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Review

Bacteriophages and phage products: Applications in medicine and biotechnological industries, and general concerns

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Since their discovery in 1928 antibiotics have transformed medicine and saved millions of lives; however, the current global appearance of antibiotic resistance and absence of new antibiotics commercialization to the health sector return to the post-antibiotic era. Today, scientists are working to revive a century old technique of using bacteriophage as an option to currently available antibiotics in management of bacterial pathogens (resistant or otherwise), and for other practical applications. Bacteriophages, specialist viral predator of bacteria, are considered to be among the most plentiful and diversified organisms in this world and were co-discovered in the early 1900s by Twort in 1915 and d'Herelle in 1917. Since then, phages were investigated by d'Herelle and others for their promising therapeutic role against medically important bacterial infections, and were commercially developed in the 1930s. However, the golden age of antibiotics, together with the scientific controversy regarding their use, the interest in phage therapy rapidly declined. As a mark of the transformed attention, the field of phage research, using intact phage and phage products, has broadened to focus on several different areas: and have been successfully applied in agriculture and food industry to control bacteria pathogens; for the detection of bacterial pathogens, as deliverance carriers for DNA and protein vaccines; and as agents for selection to libraries of proteins, antibodies (phage display). Despite these remarkable achievements, it is important to remember that phages are not infallible, and that there are real concerns that need to be addressed, like narrow host range, bacterial phage resistance, phage clearance in bodily fluids, side effects of bacterial lysis and virulence genes transfer. Up to date progress in biotechnology and synthetic biology can help conquer these methodological obstacles, and provide a more versatile approach for the current antibiotics resistance crisis.

Key words: Bacteriophages, biotechnological application, antibiotics resistance, phage products.

INTRODUCTION

Long before the discovery and widespread use of antibiotics, scientists predicted using viruses to hunt for and wipe out bacteria. Currently, as the direct and management of communicable diseases via antibiotics

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becomes constantly escalating and challenging from the emergence of multiple resistances to currently available antimicrobial and the lack of investment in new antibiotics, these viruses, called bacteriophages, are ruling fresh promoters. Based on the Food and Drug Administration (FDA), bacteriophages are described as RNA or DNA viruses which can selectively kill specific bacterial species whilst parting other, normal flora and mammalian or plant cells undamaged (Sherman, 2008). Phages are ubiquitous entities on earth- in the oceans, soil, deep sea vents, and gastrointestinal tract, and humans are regularly bared to them at elevated levels during meal and water without undesirable effects. They are also most abundant, as an estimated 10^{30}-10^{32} bacteriophages lives in the biosphere (Sulakvelidze, 2011), and a predicted 10^{23} phage infections occurs every second (Hatfull and Hendrix, 2011). Each 48 h, phages demolish nearly half the bacteria in this globe (Abedon, 2009), a vibrant progression that happens in all ecosystems (Hatfull and Hendrix, 2011).

Since their discovery, bacteria infecting viruses have been studied in numerous laboratories and practiced in a diversity of practical functions. Without a doubt, the results of phage explored were very critical in production of nearly all considerable discoveries in biological sciences, including the identification of DNA as the genetic material, the interpretation of the hereditary code, and the development of recombinant DNA techniques; these advances directed to the beginning of the novel science of Molecular Biology (Clokie and Kropinski, 2009).

Remarkably, over the last decade, the innovative admiration of bacterial virus widespread existence and commonness in life and of a renewed unrestricted and scientific attention in possible phage relevancies alongside antimicrobial resistant bacteria, as well as in a variety of essential and practical features of phage biology has directed scientists to review this century-old loom and acquire an unmarked look at phages as a “new” and potentially viable treatment choice for tricky-to-care-for bacterial pathogens (Kutter and Sulakvelidze, 2005).

In this review, the record of phage finding and its closeness to earlier investigations with phages, along with its taxonomy and lifecycle will be discussed. Also, recent literature emphasizing on phage applications in clinical and medical settings as well as in biotechnology industry, and concerns regarding their use will also be addressed. Finally, thoughts are shared on prospects for uses of phages in Ethiopia.

Early phage research: Promises and problems

Not long after d’Herelle’s first observations, the function of phage in the route of communicable disease was of vital attention. The first published report was, however, noted by Bruynoghe and Maisin in 1921 for treating staphylococcal skin disease with phages (Summers, 2005). In the summer of 1919, d’Herelle conceded broad tests of phage as prophylaxis alongside the usual infection of chickens by *Bacillus gallinarum* (causes avian typhosis). In these experiments, phage opened a high level of protection and reported these results in 1921. D’Herelle’s results were confirmed during occurrence of a similar disease in Holland by Kramer. Phage therapy was also successfully assessed by field trials via bovine hemorrhagic septicemia (*barbone*) in Indochina. Encouraged by these results, d’Herelle intensified his experiments to humans. The work that involved the interest of phage therapy was d’Herelle’s (1925) statement of successfully treating four cases of bubonic plague with antiplague phage in Alexandria, Egypt, by direct injection of phage into the infected inguinal and axillary lymph nodes (buboes). Later, d’Herelle was invited to India by the British government to work on the cholera epidemics, where cholera- specific phage administration reduced the severity and overall mortality (Summers, 2005). The commercial prospects were also quickly recognized in the West and by the 1930s companies such as L’Oreal, Eli Lilly, Medico-Biological Laboratories, Squibb and Parke-Davis, and Swan-Myers had begun to market therapeutic phages active against various bacterial infections (Monk et al., 2010).

Even with all the success stories and properties of phages that appear to promote their medical use, there are problems associated with the early phage research that has led to some clinical failures and controversies. Some of the troubles of early phage investigations (Sulakvelidze et al., 2001) that contributed to this situation are listed below:

i) Failure to establish scientific proof of efficacy.
ii) Narrow host range of phages: The researchers ’inability to carefully select specific phages against the strains of the etiological agent of the disease has led to many negative results.
iii) Impurity of phage preparations.
iv) Underprivileged solidity and feasibility of phage preparations: Some investigators used oxidizing agents or heat to eliminate live bacteria in phage preparations, which would have denatured or inactivated the phages and resulted in ineffective phage arrangements.
v) Phage resistance.
vi) Phage inactivation.

In 1934, the Council of Pharmacy and Chemistry of the American Medical Association concluded that phage therapy was of questionable value (Asheshov et al., 1934). Moreover, the advent of antibiotics with a wide spectrum of action and well-understood mechanisms, and World War II efficiently sidetracked hard works away from additional study of phage therapy in much of the Western world by the 1940s. Phage preparations was however continually used in eastern European countries

in the former Soviet Union, separately or synergistically among antibiotics (Carlton, 1999), for therapy, prophylaxis and the diagnosis of many bacterial infections. Several institutions in these countries were actively involved in therapeutic phage research and production, with activities centered in Georgia and Poland (Sulakvelidze et al., 2001).

