro or ex-vivo results and those with incomplete information were excluded. Risk-of-bias assessment was performed using the Systematic Review Centre for Laboratory Animal Experimentation tool. The main endpoints were animal survival and tissue bacterial burden, which were reported using pooled odds ratios (ORs) and mean differences with random-effects models. The I^2 measure and its 95% CI were also calculated. This study is registered with PROSPERO, CRD42022311309.

Findings Of the 5084 references screened, 124 studies fulfilled the selection criteria. Risk of bias was high for 70 (56%) of the 124 included studies; therefore, only studies classified as having a low-to-moderate risk of bias were considered for quantitative data synthesis (n=32). Phage therapy was associated with significantly improved survival at 24 h in systemic infection models (OR 0·08 [95% CI 0·03 to 0·20]; I^2=55% [95% CI 8 to 77]), skin infection (OR 0·08 [0·04 to 0·19]; I^2=0% [0 to 79]), and pneumonia models (OR 0·13 [0·06 to 0·31]; I^2=0% [0 to 68]) when compared with placebo. Animals with skin infections (mean difference –2·66 [95% CI –3·17 to –2·16]; I^2=95% [90 to 96]) and those with pneumonia (mean difference –3·35 [–6·00 to –0·69]; I^2=99% [98 to 99]) treated with phage therapy had significantly lower tissue bacterial loads at 5±2 days of follow-up compared with placebo.

Interpretation Phage therapy significantly improved animal survival and reduced organ bacterial loads compared with placebo in preclinical animal models. However, high heterogeneity was observed in some comparisons. More evidence is needed to identify the factors influencing phage therapy performance to improve future clinical application.

Funding Swiss National Foundation and Swiss Heart Foundation.

Lancet Microbe 2022; 3: e956–68

Published Online November 9, 2022 https://doi.org/10.1016/S2666-5247(22)00288-9

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Introduction

Bacterial viruses (bacteriophages or, simply, phages) have been considered for the treatment of bacterial infections for over 100 years, yet global use is rare, with most countries typically using antibiotic chemotherapy. Early clinical studies of phage safety and efficacy produced inconsistent results; they often did not include adequate controls and used crude bacterial lysates that proved unsafe.[1–3] The so-called golden age of antibiotic discovery, which started in the early 1930s when multiple classes of antibiotics were developed, largely contributed to the abandonment of phage therapy research in most countries outside of eastern Europe and the former Soviet Union.[3]

Phage therapy has re-emerged as a possible solution to the antimicrobial resistance crisis. Global estimates from 2019 have attributed close to 1·3 million yearly deaths to antimicrobial resistance,[4] with these estimates projected to increase without the development and clinical implementation of alternative treatment strategies.[5] In this context, phage therapy has obvious appeal. First, phages cause bacteria to lyse as a natural part of the viral lifecycle and this mechanism of killing is distinct from all classes of antibiotics. Second, purified phages have been shown to be safe when tested in humans.[6] Finally, phages are highly specific, often targeting bacteria at a subspecies level, which suggests that they are unlikely to have off-target effects on the human microbiota.[7]

Research in context

Evidence before this study

Phage therapy has emerged as a possible answer to the antibiotic resistance crisis. Phase 1/2 randomised controlled trials have suggested that phage therapy is safe for use in humans; however, they have yet to prove its efficacy. These findings contrast with the growing number of reports that have pointed toward phage efficacy in single case studies and small case series. These data, however, are difficult to interpret as they do not include appropriate comparator cases or groups, and they are often confounded by the additional use of standard-of-care antibiotic treatments. Preclinical animal models are a cornerstone of anti-infective drug development. The efficacy of phage therapy has been assessed in various animal models emulating a range of infectious diseases due to various bacterial pathogens. The purpose of this study is to collectively assess the efficacy of phage therapy in these model systems.

Added value of this study

Our systematic review and meta-analysis has a unique focus on assessing phage therapy efficacy in preclinical animal models using two complementary endpoints: animal survival and tissue bacterial burden. The data collectively indicate that phage therapy is efficacious; however, most studies had a high risk of bias. Using studies with a low-to-moderate risk of bias, several quantitative subgroup meta-analyses could be performed for placebo-controlled studies.

Implications of all the available evidence

A possible disconnect between research and clinical practice was identified whereby phage therapy use in preclinical studies did not align with use in humans (ie, phage composition, dosing, and time of administration). Future preclinical trials should be designed with the purpose of rationally informing the next clinical trials.

Widespread clinical use of phage therapy at present, however, remains low because randomised controlled clinical trials (RCTs) have failed to demonstrate its efficacy.[8–10] As such, the revival of phage therapy for the treatment of bacterial infections in humans has been limited to situations in which all other therapeutic options have been exhausted.[8] The efficacy and safety of phage therapy for challenging infections was systematically reviewed in 2022; however, as reported by Uyttebroek and colleagues,[6] the quality of the evidence was low-to-moderate due to the abundance of case studies. Although the review revealed the efficacy of phage therapy in humans, most reports did not include comparative controls and involved patients that were receiving concurrent antibiotic therapy, making it difficult to ascertain the direct effects of phage therapy.[9] Thus, to complement the recent systematic reviews assessing efficacy in humans,[6,10] we have performed the first systematic review and meta-analysis of phage therapy efficacy using data from placebo or untreated-controlled, preclinical (rodent) models of bacterial infection.

Methods

Search strategy and selection criteria

This systematic review and meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (appendix pp 5–7).[11]

See Online for appendix

We searched MEDLINE/Ovid, Embase/Ovid, CINAHL/EbscoHOST, Web of Science/Wiley, Cochrane Central Register of Controlled Trials, Cochrane Database of Systematic Reviews, and Google Scholar to identify relevant articles from database inception to Sept 30, 2021, without language restrictions. We used search terms related to phage therapy such as "bacteriophage", "phage", "phage therapy", and "animal model". The complete search strategy is described in the appendix (pp 2–3).

All experimental studies using animal models were included. We included studies that assessed the efficacy of phage therapy in treating established bacterial infections in terms of survival and bacterial abundance or density. We excluded articles reporting only in-vitro or ex-vivo results and those with incomplete information (eg, about the phages or bacteria used, or inoculation route). Ten reviewers (SAG-O, MP, LGV, CDSV, JL, ACQ-C, JAHV, SJT-C, DRC, and Y-AQ) screened the titles and abstracts according to the selection criteria. Discrepancies between reviewer screening decisions were resolved by consensus or, if not possible, evaluated by a third reviewer (SAG-O).

Data analysis

Four reviewers (SAG-O, MP, LGV, and DRC) independently extracted the following data: first author's name, study location, publication year, study design, animal model evaluated, type of infection, pathogen(s) assessed, number of animals evaluated, phage cocktail characteristics, inoculation and infection characteristics, antimicrobial treatment characteristics, survival rate per group, and change in bacterial abundance or density per group.

