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Safety and efficacy of phage application in bacterial decolonisation: a systematic review

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Colonisation by bacterial pathogens typically precedes invasive infection and seeds transmission. Thus, effective decolonisation strategies are urgently needed. The literature reports attempts to use phages for decolonisation. To assess the in-vivo efficacy and safety of phages for bacterial decolonisation, we performed a systematic review by identifying relevant studies to assess the in-vivo efficacy and safety of phages for bacterial decolonisation. We searched PubMed, Embase (Ovid), MEDLINE (Ovid), Web of Science, and the Cochrane Library to identify relevant articles published between Jan 1, 1990, and May 12, 2023, without language restrictions. We included studies that assessed the efficacy of phage for bacterial decolonisation in humans or vertebrate animal models. This systematic review is registered with PROSPERO, CRD42023457637. We identified 6694 articles, of which 56 (51 animal studies and five clinical reports) met the predetermined selection criteria and were included in the final analysis. The gastrointestinal tract (n=49, 88%) was the most studied bacterial colonisation site, and other sites were central venous catheters, lung, nose, skin, and urinary tract. Of the 56 included studies, the bacterial load at the colonisation site was reported to decrease significantly in 45 (80%) studies, but only five described eradication of the target bacteria. 15 studies reported the safety of phages for decolonisation. No obvious adverse events were reported in both the short-term and long-term observation period. Given the increasing life-threatening risks posed by bacteria that are difficult to treat, phages could be an alternative option for bacterial decolonisation, although further optimisation is required before their application to meet clinical needs.

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Introduction

Many bacteria of clinical significance (eg, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) cause severe infections that are difficult to treat owing to scarce antimicrobial options resulting from intrinsic or acquired resistance to antimicrobial agents.[1] Colonisation of pathogens, including these bacteria that are difficult to treat, is typically a stepping-stone for the occurrence of subsequent invasive infections in the host (human or animals).[2–4] Colonised hosts might also act as a source of infection by shedding bacteria, resulting in contamination of the environment and facilitating transmission of the pathogen to other hosts with the potential to cause subsequent infections and even outbreaks or epidemics.[5–9] Colonisation can also facilitate the transfer or exchange of genes encoding antimicrobial resistance or virulence factors among bacteria, leading to the formation of new multidrug-resistant organisms or hypervirulent strains.[5] Consequently, effective decolonisation strategies could prevent hosts from developing infections and reduce the spread of multidrug-resistant organisms.[10] However, such strategies, in particular those targeting bacteria residing on mucous membranes, are currently rare. The use of antimicrobial agents for decolonisation is largely unsuccessful and can perturb the commensal microflora and facilitate the emergence of multidrug-resistant organisms.[11,12] Decolonisation using ecological approaches by transplanting microbiome or their products is still controversial with variable outcomes.[13,14] Compared with the treatment of infections, decolonisation appears to be more challenging. Pathogens cause inflammation in infections and induce responses by the host immune system that, in turn, control infection, whereas bacterial colonisation is typically cryptic and does not cause any protective immune responses. Colonisation of pathogenic bacteria, in particular that occur in health-care or veterinary settings, often reflects the loss of colonisation resistance conferred by commensal microflora as a result of perturbation.[15,16] Factors perturbing commensal microflora such as the use of antimicrobial agents might not be avoidable, and the host might be continuously exposed to sources of pathogenic bacteria such as those present in environmental reservoirs and in other patients or infected animals.[15,17] Thus, pathogenic bacteria might continue to establish constant, difficult-to-eradicate colonisation. Hence, there is an urgent need to identify novel decolonisation strategies.

Bacteriophages (phages) are viruses that infect bacteria and are widely distributed in nature.[18] Some phages can lyse bacteria, and these lytic phages have been used to treat bacterial infections even before the application of antibiotics.[19] Phages have a narrow host spectrum, typically targeting few bacterial species or even a particular strain of a species;[20,21] thus, phages marginally perturb the commensal microflora.[22,23] Phage therapy is an alternative approach for managing bacterial infections, and research interest has been renewed in phage therapy against bacterial infections worldwide, with reports of its success in resolving bacterial infections that are difficult to treat.[24,25] Currently, most studies of phage applications involve phage therapy for bacterial infections, and the use of phages for decolonising bacteria has received less attention. Nonetheless, there have been many attempts,[26–28] including our own,[21] to use phages for decolonisation, and an increasing number of such studies have been published in the past few years. We, therefore, performed a systematic review to summarise the currently available data about the use of phages for

decolonisation against bacteria focusing on efficacy and safety. We anticipate that this systematic review will encourage more well designed, high-quality studies to be undertaken.

Methods

Search strategy and selection criteria

See Online for appendix

We performed this systematic review in accordance with PRISMA guidelines[29] (appendix pp 9–13). The study protocol (appendix pp 2–4) was published in PROSPERO, CRD42023457637. We searched PubMed, Embase (Ovid), MEDLINE (Ovid), Web of Science, and the Cochrane Library to identify relevant articles published between Jan 1, 1990, and May 12, 2023, without language restrictions. We used search terms (appendix pp 5–6) related to phage applications in bacterial decolonisation, including "phage", "bacteriophage", "phage therapy" plus "colonize", "colonise", "colonisation", "colonization", "decolonisation" and "decolonization".