One of the most remarkable large scale, placebo-controlled, randomized clinical trial studies carried out in Tbilisi implicated a careful assessment of the prophylactic outcome of a phage concoction against bacterial dysentery (Babalova et al., 1968). This study was performed with two groups of children (n- 30,769; age>7 years), where one group were given an oral administration of *Shigella* phage and the other a placebo. This resulted in a 3.8-fold decrease of dysentery incidence in the phage treated group. In general, the study and use of phage treatment in these Institutes has focused primarily on stopping and treating enteric diseases like dysentery, diarrhea, and typhoid, and purulent-septic infections (Sulakvelidze et al., 2001).

PHAGES IN MEDICINE AND BIOTECHNOLOGICAL INDUSTRY

Phages have been extensively characterized, and some of them have been exploited to develop applications in research laboratories, biotechnological industries, and the medical field. The main applications of phages and phage products are: as an alternative to antibiotics to control bacterial pathogens including food pathogens (phage therapy); to screen phage-display; as genetic tools to detect pathogenic bacteria (phage-typing). This part of the study explores some examples of these applications with the main focus on phage therapy (Verheust et al., 2010).

Role in research laboratory

In 1943 Max Delbruck and Salvador Luria studied the mutation that arises when phages (T1) infect bacteria (*Escherichia coli*) (McAuliffe et al., 2007). Their experiment revealed that phage resistance in bacteria was due to spontaneous genetic change and not a direct response to environmental factors. Later, the chemical composition of the virion (that is, proteins and genetic material) was determined, and the transduction phenomenon was discovered by Lederberg and Zinder in 1951 while working on phage P22 (a known temperate phage of *Salmonella*) (Segundo et al., 2010). Until 1952, scientists did not know which part of the virus, the protein or the DNA, carried the information regarding viral replication. Al Hershey and Martha Chase (1952) performed a series of experiments using T2 phage and concluded that DNA, not protein, was the genetic material. For discoveries concerning the replication of viruses and their genetic structure, Delbrück, Hershey and Salvadore Luria shared the Nobel Prize for Physiology or Medicine in 1969 (McAuliffe et al., 2007).

From the 1960s onwards, phage studies continued to show astonishing results such as mRNA description, nature of the genetic code, mechanism of action of the transcription factors, site specific recombination, anti-termination as a mechanism of transcriptional regulation, and the first genome to be completely sequenced (MS2 and φX174) (Segundo et al., 2010). The work with phage also led to the development of recombinant DNA techniques (as primary vectors or parts of [promotor, packaging signals, replicons]) and contributed a great variety of enzymes (such as, polynucleotide kinases, DNA ligases, polymerases, recombinases, ssDNA binding proteins, endo- and exonucleases, restriction endonucleases) employed in today's molecular biology laboratory (Clokie and Kropinski, 2009). Other phage studies led to description of new features of the replication mechanism (such as discontinuous synthesis, Okazaki fragments, rolling circle mechanism of replication, and the role of the primers), the identification of chaperonins, the characterization of transposons, the finding of overlapping genes, the description of negative feedback loops and the use of reporter phages for the medical diagnosis. On the other hand, the use of phages as gene and vaccine delivery vehicles, and the production of recombinant proteins, is a technique that is still used today (Segundo et al., 2010). In each of these cases, phage has been a cornerstone of modern molecular biology and genetics.

Bacteriophage therapy

The possibility of utilizing phages for medical action of infectious disease was recognized at the time of their finding and it became extensively termed as 'phage therapy'. Phage therapy involves the embattled use of bacteriophages that, ahead meet with precise pathogenic bacteria, and can infect and destroy them (Abedon et al., 2011).

Whole phage therapy in humans

A recent advancement in knowledge of the phage biology and their bacterial host interaction can have a thoughtful blow on the progress of secure remedial phage provisions having most favorable efficiency alongside their explicit bacterial hosts. The first application of phage as human therapeutic agents was in 1919 just when they were discovered (Summers, 1999). But nearly after a decade (that is, 1930), the advent of antibiotics overshadows phage therapy.

The majority of recent publications on phage therapy in

humans were imminent from researchers in Eastern Europe and the former Soviet Union (Sulakvelidze et al., 2001), and it was widely browbeaten for both diarrheal and the conduct of distressing infections even after antibiotics became extensively accessible. There has been no information on severe barriers related with the use of phages at such settings (Sulakvelidze and Kutter, 2005).

The most comprehensive reports on phage therapy in humans were by Slopek et al. who published a number of papers on the efficiency of phages against bacterial pathogens, including MDR mutants. The etiologic agents in these studies were *Staphylococcci*, *Pseudomonas*, *Escherichia*, *Klebsiella*, and *Salmonella* (Sulakvelidze et al., 2001). Slopek et al. (1987) reported the results of treating 550 septicemic patients with phages, after the antibiotic treatments failed. The success rate of 92% was achieved after phage therapy was initiated. In other publications, phages were reported to be effective in treating eye infections (Proskurov, 1970), staphylococcal lung infections (Kaczkowski et al., 1990), skin infections (Cislo et al., 1987), urinary tract infections (Perepanova et al., 1995), cerebrospinal meningitis in a newborn (Stroj et al., 1999), and cancer (Weber-Dabrowska et al., 2001).

A group of Central Manpower Base (CMPB) investigators in collaboration with Eliava Institute of Bacteriophages, Microbiology and Virology (EIBMV) scientists in Tbilisi, Georgia, developed a novel approach using a biodegradable polymer impregnated with lytic phages (together with ciprofloxacin and benzocaine), called PhagoBioDerm, to treat infected wounds. The product was accredited by Georgian Ministry of Health in 2000. In its present structure, is a punctured, recyclable, non-hazardous polymer combined comprising a mix of lytic bacteriophages ("PyoPhage", against *Pseudomonas aeruginosa, E. coli, Staphylococcus aureus, Streptococcus,* and *Proteus*), an antibiotic (ciprofloxacin), an analgesic (benzocaine), α-chymotrypsin, and sodium bicarbonate (Markoishvili et al., 2002; Katsarava and Alavidze, 2004). Markoishvili et al. (2002) reported the use of PhagoBioDerm to treat 107 patients infected with venous skin ulcers that had failed to respond to other treatment options. PhagoBioDerm was applied to ulcers alone or in combination with other treatment options that resulted in complete healing of ulcers in 70% of the patients. More recently, PhagoBioDerm was used to treat patients with antibiotic-resistant *S. aureus*-infected severe radiation burns caused by exposure to strontium-90 (Jikia et al., 2005). Leszczynski et al. (2006) described the use of oral phage therapy for successfully treating patient with methicillin resistant *S. aureus* (MRSA) infection.

In Russia, companies like ImBio, Immunopreparat and Biomed are currently producing phage-based preparations and various bacterial pathogens ("Bacteriophagum" and "Piobacteriaphagum") (Sulakvelidze and Kutter, 2005).