Four authors (SAG-O, MP, LGV, and DRC) assessed each study independently using the Systematic Review Centre for Laboratory Animal Experimentation Risk of Bias tool,[12] which is based on the Cochrane risk-of-bias tool and specifically adapted for animal studies. The scale assessed quality in six categories: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases. Risk of bias was evaluated on a 10-point scale and classified as low (8–10 points), moderate (4–7), or high (<4).

Studies with low-to-moderate risk of bias were considered for meta-analyses. To synthesise useful quantitative information, we applied further selection criteria, which stipulated (1) the use of mouse or rat models and (2) pathogen-setting-model combinations that were assessed at least twice. Group definitions are provided in the appendix (p 4). Pooled mean differences were used for analysing continuous variables. Mean differences and 95% CIs were used to assess differences in bacterial abundance, which were measured in decimal-log-converted colony forming units in the tissue of interest (eg, lung concentrations in pneumonia models). Odds ratios (OR) with 95% CI were used for assessing mortality risk. To minimise the heterogeneity of the results, pooling of the data was performed by type of infection model. Furthermore, considering the variability in survival data reporting, we performed multiple meta-analyses per infection model according to different follow-up timepoints that were common across the included studies (24 h, 48 h, 7 days, and 10 days). The inverse variance weighted method was used to combine summary measures using random-effects models to minimise the effect of between-study heterogeneity. Heterogeneity was evaluated using the I^2 index (low I^2<25%, moderate I^2 25–75%, or high I^2>75%) and its respective 95% CI using the *heterogi* command in STATA (version 16.1 [which was used for all statistical analyses]).[13] The method used for estimating heterogeneity variance was the restricted maximum likelihood method.[14] We assessed potential additional sources of heterogeneity using univariable random-effects meta-regression analyses by time from bacterial infection to phage inoculation, multiplicity of infection, and phage administration routes. Publication bias was appraised using funnel plots and Egger's test for assessing asymmetry. For studies reporting only median and measures of dispersion (interquartile range, range, and maximum-minimum values), we converted these values into mean and standard deviation.[15] All tests were 2-tailed; p<0·05 was considered statistically significant.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Of the 5084 references screened, 124 studies fulfilled the selection criteria and were included in the systematic review (figure 1), references for each of the included studies can be found in the appendix (pp 8–18). The included studies were published between 1963 and 2021; more than half (64 [52%] of 124) within the past 5 years (appendix p 23). Most of the experiments were performed in Europe (n=36), the USA (n=18), and China (n=16; appendix p 24).

Most studies (85 [69%] of 124 studies) used rodent models (mice or rats) for their experiments, followed by chicken models (11 studies [9%]) and *Galleria mellonella* (6 studies [5%]).

The models most commonly emulated systemic infection (47 [38%] of 124 studies), respiratory infection (28 studies [23%]), skin or burn infection (23 studies [19%]), and gastrointestinal infection (19 studies [15%]).

The most common target pathogens were *Pseudomonas aeruginosa* (31 [25%] of 124 studies), *Staphylococcus aureus* (22 studies [18%]), and *Escherichia coli* (15 studies [12%]).

The main routes of phage administration were the parenteral route (62 [50%] of 124 studies), with the intraperitoneal route being the most frequent (35 studies [28%]). Other reported administration

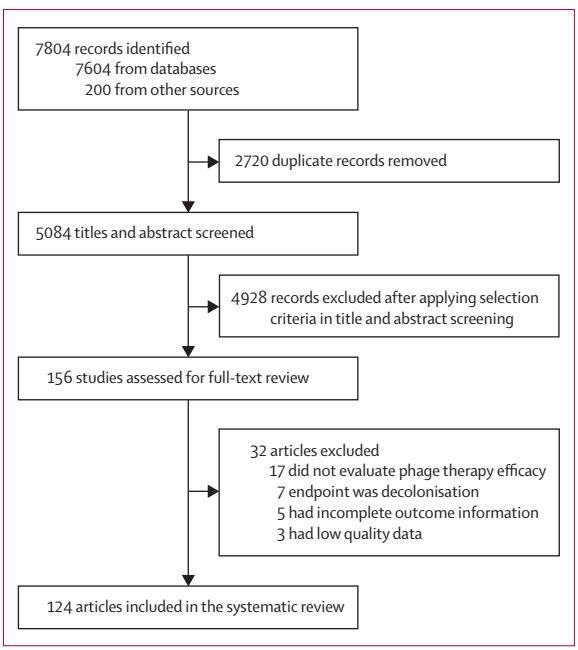


Figure 1: **Systematic review of studies assessing phage therapy in animal models**
PRISMA flow chart summarising the study selection process.

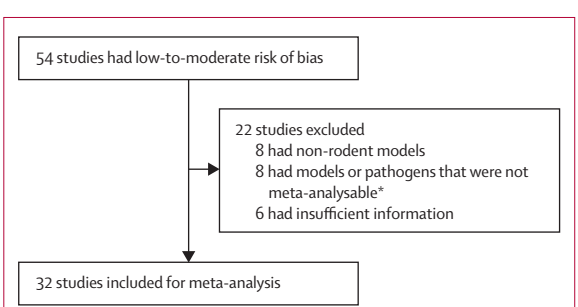


Figure 2: **Risk-of-bias analysis and inclusion criteria for meta-analyses**
*Defined as models or pathogens with fewer than two available studies evaluating them.