Two investigators independently searched the databases and retrieved basic information of manuscripts, including year of publication, study location, author name, title, and abstract, to an Excel sheet, and duplicate entries were removed. We included studies that assessed the efficacy of phage for bacterial decolonisation in humans or vertebrate animal models. We excluded studies that met any of the following criteria: (1) reporting only in-vitro or ex-vivo results; (2) with incomplete information (eg, about the phages or bacteria used or inoculation route); (3) reporting the infection model only; (4) not related to phages for bacterial decolonisation; (5) prophylactic application of phages before bacteria colonisation; (6) using a non-vertebrate model; (7) using phages that are not lytic or using phage-derived products such as endolysin and depolymerase rather than phages; and (8) review articles.

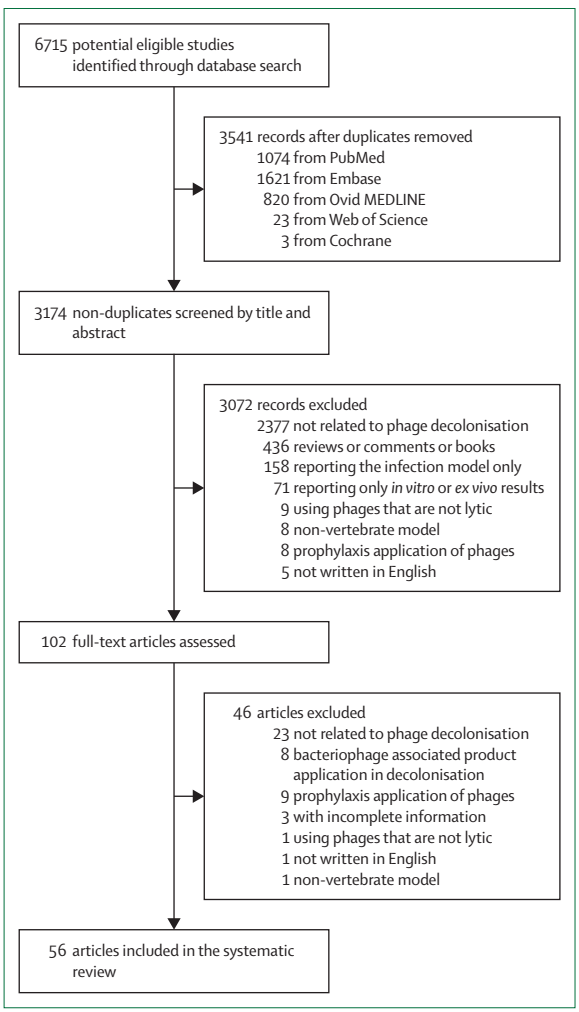

Figure: **Flowchart depicting the literature search strategy**

Data analysis

Each of the articles were preliminarily and independently screened for titles and abstracts by two investigators. Discrepancies between reviewer screening decisions were resolved by consensus or further evaluation by a third reviewer. Next, the full text of each remaining article was assessed by four reviewers according to the inclusion criteria. Finally, relevant data regarding the study population (human or the type of animal model), study design (case report, series, or randomised controlled trial), colonisation site, target bacteria, phage or phages (the number, name, source, and taxonomy) and their application (the route, frequency, dosage, and duration), concomitant measures (antimicrobial agents, vaccine, and probiotics), outcome parameters (change in bacterial loads and presence or absence of recurrence), and adverse events were retrieved and summarised in descriptive tables for each included study. Four reviewers independently assessed the risk of bias. Any disagreement was resolved by consensus. Risk of bias was not applicable to case reports. For animal studies, Systematic Review Centre for Laboratory Animal Experimentation's risk-of-bias tool was used for assessing the risk of bias.[30] The quality in six categories, including selection bias, performance bias, detection bias, attrition bias, reporting bias, and other biases, was assessed. Risk of bias was recorded as low (8–10 points), moderate (4–7 points), or high (<4 points).

Results

We identified 6694 articles by searching databases, of which 3174 were retained after removal of duplicates. After preliminary screening of titles and abstracts, we identified 102 studies for further examination. After review, we finally included 56 studies according to the predetermined selection and exclusion criteria (figure). Information about and reasons for the exclusion of the remaining 46 studies are available in the appendix (pp 14–18). All included studies were published since 2005, and most studies were from Europe (n=24), the USA (n=13), or China (n=5). Five studies reported the use of phages for bacterial decolonisation in