Phages which use host virulence factors as receptors are preferred so that if phage resistance develops the virulence of the host will concurrently be decreased (Brussow, 2012). Well characterized growth media must be used and phage lysates must be purified to remove harmful substances such as endotoxins released by lysed host cells (Boratynski et al., 2004). Sterility and stability tests are required, as are animal trials which demonstrate the safety of the therapeutic preparation (Brussow, 2012). In addition, the following criteria must be taken into account for the design of a trustworthy clinical trial: time of treatment, dosage relative to the location of the infection and the means of administration and if a single phage or mix is being used for treatment (Parracho et al., 2012). New report by Miedzybrodzki et al. (2012), on the assessment of the wellbeing and efficacy of phage therapy of 153 patients with a broad variety of infections resistant to antibiotic therapy admitted at the PTU, revealed that phage therapy can give effective treatment for a range of bacterial infections, against which all other obtainable therapies have futile, and is essentially well tolerated.

Even though most information on phage remedy over the preceding 90 years has come from Eastern Europe and the former Soviet Union, there has been an invariable attention in phage treatment in Western Europe, Africa, and the United States which has recently gained momentum. In Egypt, phages were used to treat burn wound sepsis and post-burn infections associated with antibiotic-resistant *P. aeruginosa* of humans during the 1980s and early 1990s (Abdul-Hassan et al., 1990). In Switzerland, Saphal, a tiny pharmaceutical company in Vevey, produced phages well into the 1960s. Coliphagine was used for *E. coli*, Intestiphagine for diarrheal diseases, Pyophagine for purulent skin infections and Staphagine for staphylococci (Hausler, 2006). Merabishvili et al. (2009) used a phage cocktail, BFC-1, consisting of exclusively lytic phages against the most prevalent MDR *P. aeruginosa* and MRSA bacteria in the Burn Wound Center of the Queen Astrid Military Hospital in Brussels, Belgium. The phage mixture was also subjected to all necessary safety procedures and no adverse reactions were observed. In UK, a successful double-blind, randomized, placebo controlled phase I/II clinical trial was conducted on safety and therapeutic efficacy of Biophage-PA (Bio control Ltd.) in treating 24 patients with chronic otitis caused by drug resistant *P. aeruginosa*. The study also indicated further development of Biophage-PA for large scale phase III clinical trials in treating chronic otitis as well as other infections - such as *P. aeruginosa* infected burn wounds or lung diseases (Wright et al., 2009).

In the United States, curative phage production was basically non-existent after antibiotics became extensively accessible; though, phages were applied to arrange vaccines. One such preparation ("Staph phage lysate," [SPL]) by Delmont Laboratories was used

therapeutically from the 1950s to the 1990s. The safety and efficacy of SPL was demonstrated in a clinical trial, wherein 607 cases of chronic staphylococcal infections who failed to respond to various antibiotics treatment were treated by the phage, which resulted in 80% recovery rate of patients with no side effects. Unfortunately, owing to regulatory pressure, production of SPL for human therapy was suspended in the 1990s. Several additional studies in which SPL was used to treat patients with furunculosis, pustular acne, pyoderma, eczema, bronchial asthma, and upper respiratory infections have been published (Sulakvelidze and Kutter, 2005). In Lubbock, Texas, the FDA approved the first phase I clinical trial to evaluate the safety of eight-phage cocktail ("WPP-201") developed by Intralytix to treat venous leg ulcers. The phages included were isolated from the environment, and were lytic against *P. aeruginosa, S. aureus,* and *E. coli.* This phase I study raised no concerns about the clinical safety of the "WPP-201" (Kutter et al., 2010). In 2012, a phage research team in Los Angles discovered phage 11P, which is effective on *Propionibacterium acnes* (Marinelli et al., 2012). This phage kills a broad spectrum of *P. acnes* and other acne causing skin colonizing bacteria, which would make them ideal candidates for the development of a phage-based therapy for acne. Vieira et al. (2012) reported similar observation against MDR *P. aeruginosa* skin infections in both in vitro and ex vivo (human skin) experiments.

Fish et al. (2016) reported a compassionate-use case progression of nine patients among diabetes and staph infected toe sores using staphylococcal phage Sb-1; sores cured in a middling of week seven. Another study published in 2016 reported a successful phage treatment of a patient with a history of chronic *Pseudomonas* infection associated with an aortic arch replacement surgery. In this study, a lytic phage (OMKO1) isolated from a pond were used to infect *P. aeruginosa* by using the outer membrane porinM (OprM) of the multidrug efflux systems as a receptor-binding site. This exerts selection pressure for bacteria to alter the efflux pump mechanism, thereby restoring antibiotic sensitivity to MDR *P. aeruginosa* (Chan et al*.,* 2016). These studies and many others have successfully indicated a promising future of phages as antibacterials, thereby extending the lifetime of our current antibiotics.

Whole phage therapy in veterinary medicine

Even if phage therapy has been traditionally coupled with their use in human medicine, phages have also been successfully used for veterinary application either to direct address infectious diseases in animals or for sustaining facts when pertaining for human clinical trials (Keary et al., 2013). It is also vital to reveal that animal phage therapy trials do not have the similar degree of authoritarian obstacles as human trials and are probable to put a key pattern for phage therapy in a clinical setting. The earliest use of phages in veterinary medicine was conducted by D'Herelle in 1919, who proved their efficacy in preventing and treating fowl typhoid (*S. gallinarum*) in six experimentally infected chickens (d'Herelle, 1926).

The revitalization of phage therapy in the west in the 1980s is frequently associated to the study by Smith and Huggins (1982, 1983) in the Institute for Animal Disease Research in Houghton, UK who conducted a series of restricted studies on the use of phages to treat mice with experimentally induced *E. coli* septicemia, and neonatal enteritis in calves, piglets and lambs. Ramesh et al. (1999) determined the ability of lysogenic phages to prevent a fatal, *Clostridium difficile* ileocecitis in experimentally infected 26 adult hamsters, and also studied the factors that influence the efficacy of phage therapy (e.g., persistence of phages, effect of gastric acidity on phage viability, etc.). The experiment was conducted in three groups. In order to facilitate the *C. difficile* ileocecitis, the hamsters in groups 1 and 2 were pre-treated with clindamycin; the hamsters in group 3 received saline. In addition, the therapy did not have a long-lasting protective effect in the hamsters; that is, when the surviving hamsters were pre-treated with clindamycin and rechallenged with *C. difficile*, they died within 96 h' post-challenge. Current researches on prophylactic and therapeutic use of phages against naturally occurring and experimentally induced infections of animals are summarized in Table 1.

From the business standpoint, American phage-based corporation Intralytix has produced and accredited phage-origin products efficient against *Salmonella* (PLSV-1™) and *C. perfringens* (INT-401™) in poultry (Miller et al., 2010).