	Country	Animal	Infection setting	Pathogen	Number of phages; phage name(s)	Phage administration route	Outcomes assessed	Overall results	Included in meta-analysis?	Reason for exclusion
Albac et al (2020)[16]	France	Mice	Skin or burn	*Staphylococcus aureus*	3; 1493, 1815, and 1957	Subcutaneous	Bacterial load	Phages reduce bacterial load	Yes	NA
Alemayehu et al (2012)[17]	Ireland	Mice	Respiratory	*Pseudomonas aeruginosa*	2; PHIMR299-2 and PHINH-4	Respiratory*	Bacterial load	Phages reduce bacterial load	No	Insufficient information
Cha et al (2018)[18]	South Korea	Mice	Respiratory	*Acinetobacter baumannii*	5; PBAB08, PBAB25, PBAB68, PBAB80, and PBAB93	Respiratory	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Chadha et al (2016)[19]	India	Mice	Skin or burn	*Klebsiella pneumoniae*	5; Kpn1, Kpn2, Kpn3, Kpn4, and Kpn5	Topical or superficial	Bacterial load	Phages reduce bacterial load	No	Model or pathogen <2 studies
Chadha et al (2017)[20]	India	Mice	Skin or burn	*Klebsiella pneumoniae*	5; KØ1, KØ, KØ3, KØ4, and KØ5	Intraperitoneal	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Chang et al (2018)[21]	Australia	Mice	Respiratory	*Pseudomonas aeruginosa*	1; PEV20	Respiratory	Bacterial load	Phages reduce bacterial load	Yes	NA
Chen et al (2021)[22]	China	Mice	Respiratory	*Pseudomonas aeruginosa*	2; MYY9 and HX1	Respiratory	Bacterial load	Phages reduce bacterial load	Yes	NA
Chen et al (2019)[23]	China	Shrimp	Systemic	*Vibrio vulnificus*	5; VspDsh-1, VpaJT-1, ValLY-3, ValSw4–1, and VspSw-1	Oral or enteral	Mortality	Phages reduce mortality risk	No	Non-rodent model
Chen et al (2019)[24]	China	Mice	Systemic	*Pasteurella multocida*	1; PHB01	Intraperitoneal	Mortality	Phages reduce mortality risk	Yes	NA
Cheng et al (2017)[25]	China	Mice	Systemic	*Enterococcus faecalis*	1; EF-P29	Intravenous	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Chhibber et al (2013)[26]	India	Mice	Skin or burn	*Staphylococcus aureus*	1; MR-10	Oral or enteral	Bacterial load	Phages reduce bacterial load	Yes	NA
Chhibber et al (2018)[27]	India	Mice	Skin or burn	*Staphylococcus aureus*	2; MR-5 and MR-10	Topical or superficial	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Chhibber et al (2017)[28]	India	Mice	Skin or burn	*Staphylococcus aureus*	2; MR-5 and MR-10	Intramuscular	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Chung et al (2012)[29]	South Korea	Mice	Systemic	*Pseudomonas aeruginosa*	2; MP22 and D3112	Intraperitoneal	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Dallal et al (2019)[30]	Iran	Mice	Intestinal	*Salmonella enterica*	1; SE20	Oral or enteral	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	No	Model or pathogen <2 studies
Danelishvili et al (2006)[31]	USA	Mice	Systemic	*Mycobacterium avium*	1; TM4	Intravenous	Bacterial load	Phages reduce bacterial load	No	Model or pathogen <2 studies
Danis-Wlodarczyk et al (2016)[32]	Poland	*Galleria mellonella*	Systemic	*Pseudomonas aeruginosa*	1; KTN4	Haemolymph	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	No	Non-rodent model
Debarbieux et al (2010)[33]	France	Mice	Respiratory	*Pseudomonas aeruginosa*	1; PAK-P1	Respiratory	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Dhungana et al (2021)[34]	Nepal	Mice	Systemic	*Klebsiella pneumoniae*	1; Kp_Pokalde_002	Intraperitoneal	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Dien et al (2022)[35]	Thailand	Nile tilapia	Intestinal	*Aeromonas hydrophila*	1; pAh6.2TG	Oral or enteral	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	No	Non-rodent model
Forti et al (2018)[36]	Italy	Mice	Respiratory	*Pseudomonas aeruginosa*	6; PYO2, DEV, E215, E217, PAK_P1, and PAK_P4	Respiratory	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Gill et al (2006)[37]	Canada	Cows	Skin or burn	*Staphylococcus aureus*	1; K	Intramammary	Bacterial load	No effect observed	No	Non-rodent model

(Table continues on next page)

	Country	Animal	Infection setting	Pathogen	Number of phages; phage name(s)	Phage administration route	Outcomes assessed	Overall results	Included in meta-analysis?	Reason for exclusion
(Continued from previous page)										
Green et al (2017)[38]	USA	Mice	Systemic	*Escherichia coli*	1; HP3	Intraperitoneal	Bacterial load	Phages reduce bacterial load	No	Model or pathogen <2 studies
Grygorcewicz et al (2020)[39]	Poland	*Galleria mellonella*	Systemic	*Acinetobacter baumannii*	1; vB_AbaP_AGC01	Haemolymph	Mortality	Phages reduce mortality risk	No	Non-rodent model
Henry et al (2013)[40]	France	Mice	Respiratory	*Pseudomonas aeruginosa*	8; PAK_P1, PAK_P2, PAK_P3, PAK_P4, PAK_P5, LBL3, PhiKZ, and LUZ19.	Respiratory	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Heo et al (2009)[41]	South Korea	Mice	Systemic	*Pseudomonas aeruginosa*	2; MPK1 and MPK6	Intraperitoneal	Bacterial load	Phages reduce bacterial load	No	Insufficient information
Hesse et al (2021)[42]	USA	Mice	Systemic	*Klebsiella pneumoniae*	2; Pharr and PHIKpNIH-2	Intraperitoneal	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Holguin et al (2015)[43]	Colombia	Mice	Skin or burn	*Pseudomonas aeruginosa*	1; PHI-Pan70	Topical or superficial	Mortality	Phages reduce mortality risk	Yes	NA
Horváth et al (2020)[44]	Hungary	Mice	Systemic	*Klebsiella pneumoniae*	1; vB_KpnS_Kp13	Intraperitoneal	Mortality	Phages reduce mortality risk	Yes	NA
Hsieh et al (2017)[45]	Taiwan	Mice	Systemic	*Klebsiella pneumoniae*	1; K5-4	Intraperitoneal	Mortality	Phages reduce mortality risk	Yes	NA
Hua Y et al (2018)[46]	China	Mice	Respiratory	*Acinetobacter baumannii*	1; SH-Ab15519	Respiratory	Mortality	Phages reduce mortality risk	Yes	NA
Huff et al (2004)[47]	USA	Chickens	Respiratory	*Escherichia coli*	2; SPR02 and DAF6	Intramuscular	Mortality	Phages reduce mortality risk	No	Non-rodent model
Hung et al (2011)[48]	Taiwan	Mice	Intestinal	*Klebsiella pneumoniae*	1; PHI-NK5	Oral or enteral	Mortality	Phages reduce mortality risk	No	Model or pathogen <2 studies
Huon et al (2020)[49]	France	Mice	Skin or burn	*Staphylococcus aureus*	2; PN1815 and PN1957	Topical or superficial	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Iwano et al (2018)[50]	Japan	Mice	Skin or burn	*Staphylococcus aureus*	1; PHI-SA012	Intraperitoneal	Bacterial load	Phages reduce bacterial load	No	Insufficient information
Jaiswal et al (2014)[51]	India	Mice	Intestinal	*Vibrio cholerae*	5; ATCC51352- BI, B2, B3, B4, and B5	Oral or enteral	Bacterial load	Phages reduce bacterial load	No	Model or pathogen <2 studies
Jasim et al (2018)[52]	Iraq	Mice	Systemic	*Acinetobacter baumannii*	64; reported names are AB1P1, AB1P2, AB2P1, AB3P1, AB3P2, AB3P3, AB3P4, AB4P1, AB5P1, AB6P1, AB6P2, AB9P1, AB10P1, AB10P2, AB12P1, AB15P1, AB15P2, AB17P1, AB19P1, AB19P2, AB20P1, AB21P1, AB21P2, AB22P1, and AB22P2	Intraperitoneal	Mortality	Phages reduce mortality risk	Yes	NA
Jeon et al (2019)[53]	South Korea	Mice	Respiratory	*Acinetobacter baumannii*	1; BPHI-R2096	Respiratory	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Jeon et al (2019)[54]	South Korea	Mice	Respiratory	*Pseudomonas aeruginosa*	2; BPHI-R656 and BPHI-R1836	Respiratory	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Ji et al (2020)[55]	China	Mice	Skin or burn	*Staphylococcus aureus*	1; VB_SauS_SH-St 15644	Subcutaneous	Bacterial load	Phages reduce bacterial load	No	Insufficient information
Ji et al (2019)[56]	China	Rabbits	Respiratory	*Staphylococcus aureus*	1; VB-SavM-JYL01	Respiratory	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	No	Non-rodent model