	Country	Animal	Sex	Number*	Target bacteria	Phage administration						Efficacy	
						Phage number	Combination	Route	Dosage, PFU†	Frequency	Duration	Load change	Recurrence
Loc Carrillo et al (2005)[31]	UK	Chicken	Male	NA	*Campylobacter jejuni*	1	..	Gavage	10^5, 10^7, 10^9	Once	1 d	↓	No
Cui et al (2023)[32]	China	Chicken	Female	5	*Salmonella*	1	..	Oral	10^8	qd	2 d	↓	No
Scott et al (2007)[33]	UK	Chicken	Male	9	*C jejuni*	1	..	Gavage	10^8	Once	1 d	↓	No
Zhang et al (2022)[26]	China	Chicken	Male and female (1:1)	10	*Salmonella*	1	..	Gavage	$1{\cdot}5 \times 10^{10}$	Once	1 d	↓	No
Hammerl et al (2014)[34]	Germany	Chicken	Male	10	*C jejuni*	2	Tandem	Oral	CP14 $5{\cdot}0 \times 10^8$; CP68 $5{\cdot}0 \times 10^{10}$	Once	1 d	↓	No
Pelyuntha et al (2022)[35]	Thailand	Chicken	..	10	*Salmonella*	3	Cocktail	Gavage	10^9	qd	5 d	X	No
Hurley et al (2008)[36]	USA	Chicken	..	10	*Salmonella*	1	..	Gavage	$8{\cdot}5 \times 10^6$ (d2, d3); 5×10^8 (d28)	qd	3 d	No	..
Chinivasagam et al (2020)[37]	Australia	Chicken	..	15	*Campylobacter*	4 or 2	Cocktail	Gavage	3×10^7	Once	1 d	↓	No
Toro et al (2005)[38]	USA	Chicken	..	16	*Salmonella*	3	Cocktail	Gavage	$5{\cdot}4 \times 10^6$	qd	6 d	↓	No
Kittler et al (2013)[39]	Germany	Chicken	..	9 (T1); 9 (T2); 9 (T3)‡	*C jejuni*	4	Cocktail	Water	$10^{7{\cdot}5}$ (T1); $10^{5{\cdot}8}$ (T2); $10^{7{\cdot}6}$ (T3)‡	Cont	2·6 h (T1); 1 h (T2); 6·3 h (T3)	↓ (T1) No (T2 and T3)	No
D'Angelantonio et al (2021)[28]	Italy	Chicken	..	23	*C jejuni*	2	Tandem	Gavage	10^7 or 10^8 per mL§	qd	2 d	↓	..
Andreatti et al (2007)[40]	Brazil	Chicken	..	25 (T2)¶; 40 (T3)	*Salmonella*	45 or 4¶	Cocktail	Cloacal (T2), gavage (T3)	10^9 (T2); 10^8 per cocktail (T3)¶	Once	1 d	↓	48 h later
Li et al (2022)[41]	China	Chicken	..	27	*Salmonella*	3	Cocktail	Oral	10^9	Once	6 d	↓	10 d later
Kimminau et al (2020)[42]	USA	Chicken	..	30	*Salmonella*	1	..	In food	10^8 per g	Cont	14 d	↓	No
Lim et al (2012)[43]	Korea	Chicken	..	30	*Salmonella*	1	..	In food	10^5, 10^7, 10^9 per g	Cont	21 d	↓	No
Bardina et al (2012)[44]	Spain	Chicken	Female	30	*Salmonella*	3	Cocktail	Gavage	10^{10}	bid	2 d	No	..
Vaz et al (2020)[45]	Brazil	Chicken	..	Early 32; Later 36	*Salmonella*	3	Cocktail	Water	Early $2{\cdot}9 \times 10^{10}$; Later $6{\cdot}8 \times 10^{10}$	Cont	5 d	↓	No
Wagenaar et al (2005)[46]	Netherlands	Chicken	..	36	*C jejuni*	2	Cocktail	Gavage	Phage 71 $(0{\cdot}2\text{–}4) \times 10^{11}$; Phage 69 $(0{\cdot}5\text{–}3) \times 10^{10}$	qd	6 d	↓	No
Atterbury et al (2007)[47]	UK	Chicken	..	36 (T1); 18 (T2)	*Salmonella*	1	..	Gavage	10^9 (T1); 10^{11} (T2)	qd	6 d (T1); 3 d (T2)	↓	No
El-Shibiny et al (2009)[48]	UK	Chicken	Male	45	*Campylobacter coli*, *C jejuni*	1	..	Gavage	10^5, 10^7, 10^9	Once	1 d	↓‖	No
Carvalho et al (2010)[49]	Portugal	Chicken	..	45	*C coli*, *C jejuni*	3	Cocktail	Gavage (T1); In food (T2)	1×10^6 (T1); $1{\cdot}5 \times 10^7$ (T2)	Once	1 d	↓	No
Lorenzo-Rebenaque et al (2022)[50]	Spain	Chicken	..	50	*Salmonella*	1	..	In food	..	Cont	3 w	↓	No
Kimminau et al (2022)[51]	Greece	Chicken	..	70	*Salmonella*	1	..	In food	10^8 per g	Cont	42 d	No	..
Fischer et al (2013)[52]	Germany	Chicken	..	92	*C jejuni*	4 or 1	Cocktail	In food	10^7	Cont	1 d	↓	No
Kim et al (2015)[53]	Korea	Chicken	Female	120	*Salmonella*	..	Cocktail	In food	$(0{\cdot}4$ or $0{\cdot}8) \times 10^8$ per kg	Cont	8 w	↓	..
Wang et al (2013)[54]	Korea	Chicken	Male	180	*Salmonella*	3	Cocktail	In food	$(0{\cdot}25$ or $0{\cdot}5) \times 10^8$ per kg	Cont	32 d	↓	No
Matiuhin et al (2020)[55]	Israel	Mice	..	..	*Klebsiella pneumoniae*	5	Cocktail	..	..	qd	12 d	↓	No
Federici et al (2022)[56]	Israel	Mice	Male	..	*K pneumoniae*	5	Cocktail	Gavage	1×10^9	tiw	9 d	↓	No
Titécat et al (2022)[57]	France	Mice	Male	12 (1 d); 6 (15 d)	*Escherichia coli*	7	Cocktail	Oral	$1{\cdot}4 \times 10^8$ (1 d); 2×10^9 (15 d)	Once (1 d); bid (15 d)	1 or 15 d	↓	16 d later
Maura et al (2012)[58]	France	Mice	Male	15–29**	*E coli*	3	Cocktail	Water	3×10^8 per mL§	Cont	1 d	No	..
Maura et al (2012)[59]	France	Mice	Female	4	*E coli*	3	Cocktail	Water	3×10^8 per mL§	Cont	1 d	No	..