Phage products as antibacterial agents

As an option to "classic" bacteriophage therapy, in which the intact phages are used, one can also apply phage encoded gene products. Indeed, a number of studies have revealed vast likely hood of the use of phage products as therapeutics; Fischetti (2011) was, however, the first to show the use of potent and specific enzymes produced by these viruses (Chhibber and Kumari, 2012). These highly evolved enzymes are specifically developed by phages to quickly and efficiently lyse the host cell and agree to their descendants to be unrestricted. There are two classes of cell wall hydrolase enzymes namely endolysins and virion associated hydrolase. Endolysins, or lysins are enzymes that disrupt the cell walls' integrity by hydrolyzing the four major bonds in its peptidoglycan constituent, eventually bursting the cell. They work in coincidence with a holin protein, which perforate the inner cell membrane and allow the lysin to enter through the holes to gain access to the peptidoglycan layer.

Table 1. Summary of selected phage therapy studies in animal.

Reference	Phage therapy outcome	Animal
Yen et al. (2017)	Oral administration of phage cocktail reduces *Vibrio cholerae* colonization of the intestinal tract and prevents cholera-like diarrhea	Mice, rabbit
Kishor et al. (2016)	Intralesional injection of 5×10^{11} PFU effectively treated chronic osteomyelitis caused by MRSA	Rabbit
Colom et al. (2015)	Oral dose of encapsulated phage resulted enhanced protection against *Salmonella* sp.	Chicken
Yilmaz et al. (2014)	Medullary injection of phage against MRSA associated bone infection	Rat
Mendes et al. (2013)	Phage cocktail effectively treated diabetic wounds infected with *S. saureus* and *P. aeruginosa* (that is, decreased bacterial load and improved planimetric and histological parameters)	Rat, pig
Pouillot et al. (2012)	Effective treatment of mice with sepsis and meningitis elicited by lethal neonatal meningitis *Eschelichia coli* strain (via. I.P. injection)	Rat
Alemayehu et al. (2012)	*Pseudomonas* was successfully empty (minimized by a extent of at least 3 to 4 log units) from murine lungs in 6 h (intranasal administration)	Mice
Morello et al. (2011)	Intranasal injection of phage effectively cure *P. aeruginosa* lung infections	Mice
Kumari et al. (2011)	Topical administration of phages to cure burn wound infection elicited by *Klebsiella pneumoniae*	Mice
Hung et al. (2011)	*K. pneumoniae*-mediated liver abscesses and bacteremia were effectively treated with intragastric administration of phage	Mice
Jamalludeen et al. (2009)	Effective anticipation and treatment of diarrhea owing to trial enterotoxic E. coli (ETEC) infection	Pig
Vinodkumar et al. (2008)	Completely rescued septicemic mice with MDR *P. aeruginosa*	Mice
Chhibber et al. (2008)	Phage SS treatment of *K. pneumoniae* induced respiratory infection	Mice
Watanabe et al. (2007)	Oral administration of lytic phage KPP10 effective against a gut-derived sepsis model caused by *P. aeruginosa* (66.7% survival rates)	Mice
Wang et al. (2006A)	A single I.P. injection of phage strain ØA392 showed 100% rescue rate against imipenem resistant *P. aeruginosa* (IMPR-Pa)	Mice
Wang et al. (2006B)	Rescue mice with ESBL-producing *E. coli* bacteremia	Mice
Vinodkumar et al. (2005)	Completely rescued septicemic mice with MDR *K. pneumoniae*	Mice
Huff et al. (2003)	Aerosol and intramuscular injection avoid deadly *E. coli* respiratory infections in broiler chickens	Chicken
Biswas et al. (2002)	Completely rescued mice from bacteremia elicited by vancomycin-resistant *Enterococcus faecium* (I.P. injection)	Mice

In specific Gram-positive bacteria, exogenously-applied endolysins can exert a direct effect. This feature directs to their discovery of novel antibacterials with likely applications in healthcare, veterinary, agriculture, food and biotechnology sectors (Keary et al., 2013).

To get merit of their bactericidal prospective, several researches have been investigated on the efficiency of phage lysins able of treating both systemic and mucosal infections. The second is a very noteworthy result, as mucosal membranes are a reservoir for many human pathogens including resistant strains and presently prophylactic treatment against such infections are limited to polysporin and mupirocin ointments (Fischetti, 2011). Thus, reducing or eliminating this reservoir should markedly reduce the incidence of human disease caused by those bacteria. To this effect, various animal models of mucosal colonization were used to test the efficacy of phage lysins to organisms on these surfaces. Nelson et al. (2001) reported the first successful *in vivo* endolysin trial, where an oral colonization model was developed for prophylaxis and treatment of upper respiratory infection in mice caused by group A streptococci (*Streptococcus pyogenes*) using a purified phage lysin (PlyC). Similar reduction of nasopharyngeal carriage of *S. pneumoniae* was observed using phage lytic enzymes (Loeffler et al., 2001, 2003).

In another study, a single dose of phage lysin (PlyGBS) was found to confiscate bacterial colonization from the vagina and oropharynx in a vaginal model for group B streptococci (*S. agalactiae*), the foremost reason of neonatal meningitis and sepsis infection (Cheng et al., 2005). The efficiency of phage lysins in killing pathogenic bacteria implies that they may be an important tool in controlling bio warfare bacteria. To agree on viability of this approach, a gamma lysin (PlyG) was specific for *Bacillus anthracis* and determined its *'in vitro* and *in vivo* activity against

gamma phage-sensitive anthrax bacilli. A phage lysin specific for *S. pneumonia* (cp1) was also reported to be effective in experimental pneumococcal meningitis using infant Wistar rats (Grandgirard et al*.,* 2008). Phage endolysins have also been shown to act synergistically with other bacteriolytic enzymes and antibiotics in vivo (Schmelcher et al., 2012). Garcia et al. (2010) reported effective inhibition of *S. aureus* by phage endolysin LysH5 and nisin. Recently, Micreos Human Health develops targeted antibacterial products, Staphefekt SA.100 (2013) and Staphefekt XDR.300 (2014), for human health based on its endolysin technology that specifically targets *S. aureus*, including Methicillin-resistant *S. aureus* (MRSA).

Bacteriophage-mediated biocontrol and bioprocessing

In recent year, phage-based biocontrol measurements in the food industry has become widely recognized due to their specific and effective antibacterial activity, and their long history of safe use. The concept of combating food pathogens using phages can be addressed at all stages of production in the classic 'farm to fork' continuum in the human food chain. Accordingly, phages can be used (i) to prevent diseases or reduce colonization in livestock (phage therapy), (ii) to disinfect food materials (carcasses and ready-to-eat products: biocontrol) or equipment and contact surfaces (biosanitation) and (iii) to extend the shelf life of foods (bio preservation) (Garcia et al., 2008). Several examples of the most frequently targeted foodborne pathogens for phage application throughout the food chain are summarized in Table 2.

