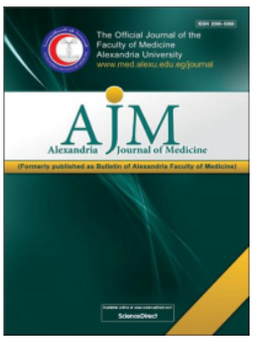

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REVIEW ARTICLE

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A review of phage mediated antibacterial applications

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ABSTRACT

Background: For over a decade, resistance to newly synthesized antibiotics has been observed worldwide. The challenge of antibiotic resistance has led to several pharmaceutical companies to abandon the synthesis of new drugs in fear of bacteria developing resistance in a short period hence limiting initial investment return. To this effect, alternative approaches such as the use of bacteriophages to treat bacterial infections are being explored. This review explores the recent advances in phage-mediated antibacterial applications and their limitations.

Methods: We conducted a comprehensive literature search of PubMed, Lib Hub and Google Scholar databases from January 2019 to November 2019. The search key words used were the application of bacteriophages to inhibit bacterial growth and human phage therapy to extract full-text research articles and proceedings from International Conferences published only in English.

Results: The search generated 709 articles of which 95 full-text research articles fulfilled the inclusion guidelines. Transmission Electron Microscopy morphological characterization conducted in 23 studies registered Myoviruses, Siphoviruses, Podoviruses, and Cytoviruses phage families while molecular characterization revealed that some phages were not safe to use as they harbored undesirable genes. All *in vivo* phage therapy studies in humans and model animals against multidrug-resistant (MDR) bacterial infection provided 100% protection. *Ex vivo* and *in vitro* phage therapy experiments exhibited overwhelming results as they registered high efficacies of up to 100% against MDR clinical isolates. Phage-mediated bio-preservation of foods and beverages and bio-sanitization of surfaces were highly successful with bacterial growth suppression of up to 100%. Phage endolysins revealed efficacies statistically comparable to those of phages and restored normal ethanol production by completely eradicating lactic acid bacteria in ethanol fermenters. Furthermore, the average multiplicity of infection was highest in *ex vivo* phage therapy (557,291.8) followed by *in vivo* (155,612.4) and *in vitro* (434.5).

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Antibiotics resistance; bacteriophages; phage therapy; phage mediated biocontrol; phage efficacy

1. Background

Currently, the world populace is deemed to be at a great risk as a result of the ever-escalating prevalence of antibiotic resistance bringing about an epoch where many familiar bacterial infections are becoming increasingly hard to treat [1]. Similar to many other developing countries, Sub-Saharan Africa is experiencing an elevated burden of bacterial infectious diseases which calls for the overuse of antibiotics and consequently emergence of resistant microorganisms [1,2]. The development of antibiotic resistance is also contributed by self-medication with uncontrolled over-the-counter access to drugs without any guidance from qualified medical practitioners. In addition, there is excessive application of antibiotics in poultry, aquaculture, and livestock production. The unrestricted access and use of antibiotics for animal disease treatment and prophylaxis as well as growth promotion have been implicated as one of the major drivers for antibiotic resistance that may spillover to humans [3–5]. Infectious food and water-borne illnesses are acquired through the consumption of contaminated food and water; and are the major cause of mortality and morbidity worldwide owing to their extensive and spontaneous transmission [6,7]. It was estimated that water, sanitation, and hygiene (WSH) associated infectious diseases are accountable for 4.0% of the worldwide deaths and 5.7% of the universal disease burden [7,8]. Furthermore, WHO reported that 600 million or 1 in 10 people fall ill worldwide as a result of foodborne infections and more than 91 million people affected are in Africa [6].

The rate at which drug resistance emerges has resulted in big pharmaceutical companies backing away from developing new antibiotics since the latter

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are rendered non-effective within a short period, making the venture not cost-effective [9]. Therefore, affordable alternative approaches such as the use of probiotics, phytomedicines, and bacteriophages to manage bacterial infections and control the emergence of antibiotic resistance are highly commendable.

Bacteriophages (phages) are natural enemies of bacteria which are the most abundant replicating entities on earth. Phages are viruses that specifically attack and multiply in bacterial cells and have no effect on other cell types. They are self-replicating and self-limiting as long as the specific bacterial host cells exist. Similar to other viruses, their genomes may either be double-stranded or single-stranded DNA or RNA [10]. Phages have either a lytic or lysogenic type of replication cycle. The lytic cycle, also referred to as the virulent cycle, results in the production of progeny viruses that are released through cell lysis. The lysogenic or temperate cycle results in the incorporation of the phage genome into the host chromosome without the production of new virus particles. Depending on some circumstances, some phages can exhibit both replication cycles [10]. Lytic phages are applied as bacterial growth inhibitors, which can be categorized as phage therapy or phage-mediated decontaminants. For therapy, phages are mainly used like antibiotics, whereas for decontamination, they are applied as disinfectants. Literally, phage therapy is the application of phages as therapeutic agents more especially in a clinical context to treat bacterial infections while phage-mediated biocontrol can be defined as the use of phages to suppress bacterial growth on non-living surfaces. Safety and efficacy of phage therapy or phage-mediated biocontrol relies on isolation and use of only professional lytic phages, which are obligately lytic or virulent but they are neither temperate nor directly linked to temperate phages [11]. Phage therapy is a proven eco-friendly alternative approach to prevent and control pathogenic bacterial infections [12,13].

Phages were used to treat bacterial infections in Europe during the pre-antibiotic era. However, with the discovery of antibiotics and the substandard medical trials conducted in the western world without putting into consideration that phages were specific, phage therapy was shortly after deemed impotent in the treatment of bacterial infections. Nevertheless, phage therapy continued to be used for the treatment of bacterial infections in the Soviet Union since 1940 [14]. The advantages of phage applications, such as disruption of bacterial biofilms and nondependency on the drug resistance status of the organisms, have rekindled their use as antibacterial agents [15,16]. Furthermore, renewed attention to phage therapy has been registered due to an overall decline in the total reserves of effective antibiotics. Hence, phage therapy clinical trials and experiments in poultry, aquaculture, crop husbandry, model animals, *in vitro* model systems, and humans have been widely carried out [17,18]. Currently, the notable human phage therapy under application is the compassionate use of phages as individualized therapeutic options to manage MDR bacterial infections unresponsive to all classes of conventional antibiotics [19]. Furthermore, phage preparations have been used and experimented with as diagnostic tools for bacterial infections to supplement the available methods [12].

For use as decontaminants, several studies have been conducted to evaluate the efficacy of phages as biocontrol agents against food and beverage borne pathogens [20]. Phages have been experimented with in biosanitization of equipment surfaces to eradicate biofilms in food industries [21]; and bio-preservation of perishable processed foods to increase shelf-life. Some phage-specific enzymes; such as lysins which degrade the cell wall of gram-positive bacteria, have been applied to processed foods to enhance their safety for human consumption [18,22–24]. The use of bacteriophages in food products in the US, Europe, and Australia has been reported [25]. Indeed, some phage preparations have been approved in the USA and are commercially available; such as LISTEX P100; LMP-102^{TM}, Listshield^{TM}, ECP-100^{TM} (Ecoshield^{TM}), SALMONELEX^{TM}, AgriPhage^{TM}, and Biophage-PA [26].

This review expounds on the current level, limitations, and prospects of phage applications such as enhancing food safety and fermentation of biofuels; phage therapy clinical trials and experiments in humans and model animals; animal and plant disease control and environmental bioremediation.

2. Methods

2.1. Literature search strategy

A comprehensive literature search of PubMed, Lib Hub, and Google Scholar databases was conducted from January 2018 to November 2018. The search key words used were “application of bacteriophages to inhibit bacterial growth” and “human phage therapy,” Figure 1.