(Table 1 continues on next page)

	Country	Animal	Sex	Number*	Target bacteria	Phage administration						Efficacy	
						Phage number	Combination	Route	Dosage, PFU†	Frequency	Duration	Load change	Recurrence
(Continued from previous page)													
Wolfoviz-Zilberman et al (2021)[60]	Israel	Mice	Female	5	*Streptococcus mutans*	1	..	Oral swab††	10^8 per mL	Q48h	42 d	↓	No
Buttimer et al (2022)[61]	Ireland	Mice	..	8	*E coli*, *Enterococcus faecalis*	6	Cocktail	Gavage	2×10^9	Biw	9 w	No	..
Javaudin et al (2021)[62]	France	Mice	Male	8	*E coli*	4	Cocktail	Oral and rectal	Oral 4×10^7; rectal 2×10^7	qd	3 d	No	..
Fang et al (2022)[21]	China	Mice	..	8	*K pneumoniae*	2	Cocktail	Water or rectal	P39 10^9 per mL§; P24 10^9	P39 Cont; P24 qd	7 d (P39); 3 d (P24)	↓	No
Porter et al (2022)[63]	USA	Mice	Female	16	*E coli*	5	Cocktail	Gavage	10^6–10^8	qd	5 d	↓	10 d later
Liu et al (2022)[27]	China	Mice	..	16	*K pneumoniae*	2	Cocktail	Oral and IG inject	10^9	qd	3 d	↓	21 d later
Mai et al (2015)[23]	USA	Mice	Male	20	*Shigella*	5	Cocktail	Gavage	$1{\cdot}0 \times 10^9$	Once	1 d	↓	..
Galtier et al (2016)[22]	France	Mice	Female	30	*E coli*	3	Cocktail	Gavage	6×10^5 or 10^7	Once	1 d	↓	..
Galtier et al (2017)[64]	France	Mice	Female	70	*E coli*	3	Cocktail	Gavage	3×10^7	bid	1 d	↓	No
Albino et al (2014)[65]	Brazil	Pig	..	6	*Salmonella*	6	Cocktail	Gavage	10^3, 10^5, 10^7, 10^9 per mL§	Once	1 d	No	..
Wall et al (2010)[66]	USA	Pig	Male	6 (T1); 8 (T2)	*Salmonella*	15	Cocktail	Gavage	5×10^9 (T1); $1{\cdot}5 \times 10^{10}$ (T2)	q2h	6 h	↓	No
Saez et al (2011)[67]	USA	Pig	..	7	*Salmonella*	14	Cocktail	Gavage	$5{\cdot}0 \times 10^{11}$	q2h	6 h	↓	No
Ahmadi et al (2016)[68]	USA	Quail	..	25	*Salmonella*	1	..	Gavage	10^5	qd	3 d	No	..
Raya et al (2006)[69]	USA	Sheep	..	4	*E coli*	1	..	Gavage	10^{11}	Once	1 d	No	..
Raya et al (2011)[70]	USA	Sheep	..	4	*E coli*	2	Cocktail	Gavage	10^{11}	Once	1 d	↓	No
Callaway et al (2008)[71]	USA	Sheep	..	10	*E coli*	8	Cocktail	Gavage	10^9	qd	2 d	↓	No

d=day. h=hour. w=week. PFU=plaque forming unit. ↓=decreased but not eradicated. X=eradicated. T=trial (experiment). IG inject=intragastric injection. bid=twice daily. biw=twice per week. Cont=continuously. Once=a single dose. q2h=every 2 h. q8h=every 8 h. q48h=every 48 h. qd=once daily. tiw=three times weekly. *Animal number in the phage administration group. †The data below are the total number of phages given to each animal at a time, unless otherwise noted. ‡Trial 1, trial 2, and trial 3 were conducted in three different farms. §The specific dosage is not available. ¶Trial 1 was performed in vitro. In trial 2, 10^9 PFU per chick of phage cocktail WT45Ø or combined with 2×10^6 CFU per chick of probiotic via cloacal administration significantly reduced *Salmonella* Enteritidis counts. In trial 3, the treatments via oral gavage with 10^8 phage cocktail CB4Ø PFU per chick, 10^8 WT45Ø PFU per chick, or a combination of both significantly reduced *Salmonella* Enteritidis counts. The WT45Ø phage cocktail contained 45 phages, and the CB4Ø phage cocktail contained 4 phages. ||Only treatment with 10^9 PFU of phage CP220 resulted in a significant reduction in mean caecal and lower intestinal *Campylobacter* counts. **The specific animal number in the phage treatment group is not available. ††This study was performed using a murine caries model, in which mice were treated every 48 h by oral swab with SMHBZ8 phage suspension (approximately 10^8 PFU/mL).

Table 1: **Studies reporting the efficacy of phages for decolonisation of the gastrointestinal tract in animals**

humans, whereas all other studies (n=51, 91%) were done in animal models, among which chickens (n=26) were the most commonly used, followed by mice (n=16) and other mammals (pigs, sheep, or rabbits; n=8). The gastrointestinal tract was the most common site of bacterial colonisation (n=49, 88%; 47 animal studies and two clinical reports), and other bacterial colonisation sites studied included central venous catheters, lung, nose, skin, and urinary tract.

Each of the 51 animal studies was subjected to an assessment of risk of bias. 17 studies (33%) had a high risk of bias primarily due to the dearth of specific reporting methodology (eg, the method and use of randomisation or blinding; appendix pp 19–21). The remaining 34 studies (65%) were classified with a moderate risk of bias (appendix pp 19–21). Of the five studies reporting clinical application of phages in humans, four were case reports and one was a randomised controlled trial with incomplete information, which were not applicable to risk assessment. We also reviewed the included studies for the sex factor (results available in the appendix p 7).