(Table continues on next page)

	Country	Animal	Infection setting	Pathogen	Number of phages; phage name(s)	Phage administration route	Outcomes assessed	Overall results	Included in meta-analysis?	Reason for exclusion
(Continued from previous page)										
Jia et al (2020)[57]	China	Carp	Systemic	*Citrobacter freundii*	1; IME-JL8	Intraperitoneal	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	No	Non-rodent model
Jiang et al (2020)[58]	China	Mice	Systemic	*Acinetobacter baumannii*	1; Abp9	Intraperitoneal	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Jun et al (2014)[59]	South Korea	Mice	Systemic	*Vibrio parahaemolyticus*	1; pVp-1	Intraperitoneal	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Kaabi et al (2019)[60]	Iraq	Mice	Systemic	*Escherichia coli, Klebsiella pneumoniae, Haemophilus influenzae, Pseudomonas aeruginosa, Citrobacter freundii, and Moraxella catarrhalis*	29; name not reported	Intraperitoneal	Mortality	Phages reduce mortality risk	No	Insufficient information
Kaur et al (2021)[61]	India	Mice	Skin or burn	*Staphylococcus aureus*	1; MR5	Subcutaneous	Bacterial load	Phages reduce bacterial load	Yes	NA
Kifelew et al (2020)[62]	Australia	Mice	Skin or burn	*Staphylococcus aureus*	3; J-Sa36, Sa83, and Sa87	Topical or superficial	Bacterial load	Phages reduce bacterial load	Yes	NA
Kim et al (2021)[63]	South Korea	Mice	Systemic	*Vibrio vulnificus*	1; VVP001	Intraperitoneal	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
McVay et al (2007)[64]	USA	Mice	Skin or burn	*Pseudomonas aeruginosa*	3; Pa1 (ATCC 12175-B1), Pa2 (ATCC 14203-B1), and Pa11 (ATCC 14205-B1)	Intramuscular, subcutaneous, or intraperitoneal	Mortality	Phages reduce mortality risk	Yes	NA
Prazak et al (2022)[65]	Switzerland	Rats	Respiratory	*Staphylococcus aureus*	4; 2003, 2002, 3A, and K	Respiratory and intravenous	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Prazak et al (2019)[66]	Switzerland	Rats	Respiratory	*Staphylococcus aureus*	4; 2003, 2002, 3A, and K	Intravenous	Mortality and bacterial load	Phages reduce mortality risk and bacterial load	Yes	NA
Takemura-Uchiyama et al (2014)[67]	Japan	Mice	Respiratory	*Staphylococcus aureus*	1; S13	Intraperitoneal	Mortality	Phages reduce mortality risk	No	Insufficient information
Tóthová et al (2011)[68]	Slovakia	Mice	Urinary	*Cronobacter turicensis*	2; name not reported	Intraperitoneal	Bacterial load	Phages reduce bacterial load	No	Model or pathogen <2 studies
Trigo et al (2013)[69]	Spain	Mice	Skin or burn	*Mycobacterium ulcerans*	1; Mycobacteriophage D29	Subcutaneous	Bacterial load	Phages reduce bacterial load	No	Model or pathogen <2 studies

NA=not applicable. *Nebulised, intranasal, or intratracheal.

Table: **Studies assessing phage efficacy in pre-clinical models of bacterial infection with low-moderate risk of bias**

routes were respiratory (ie, nebulised, intranasal, and intratracheal; 24 studies [19%]), oral or enteral routes (22 studies [18%]), and superficial or topical routes (12 [10%]). *G mellonella* and *Caenorhabditis elegans* models emulated systemic infection processes, with oral or enteral, and parenteral routes being the routes of phage administration in these models. A wide variety of bacteriophages were tested alone (71 [57%]), or in cocktails (53 [42%]), with an mean of 2·5 phages (range 2·0–64·0) evaluated per study. Most phages were isolated from sewage or wastewater (38 studies [31%]). Most studies used a single dose of phages (98 studies [79%]; overall mean 1·7 [range 1·0–14·0]).

The 124 studies included more than 7570 animal subjects. An exact number could not be calculated because 22 (18%) studies did not clearly report the

sample size. Most studies concluded that phages were effective at reducing tissue bacterial burden (n=83/91) or reducing the risk of mortality (n=73/78).

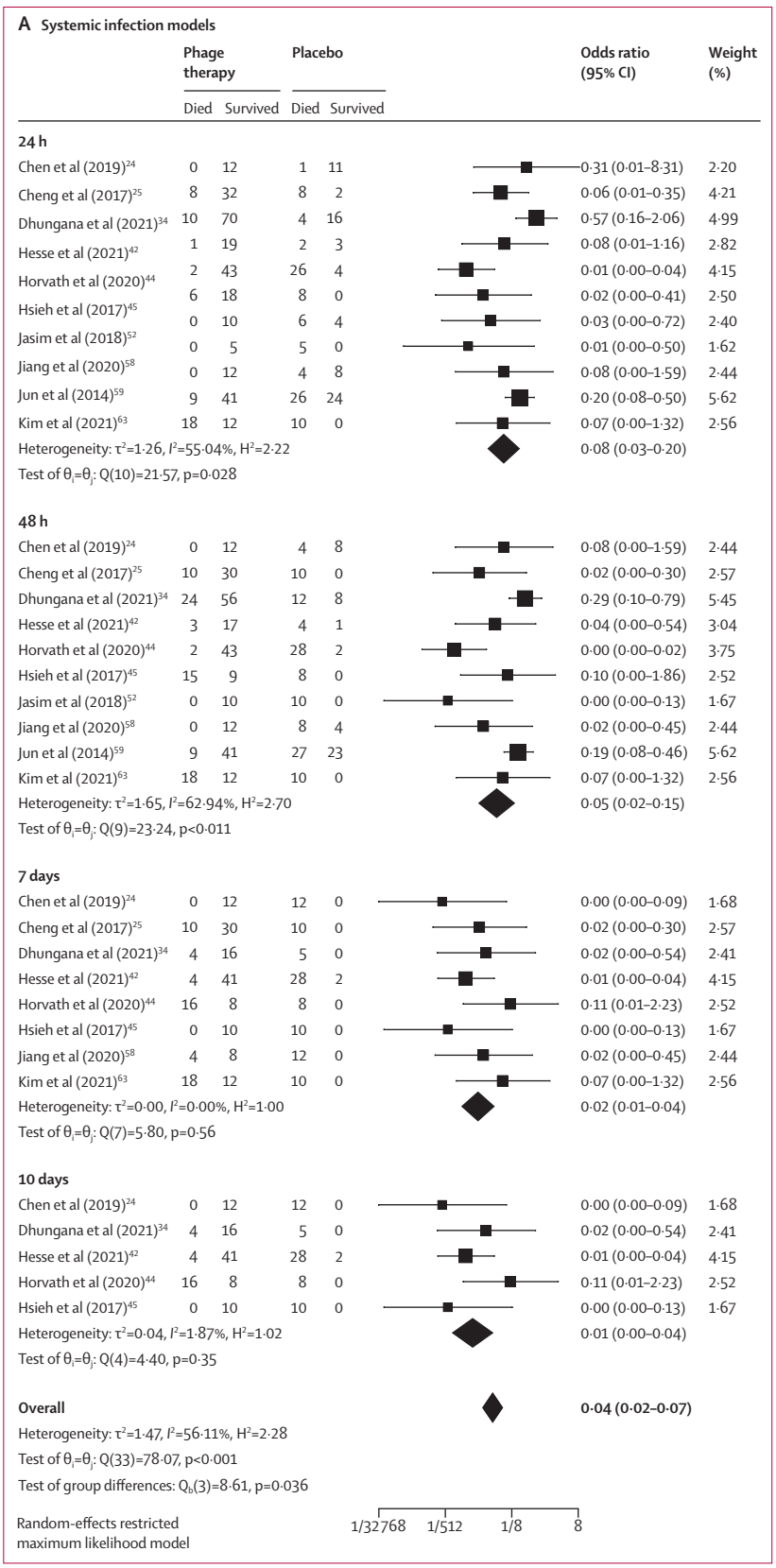
Before quantitatively assessing the efficacy of phage therapy, each of the 124 studies was subject to a risk-of-bias assessment (low, moderate, or high; appendix pp 8–18). Most studies (70 studies [57%]) had high risk of bias primarily due to the omission of information about the methodology (eg, the method and use of randomisation or blinding; appendix pp 8–18). The remaining 54 studies (44%) were classified as having low-to-moderate risk of bias and were considered for the quantitative synthesis of the review (figure 2).