3. Study selection criteria

The search targeted articles published in English without restriction on year of publication in an attempt to capture all available literature about the application of phages as antibacterial agents worldwide, Figure 1. In addition, only full-text research articles and proceedings from the International Conference on Prevention & Infection Control were selected, Table 1–5, S1. Review articles were excluded from this search. To avoid bias, all the seven coauthors were involved in the selection process. Articles were assigned to the different coauthors blindly, review reports on the

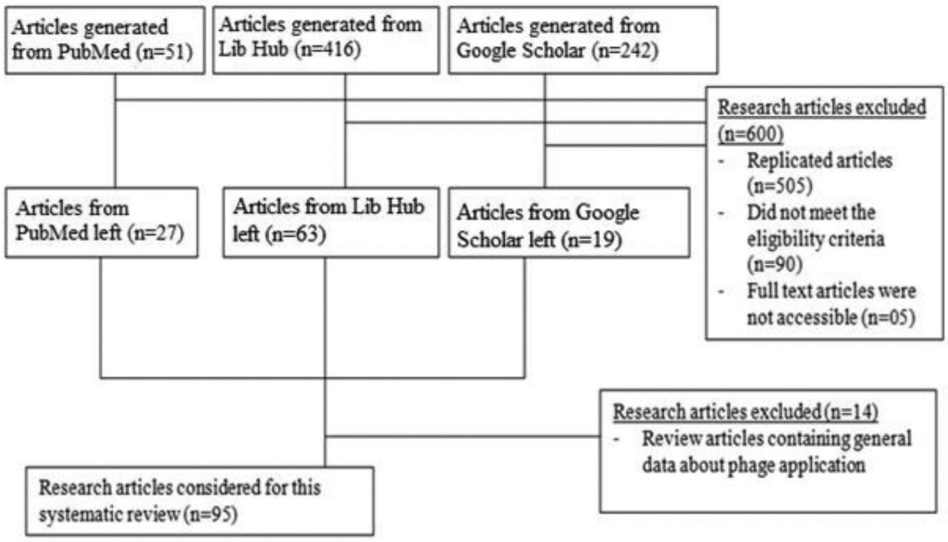

Figure 1. Selection process of research articles for inclusion in this review.

Table 1. *In vivo* human phage therapy trials.

Phage therapy in humans	Phage type	Source of phages	Pathogens targeted	Serovar/ pathotype	efficacy	Ref
Treatment of diabetic toe ulcers	Staphylococcal phage Sb-1	Eliava Institute	*S. aureus (MRSA and MSSA)*	-	100%	[93]
Treatment of GIT MRSA infection	polyvalent *S. aureus* bacteriophages	L. Hirszfeld Institute collection	*S. aureus (MRSA)*	-	100%	[94]
Treatment of burn infections	-	J. Soothill	*P. aeruginosa*	-	100%	[95]
Treatment of infected venous stasis ulcers and other poorly healing wounds	Pyophage in PhagoBioDerm films	Eliava Institute	*P. aeruginosa, E. coli, S. aureus, Proteus, and Streptococcus*	-	76%	[49]
Treatment of corneal abscess and interstitial keratitis	*S. aureus* bacteriophage SATA-8505	ATCC	VRSA	-	100%	[96]
Treatment of burn wound infection	Cocktail of *P. aeruginosa* phages 14/1 (*Myoviridae*) and PNM (*Podoviridae*) and *S. aureus* phage ISP (*Myoviridae*)	Merabishvili et al 2009	*S. aureus and P. aeruginosa*	-	0%	[69]
Treatment of chronic otitis antibiotic-resistant *P. aeruginosa* Infection	Biophage-PA	NCIMB	MDR *P. aeruginosa*	-	80%	[97]
Treatment of *P. aeruginosa* UTI	PA Phage cocktail (Pyophage #051007)	Eliava Institute	MDR *P. aeruginosa*	-	100%	[98]
Treatment of acute bacterial diarrhea	T4-like coliphages cocktail	Microgen-Russia	*E. coli*	-	0%	[70]
Treatment chronic bacterial prostatitis	IIET bacteriophage collection	IIET bacteriophage collection sewage, environmental, or drinking water	*Enterococcus faecalis*	-	100%	[99]
Phage safety analysis	Phage cocktail Coli Proteus	Microgen Russia	*E. coli and proteus*	-	-	[30]

merits and demerits of the studies as per inclusion criteria were submitted to the lead researcher (JLN) and the entire selection process was conducted based on the review reports by all the seven coauthors. In case of any disagreement, powers were entrusted to the most experienced researchers in bacteriophages (JLN, DKB, and FE) to make the final decision.

4. Data extraction

A database was created in which the field of phage application, type of phage or phage part used, source of phages, level of phage application, type of bacteria and strain or serovar challenged, level of phage efficacy, physiochemical properties of phages, the multiplicity of infection (MOI) of phages and methods used in the characterization of phages were included. Studies where MOIs were not reported but the number of plaque-forming units/mL (PFU/mL) and the number of colony-forming units/mL (CFU/mL) given, MOIs were computed by dividing the PFU/mL by CFU/mL units (O'Flynn et al., 2004). To compare the MOI of different investigations, all studies were grouped into three categories namely; *in vivo* phage therapy, *ex vivo* phage therapy, and in vitro phage therapy.

Table 2. Phage-mediated biocontrol of bacterial growth in ethanol fermentation, foods, and beverages.