47 published studies addressed the use of phages for decolonisation of specific bacteria in the gastrointestinal tract of animals (table 1).[21–23,26–28,31–71] The target bacteria were *Salmonella* spp (n=20), *E coli* (n=10), *Campylobacter* spp (n=10), *K pneumoniae* (n=4), *Shigella* spp (n=1), *Streptococcus mutans* (n=1), and the combination of *E coli* and *Enterococcus faecalis* (n=1). In 14 studies, a single phage was used, whereas six studies used two, 11 studies used three, and 15 studies used four or more phages. The remaining study used a cocktail but did not specify the number of phages included. Of the studies using at least two phages for decolonisation, 31 administered a cocktail of phages and two used phages in a two-step tandem approach. In most studies (n=42), phages were administered orally—ie, via gavage, as a supplement to the basal diet or drinking water. A combination of oral and rectal routes was used in two studies, whereas cloacal administration (for chickens) or intraperitoneal injection was used in one study. In addition, an oral swab containing a phage suspension was used in one study against *S mutans* colonisation of teeth. The used dose of phages varied widely ranging from 10^5 to 10^{11} plaque forming units (PFU) per animal. In all studies using murine models (n=14), ten described the specific dose of phages at 10^7 to 10^9 PFU per animal. However, one of the ten studies also used a dose of 10^5 PFU per animal for a subgroup of mice. In other mammals (pigs or sheep, n=6), five studies specified phage doses, ranging from 10^9 to 10^{11} PFU per animal. In chicken models (n=26) and a quail model, phages were used in a much wider dose ranging from 10^5 to 10^{11} PFU per animal. Furthermore, the frequency and duration of phage administration in different studies varied remarkably from a single dose of a phage preparation to the addition of phages into animal basal diet for 8 weeks. The timing of phage administration ranged from immediate administration to 37 days after successful gastrointestinal tract colonisation of target bacteria.[26,28] The taxonomy of used phages was specified in 28 of the 47 studies. Of studies using at least two phages (n=34), 14 used phages of different viral families and eight used phages of the same family; the taxonomy of used phages was not specified in the remaining 12 studies (appendix pp 22–25). As for efficacy, only one study reported that a phage cocktail comprising three lytic phages (without specifying the taxonomy) was used to eliminate the target bacteria (*Salmonella*) from the gastrointestinal tract;[35] 36 (77%) studies reported a statistically significant reduction of target bacteria in the gastrointestinal tract after phage administration. Among these 36 studies, four used phages in combination with other measures: probiotics in two, penicillin in one, and a product consisting of a defined culture of seven microbial species for competitive exclusion in one.[38–40,63] In five studies, phages showed effectiveness in reducing bacteria colonisation in a short timeframe, but the target bacteria recurred in the gastrointestinal tract during follow-up.[27,40,41,57,63]

We found two studies involving the use of phages targeting nasal colonisation of meticillin-resistant *Staphylococcus aureus* (MRSA).[72,73] Two phages (P68 and K*710) were administered to piglets via an intranasal drip after MRSA colonisation daily for 5 days, and the abundance of MRSA was measured daily for 7 days.[73] No significant differences were observed during or after phage administration ($p>0·05$, ANOVA). In the other study,[72] a Myoviridae family phage (MR-10),[74] mupirocin, or both in combination was administered by an intranasal drip after MRSA colonisation in female murine models. Compared with that in the untreated group, mice administered phages twice (at an interval of 24 h) showed reduced bacterial counts ($p<0·01$) of 2·8 log colony-forming units (CFU)/g on day 2 and 1·14 log CFU/g on day 7. By contrast, MRSA was completely eradicated from the nasal tissue in mice receiving the phage and mupirocin combination on day 5.

One study described the use of phages against female murine skin colonisation by meticillin-susceptible *S aureus* strain ATCC 25 923.[75] When Myoviridae phage pSa-3 was administered to mice topically for 1 day, no significant reduction was seen in bacterial load on the murine skin. When the phage administration was extended to 3 or 5 days, the bacterial load on murine skin was significantly reduced (approximately 9×10^6 *vs* approximately 3×10^6 CFU/mL, $p=0·013$). Phage application in combination with Tween 20, a surfactant able to disrupt bacterial aggregation, results in further reduction of bacterial loads (approximately $1·5 \times 10^6$ CFU/mL) compared with the use of phage alone.[75]

Only one study described the use of phages with the aim to reduce bacterial intraluminal colonisation on central venous catheters in rabbit models.[76] Phage K is a polyvalent *Staphylococcus* phage of the Myoviridae family and can lyse nine *Staphylococcus* species. Phage K was administered to female rabbits installed with central venous catheters inoculated with meticillin-susceptible *S aureus*. Compared with that in the control group, a catheter lock solution consisting of phage K (0·3 mL of 10^8 PFU/mL) residing for 24 h significantly reduced bacterial colonisation ($1·2 \times 10^5$ *vs* $7·6 \times 10^3$ CFU/cm^2, $p=0·016$) and biofilm formation (5/5 *vs* 1/5 in