Regarding the 54 low-to-moderate risk of bias studies, most studies (n=29) were performed in Asia (appendix p 25). The temporal trends remained similar to the overall collection (appendix p 25), rodents remained the main animal host (47 [87%] of 54 studies) and systemic infection was the most frequently assessed infection setting (19 [35%]). *S aureus* was the main bacterial pathogen (15 [28%]), followed by *P aeruginosa* (12 [22%]; appendix p 25).

After applying further selection criteria for inclusion in the meta-analyses, 32 studies assessing 1422 animals fulfilled the criteria for inclusion in the quantitative synthesis (figure 2; table).[16–69] These 32 studies were performed between 2007 and 2021, with *P aeruginosa* (9 studies [28%]) and *S aureus* (9 studies [28%]) being the most frequently evaluated pathogens. Moreover, a total of 30 different bacterial strains were analysed, most of them being clinical strains (n=26; 87%). A total of 123 different phages were evaluated, with a mean of two phages used per study (SD 1·4; range 1·0–64·0). From these, 58 phages were classified into a family with the Myoviridae family being the most abundant (n=22; 38%), followed by Siphoviridae (n=14; 24%), and Podoviridae (n=12; 21%; appendix p 26). Most of the evaluated phages were classified as lytic (n=57; 98%; appendix p 19–22).

25 studies reported survival outcomes (1277 animals, mean of 51 mice or rats per study [SD 43]; figure 3). Most studies reported survival at 24 h (24 [96%] of 25 studies), with additional timepoints including 48 h (22 studies [88%]), 7 days (16 studies [64%]), and 10 days (10 [40%]). All 25 studies that reported survival outcomes assessed the efficacy of phage therapy compared with placebo (or untreated controlled), only four studies (16%) compared phage therapy with antibiotics,[23,56,66,70] and two studies (8%) compared phage therapy with a combination of phages and antibiotics.[66,70] Therefore, our analyses were focused on evaluating the efficacy of phage therapy compared with placebo in systemic models, skin or burn infection models, and pneumonia models.

11 (34%) of 32 studies evaluated the efficacy of phage therapy for the treatment of systemic infections using survival as an endpoint and placebo as a comparator (figure 3A). Most of the studies assessed Gram-negative



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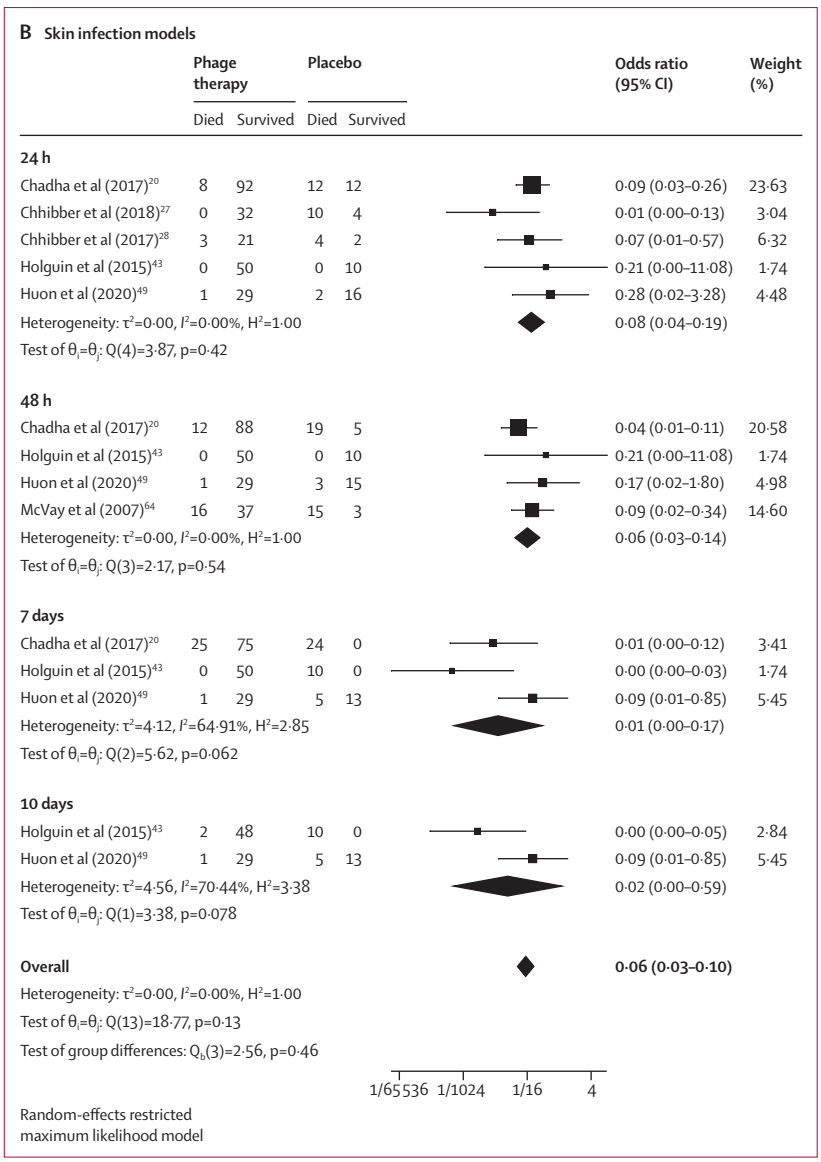

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pathogens (10 [90%] of 11 studies), the most frequent of which was *Klebsiella pneumoniae* (4 studies [36%]). Most studies evaluated a single phage in their experiments (8 [73%]; table).