Field of application	Phage/phage part used	Source of phages/part	Level of application	Bacteria type controlled	Bacteria serotype/Strain	Efficacy	Ref.
Ethanol fermentation	Streptococcal phage LambdaSa2 (λSa2) endolysin	EMD Biosciences, San Diego,CA)	Laboratory experiment	*Lactobacillus, staphylococci, and streptococci*		77.3%	[100]
	LysA, LysA2, LysgaY and λSa2 endolysin proteins	Subcloned into the pET21a E. coli expression vector					
Ethanol fermentation	Lytic enzymes LysA and LysA2	endolysins genes expressed in *Saccharomyces cerevisiae*	Laboratory experiment	*Lactobacillus fermentum, Lactobacillus brevis, and Lactobacillus mucosae.*		~ 90%	[101]
Ethanol fermentation	EcoSau and EcoInf	Wastewater influent	Laboratory experiment	*L. fermentum*		100%	[37]
Ethanol fermentation	ATCC® 8014-B1™ (phage B1) and ATCC® 8014-B2™ (phage B2)	ATCC	Laboratory experiment	*Lactobacillus plantarum ATCC® 8014™*	ATCC® 8014™	99%	[92]
Dairy (Cheese)	Phage P100	Dairy plant sewage effluent	Laboratory experiment	*L. monocytogenes*	WSLC 1001	100%	[39]
Dairy (Milk)	vB_SauS-phiIPLA35	Dairy environment	Laboratory experiment	*Staphylococcus aureus*		100% for	
cocktail	[102]						
Dairy (Milk) and control of *E. coli* biofilms	BECP2 and BECP6 phages	Sewage	Laboratory experiment	*E. coli*	O157:H7	90%	[103]
Dairy (Milk fermentation)	Coliphages DT1 and DT6	Feces	Laboratory experiment	*E. coli*	O157:H7 STEC	100%	[104]
Dairy (Cheese)	phage A511	-	Laboratory experiment	*Listeria monocytogenes,*		90%	[105]
Fruits (Cucumber, Apple, and Tomatoes)	T7 bacteriophages		Laboratory experiment	*Escherichia coli*	BL21	99.9%	[106]
Fresh-cut fruits and vegetables	LM-103 and LMP-102,	Intralytix, Inc. (Baltimore, Md.).	Laboratory experiment	*Listeria monocytogenes,*		99.9%	[107]
Dairy, poultry, beef products, sea food, and vegetables	A511 and P100	-	Laboratory experiment	*Listeria monocytogenes*	WSLC 1001 (serovar 1/2 c) and Scott A (serovar 4b)	100%	[108]
Beer industry	Myophage SA-C12	Fresh silage	Laboratory experiment	*Lactobacillus brevis*	8840 (NCIMB culture collection),	100%	[109]
Chicken cuts	FSP-1 and FSP-3/PSZ1 and/PSZ2		Laboratory Experiment	*Salmonella enterica*	Strain S49	92%	[110]
Spinach	-	Feedlot cattle feces	Laboratory Experiment	*Escherichia coli*	O157:H7	100%	[111]
Oysters	Siphoviridae phage pVp-1,	-	Laboratory experiment/trial on oysters	*Vibrio parahaemolyticus*	CRS 09–17	~99.999%	[112]
Fermented Soy bean paste	BCP1-1 and BCP8-2	Fermented food products	Laboratory experiment	*Bacillus cereus*	ATCC27348, ATCC21768, ATCC13061	100%	[113]
Bioactive packaging materials (meat and alfalfa seeds and sprouts)	LinM-AG8, LmoM-AG13, and LmoM-AG20, while the E. coli O104:H4 EcoM-HG2, EcoM-HG7 and EcoM-HG8 (Myoviridae)	Canadian Research Institute for Food Safety	Laboratory experiments	*Listeria monocytogenes and Escherichia coli*	*E. coli* O104:H4, LJH391 serotype 1/2b,	100%	[114]
Infant formula milk	ESP 1–3 and ESP 732–1	Sewage	Laboratory experiment	*Enterobacter sakazakii*	ATCC 29,544, 236/04, 732/03	100%	[115]
Infant formula milk	IeB, IeE and IeN	Slurry	Laboratory experiment	*Cronobacter sakazakii*	*C. sakazakii* ATCC BAA 894, C. sakazakii ATCC BAA 894 LUX	100%	[40]

(*Continued*)

Table 2. (Continued).

Field of application	Phage/phage part used	Source of phages/part	Level of application	Bacteria type controlled	Bacteria serotype/Strain	Efficacy	Ref.
Pork, milk, and kitchenware	fHe-Yen3-01 *(Podoviridae)* fHe-Yen9-01, fHe-Yen9-02, fHe-Yen9-03 (Myoviridae)	Sewage	Laboratory experiment	*Yersinia enterocolitica*	O:3 strain 6471/76 and O:9 strain Ruokola/71	100%	[41]
Milk, sausage, and lettuce	LPST10, LPST18, and LPST23(Siphoviridae family)	Waste water, sewage, farm ditch, poultry house	Laboratory experiment	*Salmonella strains Typhimurium and Salmonella Enteritidis*	*Salmonella* Typhimurium ATCC 14,028	64.1%	[116]
Active food packaging system (cellulose acetate films)	BFSE16, BFSE18, PaDTA1, PaDTA9,PaDTA10 and PaDTA11	Chickenfeces, poultry exudates, and swine feces	Laboratory experiment	*Salmonella enterica subsp.*	Enterica serovar Typhimurium ATCC 14,028	100%	[117]
Bioctive food packaging system	BFSE16, BFSE18, PaDTA1, PaDTA9, PaDTA10 and PaDTA11	Poultry exudates and swine feces	Laboratory experiment	*Salmonella enterica subsp.*	Enterica serovar Typhimurium ATCC 14,028.	~ 99.99%	[118]
Sea food (Cockles)	phT4A, ECA2	Sewage	Laboratory experiment	*Escherichia coli*	ATCC 13,706),	90%	[119]

Table 3. In vitro phage therapy against clinical isolates assays.

Field of application	Phage/phage part used	Source of phages/part	Level of application	*Bacterial targeted*	Strain/serovar/pathotype	Level of efficacy	Ref.
Phage activity against STEC and EHEC clinical isolates	CA911, CA933P, MFA933P and MFA45D	Minced meat, pork sauasage & bovine feces.	Laboratory experiment	*Escherichia coli*	STEC and EHEC	100%	[44]
Phage-Antibiotics synergism against *E. coli* biofilm	T4 bacteriophage ATTC 11,303-B4	LGC Standards, Middlesex, UK)	Laboratory experiment	*E. coli biofilms*	*E. coli* 11,303	100%	[120]
Phage activity against *Bacillus pumilus*	Phage FBa1, FBa2, and FBa3	River water	Laboratory experiment	*Bacillus pumilus*		100%	[121]
Phage activity against *Salmonella Typhimurium*	phSE-1, phSE-2, and phSE-5 (family *Siphoviridae)*	Sewage	Laboratory experiment	*Salmonella Typhimurium*	*Enterica* serovar *Typhimurium*	99%	[42]
Phage activity against *S. aureus* clinical isolates	Sb-1	-	Laboratory experiment	*Staphylococcus aureus*	-	100%	[122]
Phage activity against *Pseudomonas fluorescens* biofilms	Phage IBB-PF7A	sewage treatment plant	Laboratory experiment	*P. fluorescens* biofilms		91%	[123]
Phage activity against *SalmonellaTyphimurium*	P22-B1, P22, PBST10, PBST13, PBST32, and PBST 35)	ATCC and Hankuk University of Foreign Studies	Laboratory experiment	*SalmonellaTyphimurium*	KCCM 40,253, ATCC 19,585, ATCC 19,585, and CCARM 8009.	54%	[124]
Phage activity against *Staphylococcus aureus* biofilm	Phage DRA88 and SAB4328-A	Sewage	Laboratory experiment *and ex vivo*-burn models	*Staphylococcus aureus biofilm*	RN6390B, RN6911, B4328, MSSA 3, MSSA 10, MRSA 82	Reduced biofilm formation	[125]
Phage activity against *Pseudomonas aeruginosa* biofilm	DL 52, DL 54, DL 60, DL 62, DL 64, and DL 68	Crude sewage	Laboratory experiment	*P. aeruginosa biofilm*	PAO1, PA45311, PA45291, PA45235	95%	[35]
Phage activity against *Staphylococcus aureus* biofilm	DRA88 and phage K	Crude sewage	Laboratory experiment	*Staphylococcus aureus biofilm*	1598, MRSA 252, & H325	100%	[36]
Phage therapy against *Staphylococcus aureus*	phage K and phage 92	ATCC	Laboratory experiment	MRSA/MSSA	MRSA (N315, COL, Mu50) ATCC 6538	100%	[126]
Ex vivo Phage activity against catheter MRSA biofilm	phage K	ATCC	Laboratory experiment (Catheter)	MRSA biofilms	*Staphylococcus aureus* 46,106	99%	[127]
Ex vivo Phage activity against catheter *Proteus mirabilis* and *Escherichia coli* biofilms	Escherichia coli T4 phage ATCC 11,303-B4	Bacteriofag coli-proteic, Microgen Pharma, Russia	Laboratory experiment (Catheter)	*Proteus mirabilis* and *E. coli* biofilms	*E. coli* ATCC 11,303 and *P. mirabilis* 13 HER1094	99.99%	[128]
Phage activity against *Pseudomonas aeruginosa* biofilm	-	Hospital environmental dirt, sewage disposal, and cattle waste effluents	Laboratory experiment	*P. aeruginosa* biofilms	-	50%	[129]
Phage therapy against *Staphylococcus aureus*	P128 proteins	Inducible T7 expression system in E. coli ER2566 strain	Laboratory experiment	*Staphylococcus aureus*	BK#13,725, BK#9894, BK#13,993	99.99%	[130]
Phage activity against *K. pneumoniae* biofilms	KPO1K2 and NDP, depolymerase, and nondepolymerase producing phages	-	Laboratory experiment	*K. pneumoniae biofilms*	B5055 (O1:K2)	Significant eradication	[131]
phage activity against MRSA & MSSA	.	Hospital environmental dirt, sewage disposal, and cattle waste	Laboratory experiment	*MRSA and MSSA*		100% for MSSA and 78% MRSA	[132]
Phage activity against MDR *Acinetobacter baumanni*	Phage AB2	Sewage	Laboratory experiment	MDR *Acinetobacter baumanni*	*A. baumannii* M3237	99.90%	[56]
Phage activity against MDR *P. aeruginosa*		Sewage	Laboratory Experiment	MDR *P. Aeruginosa*	-	100%	[133]
Phage therapy assay against *Pseudomonas aeruginosa* and *Staphylococcus spp* clinical isolates	Intesti and Pyobacteriophag	Eliava BioPreparations, Tbilisi, Georgia	Laboratory experiment	*P. aeruginosa* and Staphylococcus spp.	-	100%	[134]
Phage bacterial lytic activity against resistant *S. aureus*	SA11 (Siphoviridae family	Hankuk University of Foreign studies	Laboratory experiment	***Resistant S. aureus***	ATCC 13,301 and CCARM 3080	99.99&	[135]
Phage bacterial lytic activity against *S. aureus* biofilms isolated from orthopedic Implant	StaPhage (Myoviridae)	AusPhage Pty Ltd and sewage water	Laboratory experiment	*Staphylococcus aureus* biofilms	ORI16C02N and ORI16025	98%	[136]
Phage activity against *P. aeruginosa* biofilms	*P. aeruginosa* phage M4	Health Protection Agency, Colindale, United Kingdom	Laboratory experiment *(In vitro* model system)	*Pseudomonas aeruginosa* biofilms	M4	99.9%	[137]