	Animal	Target bacteria	Colonisation site	Phage administration			Adverse events
				Phage number	Dosage and duration	Route	
Pelyuntha et al (2022)[35]	Chicken	*Salmonella*	GI	3	10^9 PFU qd for 5 d	Gavage	No; these did not cause any cytotoxicity to human fibroblast cells or Caco-2 cells
Wagenaar et al (2005)[46]	Chicken	*Campylobacter jejuni*	GI	2	(0·2–4) × 10^{11} PFU (phage 71) qd for 4 d; (0·5–3) × 10^{10} PFU (phage 69) qd for 4 d	Gavage	No
Kim et al (2015)[53]	Chicken	*Salmonella*	GI	..	0·4 or 0·8 × 10^8 PFU/kg Cont for 8 w	In food	No; laying performance and egg quality were not affected
Federici et al (2022)[56]	Mice	*Klebsiella pneumoniae*	GI	5	1 × 10^9 PFU tiw for 9 d	Gavage	No
Titécat et al (2022)[57]	Mice	*Escherichia coli*	GI	7	1·4 × 10^8 PFU once for 1 d or 2 × 10^9-PFU bid for 15 d	Oral	No; long-term phage administration did not induce dysbiosis
Hyman et al (2010)[21]	Mice	*K pneumoniae*	GI	2	10^9 PFU/mL[a] (P39) for 7 d; 10^9 PFU qd (P24) for 3 d	Oral and rectal	No
Mai et al (2015)[23]	Mice	*Shigella*	GI	5	1·2 × 10^9 PFU once	Gavage	No; side-effects or distortions in the overall GI microbiome were not identified
Galtier et al (2016)[22]	Mice	*E coli*	GI	3	6 × 10^5 or 10^7 PFU once	Gavage	No; microbiome diversity was not directly affected by bacteriophages
Ahmadi et al (2016)[68]	Quail	*Salmonella*	GI	1	10^8 PFU qd for 3 d	Gavage	Possible; phage administration strongly affected ileal bacterial proportions
Lebeaux et al (2021)[77]	A patient	*Achromobacter xylosoxidans*	Lower airway	4	10^{10} PFU tid for 15 d	Bronchoscopy (d1); nebulisation (d2-d15)	No
Kim et al (2021)[78]	Patients	*E coli*	Urinary tract	1	..	..	No
Corbellino et al (2020)[25]	A patient	*K pneumoniae*	GI, urinary tract, ureteral stent	1	10^7 PFU q12h for 3 w	Oral and rectal	No; phage was well tolerated, and the patient did not experience adverse effects
Kvachadze et al (2011)[80]	A patient	*Pseudomonas aeruginosa*, *Staphylococcus aureus*	Lung	..	..	Nebulisation	No
Leszczyński et al (2006)[79]	A patient	*S aureus*	GI	3	7 × 10^8 PFU/mL tid for 4 w*	Oral	No
Kim et al (2020)[75]	Mice	*S aureus*	Skin	1	10^7-PFU bid for 3 or 5 d	Topical	No

d=day. h=hour. w=week. GI=gastrointestinal tract. PFU=plaque forming unit. bid=twice daily. Cont=continuously. Once=a single dose. q12h=every 12 h. qd=once daily. tid=three times daily. tiw=three times weekly.
*The specific dosage is not available.

Table 2: **Studies reporting the safety of phage administration for decolonisation**

presence of biofilm, p=0·048) on the surface of the central venous catheters.[76]

Four case reports and one clinical trial described the use of phages for bacterial decolonisation (table 2).[25,77–80] LBP-EC01, the first CRISPR-engineered phage cocktail, has completed a phase 1b trial, investigating lower urinary tract colonisation by *E coli*. 36 individuals were enrolled in this randomised double-blind study.[78] LBP-EC01 could reduce bacterial load by 2–3 log (100–1000-fold) in the urine compared with the results seen with a placebo.

A 57-year-old patient with Crohn's disease received custom-made phage therapy after detection of gastrointestinal and urinary tract colonisation with a carbapenem-resistant *K pneumoniae* (CRKP) strain. 10^7 PFU of a Myoviridae phage, vB_KpnM_GF, was administered orally every 12 h for 3 weeks. During the first 2 weeks, 10^7 PFU phages were also administered daily via the rectum. From 15 days after initiating phage therapy, CRKP was no longer detected by cultures of urine samples, rectal swabs, or ureteral stents, suggesting successful decolonisation.[25]

A 12-year-old boy with cystic fibrosis, who received a double lung transplant, showed colonisation with *Achromobacter xylosoxidans*, *Aspergillus fumigatus*, and *P aeruginosa* before transplantation and developed persistent lung infection and airway colonisation due to *A xylosoxidans*.[77] The patient was administered phage cocktails against *A xylosoxidans* by nebulisation three times daily for two rounds. In the first round, a cocktail comprising three custom-made lytic phages (taxonomy unknown) for 2 days did not eradicate *A xylosoxidans*, and therefore, in the second round, a fourth lytic phage (taxonomy unknown) was added into the cocktail, which was instilled in each pulmonary lobe by bronchoscopy followed by nebulisation three times daily for 14 days. *A xylosoxidans* was no longer detected 6 months after the administration of phages was stopped.[77] However, the possibility that the disappearance of *A xylosoxidans* resulted from the improvement of host factors such as mucous immunity rather than the effects of phages cannot be excluded.