We observed a significantly lower risk of mortality for mice or rats treated with phages when assessed at 24 h (OR 0·08 [95% CI 0·03–0·20]; I^2=55% [95% CI 8–77]), 48 h (OR 0·05 [0·02–0·15]; I^2=63% [23–81]), 7 days (OR 0·02 [0·01–0·04]; I^2=0% [0–68]), and 10 days (OR 0·01 [0·00–0.04]; I^2=2% [0–81]; figure 3A). Finally, we observed a potential reporting bias in the studies assessing phage therapy efficacy at 48 h (Egger's test p value=0·028), but the funnel plots and Egger's tests for comparisons at 24 h, 7 days, and 10 days did not suggest the presence of this bias (appendix p 27).

Six (19%) of 32 studies evaluated the efficacy of phage therapy for the treatment of skin or burn infections using survival as an endpoint and placebo as a comparator (figure 3A). Three (50%) of the studies used *S aureus* as the causal pathogen,[20,27,70] and the other three assessed Gram-negative bacteria.[19,64,71] In contrast to studies assessing systemic infection, most studies evaluated multiple phages in their experiments (5 [83%] of 6 studies), with an average of 4·2 per study (table). We observed significantly lower mortality of phage-treated mice or rats assessed at 24 h (OR 0·08 [95% CI 0·04–0·19]; I^2=0% [95% CI 0–79]), 48 h (OR 0·06 [0·03–0·14]; I^2=0% [0–85]), 7 days (OR 0·01 [0·00–0·17]; I^2=65% [0–90]), and 10 days (OR 0·02 [0·00–0·59]; I^2=70% [95% CI could not be calculated due to the small number of studies, degrees of freedom <2]; figure 3B). Regarding reporting bias, only the studies that made comparisons at 7 days showed a potential bias; nevertheless, the small number of studies (n=3) might lead to imprecise estimates (Egger's test p=0·019; appendix p 27).

Eight (25%) of 32 studies evaluated the efficacy of phage therapy for the treatment of experimental pneumonia using survival as an endpoint and placebo as a comparator (figure 3C). Most studies used Gram-negative bacteria as the causal pathogen (six [75%] of eight studies),[18,33,36,40,46,54] mainly *P aeruginosa* (four [50%] of eight).[33,36,40,54] The remaining two studies (25%) assessed *S aureus*.[65,66] Most studies assessed multiphage cocktails (five [63%] of eight). The mean number of phages used per study was 4·2 (range 1·0–64·0; table). A significantly lower mortality risk was observed in the mice or rats treated with phages when assessed at 24 h (OR 0·13 [95% CI 0·06–0·31]; I^2=0% [95% CI 0–68]), 48 h (OR 0·08 [0·04–0·19]; I^2=0% [0–68]), 7 days (OR 0·11 [0·04–0·33]; I^2=37% [0–79]), and 10 days (OR 0·04 [0·01–0·16]; I^2=0% [0–90]; figure 3C). The asymmetry of the funnel plot and the significant value of the Egger's test (p=0·028) suggested a reporting bias in the studies comparing phage therapy efficacy at 7 days; however, this result should be interpreted with caution due to the small number of studies (appendix p 27).

Subgroup meta-analyses specific to the pathogen, infectious setting, and animal model were performed, evaluating the efficacy of phage therapy for skin or burn infections due to *S aureus* and for pneumonia due to *P aeruginosa*.

Seven studies (380 mice or rats) evaluated the effectiveness of phage therapy for the treatment of skin or burn infections caused by *S aureus* in terms of bacterial load reduction at the site of infection. Phage therapy was associated with a significantly lower bacterial load in the skin compared with placebo at day 5±2 after infection (mean difference –2·66 [95% CI –3·17 to –2·16]; I^2=95% [95% CI 90 to 96]; figure 4A). There were, however, no significant differences in bacterial load at this timepoint when comparing phage therapy to antibiotic therapy (mean difference –0·25 [–0·87 to 0·37]; I^2=98%

[98 to 99]; figure 4B). Similarly, the difference in bacterial load for animals treated with phage therapy and those treated with a combination of phages and antibiotics was not significant (mean difference 0·70 [–0·62 to 2·03]; I^2=98% [98–99]; figure 4C). Funnel plots and Egger's tests did not indicate the presence of reporting bias in comparisons of phage therapy versus placebo and phage therapy versus antibiotics. However, a potential bias was suggested for the comparison of phage therapy versus phage therapy plus antibiotics despite the small number of studies evaluated (appendix p 28).

Only three studies (37 mice or rats) compared phage therapy with placebo using bacterial loads in the lungs as an endpoint (figure 4D). Bacterial burdens were significantly lower at day 5±2 after infection for phage therapy treatment groups (mean difference –3·35 [95% CI –6·00 to –0·69]; I^2=99 [95% CI 98–99]). Finally, although the analyses did not suggest reporting bias, no robust result was achieved due to the small sample size (appendix p 28).

An additional analysis of factors potentially associated with phage therapy efficacy is available in the appendix (p 4). In summary, time from bacterial inoculation to phage administration was observed to be significantly associated with phage therapy efficacy at most follow-up times (24 h p=0·0051, 48 h p=0·023, and 10 days p=0·025), and no evidence was observed for the multiplicity of infection and the phage administration route.

Discussion

To our knowledge, this report is the first systematic review and meta-analysis to assess phage therapy efficacy in terms of mortality and microbiological outcomes in preclinical models of disease. Phage therapy was found to be highly effective at reducing mortality and tissue bacterial burdens when assessed in animal models, however, the quality of the collective synthesis of evidence (n=124 studies) was low because most studies had a high risk of bias. Factors contributing to the high risk of bias included inadequate reporting of experimental methods (eg, randomisation and blinding) and inappropriate statistical approaches, among others. When studies with low-to-moderate risk of bias were quantitatively assessed in subgroup meta-analyses, phage therapy significantly improved outcomes in all settings analysed (ie, lowered mortality in systemic infection, skin or burn infections and pneumonia, and reduced bacterial loads in the context of skin or burn infection due to *S aureus* and pneumonia due to *P aeruginosa*). The current results from rodent models align with similar studies assessing phage efficacy in pigs and poultry.[72,73]

Animal models of infection have been a cornerstone of anti-infective drug development. It is probable, however, that in the case of phage therapy there is a disconnect between research and clinical application, whereby preclinical models have failed to adequately guide phage implementation in humans based on dosing, phage therapeutic design, and time to treatment.