Table 4. *In vivo* and *ex vivo* phage therapy experiments in animal models/tissues, fish, plants, poultry, piggery, and bees.

Field of application	Phages	Source of phages	Level of application	Target bacteria	Target bacteria strain/ pathotype	Level of efficacy	Ref.
Phage therapy in model organisms							
Treatment of *P. aeruginosa* infection in insect	PA5oct and KT28	Natural wastewater treatment plant	*In vivo*-insect model	*P. aeruginosa*	PA PAO1 and 0038	93.60%	[138]
Treatment of *Burkholderia pseudomallei* in mice	Phage C34	Sea water	*In vivo*-mouse model	*Burkholderia pseudomallei*	-	33.30%	[139]
Treatment of *S. aureus* infection in BALB/C mice	MR-10	-	*In vivo*-mouse model	*S. aureus*	ATCC 43,300 (MRSA) and ATCC 29,213 (MSSA)	100%	[140]
Treatment of *S. aureus osteomyelitis* in Rabbits	SA-BHU1, SA-BHU2, SA-BHU8, SA-BHU15 and SA-BHU21, SA-BHU37, SA-BHU47)	River, pond, and sewage	*In vivo*-Rabbit model	MRSA	-	100%	[58]
Treatment of GIT pathogenic *E. coli* infection in white rats	EHEC-specific coliphage	http://www.sumobrain.com/patents/wipo/Methodsbacteriophage-design/WO2010064044A1.pdf.	*In vivo*-mouse model	*E. coli.*	EHEC and non-EHEC *E. coli*	99.9%	[141]
Treatment of *A. baumannii* infection in Mouse Model	B C62 of *Myoviridae family*	Sewage water	*In vivo*-mouse model	Carbapenem resistant *A. baumannii*		100%	[34]
Treatment of *A. baumannii* pneumonia in BALB/c mice	vB_AbaM-IME-AB2 (IME-AB2),	Sewage	*In vivo*-mouse model	*A. baumannii* clinical isolates (MDR and sensitive)	-	100%	[59]
Treatment of *P. aeruginosa* keratitis in mice	ϕR18 and ΦS12-1	Sewage	*In vivo*-mouse model	*P. aeruginosa*	-	99.78%	[142]
Prevention of *V. cholerae* infections in mouse and rabbits	Vibrio phages ICP1, ICP2, and ICP3	Human feces	*In vivo*-mouse and rabbit models	*V. cholerae*	AC 53, AC2846, and AC4653	100%	[143]
Treatment of *S. Enteritidis* infection in *Caenorhabditis elegans* worms	ΦSP-1 and ΦSP-3	Chicken feces	*In vivo*-worm model	*Salmonella enteritidis*	S49	94.8%	[144]
Treatment of PDR *A. baumannii* infections in Mice and human cells	Abp1	Sewage	*In vivo*-mice model and *Ex vivo*-human HeLa cells	PDR *A. baumanni*	-	100%	[60]
Treatment of *Pseudomonas aeruginosa* skin infections	Phage PA709 characterized	Sewage water	*Ex vivo*-human skin	MDR *P. aeruginosa*	MDR *P. aeruginosa* 709	99.99%	[145]
Treatment of *K. pneumoniae* wound infections in BALB/c mice	phage Kpn5	Sewage	*In vivo* experiment	*Klebsiella pneumoniae*	B5055	100%	[146]
Crop protection							
Biocontrol of potato bacterial wilt	P-PSG-3, P-PSG-4, P-PSG-1, P-PSG-8 to P-PSG-12	water	Field trial	*Ralstonia solanacearum*	PS-X4-1, PS-X10-2, and PS-X13-1	80% *in vivo* and 98% *in vitro*	[147]
Biocontrol of alfalfa seeds spoilage Salmonella enterica	Phages SSP5 and SSP6	sewage	*In vitro* and *in vivo* laboratory experiments	*Salmonella enterica*	*S. oranienburg*	0%	[148]
Biocontrol of tomato bacterial wilt	RsPod1EGY	Soil	*In vitro* and *in vivo* laboratory experiments	*Ralstonia solanacearum*	K3, K9, K10, K11, K12, K16, K17, and K19	100%	[149]
Phage biocontrol of antibiotics resistant *Dickeya dadantii* which causes potato tuber rot	*Myoviridae family*	Caspian Sea water	Laboratory experiment/ field Trial	*Dickeya dadantii*		100% in *vitro* 88.9% for trial	[150]
Acquaculture							
Aquaculture	H20-*Siphovirus* and KVP40-*Myovirus*	Sea water	Laboratory experiment	*Vibrio anguillarum*	**(BA35 and PF430-3)**	Reduced biofilms	[151]

(*Continued*)

Table 4. (Continued).