Incidents in which phage-interfered bio-control of plant pathogens has been fruitfully tried include use against *Xanthomonas pruni*-related bacterial spot of peach trees, cabbage, and peppers; to control *Ralstonia solanacearum* infection of tobacco; to control soft rot and fire blight associated with *Erwinia*; and against bacterial leaf spot of mung beans; to disinfect *Streptomyces scabies*-infected potato seed-tuber (Goodridge and Abedon, 2003).

The application of phages for use in the food industry is currently strengthened by the commercial production of phage-based products approved by Environmental Protection Agency (EPA), United States Department of Agriculture (USDA) and Food and Drug Administration (FDA). List Shield™ (LMP-102) was one of the first phage-based products developed by Intralytix Inc. and was approved by FDA as a phage cocktail that was designed to control *L. monocytogenes* in RTE foods (Bren, 2007). This phage cocktail also obtained the 'generally regarded as safe' (GRAS) status from FDA. Intralytix Inc. has also produced EcoShield™ (against *E. coli* O157 contamination on ground meats) and SalmoFresh™ (against *S. enterica* in foods) (Lu and Koeris, 2011). Furthermore, other products from various companies have been accepted by the US FDA and given endorsement under the GRAS system. Micreos Inc. (Netherlands) developed Listex™ P100 (targeting *L. monocytogenes* on cheese, meat, fish) and Salmonelex™ (targeting *Salmonella* contamination of meet) (Fan and Tong, 2012). Other commercial phage products include: phages targeting spoilage pathogens of washed, packed potatoes (Biolyse®, APS Biocontrol Ltd.), against *X. campestris* pv. *vesicatoria* and *P. syringae* pv. *Tomato* (Agriphage, Omnilytics), and as animal feed for the control of *Salmonella* in poultry (BioTector, CheilJedang Corporation). These findings and more (Table 2) have all shown the recognition for the safe use of phages on food destined for human consumption.

Phage display

The notion of phage exhibit was introduced via the pioneering work of Smith (1985), who proposed a reputable method for presenting foreign polypeptides on the surface of filamentous phage of *E. coli*. Since then, phage display technology based on these lysogenicphages (e.g., M13, f1, or fd) has developed into a broadly used technique for selecting peptides and proteins with preferred functions and properties from molecular libraries (Paschke, 2006).

In phage display, exogenous DNA is fused with phage coat protein genes, and the desired foreign protein is expressed as part of the relevant coat proteins and are thus "displayed" on the surface of the mature virion as a fusion protein. The remaining unbound or weakly-bound phages are washed off, whereas the bound phages are eluted and amplified by bacterial reinfection. Repeated rounds of biopanning (usually 3-5) lead to rapid identification of phage clones displaying peptides with the highest affinity (via DNA sequencing) (Aghebati-Maleki et al., 2016). This approach has led to the development of Phage Antibody technology where antibody-derived peptides are displayed by fusion to phage coat proteins. The most significant advance provided by this method is that there is no need for the target ligand to be immuno-reactive in a given host and toxic molecules can be used as the target ligand (Rees and Loessner, 2005).

In general, this sort of knowledge has been used for medical or pharmaceutical applications, with the phage display system being used exclusively to recognize a peptide with the preferred binding character (Sidhu, 2000). These peptides can be used as therapeutic agents by inhibiting receptor-ligand interaction or acting as agonist. Dickerson et al. (2005) treated cocaine addiction in a rodent model via phage display of "cocaine-sequestering antibodies". These phage particles have the capacity to penetrate the central nervous system and sequester cocaine, thereby attenuating its psychoactive

Table 2. Bacteriophages as bio-control mediators for food safety applications.

Pathogen	Reported outcome	References
E. coli O157:H7	Reduced number of verotoxigenic *E. coli (*VTEC) from the surface of lettuce by more than 90%	Ding et al. (2016)
	Reduction (3.3 log unit) of pathogen from the surface of cucumber and eggs to undetectable levels	El-Shibiny (2016)
	Significant viable cell reduction of EPECand STEC on meat	Tomat et al. (2013)
	Eradication from food surfaces (steel, ceramic chips) after 10 min at 37°C and following 1 h at 23°C	Viazis et al. (2011)
Salmonella	Mean bacterial reduction of 3.02 Log_{10} CFU/tomato at 10°C and 0.7 Log_{10}CFU/tomato (at 20°C) from tomato surface at day seven	López-Cuevas et al. (2016)
	Momentous decline in bacterial count (1.5-4 log unit) in chicken breast, pasteurized whole milk and Chinese cabbage	Bao et al. (2015)
	Noteworthy bacterial reduction in raw and smoked salmon tissues	Galarce et al. (2014)
	A significant bacterial reduction was found in pig skin, chicken breasts, and lettuce	Spricigo et al. (2013)
	Decline of colonization by 99.0 to 99.9% in the tonsils, ileum, and cecum of pigs	Wall et al. (2010)
Campylobacter	Reduction levels by 2 log CFU/g in broiler chickens feces	Carvalho et al. (2010)
	Reduction of 2 log CFU/g in cecal content of broiler chickens after 48 h	El-Shibiny et al. (2009)
	Significant host inactivation in raw and cooked beef	Bigwood et al. (2008)
	Decreased bacterial load in chicken skin after slaughter	Wagenaar et al. (2005)
Listeria monocytogenes	Reduce bacterial counts in Brazilian fresh sausage	Rossi et al. (2011)
	Significant reductions of biofilm cells from the stainless steel coupons (3.5 and 5.4 log unit) following 24 h phage treatment	Soni and Nannapaneni (2010)
	Significant reductions (2.3-5 log unit) in cabbage, iceberg lettuce, chocolate milk, and mozzarella cheese brine; In liquid foods, eradication of bacterial cells.	Guenther et al. (2009)
Staphylococcus aureus	Phage cocktail resulted to untraceable limits of *S. aureus* after 6 h in fresh cheese and permanent reductions in hard cheese	Bueno et al. (2012)
	Effective inhibition by phage endolysin and nisin in pasteurized milk	Garcia et al. (2010)
	Phage cocktail eliminates up to 6 log units of *S. aureus* in acid and rennet-coagulated curd	Garcia et al. (2007)

effects. As of this year, at least eight phage display-derived antibody and peptide drugs have been approved for use, and many more are in phase III clinical trials. Their application range from prevention and treatment of anthrax (Raxibacumab), to treatment of hereditary angioedema (Adalimumab), wet age-related macular degeneration (Ranibizumab), certain autoimmune disorders (Romiplostim Belimumaband Adalimumab) and cancer (Ramucirumab and Necitumumab) (Nixon et al., 2014). The phage display has also been successfully used to develop a range of detection assays, including detection of *Mycobacterium avium* subspecies *paratuberculos* which is from milk samples (Stratmann et al., 2002), the identification of a variety of viruses (Petrenko and Vodyanoy, 2003) and to develop an assay for the species-specific detection of *Bacillus* spores (Turnbough, 2003).