In the preclinical trials assessed in this Article, most used a single phage (57%) that was administered as a single dose (79%). By contrast, although the best approach to phage dosing in humans is not yet clear,

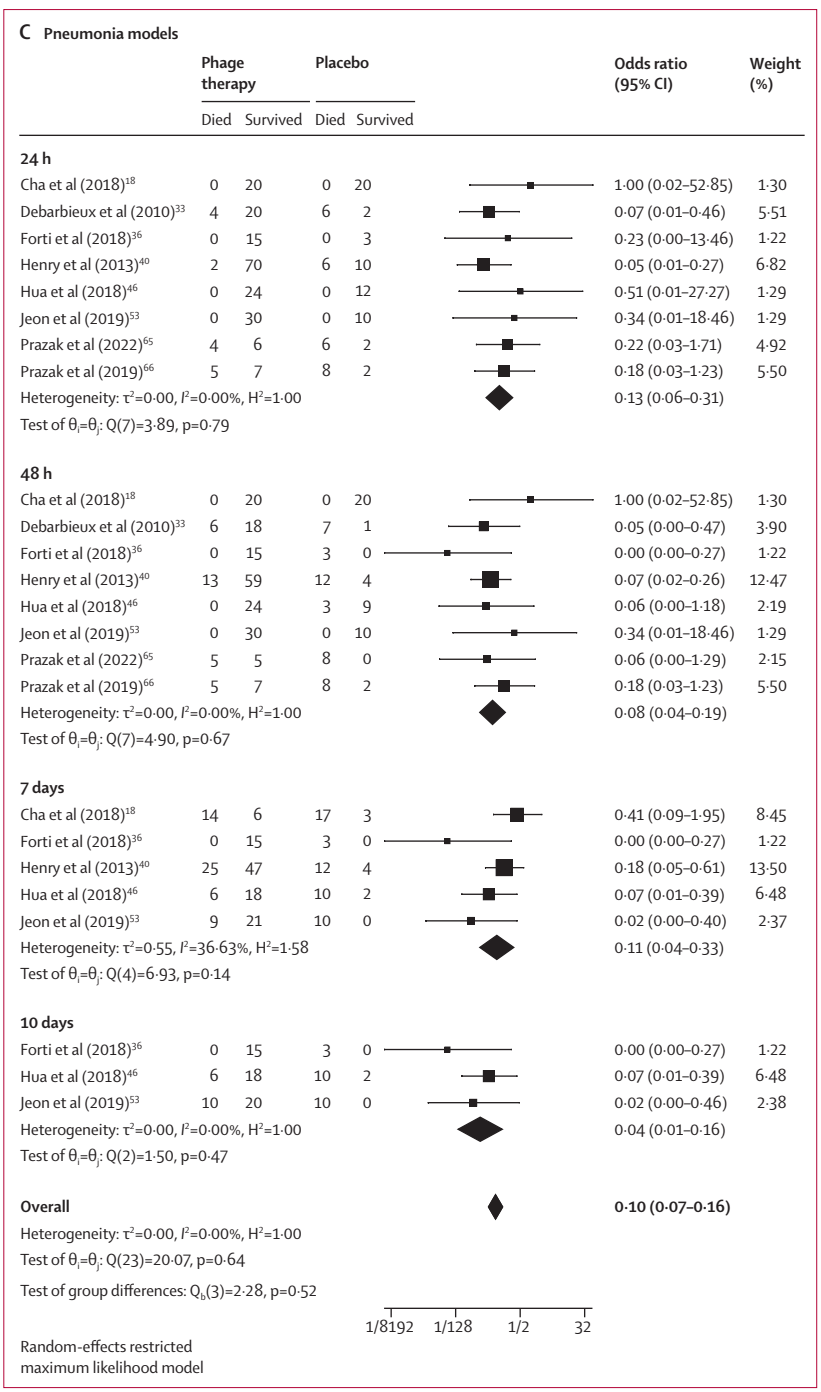

Figure 3: **Forest plots evaluating odds-ratios for the effect of phage therapy compared with placebo in experimental infection models at different follow-up times**
(A) Systemic infection models. (B) Skin or burn infection models. (C) Bacterial pneumonia models.