Field of application	Phages	Source of phages	Level of application	Target bacteria	Target bacteria strain/ pathotype	Level of efficacy	Ref.
Phage therapy in model organisms							
Aquaculture (treatment of ulcerative lesions in catfish)	PA phages	Waste water	Field trial	Pseudomonas aeruginosa (MDR)	-	100%	[152]
aquaculture	VP-2 and VA-1 phage	Sewage water	Laboratory experiment and trial	*Vibrio*. anguillarum		100%	[13]
Aquaculture	FpV-4, FpV-9, and FpV-21	pond water	Laboratory Experiment	*Flavobacterium psychrophilum*		Reduced bacterial growth	[153]
Treatment of *Vibrio parahaemolyticus* shrimp infections	V. parahaemolyticus phages (Myoviridae family)	Shrimp pond water suspended sediment	Laboratory experiment/ trial	Vibrio parahaemolyticus	N1A and N7A	90%	[154]
Phage therapy in poultry							
In vivo phage therapy against Campylobacter spp. infections in broiler chicken	typelll phages NCTC12672, 12,673, 12,674, and 12,678 of the British phage typing scheme	Lohmann Animal Health, GmbH.	Field trial	*Campylobacter* spp.		99%	[155]
In vitro phage therapy against APEC	Phage ØEC1	Chicken feces	Laboratory experiment	*E. coli*	APEC O78:K80	99.99%	[156]
Phage therapy in piggery							
In vivo phage therapy of *Staphylococcus aureus* nasal infection in pigs	phage K*710 and P68	Novolytics Ltd	Trial using animal model	*Staphylococcus aureus* (MRSA)	ST398, spa type t011, SCCmec type V	0%	[71]
Phage therapy in apiculture							
In vivo phage therapy of American foulbrood caused *by Paenibacillus larvae*	Siphoviridae (HB10c2)	Glue-like liquid of a beehive	Laboratory experiment and field Trial	*Paenibacillus larvae*	ERIC I DSM 7030 and ERIC II DSM 25,430	2%	[43]

Table 5. Phage application in biosanitization.

Field of application	Phage	Phage source	Level of application	Target bacteria	Bacteria strain/pathotype	Level of efficacy	Ref
Water and sewage treatment							
Water purification	vB_AspP-UFV1 (Podoviridae)	Sludge of wastewater	Laboratory experiment	*A. soli, Pseudomonas sp.*, and *Brevundimonas sp.*	AO1-02, AO2-07, AO1-30, and AO1-33	Significant biofilm control	[157]
Coliform phage biocontrol in sewage	Coliphage (Myovirus and Podovirus)	River water	Laboratory experiment	*E. coli*	*E. coli* SBSWF27	95.40%	[158,159]
Biosanitization							
Hospital anitizer	Staphylococcal phage and Pyophage; GA,	Eliava Institute	Laboratory experiment	*S. aureus, E. coli*, and *P. aeruginosa*	*S. aureus* (SA2-R73), *E. coli* (EC-R60), and *P. aeruginosa* (PAV6).	90%	[86]
Hospital sanitizer	AB1, AB2, AB6, AB7 phages	Sewage or river water	Laboratory experiment and trial	*CR A. baumannii*	-	47.50%	[87]
Hospital sanitizer	Pyobacteriophag polyvalent	Research and Production Association "Microgen" (Russia))	Trial	*staphylococci, streptococci, enterococci, proteus, klebsiella* (*pneumoniae*, and *oxytoca), P. aeruginosa* and *E. coli*	-	100%	[88]
Phage cream and sanitizers	Polyvalent Anti-*Staphylococcus* Phage K	ATCC 19,685-B1	Laboratory experiment and trial	*MDR Staphylococcus aureus* (MRSA and VRSA)	-	100%	[89]
Use of phages in semi solid creams for control of *Propionibacterium acnes* growth	PAC1 to PAC10	*P. acnes* strains isolated from facial skin swabs	Laboratory experiment	*Propionibacterium acnes*	A1, A2, or E8	100%	[90]

5. Data analysis

Data analysis was performed using Tukey's multiple comparisons test in STATA version 2018.1 to establish whether; (a) the number of studies that reported *in vivo* human phage therapy efficacy of 100% was more pronounced than the number of studies that recorded efficacy lower than 100%, (b) phages are more efficient inhibitors of bacterial growth in ethanol fermenters than phage endolysins, (c) there is a considerable difference in *in vitro* phage therapy outcomes against different species of clinical bacterial isolates, (d) the outcomes of phage-mediated biocontrol in different fields are momentously dissimilar, (e) MOIs used for *ex vivo* phage therapy/phage-mediated biocontrol experiments, *in vivo* phage therapy and in vitro phage therapy are soundly similar. A P value of ≤ 0.05 indicated a significant statistical difference. For comparison of phage therapy and phage-mediated biocontrol efficacy across the different fields, only fields that had three or more studies reporting phage therapy efficacy in percentages were considered for Tukey's multiple comparisons test to prevent skewing of data.

6. Results and discussion

6.1. Literature search

A total of 709 articles were generated through an electronic database literature search conducted between January and November 2018. The databases were PubMed, Lib Hub, and Google Scholar, which yielded 51, 416, and 242 articles, respectively. Following the removal of duplications, 204 articles were screened on the basis of their titles and abstracts. Of the 204 articles; 90 did not meet the specified inclusion criteria; and five full-text articles were not accessible. Finally, 109 full-text articles were reviewed, of which 95 full-text research articles fulfilled the inclusion guidelines for this review, Figure 1. Studies included in this review were grouped into *in vivo* human phage therapy, *in vivo* phage therapy in model organisms, phages as biocontrol agents in biofuels fermentation, phages as biocontrol agents in foods and beverages, in vitro phage therapy experiments using clinical isolates, *in vivo* phage therapy in crop protection, application of phages as biocontrol agents in water purification, *in vivo* phage therapy in aquaculture, *in vivo* phage therapy in apiculture, *in vivo* phage therapy in a piggery *in vivo* and *in vitro* phage therapy in poultry, application of phages as bio-sanitizers, and in vitro use of phages as biocontrol agents in creams, Table 1–5, Figure 3.

Phage characterization; a prerequisite for phage-mediated biocontrol of bacterial growth and *in vivo* phage therapy

Phage-mediated biocontrol and phage therapy rely on the ability of lytic phages to infect bacterial host cells, hijacking the host metabolism and utilizing it to produce their progeny. As a result, the lytic phages lyse bacteria cells to release multiple phage virions which spread to infect other host cells [10]. Contrary to that, after infecting the bacterial host cells, lysogeny phages incorporate their genetic material into the host genome resulting in their permanent existence as prophages within host cells and all their offspring.

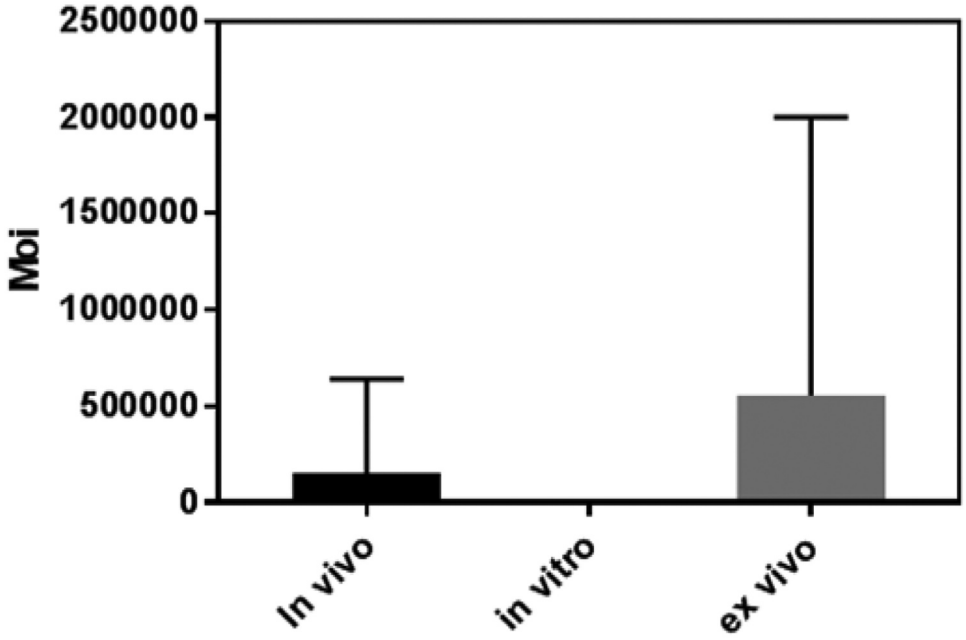

Figure 2. Comparison of mean MOIs between *in vivo*, *in vitro*, and *ex vivo* phage therapy. Tukey's multiple-comparison test was used to compute and compare MOIs *P* value of 0.0002 < 0.05 generated indicating significant variation between *ex vivo*/*in vivo* PT and *in vitro* PT.

