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).

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...
Phages 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 bio-sanitization 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-102TM, ListshieldTM, ECP-100TM (EcoshieldTM), SALMONELEXTM, AgriPhageTM, 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
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 (OFlynn 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 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. Sootill       | 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     | IIEF bacteriophage collection       | IIEF             | Enterococcus faecalis                                   | 100%                       | [99]|
| Phage safety analysis                        | Phage cocktail Coli Proteus         | Microgen Russia  | E. coli and proteus                                      | -                          | [30]|

Figure 1. Selection process of research articles for inclusion in this review.
| 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 (Sa6) endolysin | EMD Biosciences, San Diego, CA | Laboratory experiment | Lactobacillus, staphylococci, and streptococci | 77.3% | [100] |
| Ethanol fermentation | LysA, LysA2, LygA V and X Sa6 endolysin proteins | Subcloned into the PET21a E. coli expression vector | Laboratory experiment | Lactobacillus fermentum, Lactobacillus brevis, and Lactobacillus mucosae | ~ 90% | [101] |
| Ethanol fermentation | Lytic enzymes LysA and LysA2 | Endolysins genes expressed in Saccharomyces cerevisiae | Laboratory experiment | L. fermentum | 100% | [37] |
| Ethanol fermentation | EcoSau and EcolIn | Wastewater influent | Laboratory experiment | Lactobacillus plantarum ATCC® 8014™, ATCC® 8014™ | 99% | [92] |
| Ethanol fermentation | ATCC® 8014-B1™ (phage B1) and ATCC® 8014-B2™ (phage B2) | ATCC | Laboratory experiment | L. monocytoegens, WSLC 1001 | 100% | [39] |
| Dairy (Cheese) | Phage P100 | Dairy plant sewage effluent | Laboratory experiment | Staphylococcus aureus | 100% for | |
| Dairy (Milk) | vB_SauS phiPLA35 | Laboratory experiment | | | |
| Dairy (Milk) and control of E. coli biofilms | BECP2 and BECP6 phages | | | | 90% | [103] |
| Dairy (Milk fermentation) | Coliphages DT1 and DT6 | Feces | Laboratory experiment | E. coli | O157:H7 | 100% | [104] |
| Dairy (Cheese) | phage AS11 | - | Laboratory experiment | Listeria monocytogenes, | 90% | [105] |
| Fruits (Cucumber, Apple, and Tomatoes) | T7 bacteriophages | Intralytx, Inc. (Baltimore, Md.) | Laboratory experiment | Escherichia coli | BL21 | 99.9% | [106] |
| Fresh-cut fruits and vegetables | LM-103 and LMP-102, | | Laboratory experiment | Listeria monocytogenes, | 99.9% | [107] |
| Dairy, poultry, beef products, seafood, and vegetables | AS11 and P100 | Myophages SA-C12 | Laboratory experiment | Listeria monocytogenes, WSLC 1001 (serovar 1/2 c) and Scott A (serovar 4b) 8840 (NCMB culture collection). | 100% | [108] |
| Beer industry | FSP-1 and FSP-3/PSZ1 and/PSZ2 | Fresh silage | Laboratory experiment | Lactobacillus brevis | 8840 (NCMB culture collection). | 100% | [109] |
| Chicken cuts | - | Feedlot cattle feces | Laboratory experiment | Salmonella enterica | Strain S49 | 92% | [110] |
| Spinach | - | Laboratory experiment | | | | |
| Oysters | Siphoviridae phage pVp-1, | Laboratory experiment/trial on oysters | | | | |
| Fermented Soy bean paste | BCP1-1 and BCPB-2 | Fermented food products | Laboratory experiment | Bacillus aerues | ATCC27348, ATCC21768, ATCC13061 | 100% | [113] |
| Bioactive packaging materials (meat and alfalfa seeds and sprouts) | LimMAG8, LomMAG13, and LomMAG20, while the E. coli O104:H4 EcoM-HG2, EcoM-HG7 and EcoM-HG8 (Mycoviridae) | Canadian Research Institute for Food Safety | Laboratory experiments | Listeria monocytogenes and Escherichia coli | 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 | Ice8, Ice and IceN | Slurry | Laboratory experiment | Citrobacter sakazakii | C. sakazakii ATCC BAA 894, C. sakazakii ATCC BAA 894 LUX | 100% | [40] |

(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-| Sewage                | Laboratory            | *Yersinia enterocolitica*              | O:3 strain 6471/76 and O:9 stain   | 100%     | [41] |
|                                      | Yen9-03 (*Myoviridae*)                                     |                       | experiment            |                                        | Ruokola/71                          |          |      |
| Milk, sausage, and lettuce           | LPST10, LPST18, and LPST23(*Siphoviridae family*)          | Waste water, sewage,  | Laboratory            | *Salmonella strains Typhimurium* and   | *Salmonella Typhimurium*            | 64.1%    | [116]|
|                                      |                                                            | farm ditch, poultry   | experiment            | *Salmonella Enteritidis*               |                                    |          |      |