Phages as vaccines delivery vehicles

Vaccinologists are now evaluating a novel and exciting role of bacteriophages as vaccine delivery vehicles, and the idea gained popularity owing to the inherited character of phages to stimulate both the cellular and humoral arms of the immune system. Merril et al. (1971) was the first to demonstrate phages ability to deliver gene(s) to mammalian cells. In his study, a λ phage carrying a galactose transferase gene was successfully used to induce the enzyme activity in galactose transferase deficient human fibroblast cells. The first use of phage to elicit an immune response was, however, explained by de la Cruz et al. (1988) who cloned repeat regions of the circum-sporozoite protein gene of *P. falciparum* into the minor coat protein genes (pIII) of phagef1. The recombinant phages were found to be immunogenic.

Currently, two different phage vaccines strategies can be employed to deliver antigen. The first method involves the use of phage particles in

a straight line transport of the vaccine antigens expressed on their surfaces, designated as “phage display vaccines”. This in general is succeeding by expressing antigens as fusion products of one of the main surface proteins of phage virion. But in case of “phage DNA vaccines”, sequences that are essential for the vaccine antigen synthesis are incorporated into the phage genome under the control of strong eukaryotic promoters and the phage would then act as vehicle for the delivery of foreign DNA in mammalian cells where it is expressed (Clark and March, 2004a). Thus, such phage constructs pose the additional ability of delivering the antigen gene directly to the immune reactive cells like dendritic cells, Kupffer cells, etc (Zanghi et al., 2007).

Phage display vaccine

A branching phage M13 has been successfully used to display foreign antigen peptide in several cases. For example, Fang et al. (2005) used melanoma-specific tumor antigen (MAGE-A1) displaying M13 phage to create cancer vaccines effectual in dropping tumor intensification in mice. The limitation of this system is that it is either ineffective in displaying larger proteins (gpVIII) or has a low copy number (gpIII) (Smith, 1985). Unlike M13, T4 is considered as the better display platform because of their highly immunogenic virion proteins (that is, Soc and Hoc) that are not only capable to display peptides of different sizes and sequences but also act as good adjuvants (Li et al., 2006a). T7 is another phage capable of displaying proteins and peptides together with antigens. Lewis lung cancer vaccine primed by T7 phage display of vascular endothelial growth factor (VEGF) has been effectively used to induce strong immunogenic response and inhibit cancer growth (Li et al., 2006b). Phage lambda (λ) also allows displaying foreign genes fused to λ capsid protein gene D (gpD); multiple copies (~450) of the same foreign antigen can display as D fusion on λ surface (Gupta et al., 2003), which is effective in eliciting a strong immune response (Clark and March, 2004b). Production of successful λ display antigen to produce neutralizing antibodies against infectious porcine circovirus infection has been shown by Hayes et al. (2010), using gpD fusion antigen on phage λ.

Phages for the detection and typing of bacteria

Phages are ideal for precise identification of bacterial strains and detection of pathogenic bacteria due to their highly specific targeting capabilities (Clark and March, 2006). The ease of use and cheapness of phage typing also contribute to the fact that it is still among the most commonly used methods for strain identification (e.g., *Salmonella, Listeria,* and *Staphylococcus* (Rees and Loessner, 2005).

This loom has been rationalized, and diverse correlated methodologies have been projected to augment the sensitivity of detection. These include the use of phages DNA encoding a reporter gene like luciferase (*lux)*(Kodikara et al., 1991) and green fluorescent protein (*gfp*) (Funatsu et al., 2002), which are expressed only once introduced into metabolically active target bacteria. Detection in Lux reporter phage assay is based on the linear relationship between the number of ATP molecules hydrolyzed and the quantity of light produced by the luciferase. The bioluminescence may be rapidly measured in a bio-luminometer; thus, quickly identifying the bacterial strain in the sample (Rees and Loessner, 2005). Similarly, fluorescently labelled phage prepared by cross-linking dyes to its surface can be used to detect bacteria; following adsorption phage to the host cells and detection of the bound fluorescent signal (via epifluorescence microscopy, flow cytometry, or confocal microscopy) (Hennes and Suttle, 1995; Goodridge et al., 1999).

Another phage-based approach to bacterial detection is to identify the released intracellular components such as adenylate kinase and β-D-galactosidase (Corbitt et al., 2000), or virion particles upon specific lysis of bacteria. The later method forms the basis for so-called “phage amplification assays,” which have been applied for the rapid detection and identification of specific pathogenic bacteria including *Salmonella*, *E. coli* O157:H7 and *M. tuberculosis* (Favrin et al., 2003; Mole and Maskell, 2001). The increased phage progeny in this method can be detected by plaque assay or other endpoint methods (Rees and Loessner, 2005).

Commercial phage-based products for detection of pathogens have also been developed including FAST Plaque-Response™ (Detection of rifampicin resistance in *M. tuberculosis*), FAST PlaqueTB™ (Detection of *M. tuberculosis),* and MRSA/MSSA blood culture test (Detects *S. aureus* methicillin resistance/susceptibility) (Monk et al., 2010).

GENERAL CONCERNS AND POSSIBLE SOLUTIONS

Despite their remarkable achievements in many areas, it is important to remember that bacteriophages are not infallible, and that there are real concerns that need to be addressed about the effects of putting phages into practice. The major concerns associated with the use of phages in clinical practices are discussed and possible solutions are suggested here below.

Bacteriophage specificity

Because of phages’ high host specificity, the development of clinical assays to rapidly identify the etiological agent and their susceptibility to phages are

necessary prior to phage administration. Mutations or other changes in the target bacteria could lead to the loss of specific recognition/interaction between the phage and the host. (Re) selection from a panel of phages or the use of polyvalent phage cocktails active against the majority of strains of the etiologic agent would then be necessary to address the limited host range of any single phage (Van der Vlugt and Verbeek, 2008).

Resistance to bacteriophages

In order to survive the constant onslaught of phage, bacteria have evolved phage resistance through mechanistically diverse defense strategies that act at every stage of the phage life cycle. The "innate" strategies used by bacteria to evade phage predation are: blocking phage adsorption to bacterial surface receptors (via mutation or masking receptors); inhibiting the injection and/or integration of phage genomes (via Super infection exclusion (Sie) systems); degradation of phage DNA by Restriction-Modification (RM) systems (via restriction endonuclease and a cognate methyl transferase); and abortive infection systems (Abi) (that is, death of the infected cell to protect other clonal population from predation) (Seed, 2015). The other system, called CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats), provide sequence-specific adaptive immunity by integrating short virus sequences in the bacteria's CRISPR locus. Thus, allowing the bacteria to recognize and clear infections (Rath et al., 2015). However, the rate of developing resistance to phages (~10^{-7}/host cell) is approximately 10-fold lower than to antibiotics (Tanji et al., 2004). Phage mixtures constitute the traditional method used to deal with this problem but have shown mixed results (Sulakvelidze et al., 2001). Another approach involves the use of phages that are designed to target one or more virulence factors of a pathogenic bacterium as receptor binding site. Phage resistant, via receptor loss or modification, is expected to occur only rarely, and with detrimental effects on the ability of the resistant strain to cause disease (Orndorff, 2016).