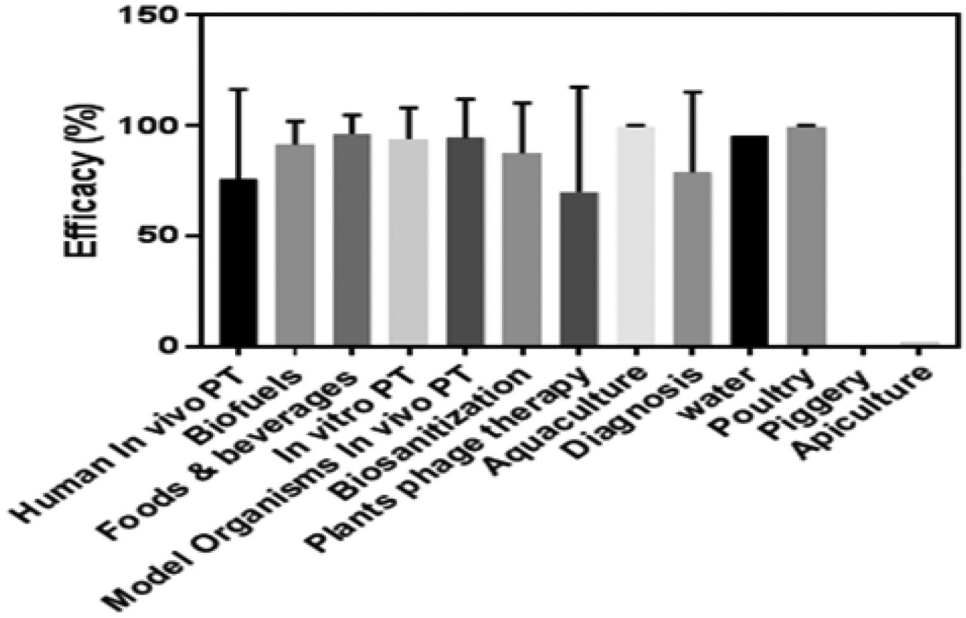

Figure 3. Comparison of phage therapy (PT), phage-mediated biocontrol/diagnosis mean efficacy percentages. Tukey's multiple-comparison test was used to calculate and compare the mean percentage efficacies generating a *P* value of 0.148 > 0.05 after exclusion of fields with less than three studies (water, piggery poultry, and apiculture).

Phages neither replicate into virions nor lyse bacteria throughout their lysogeny life time, hence called temperate phages [10]. Furthermore, the integration of the phage nucleic acids into its host bacterium protects the temperate phage genome and has the ability to modify the phenotype of the host bacterium cell [27]. Unfortunately, temperate phages might harbor toxin encoding genes, virulent genes, and genetic determinants of antibiotic resistance acquired from other bacterial hosts. Therefore, temperate phages may transform the phenotype of the host bacteria and all their progeny from avirulent/less virulent and antibiotic susceptible strains to highly virulent and antibiotic-resistant strains [28,29]. Appropriately professionally isolated and characterized phages must be used to prevent horizontal gene transfer of undesirable genes through phage-mediated biocontrol and phage therapy [18,30,31]. Therefore, phages must be characterized morphologically by TEM and SDS PAGE protein profiling to establish their families or if they are novel phages followed by molecular characterization by WGS to confirm their families and to detect any integrase, toxin, and virulent genes in addition to antibiotic resistance genes by cross-referencing with known phage genomes, virulent factors, toxin genes, and antibiotic-resistant genes libraries. A cheaper but less-sensitive alternative to detect the presence of known integrase gene, virulent factors (VF) and genetic determinants of antibiotic resistance in phages is PCR amplification using conventional integrase gene VF, toxin genes, and antibiotic resistance genes primers. However, PCR amplification has limitations as it will not detect any possible novel VF and antibiotic resistance genes harbored by phages hence making molecular characterization of phages by WGS a prerequisite prior to phage-mediated biocontrol of bacterial growth and *in vivo* phage therapy [32,33]. However, only 12.6% (12) of the studies included in this review conducted WGS. Bioinformatics analyses and annotation demonstrated that myophages BϕC62 [34], DL52, DL60 and DL680 [35], DRA88 and phage K [36], EcoInf [37], coliphagesϕAPCEc01, ϕAPCEc02 and ϕAPCEc03 [38], Phage P100 [39], leB, leE and leN [40]:, podophages DL54, DL 62 and DL 64 [35]:, fHe-Yen3-01 [41] and siphophages EcoSau [37], phSE-1, phSE-2 and phSE-5 [42], fHe-Yen3-01, fHe-Yen9-01, fHe-Yen9-02 and fHe-Yen9-03 [41] were safe to use since they harbored no undesirable genes while siphophage HB10c2 had a gene encoding a putative beta-lactamase like protein [43]. Additionally, PCR detected Stx I and II proteins encoding genes and lysogeny module genetic determinants in phages CB60P, MFA60N, CCO103, CBO103, and CCO113 [44], Table S1. If such phages are used in phage therapy and phage-mediated biocontrol, they can facilitate the horizontal flow of undesirable genes. This exorbitantly underlines the importance of screening phages using very sensitive tools like WGS. Nevertheless, only 36.8% (39) research articles included in this review attempted to characterize phages; 3.2% (3) used PCR to detect VFs and lysogeny modules while only 12.6% (12) studies carried out WGS to fully illustrate the phage genomes indicating that there is still a big gap in ensuring phage therapy safety as per all the reviewed articles that were in English, though all the phages used for *in vivo* human phage therapy were previously characterized by committed phage research hubs. Furthermore, the morphology of phages was

determined by transmission electron microscopy (TEM) in only 24.2% (23) studies. Basing on morphology, the phages belonged to various families as follows: Myoviridae; Siphoviridae; and Podoviridae in twenty, nine and ten studies respectively. Whereas, one study in each case reported phages as B1 morphology, Phage-like particle, and Cytoviridae family, Table S1.

7. Phage stability

Establishing the abiotic conditions affecting phage activity and/or viability was done in 16.8% (16) studies. This is an important criterion for selection since phage viability, occurrence, and storage are affected by temperature, pH, humidity, salinity, and other environmental conditions. Deviation from the favorable physicochemical factors can lead to the destruction of phages' structural elements, protein envelope, and loss of genetic material thereby inactivating the phages [45,46]. These phages are isolated from natural environments such as sewage, hospital, and animal farm effluents, water bodies, foods, and beverages and evaluated for *in vitro, in vivo,* and *ex vivo* phage therapy and phage-mediated biocontrol where the prevailing physicochemical factors are completely different, Table 1–5. Hence, the need to establish the optimum conditions for the highest phage efficacy. However, such drawbacks can be mitigated by isolation of phages from local geographical locations and similar hosts as for *in vivo* phage therapy accompanied by assessing phage stability via exposing them to different physicochemical factors. Furthermore, during the preparation of commercial phage-based remedy, physicochemical properties are supposed to be investigated as they determine the shelf-life of phages [47]. Despite that concern, only 9 (9.5%) and 7 (7.4%) out of 95 research articles included in this review evaluated the thermal and pH stability of phages, respectively, Table S1. This partly explains why some research articles reported very low or 0% phage efficacy in *in vivo* studies.