| Active food packaging system (cellulose acetate films) | BFSE16, BFSE18, PaDTA1, PaDTA9, PaDTA10 and PaDTA11       | Chickenfece, poultry   | Laboratory            | *Salmonella enterica subsp.*           | Enterica serovar Typhimurium ATCC 14,028 | 100%     | [117]|
|                                      |                                                            | exudates, and swine   | experiment            |                                        |                                    |          |      |
| Bioactive food packaging system      | BFSE16, BFSE18, PaDTA1, PaDTA9, PaDTA10 and PaDTA11       | Poultry exudates and swine | Laboratory          | *Salmonella enterica subsp.*           | Enterica serovar Typhimurium ATCC 14,028 | ~ 99.99% | [118]|
| Sea food (Cockles)                   | pHT4A, ECA2                                                 | Sewage                | Laboratory            | *Escherichia coli*                     | ATCC 13,706,                        | 90%      | [119]|
|                                      |                                                            |                       | experiment            |                                        |                                    |          |      |
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, MA403S and MF445D | Minced meat, pork sausage & bovine feces | Laboratory experiment | Escherichia coli | STEC and EHEC | 100% | [44] |
| Phage-antibiotics synergism against E. coli biofilm | T4 bacteriophage ATCC 11,303-84 | 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 | Salmonella Typhimurium | Enterica serovar Typhimurium | 100% | [121] |
| Phage activity against Staphylococcus aureus (family | Phage IBB-PF7A | Sewage treatment plant | Laboratory experiment | Staphylococcus aureus | - | 100% | [122] |
| Phage activity against Pseudomonas fluorescens | Phage B5055 | ATCC and Hankuk University of Foreign Studies | Laboratory experiment | P. fluorescens biofilms | KCCM 10,585, ATCC 99,585, and CCARM 8009. | 54% | [124] |
| Phage activity against Staphylococcus aureus biofilm | Phage DRA88 and SAB4328-A | Sewage | Laboratory experiment | Staphylococcus aureus biofilm | Reduced biofilm formation | 95% | [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 | PA01, PA4531, PA45291, PA45292, 1598, MRSA 252, & H325 | 100% | [36] |
| Phage activity against Staphylococcus aureus biofilm | DRA88 and phage K | Crude sewage | Laboratory experiment | Staphylococcus aureus biofilm | MRSA/MSSA | 100% | [126] |
| Phage activity against Staphylococcus aureus | Phage K and phage 92 | ATCC | Laboratory experiment | Staphylococcus aureus biofilm | ATCC | 100% | [127] |
| Ex vivo Phage activity against catheter MRSA biofilm | phage K | ATCC | Laboratory experiment | Staphylococcus aureus biofilm | ATCC | 100% | [128] |
| Ex vivo Phage activity against catheter Proteus mirabilis and Escherichia coli biofilms | Escherichia coli 11,303-84 | Crude sewage | Laboratory experiment | Proteus mirabilis and E. coli biofilms | - | 50% | [129] |
| Phage activity against Staphylococcus aureus | P128 proteins | Inducible T7 expression system in E. coli ER2566 strain | Laboratory experiment | Staphylococcus aureus | BKO13,725, BK9989, BKO13,993 | 99.99% | [130] |
| Phage activity against K. pneumoniae biofilms | KPO12K and NDR, depolymerase, and nondepolymerase producing phages | Hospital environmental dirt, sewage disposal, and cattle waste | Laboratory experiment | K. pneumoniae biofilms | B5055 (O1-K2) | 99.99% | [131] |
| Phage activity against MRSA & MSSA | Phage AB2 | Hospital environmental dirt, sewage disposal, and cattle waste | Laboratory experiment | MRSA and MSSA | MRSA and MSSA | 100% for MSSA and 78% for MRSA | [132] |
| Phage activity against MDR Acinetobacter baumannii | Phage AB2 | Sewage | Laboratory experiment | MDR Acinetobacter baumannii | A. baumannii M3320 | 99.9% | [56] |
| Phage activity against MDR P. aeruginosa | Phage AB2 | Sewage | Laboratory experiment | MDR P. aeruginosa | P. aeruginosa | 100% | [133] |
| Phage therapy assay against Pseudomonas aeruginosa and Staphylococcus spp clinical isolates | Intesti and Pyrobacteriophag | Elawa BioPreparations, Tbilisi, Georgia | Laboratory experiment | - | - | 100% | [134] |
| Phage bacterial lytic activity against resistant S. aureus | SA11 (Siphoviridae family) | Hankuk University of Foreign studies | Laboratory experiment | Staphylococcus aureus | ATCC 13,301 and CCARM 3080 | 99.99% | [135] |
| Phage bacterial lytic activity against Staphylococcus aureus biofilms isolated from orthopedic implant | Staphage (Myoviridae) | AusPhage Pty Ltd and sewage water | Laboratory experiment | Staphylococcus aureus biofilms | OR11602N and OR16025 | 98% | [136] |
| Phage activity against P. aeruginosa biofilms | P. aeruginosa phage M4 | Health Protection Agency, Colindale, United Kingdom | Laboratory experiment | Staphylococcus aureus 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 | PAOct and KT28 | Natural wastewater treatment plant | In vivo-insect model | P. aeruginosa | PA PAO1 and 0038 | 93.60% | [138] |