Bacteriophage as mediators of virulence genes transfer

Since viruses tend to swap genes with each other and other organisms, there is a good chance that some phages may be involved in the transfer of toxins and virulence factor genes to the bacteria (Abhilash et al., 2008). Broudy and Fischetti (2003) reported in vivo phage-associated conversion of Tox^- *S. pyogenes* into Tox^+bacteria. Other phage associated toxins includes: cholera toxin encoded by CTXϕ phage (Davis and Waldor, 2003), botulinum toxin encoded by *Clostridial* phage c-st (Sakaguchi et al., 2005), shiga-toxin encoded by λ phages of enterohemorrhagic *E. coli* (EHEC, culprit of the 2010 *E. coli* epidemics in Germany) (Garcia-Aljaro et al., 2006).

Moreover, transport of antibiotic resistance genes by phages has also been documented for bacterial species: transduction of Gentamicin and Tetracycline resistance among enterococci; the transfer of antibiotic resistance plasmids in MRSA or the carriage of β-lactamase genes by phages in *E. coli*. Another study of phage DNA from hospital and urban treated effluents using qPCR assays showed the presence of resistant genes to β-lactam antibiotics (bla_{TEM}, bla_{CTX-M} and bla_{SHV}) and reduced susceptibility to fluoroquinolones (*qnrA*,*qnrB* and *qnrS*) (Marti et al., 2014). A study conducted in Ethiopia on antibiotic resistance pattern of *S. aureus from synthetic sponges had 7* multi drug resistance (MDR) patterns which were detected (Tesfaye et al*.,* 2015).

Therapeutic use of phages having virulence genes in their genomes constitutes a risk of transfer of the pathogenic properties to the bacteria of the human normal biota; thus care should be taken to identify potentially risky phages via comparative genomics. This approach is, however, restricted by the large number of unidentified ORFs in phage genomes (Merril et al., 2006). A novel approach involves restoring antibiotic sensitivity of pathogens using antibiotic resistance plasmid-hunting lytic phages (Jalasvuori et al., 2011), or via lysogenic-phage mediated delivery of sensitive genes (Edgar et al*.,* 2012), or delivering of CRISP-Cas system to destroy antibiotic resistance-conferring plasmids (Yosef et al., 2015).

Bacterial cell lysis

A concern with any bacteriophage induced lysis is that the rapid and massive destruction of bacteria in vivo may release cellular toxins (e.g., endotoxins and super antigens) that stimulate inflammatory reactions which in turn may lead to increased morbidity and mortality. In order to reduce the risk of this happening, phages have been selected or engineered to be lysis-deficient and/or non-replicative. Hagens et al. (2004) engineered *P. aeruginosa* filamentous phage Pf3 to express restriction endonucleases (RE) by replacing its export protein gene with RE gene. This non-replicative phage efficiently killed the host in vitro, while endotoxin release was kept to a minimum. Another novel strategy, developed at MIT, make use of "phagemids": engineered phages that delivers a plasmid instead of a whole genome. In a phagemid, the plasmid is encoded with genes that kill the bacteria without the cell being lysed. These scientists have had a high degree of success in treating mice for peritonitis with no resistance development (Krom et al., 2015).

Rapid clearance of bacteriophages

Bacteriophages, being foreign particle, tend to be rapidly cleared from the circulation. It had been discovered that the phages were rapidly cleared by the spleen, liver and other filtering organs of the reticulo endothelial system (RES) (Geier et al., 1973). To circumvent this problem, Merril et al. (1996) developed a method (called “serial passage” method) for serially passaging phage through animals to isolate mutant strains that circulate for longer periods of time in vivo. This is due to the fact that most isolates contained mutations in the major phage coat protein that were postulated to enable escape from clearance by the RES organs and to thereby remain in the circulation for longer periods of time (Geier et al., 1973).

Consumer perception problems

In addition to the above mentioned concerns, there is also a need to overcome the understandable stigma among consumers. This situation would be resolved via targeted educational campaigns to raise awareness and acceptance of phages, and also through the use of phages encoded products, such as endolysins (Borysowski et al., 2006).

PROSPECTS FOR FUTURE USES OF PHAGES IN ETHIOPIA

Communicable diseases are chief root of illness and death in Ethiopia. Next to nutritional troubles, they give rise to 83% of health tribulations in Ethiopia (FMOH, 2015). Acute respiratory infection, diarrheal disease and tuberculosis (TB) are among the top ten major infectious diseases caused by bacteria (Moges et al*.,* 2014). Salmonella isolates from ready to eat foods in Jigjiga city, Ethiopia catering establishments resisted at least three and more antibiotics (Tesfaye et al*.,* 2017). In addition to these main killers is the considerable worldwide trouble of hospital origin infections (e.g., *E. coli, K. pneumoniae,* and *S. aureus*) caused by resistant pathogens. Over the last 60 years, the use of antibacterial drugs has contributed to the dramatic fall in morbidity from these infectious diseases (WHO, 2001a). In recent years, however, the resistance of pathogenic bacteria to commonly used antimicrobials has been a great public health concern. Some of the major outcomes of resistance comprise amplified death and illnesses, health care costs, and loss of productivity in animals (WHO, 2001b). The consequences of this emerging antimicrobial resistance might be considered as harder in resource deprived backgrounds like Ethiopia.

In Ethiopia, antimicrobials share the highest proportion of the total drug budget and they are repeatedly the largest group of drugs sold (Andualem, 2002). Though, their prevalent accessibility has had numerous positive and negative connotations, one of this is their application for minor infections, overuse by health care providers and consumers, abuse due to lack of access to suitable treatment and under use owing to lack of money to pursue a standard treatment course that will extra go faster the development of resistance to frequently used antimicrobials. Thus, successful anticipation and inhibition of infectious diseases of both human and animals in Ethiopia are compromised by enlarge in resistance to the antimicrobial drugs (Drug Administration and Control Authority (DACA), 2009).

Although large-scale studies have not yet been performed, the available reports do indicate that various clinically important species such as *M. tuberculosis*, *E. coli, Shigella, Salmonella* and *S. aureus* are getting gradually more resistant to common antibiotics together with ampicillin, amoxicillin, penicillin, tetracycline and trimethoprim-sulfamethoxazole (SXT) (Reda et al., 2011; Godebo et al., 2013; Moges et al., 2014; Federal Ministry of Health - FMOH, 2015). According to WHO’s (2014) report, the national level of fluoroquinolones resistant nontyphoidal Salmonella and MRSA were 14 and 31.6%, respectively. The level of resistance of *E. coli* to third-generation cephalosporins were 53% ceftazidime (caz) and 70% ceftriaxone (cro), and to fluoroquinolones were 71%. Carbapenems typically continue the only accessible treatment alternative. Like *E. coli, K. pneumoniae* also showed resistance to third-generation cephalosporins (14% (caz) and 20% (cro)) (WHO, 2014).