8. Specificity of phages

Specificity restricts phage infections to only certain bacteria with corresponding receptors to which they can bind; this determines the phage's host range [48]. For that reason, the application of phage therapy relies on an accurate characterization of all the strains, pathotypes, and serotypes of the target bacteria. Interestingly, if phage therapy overcomes the current obstacles hindering its approval universally, single phage and phage cocktail formulations must be designed indicating the pharmaceutical dosage and the phage host range for a given bacteria which calls for robust characterization of given target host bacteria. Conversely, this review identified gross deviation from the recommended procedure if meaningful phage therapy outcomes are to be achieved as only 55.8% (53) of studies reviewed attempted to use identified bacterial host strains, serovars, and pathotypes, Table 1–5. Worst still, no human *in vivo* phage therapy trial reported characterization of the target bacteria to their strains, pathotypes, and serotypes. Nevertheless, the spectrum and efficacy of phages can be enhanced by the use of phage cocktails. Phage cocktails also present another advantage of preventing phage resistance [49,50].

9. Multiplicity of infection (MOI)

MOI is defined as PFU/CFU ratio [51]. MOI is an imperative factor to be considered for prospective phage therapy application. Increasing the PFU/CFU ratio enhances the probability of phage particles infecting their host bacteria. Therefore, *in vivo* and *ex vivo* phage therapies require higher MOIs than *in vitro* phage therapy as it is harder for phages to locate and infect their hosts within living tissues, surface of foods, and other materials being infected by phages. Some studies recommend an MOI of over 100 for *ex vivo* and *in vivo* phage therapy and less than 10 for *in vitro* phage therapy [52]. This is in agreement with the studies incorporated in this review that reported MOI. The average MOI was highest in *ex vivo* experiments (557,291.8), followed by *in vivo* phage therapy (155,612.4) and *in vitro* phage biocontrol experiments had the lowest average MOI of 434.5 significantly different from *ex vivo* and *in vivo* MOIs, Table S1 and Figure 2. Contrary to this, other studies disregard the term MOI as it only describes the phage quantities administered during dosing in relation to the population of the target bacteria but does not put into consideration the fact that; some phages fail to penetrate tissues/materials and get inactivated before adsorbing to the host cells, the host cell population is liable to change before phage application, the bacterial population may not easily be determined in case of infections and physicochemical factors such as temperature, pH, salinity, and humidity may inactivate phages before adsorption. As a result, MOI input may differ from the actual effective MOI [53]. Furthermore, to increase the prospect of phages adhering and infecting their hosts; for experimental-induced infections a very high MOI of $>10^5$ is recommended [54] whereas *in vivo* phage therapy of natural infection a very high titer value of $> 1 \times 10^8$ PFU is appropriate as bacterial host cells are lysed by simply adsorption of phages before injection of their nucleic acids into the host cells and replication [52,54]. However, phages are immunogenic when applied at very high doses [55]; therefore, the host immune system may identify and inactivate them. Additionally, the MOI against biofilm infections should be higher as indicated by the studies reviewed which compared optimum MOI against bacterial suspension or free-living bacteria to that against biofilms and/or immobile bacteria, Table S1 and Figure 2. In *in vitro*

experiments, MOIs of 0.1, 1, and 10; and 100, 1,000, and 10,000 [56]; 0.1 and 10 [36] were administered against bacterial planktons and biofilms, respectively. It is worth mentioning that in addition to high MOI, the most suitable phages for phage-mediated management of biofilm infections should encode polysaccharide depolymerase which degrades the biofilm polysaccharide matrix to ease phage interaction with the host cells in the lower layers of the matrix [57].

10. Efficacy of phage therapy against drug resistant and sensitive bacterial infections and isolates

In vivo human phage therapy studies reported mixed levels of efficacy ranging from 0% to 100%. The mode and median efficacies were 100% while Tukey's multiple comparison test generated a P value of 0.009 < 0.05 indicating that phage therapy efficacies of 100% were more pronounced than efficacies lower than 100% in all the *in vivo* human phage therapy. Interestingly, efficacies of 100% were scored when treating MRSA diabetic foot ulcers, GIT MRSA infection, VRSA corneal abscess and interstitial keratitis, and MDR *Pseudomonas aeruginosa* UTI with phages. Furthermore, *in vivo* phage treatment of MRSA *osteomyelitis* in Rabbits [58], carbepenem resistant *Acinetobacter baumanii* infection in mice [34], MDR *Acinetobacter baumanii* pneumonia in mice [59] and pan drug resistant (PDR) *Acinetobacter. baumannii* infections in mice [60] provided 100% protection to model animals against the super bugs while *in vivo* phage therapy of MDR *Pseudomonas aeruginosa* ulcerative lesions in catfish species achieved 100% success. It is also worth noting that *ex vivo* phage therapy against MDR *Pseudomonas aeruginosa* skin infections, MRSA biofilms induced onto porcine skin burns, and PDR *Acinetobacter baumanni* human HeLa cells infections recorded overwhelming success. *In vitro* phage therapy against MRSA, MDR *Acinetobacter baumanni*, MDR *Pseudomonas aeruginosa* scored an inhibitory efficacy ranging from 78% to 100% with an average of 95.4%. Data from around the globe show an overall decline in the total reserves of antibiotics efficacy: resistance to all first-line and last-resort antibiotics is increasing [3]. For instance, in sub-Saharan Africa, India, Latin America, and Australia, MRSA incidence is still intensifying [3,6162], and estimated at 47% in India in 2014, and 90% in Latin American hospitals in 2013 [61][]. MRSA causes 35–46% of wound complication in Mulago referral hospital [63,64]. The increased prevalence of community acquired *E. coli* isolates coding for extended-spectrum beta lactamases competent of hydrolyzing approximately all beta lactams antibiotic except carbapenems has been reported globally [65]. In more than a decade, carbapenem resistance in *Enterobacteriaceae* bacteria has been observed yet Carbapenems such as imipenem, ertapenem, meropenem, and doripenem are the newest synthesized molecules with the broadest spectrum of activity and consequently considered the first-line therapy antibiotics in the treatment of multi-resistant gram-negative bacterial infections [66,67]. The magnitude of MDR *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is a great threat to the health sector worldwide [68]. The promising outcomes of *in vivo, ex vivo,* and *in vitro* phage therapy of MDR bacterial infections and isolates exhibit that phage therapy if employed appropriately is more effective than antibiotics and therefore can replace or supplement antibiotics as a routine in the management of both resistant and sensitive bacterial infections. However, limited success was attained when treating *S. aureus* and *P. aeruginosa* wound infection in humans, acute human *E. coli* infections, MRSA nasal infections in pigs and American foulbrood caused by *Paenibacillus larvae* [43,69–71]. This is in contrary to the *in vitro* experiments carried out in two of the studies where total eradication of the bacteria was achieved [43,71]. This can be attributed to the change in physiological conditions: loss of phage viability due to deviation from their optimum temperature and pH in unnatural environments [46].