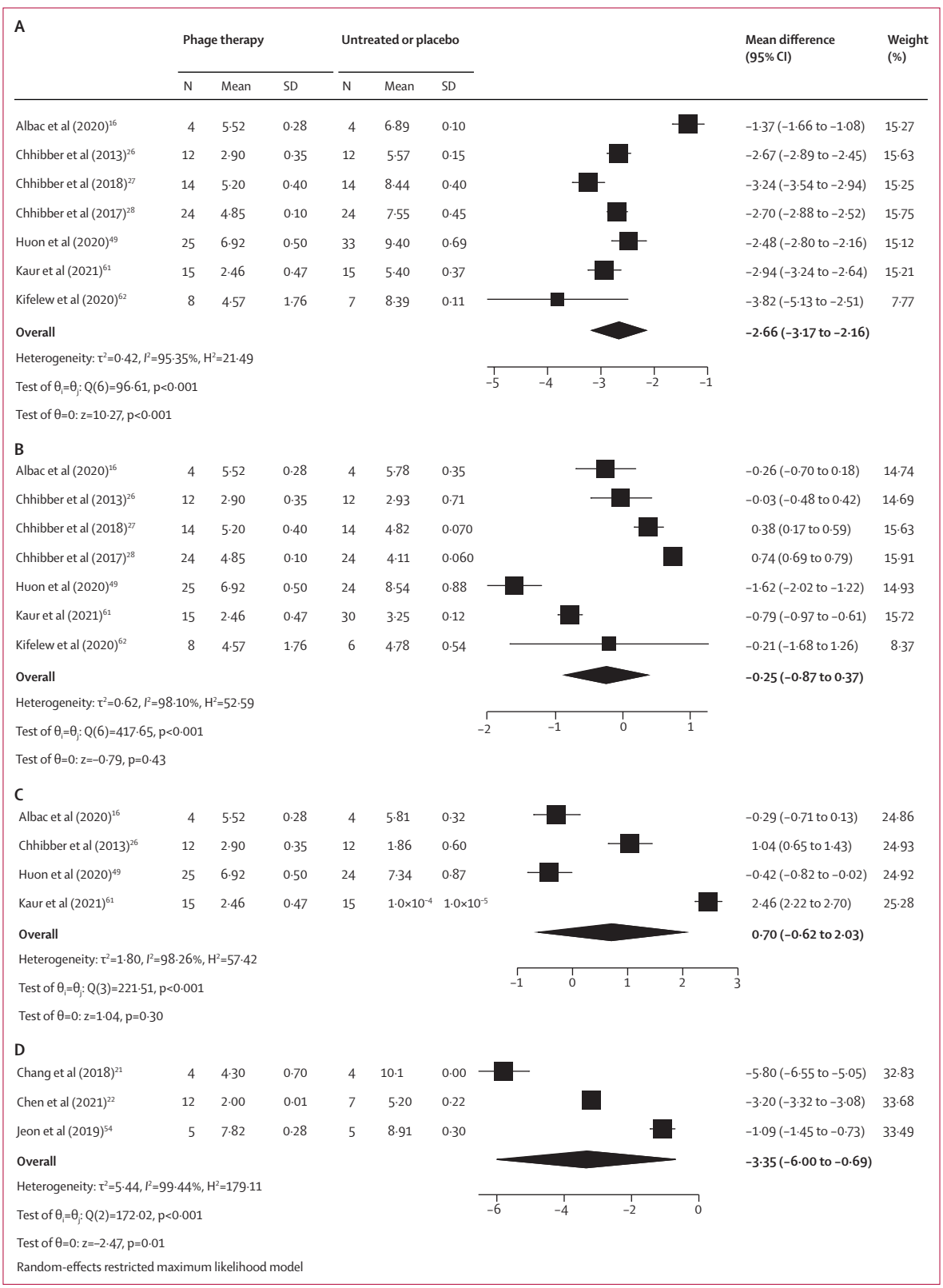

Figure 4: **Forest plots evaluating the mean difference of bacterial loads measured at a mean follow-up of 5 ± 2 days in preclinical trials assessing phage therapy**

Comparisons between placebo-treated and phage-treated animals (A), phage-treated and antibiotic-treated animals (B), and phage-treated and phage-and-antibiotic-treated animals in models of skin/burn infections due to *Staphylococcus aureus* (C). (D) Comparisons between placebo-treated and phage treated animals in models of pneumonia due to *Pseudomonas aeruginosa*.

each of the three recent phase 1/2 RCTs investigated multiphage cocktails that were administered repeatedly (either once daily,[74] twice daily,[75] or three times daily[76]).

The selection of the phage(s) used for treatment in preclinical studies was typically determined rationally by elaborate in-vitro testing, which involved screening the infective isolate against a panel of phages and looking for the best one(s). Although this approach fits with the notion of personalised phage therapy,[77] it is not conducive to a conventional or standard RCT, where the therapeutic product is established and validated by ethical committees before the start of the trial.

Most preclinical studies targeted acute infection settings (systemic, 38%; respiratory, 23%), and our analysis revealed that the time-to-treatment likely influenced phage efficacy (appendix p 4). In acute settings, especially in sepsis and septic shock, an adequate anti-infective therapy should be initiated as soon as possible, which justifies the empirical use of broad-spectrum antibiotics prior to microbiological documentation (52·6% of patients with hospital acquired bloodstream infection; n=1156; EUROBACT international cohort[8]). This approach is not feasible for narrow spectrum therapies such as phage therapy, where sufficient time for the identification of the causative pathogen and an accurate determination of phage sensitivity is required to maximise the chance of efficacy.

Collectively, the data from rodent models represent the best-case scenario for effective phage therapy (a tailored therapeutic product applied shortly after the induction of infection). Future preclinical trials should be designed to better emulate the settings whereby phage therapy is likely to be most valuable for human use. This approach includes testing established phage therapeutics (1) using different dosing strategies (while evaluating mechanisms to ensure optimal stability at the supplied phage concentrations), (2) against multiple diverse strains from the target species in vivo, (3) in long-term or chronic infection settings (ie, prosthetic joint infections, osteomyelitis, and chronic wound infections), and (4) considering and comparing different administration routes. Additionally, despite not having observed a significant role of multiplicity of infection and route of phage administration on phage therapy efficacy in this Article (appendix p 4), the small number of studies and animals included in these analyses highlights the need to address these aspects in future preclinical trials. Finally, the current study revealed relevant variations in phage therapy efficacy depending upon the follow-up period measured, with larger relative effect sizes in prolonged follow-up periods (7–10 days). Considering that a substantial proportion of the included studies performed only short follow-ups, future studies should consider longer periods to better assess phage efficacy. At the very least, all future studies assessing phage therapy in animal models should adhere to guidelines for reporting in-vivo experiments to minimise the risk of bias.[12,78]

A key strength of the current analyses, when compared with recent systematic reviews on phage efficacy in humans,[6,10] was that each of the studies with low-to-moderate risk of bias was placebo-controlled, phage efficacy was not confounded by adjunct antibiotic use, and the sample size was comparatively high, with 1422 animals assessed. Additionally, most of the studies included in the meta-analysis (30 studies [94%]) were published during the past decade (2012–22), which can reduce the risk of bias in the analyses.[76] Moreover, compared with other meta-analyses evaluating phage therapy in animals that only assessed bacterial loads after oral phage administration,[72,73] we were able to evaluate survival and different phage delivery methods. However, despite attempts to minimise sources of heterogeneity by including only studies using well described rodent models and assessing subgroups (infection setting, time of follow-up, and outcomes), there was moderate-high heterogeneity across some comparison groups, particularly considering microbiological outcomes; this heterogeneity represents a major limitation of our study. Additionally, by applying stringent selection criteria for each comparison, heterogeneity might have been influenced by the sample size effect, as the I^2 statistic can be biased in comparisons with small sample sizes.[79] Therefore, the estimates derived from pooled analyses with high heterogeneity might be less precise than those with low heterogeneity; however, a clear trend in favour of phage therapy was observed in most of the studies analysed.[80] Another limitation was the small number of studies included in most of the analyses, which might limit the optimal assessment of publication bias. Both Egger's test and funnel plots might be underpowered to detect bias when the number of studies is less than 10, which could lead to erroneous conclusions. Moreover, although useful for exploring data and generating new hypotheses, the results from meta-regression analyses should be interpreted cautiously. Even when a large number of studies are analysed, meta-regression analyses have little power to identify associations that are not large in magnitude.[81,82] Finally, important factors involved in the therapeutic efficacy of phage therapy, such as specific phage characteristics and inflammatory markers, among others, could not be evaluated. We emphasise the need to ensure optimal reporting of information on the phages used in the different studies, which will allow us to obtain more solid conclusions on the efficacy of phage therapy in the different contexts in which it is used.

In conclusion, phage therapy has proven effective for the treatment of bacterial infection in rodent models. Harnessing the knowledge gained from preclinical studies to demonstrate efficacy in human clinical trials is the next important frontier.

Contributors

SAG-O, DRC, TM, and Y-AQ conceptualised the study. SAG-O coordinated the study. TM and Y-AQ supervised and provided resources. SAG-O, MP, LGV, CDSV, JL, ACQ-C, JAHV, SJT-C, and DRC contributed

to data collection and curation. SAG-O and TM performed the formal analysis and visualisation. SAG-O and DRC prepared the first draft of the manuscript. MP, JAHV, TM, and SAG-O accessed and verified all the data reported in the study. All authors reviewed, edited, and approved the final version. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

All data pertaining to risk of bias is available in the appendix (pp 8–18). Extracted data from the 54 studies with low-to-moderate risk of bias are available in the table. Extracted data from the studies with high risk of bias will be made available from the corresponding authors upon request.

Acknowledgments

Y-AQ. was supported by the Swiss National Foundation (320030_176216 and CR31I3_166124). DRC was supported by the Swiss Heart Foundation (FF20114). We want to thank Christa Niehot for her support in performing the systematic literature searches.

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