| Treatment of *P. aeruginosa* infection in insect | 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 (MRA) and ATCC 29,213 (MSSA) | 100% | [140] |
| Treatment of *S. aureus* osteomyelitis in Rabbits | SA-BHU1, SA-BHU2, SA-BHU15 and 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 | Carbenapenem resistant *A. baumannii* | - | 100% | [34] |
| Treatment of *A. baumannii* pneumonia in BALB/c mice | vβ8_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 mouse | 49R18 and Ø512-1 | 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* | AC53, AC2846, and AC4653 | 100% | [143] |
| Treatment of *S. Enteritidis* infection in *Caenorhabditis elegans* worms | Φ5P-1 and Φ5P-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-mouse model and Ex-vivo-human HeLa cells | PDR *A. baumannii* | - | 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 | BS593 | 100% | [146] |
| Crop protection | 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 potato bacterial wilt | Phages SSP5 and SSP6 | sewage | In vitro and in vivo laboratory experiments | *Salmonella enterica* | S. oranienburg | 100% | [148] |
| Biocontrol of alfalfa seeds spoilage | RsPod1EGY | Soil | In vitro and in vivo laboratory experiments | *Ralstonia solanacearum* | K3, K9, K10, K11, K12, K16, K17, and K19 | 100% | [149] |
| Biocontrol of tomato bacterial wilt | Myoviridae family | Caspian Sea water | Laboratory experiment | Dickeya dadantii | - | 100% | [150] |
| Aquaculture | H2O-Siphovirus and KVP40-Myovirus | Sea water | Laboratory experiment | Vibrio anguillarum (BA55 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. |
|-----------------------|--------|------------------|----------------------|-----------------|----------------------------------|------------------|------|
| 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 | typ2ll 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] |
| Phage therapy in piggy | Phage ØEC1 | Chicken feces | Laboratory experiment | E. coli | APEC O78:K80 | 99.99% | [156] |
| Phage therapy in apiculture | phage K*710 and P68 | Novolytics Ltd | Trial using animal model | Staphylococcus aureus (MRSA) | ST398, spa type t011, SCCmec type V | 0% | [71] |
| 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 DS M 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  | v8, 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 sanitizer          | Staphylococcal phage and Pyophage; GA, AB1, AB2, AB6, AB7 phages | Eliava Institute              | Laboratory experiment      | *S. aureus, E. coli, and P. aeruginosa*              | *S. aureus (SA2-R73), E. coli (EC-860), and P. aeruginosa (PAV6).* | 90%               | [86]     |
| Hospital sanitizer          | Pyobacteriophage polyvalent    | Sewage or river water         | Laboratory experiment and trial | *Staphylococcus, streptococci, enterococci, Proteus, Klebsiella* (pneumoniae, and oxytocca), *P. aeruginosa and E. coli* | *MDR Staphylococcus aureus (MRSA and VRSA)* | 100%              | [88]     |
| Phage cream and sanitizers  | Polyvalent Anti-Staphylococcus Phage K | ATCC 19,685-B1               | Laboratory experiment and trial | *MDR Staphylococcus aureus*                          |                                                 | 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.
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 B\textphi C62 [34], DL52, DL60 and DL680 [35], DRA88 and phage K [36], EcoInf [37], coliphages\textphi APCEc01, \textphi APCEc02 and \textphi APCEc03 [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 lysogenic module genetic determinants in phages CB60P, MFA60N, CBO103, 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 lysogenic 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 >10³ is recommended [54] whereas in vivo phage therapy of natural infection a very high titer value of > 1 x 10⁸ 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, VRSAs corneal abscess and interstitial keratitis, and MDR *Pseudomonas aeruginosa* UTI with phages. Furthermore, *in vivo* phage treatment of MRSA *osteomyelitis* in Rabbits [58], carbapenem resistant *Acinetobacter baumannii* infection in mice [34], MDR *Acinetobacter baumannii* 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 baumannii* human HeLa cells infections recorded overwhelming success. *In vitro* phage therapy against MRSA, MDR *Acinetobacter baumannii*, 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 [3,61,62], 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
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, bio-disinfection using phages as demonstrated by this review is promising; for instance, phage-mediated biocidal decontamination 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 baumannii 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 disinfection 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 efficiency 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 integrate 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
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-preservation, 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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