11. Endolysins versus phage particles

Phages code tail spike proteins for identification and adhesion to receptors on the host cell surface. The tail spikes proteins are often incorporated with peptidoglycan hydrolases that locally hydrolyze the bacterial cell wall peptidoglycan, thus creating an opening for injection of phage nucleic acids which marks the initiation of the infection process [72]. An additional type of phage-derived enzymes; the peptidoglycan hydrolases called endolysins degrade the peptidoglycan liberating the progeny virions from the host cell at the end of the lytic phage cycle [73]. Gram-positive bacteria do not possess a shielding outer layer thereby making exogenous application of endolysins achieve speedy and effective lysis. This property makes endolysins promising possible alternative antimicrobial agents [23]. Several studies have reported endolysins as potential therapeutic agents with high efficacy and safety [74]. In addition, endolysins possess an added advantage over conventional antibiotics as; they exhibit great specificity exerting selective pressure on target pathogenic bacteria populations [75,76], emergence of resistance against endolysins is implausible given that phage (endolysins) coevolve with their host bacteria, the host receptor site where endolysins bind are highly conserved thereby making their alteration highly detrimental to the host bacterium [76,77]. Furthermore, endolysins degrade the cell wall externally without the burden of entering the bacterial cell hence evading the common antibiotic resistance mechanisms such as the active efflux pump and decreased membrane permeability [78]. A lot of ethical and safety concerns have been vehemently expressed about the use of live viruses as therapeutics in

the treatment of bacterial infections; currently, the immediate hope lies in the use of phage endolysins in the near future to combat the increasing antibiotic resistance. Fortunately, to meet the high demand, endolysins can be produced using recombinant DNA technology [79–81]. This review compared the use of phages and endolysins to suppress bacterial growth during ethanol fermentation. Phages demonstrated superior efficacy than recombinant phage endolysins with mean efficacy of 99.5% for phages and 83.6% for phage endolysins but not significantly divergent as revealed by one-way ANOVA (P value of 0.13 > 0.05). This clearly supports the use of phages and endolysins hand in hand as therapeutic agents.

12. Application of phages in Biosanitization and Biopreservation

Infectious food and water-associated diseases are the major causes of mortality and morbidity worldwide [6,7]. Irrational use of antibiotics in livestock has resulted in antibiotic resistance which spillover to humans through contaminated food, water, and environment [-3–5,67]. Fortunately, in 2006 the US Food and Drug Administration (FDA) approved the utilization of 6 independently purified LMP-102 phages as biopreservative antimicrobial agents in RTE meat and poultry products against *Listeria monocytogenes* [82]. In this review, the literature search yielded 21 (22.1%) research articles reporting foods and beverages phage-mediated biopreservation with average, mode, median efficacy of 96.5%, 100%, and 100%, respectively. In a water decontamination study, phages eradicated 95.4% of the coliform. This is a clear indicator of the potency of phages as biopreservative and bio-decontamination agents and consequently their approval to preserve food and decontaminate water following robust characterization should be considered to prevent transmission of antibiotic-resistant and susceptible food and water-associated infection.

Furthermore, the hospital environment polluted by infected patients with antibiotic-resistant bacteria is incriminated as the main route of transmission [83,84]. This has been a result of the emergence of bacterial resistance to the conventional disinfectants [83]. The possibility of a horizontal flow of mobile genetic elements encoding antibiotic resistance from clinical to environmental bacteria within the hospital is high hence advancing the evolution of new antibiotic-resistant bacterial strains [85]. On a good note, biodisinfection using phages as demonstrated by this review is promising: for instance, phage-mediated biosanitization eradicated 90% of *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* tainted on plastic, glass, and ceramic materials mimicking hospital surfaces [86] while phage-mediated sterilization trial of the ICU reduced the prevalence of carbapenem-resistant *Acinetobacter baumanii* by 47.5% [87]. In another phage sanitization trial, phages completely eliminated *staphylococci, streptococci, enterococci, proteus, Klebsiella pneumoniae, Klebsiella oxytoca, Pseudomonas aeruginosa,* and *Escherichia coli* from the hospital environment [88] while phage-based sanitization cream completely inhibited MRSA and *Propionibacterium acnes* growth [89,90]. With those laudable bio-sanitization results, the use of phages to complement conventional disinfection strategies could exhibit valuable outcomes.

13. Phages and endolysin as alternative antibacterial decontamination agents

Lactic acid bacteria (LAB) are by far the commonest bacterial contaminants of biofuel production facilities and are believed to hamper the ethanol fermentation process hence limiting ethanol production. Ethanol fermentation presents an environment of high ethanol concentration, low pH, and low oxygen concentration thereby favoring the growth of *Lactobacillus sp* which are well adapted to survive under such conditions. Currently, there is no appropriate strategy to combat ethanol loss due to LAB contamination as all possible measures have limitations [91]. Contrary to that, the four experimental studies which employed phages and endolysins to control LAB growth during ethanol fermentation analyzed in this review demonstrated eye-catching bacterial growth suppression outcomes with mean efficacy of 91.6%. Most importantly, phage and endolysins mediated ethanol fermentation facility decontamination restored normal ethanol yield without losing their viability [37,92]. Because of the promising results, to eliminate the use of antibiotics for decontamination in the ethanol fermentation business, phages and endolysins should be considered as alternatives.

14. Limitations

Hypothetically, all bacteria can be lysed by at least one type of bacteriophage. In the light of this, phages are considerably more efficacious than antibiotics. However, phage antibacterial applications have limitations. Most phages have demonstrated a broad spectrum hence can lyse both the target pathogenic strains and potentially beneficial bacterial strains. Additionally, it is difficult to isolate phages without any undesirable genes such as antibiotic-resistant genes, bacterial virulent genes, and integrase genes. Phages with such genes may contribute to the development of highly pathogenic antimicrobial-resistant bacteria. Furthermore, phage-based therapeutic formulation and stabilization is still a challenge as previous studies reported that the stability of phage formulations for clinical use is stringently influenced by the phage type. Thus, each phage type requires its unique stabilization strategy and this is extremely complicated for phage cocktail formulations. The

evolution of bacterial resistance against phages mainly mediated by loss or alteration of the bacterial phage receptors and bacterial secretions that prevent phage adsorption has been implicated as another limitation affecting phage therapy. Inactivation of phages by the immune system has also been reported as a drawback of phage therapy.

15. Conclusion

The high prevalence of MDR infections has resulted in familiar bacterial diseases becoming difficult to treat. Moreover, hospital-associated infections (both sensitive and MDR) are mainly acquired through contaminated surfaces and medical equipment. However, phage-mediated bio-sanitization, *in vivo, ex vivo,* and *in vitro* phage therapy experiments and trials analyzed by this review showed that phages can mitigate the burden caused by MDR infections and contamination of hospital surfaces as well as medical devices. Furthermore, water and food-borne bacterial infections have been implicated as the major cause of mortality and morbidity globally and LAB as the main cause of yield loss in the biofuels fermentation industry. Analysis of phage/endolysin mediated bio-preservation and bio-decontamination studies by this review showed that phages and endolysins were highly effective. Thus, phage technology presents an opportunity for developing alternative therapeutic, bio-preservative, bio-decontamination, and bio-sanitization approaches. Despite the undisputable efficacy of phage therapy and phage-mediated biocontrol, rigorous investigations using highly sensitive techniques should be carried out to ensure that only appropriate professionally lytic and safe phages are used. Thus, for low- and middle-income countries, there is a need to develop affordable and appropriate methods for screening of phages for undesirable genes. Moreover, the challenge of immunogenicity that may be associated with *in vivo* application of phages needs to be explored further.

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Authors' contributions

This work was carried out in collaboration between all authors. Jesca L. Nakavuma (JLN), Dennis K Byarugaba

(DKB), Robert Tweyongyere (RB), and Francis Ejobi (FB) conceptualized this project and designed the format for this review. Kenneth Ssekatawa (KS), Edward Wampande (EW), Charles Kato Drago (CKD) & JLN performed the literature search and data analysis. All authors drafted the section of the literature review. KS, JLN, and CKD wrote the first draft of the manuscript and managed manuscript revisions. All authors read and approved the final manuscript.

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