In a case report,[79] a health-care worker developed a urinary tract infection due to MRSA, and the gastrointestinal tract was identified as the source of the urinary tract infection as the bacterium was recovered from a rectal swab but not from the throat nor the nostrils. Three lytic phages of Styloviridae family were obtained by screening a panel of pre-existing phages in an institute for phage research to constitute a

cocktail, which was administered orally three times daily for 4 weeks. After 1 week of phage administration, MRSA was not detected from rectal swabs for the next 6 months.[79]

A 7-year-old girl with cystic fibrosis showed airway colonisation with *P aeruginosa* and *S aureus*. A commercially available cocktail Pyophage in combination with Sb-1, a staphylococcal phage of Myoviridae,[80] was administered five times using a nebuliser. The bacterial loads of the two bacteria decreased markedly but remained detectable, although at low levels (at approximately 10^3 to 10^5 CFU/mL for *S aureus* and 10 to 100 CFU/mL for *P aeruginosa*) after administration of phages. Notably, over the 2-month follow-up period, the *S aureus* counts in sputum samples showed a steady decline and fell below detectable counts after 1 month. However, *P aeruginosa* counts remained relatively constant throughout the 12-month follow-up.

15 studies (ten animal studies and five clinical reports) using phages for decolonisation have specifically reported the safety of phage administration (table 2).[21–23,25,35,46,53,56,57,68,75,77–80] In these studies, phage administration was well tolerated with no immediate adverse health effects. In two studies, the administration of phage cocktails was shown not to perturb the bacterial community in a short time.[22,57] A study using a murine model also reported that long-term (15 days) phage administration did not induce dysbiosis of the gastrointestinal microbiome.[57] Conversely, one study reported that the oral use of a phage of Siphoviridae strongly influenced the proportion of several bacterial species in the ileal microbiome of quails.[68]

Discussion

In this systematic review, we evaluated published data on the safety and efficacy of phages for bacterial decolonisation. We included 56 studies comprising five clinical reports and 51 animal studies. During the revision of this review, we found three additional relevant studies on this topic that were published since May 13, 2023, and all used phages for murine gastrointestinal tract (appendix p 7).[81–83] In most published studies, including the three recently published ones,[81–83] the load of target bacteria at the colonised site decreased after phage administration, underscoring the potential of phages to be used for bacterial decolonisation to prevent infections in both humans and animals. However, the amount of reduction in bacterial load varies markedly across studies, and only few studies have reported the eradication of the target bacteria. This finding suggests that the use of phages for bacterial decolonisation needs to be improved.

First, the efficacy of phages depends on several factors such as the host spectrum, lytic activity of phages, their number, dosage, route, timing, frequency, duration of phage administration, any concomitant measures, bacterial load, and propensity of developing phage resistance of the target bacteria. In non-emergency situations, phages and their interaction with target bacteria need to be well characterised for establishing an optimal approach and strategy for decolonisation before administration of phages. Unfortunately, such critically important data are largely absent or incomplete in many published studies.[38,40,53,54,71]

Second, owing to the rapid emergence of resistance in bacteria after exposure to phages, the use of a single phage for decolonisation in studies[21,27,52,77] is not an optimal approach. Cocktails comprising multiple phages might delay the emergence of phage resistance and could, therefore, enhance the effect for decolonisation.[21,52] However, combining multiple phages of the same species or of the same genus is unlikely to expand the host spectrum nor enhance killing and can lead to non-optimal cocktails. We noticed that many studies used cocktails without specifying the taxonomy of the included phages and might contain abundant phages,[40,57,63] which could be a factor for a compromised effect or recurrence of bacteria after phage administration for decolonisation.[41,57,63] Nonetheless, the occurrence of phage resistance appears to be inevitable,[84,85] representing a major bottleneck of phage therapy or administration for decolonisation. In addition, multiple pathogenic bacteria can colonise at the same site and, as aforementioned, the risks of bacterial colonisation might be continuously present, hindering successful decolonisation.[61] Therefore, more naturally occurring phages against major target bacteria should be identified in advance to generate a large phage bank, enabling high-throughput screening, and to construct an optimal working cocktail. Alternatively, phages could be freshly isolated using target bacterial strains, including mutants, which have developed resistance to previously administered phages in a timely manner or phages could be modified against major target bacteria in advance to expand the host spectrum and overcome resistance to the original phages. Phage resistance that has emerged in decolonisation might also compromise or diminish its efficacy of treating infections caused by the target bacteria. Therefore, phages or phage cocktails used for decolonisation should differ from those for therapy.

Third, in addition to phage resistance, the mucosal layer is spatially heterogeneous and provides spatial refuges for target bacteria.[86] The presence of mucins, glycoproteins, lipids, and DNA molecules on mucosal layers can limit phage diffusion to the mucus and result in the uneven spatial distribution of phages in mucosal tracts, such as the gastrointestinal tract.[86,87] This area represents a major challenge for the use of phages for decolonisation. Nonetheless, approximately 25% of sequenced tailed dsDNA phages (Caudovirales) encode immunoglobin-like proteins, which can enhance phage adhesion to mucus membranes by binding to mucin glycoproteins, thus increasing the chance of preying on the target bacteria.[88] As such, use of phages containing immunoglobin-like proteins might elevate the decolonisation efficacy. In addition, several studies have used phages combined with other countermeasures such as probiotics, antimicrobial agents, surfactants, and vaccines.[23,39,40,51,63,75,77] Such combinations of phages and other measures warrant further studies to evaluate the effect

of each individual measure and as a combined countermeasure to develop the optimal approach with maximum efficacy and safety.