Antimicrobial resistance has a blow on animal health and influences assembly costs. In the case of human beings, in some occasions there are only some options for the treatment of infections in fauna (Vaarten, 2012). As an example, *S. aureus*, for the most part frequently cause mastitis, which has provided 100% resistance against nitrofurantoin, bacitracin, and sulfamethoxazole. Several Salmonella strains have developed resistance to normally given antimicrobials in infectious diseases. *C. jejuni, S. aureus, S. epidermidis, S. agalactiae, B. cereus, E. coli, K. pneumoniae, E. aerogenes* and other bacterial species showed that resistance ranging from 17.3 to 100% were isolated from ready to eat meat and milk (DACA, 2009). Abebe et al*.* (2014) reported two predominant serovars of Salmonella (that is, typhimurium and enteritidis) from cattle that are 100% resistant to at least one antibiotic (of which 71.4% are multidrug-resistant (MDR)).

Given the severity of bacterial infection in Ethiopia, and the complex multifactorial problem presented by antimicrobial resistance (AMR) on our societies, it is thought that the bacteriophage concept should be taken into consideration as a valuable instrument to resolve the current state of affairs. In Ethiopia, nonhuman applications of phages and phage products will represent the first and widest use of these agents, which include

use in: veterinary, agricultural (to control phytopathogens), and food hygiene and food safety.

Veterinary medicine: bacteria infecting viruses, numerous vital compensation along with more antibiotics, together make their use in diverse livestock industries potentially very attractive. For instance:

i) Because of their specificity, their use is not probably to choose for phage resistance in untargeted species.
ii) Because of difference in bacterial mechanisms of resistance to phages and antibiotics, phages resistance will not affect antibiotic efficacy against human infections.
iii) Since they are cheaper and faster to produce, and also more readily modifiable, effective phage preparations are quickly attained against rising phage resistant mutants microbes.

In Ethiopia, a multitude of disease causing microbes could be embattled amid phages, however preliminary phage studies would be more likely to focus on the prevention or control of major infectious diseases that result in high morbidity and mortality in livestock industries, and which are transmissible to humans. These zoonotic diseases include anthrax, pasteurellosis, salmonellosis, tuberculosis, and brucellosis (Hooper, 2016). Phage components could also be industrialized against additional bacterial infections of immense concern, like blackleg and bovine mastitis that should have a considerable practical applicability in the dairy industry. The use of such preparations will not only decrease the prophylactic and therapeutic use of antibiotics in farm animals, except also it may potentially have some growth encouraging effect in animals (e.g., by falling antibiotic-induced dysbiosis), which possibly will aid diminish or eradicate the use of GPAs in various livestock industries. Consequently, reducing the tremendous economic losses associated with livestock industry.

Agriculture is one of the most vital sectors for economy in Ethiopia and the bacterial diseases are major threat to the agriculture food production, which makes their control even more difficult due to resistance to bactericides. Phages can represent biological control agents that not only destroy phytopathogens but also reduce the use of antibiotics and pesticides.

The initial attractive applications of phages in Ethiopia will probably be for relevant admin, since hurdle of translocation is not present and its applicability is therefore less complicated (Tsonos et al., 2014). Phages could also play a significant part in the prevention and treatment of diarrheal disease, wherein many causative agents' are resistant bacteria and considering the poor sanitation, the control of those epidemics such as cholera for example quickly run out of control. But the most optimal targets for their application could be to diseases caused by MDR pathogens (MRSA, Extended spectrum beta-lactamase (ESBL) enterobacteria, vancomycin-resistant enterococcus (VRE) and *C. difficile*) where there are little/no other treatment options left and organisms that are tricky to eliminate, like, those proliferating in biofilm. In general, phages are not the panacea to control bacterial infections; however, when carefully evaluated and in combination with other strategies (like antibiotics) they have tremendous potential.

Though there is immense possibility for phage usage, substantial effort have to be made prior to phages approval in Ethiopia, and the country will also require creating proper authoritarian guidelines ahead of entirely realizing the public health reimbursement of phages. Since it is a new concept, phages are hurdles that cause cause panic and misconceptions. Thus, can clarify delusions and increase recognition of this latest class of antibacterial products. Facilitating workshops to offer information regarding the possible settlement of phages, deal with safety concerns, and get contribution on how phages could be duly applied within a local setting.

It is believed that bacteriophage holds a great potential and should be exploited in Ethiopia, and thus collection of phages against various bacterial hosts from environmental samples including extreme habitats is of the utmost importance and should be encouraged. Subsequently, credible studies must be setup to congregate the necessary data with respect to the wellbeing and effectiveness of phage treatment and evolutionary outcomes of its limitless application.

CONCLUSION

Facts earlier specified provide a glimpse on diversity and abundance of bacteriophages in the biosphere and how it evolved through the years following their discovery: from early enthusiasm to critical skepticism and rejection, and now to the current attention and reassessment. It also gives detail on the wide range of applications of phages and their products in arena of biotechnology and medicine, ranging from diagnosis of diseases and its prevention, to the treatment. This versatility gives optimism that phages could be practical to human beings in various ways. They have also been a cornerstone of modern molecular biology and genetics. Through a combination of bacteriophages, it could be used to simply treat a broad diversity of antibiotics resistant pathogens. By virtue of being specific, a lytic phage can be used independently to treat infection via lysis without harming any helpful bacteria or mammalian cells. Easy accessibility and commonness of phages could enable us to exploit the peptides against bacteria that have been observed on the phage surface. Similarly, a protective antigen could be delivered as a DNA or phage display vaccine. Phages have also been recognized in the food industry to combat food pathogens and extend the shelf life, and to reduce colonization or eliminate bacterial infection in livestock, plants and fruits.

Prospective applications comprises that in the developing world like Ethiopia to the newly emerging outbreaks of human and animal origin that are resistant to antibiotics or in response to food safety to control food borne pathogens- every instances of where assets might be extended to the boundary, and where the hurry, low cost and ease of production of bacteriophage-based medicines would be preferably appropriate. Despite their remarkable achievements, there are a few considerations about the use of phages that need to be addressed. It includes the high host specificity of phages, bacterial resistance to phages, the issue of lysogenic phages as mediators of virulence genes transfer, as well as their rapid clearance by the immune system. There is also necessity for prevailing over the reasonable disgrace among consumers concerning safety of deliberate utilization of phages. Nevertheless, with humanizing ecological perceptive and with the tools of biotechnology and synthetic biology offering the solutions, phage applications in the environment and in medical practice can be handled responsibly and may provide a solution for the current antibiotics resistance crisis. Furthermore, we must learn from past mistakes that led to the antibiotic crisis if phages are to replace all or some of the current applications of antibiotics in medicine and agriculture. However, for now there is sufficient data and it is hoped that further studies is necessary in the arena of bacteriophage.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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