Fourth, although oral is the most common route for phage administration, phages might not be able to tolerate gastric acid, resulting in an attenuated effect. The use of microencapsulated phages, as seen in several studies,[26,66,67] might aid decolonisation. The addition of agents to neutralise gastric acid, such as $CaCO_3$, to protect phages in the stomach could be another approach.[31,48,52] In addition, several studies have shown that surfactants can inhibit bacterial self-aggregation and even disrupt bacterial biofilms,[89,90] thus enhancing the decolonisation efficacy of phage therapy.[75]

15 studies also reported the safety of phage administration.[21–23,25,35,46,53,56,57,68,75,77–80] In general, phage administration for decolonisation is well tolerated, with no obvious adverse reactions reported, consistent with findings of phage therapy for bacterial infection studies.[24,91] However, phages were mostly administered orally in the studies, and data on other routes of administration are largely lacking.[21–23,25,35,46,53,56,57,68,75,77–80] In addition, indicators used for evaluating phage safety in published studies are inconsistent and unreliable. Several studies have only reported that health-related adverse effects were not observed or immediate tolerance was acceptable after phage therapy, without providing data on more indicators such as blood-related parameters and gastrointestinal tract microbiome.[25,46] As the use of phages is increasingly described in the literature, an approach towards standardised reporting with a set of indicators needs to be established for comprehensive, rigorous examination of safety. Furthermore, at least one study has described the alteration in the proportion of some bacteria of the gastrointestinal tract microbiome after long-term administration of phages for bacteria decolonisation.[68] This finding might not be surprising as when one bacterium decreases in quantity or is eradicated, other bacteria could occupy the resulting space with an increased load, which could have a stronger effect on the wider microbiome ecosystem. Nonetheless, the long-term impact of phage administration on commensal microbiome warrants further studies.

This systematic review has some limitations. First, few studies on this topic are available. Most available studies were case reports or animal studies with small sample numbers and moderate to high risks of biases (for animal studies), resulting in a lower quality of evidence that hinders drawing robust conclusions. Moreover, many unsuccessful attempts in phage decolonisation might not have been published, resulting in publication bias. Second, the included studies are remarkably heterogeneous in many aspects such as the number and type of phages used; the target bacterial species or strains; the colonisation site; the administration dosage, route, frequency, and duration; and the animal type. Considering the heterogeneity, we were unable to perform a meta-analysis. Third, there are few human reports, and the only clinical trial on this topic consists of a small sample size and is presented only as a poster without detailed data currently available. For each type of animal model tested, the number of studies is also less. In addition, most phage decolonisation studies were performed in poultry or livestock targeting a specific set of gastrointestinal tract pathogens such as *Campylobacter*, *Salmonella*, and O157-type *E coli*. This application can protect animal husbandry from devastating bacterial infections and is likely to reflect the interest of replacing antibiotics.[92] This application could also prevent bacterial infections from entering the food chain to cause food-borne diseases and outbreaks and therefore has implications from the One Health perspective.[92] In contrast, some studies were performed in mice, typically targeting clinically significant bacterial pathogens such as *K pneumoniae* and particular *E coli* strains causing human diseases or colonisation, aiming for later clinical applications. However, researchers should consider that humans and mice have different gastrointestinal tract microbiomes[93] and that the decolonisation effects in mice might not be generalisable to humans. In addition, the application of phages in humans typically requires higher standards for preparation, incurs increased financial costs, and is subjected to stricter regulations,[94] representing a more difficult challenge than that observed in animals.

Despite the limitations and concerns outlined, we believe that this systematic review provides a much needed overview regarding the application of phages for decolonisation as a preventive approach to combat transmission of infections, in addition to therapy for bacterial infections. The summary of currently available studies highlights that phages could become an option for decolonising pathogenic bacteria or at least significantly reducing their loads to minimise risks of developing subsequent invasive infections for the host and mitigate transmission that causes infections for others. However, the use of phages for decolonisation is still understudied, and many challenges remain to be overcome. Compared with antimicrobial agents, phages typically have a narrower host spectrum but are more prone to induce resistance in shorter time.[19] The development of phages for decolonisation applications might require a more precise medicine-like approach and should consider individual-based factors such as the target bacterial strain and its interaction with potential phages, the emergence of resistance and the corresponding countermeasures, the colonisation site, and the host status. In future studies, investigators might need to perform more assays with rigorous examinations before introducing applications to hosts, improve design, enrol more participants, and identify which hosts would benefit most from phage therapy. To improve research quality and comparative analysis across studies, future studies of phage decolonisation should provide essential information about the host, the target bacteria, the phages used, the route of phage administration, the efficacy, and their safety (appendix pp 7–8). Conversely, decolonisation is a measure addressing a lagging scenario as bacterial

colonisation has already occurred. A better strategy would be to prevent such colonisation in advance, which would broaden phage applications and represent a new area warranting further study.

The use of phages for decolonisation is generally safe with no obvious adverse reactions seen on the basis of currently available data. Given the global threat posed by bacteria that are difficult to treat and the significance of colonisation in subsequent infection and pathogen transmission, phages might be a potentially effective alternative targeting decolonisation of bacteria, an approach that warrants further studies and rigorous evaluation.

Contributors

ZZ supervised the project. ZZ and AM designed the outline. QF and YH formulated the search strategy and pooled the literature. QF, YH, XY, YF, LZ, and HL screened the titles and abstracts. QF, YH, XY, and YF assessed the full text of included studies. QF, YH, XY, YF, GY, AM, and ZZ analysed and interpreted the data. QF, YH, XY, YF, and GY drafted the manuscript and verified all the data reported in the study. ZZ and AM revised the manuscript. All authors approved the final version.

Declaration of interests

We declare no competing interests.

Data sharing

All data supporting the finding of this study are in the manuscript and its appendix file.

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