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Review on Bacteriophage and its Application in the Combat of Zoonotic Bacterial Pathogens

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Abstract: The high prevalence of certain bacterial diseases in animals and their economic impact at the productive and public health levels, have directed attention towards the search for new methods of control and prevention, alternative or complementary. Moreover, limiting the choice of control strategies, the current raining and challenges of antimicrobial resistance profile of pathogen and the cost of drug discovery could push to look into an alternative of pathogen mitigation. The aim of this paper is to review on the renewed interest on the application of bacteriophages, viruses that kill bacteria, as potential biological antimicrobial agents against zoonotic bacterial pathogens. The progressive increase in the number of multi-drug resistant bacteria on the use of antibiotics in treatment of livestock have led to the growth of research on the use of bacteriophages to combat bacterial infections in humans and animals. The high success rate and safety of phage therapy in compares on with antibiotics are partly due to their specificity for selected bacteria and the ability to infect only one species, serotype or strain. This mechanism does not cause the destruction of commensally bacterial flora. Phages are currently being used with success in humans and animals in targeted therapies for slow-healing infections. Bacteriophage application trials have shown that 10^2-10^3 plaque forming unit (PFU) is adequate to counter 10^6-10^9 CFU per milliliter proliferation threshold of bacteria in vivo. For instance, Colo-jel, Ento-jel and Staphylo-jel are suggested against targeted bacteria like, *Staphylococci*, *Streptococci* and *Escherichia coli*. Phagoburn contains bacteriophage cocktail targeting pathogenic strain of *E. coli* and *Pseudomonas aeruginosa*. In general, they have also found application in eliminating pathogens from the surface of foods of animal and plant origin.

Key words: Antimicrobial Resistance · Bacteriophage · Pathogens · Phage Therapy

INTRODUCTION

Bacteriophages (or phages, viruses that infect bacteria) are the most abundant entities on our planet, being harmless for all organisms including humans except for their target bacterial hosts, infecting every type of bacterium in every known environment and being among the major drivers of bacterial adaptive evolution [1].

The search for an appropriate mechanism for the control of several bacterial pathogens of veterinary medical importance, especially those involving an impact on human and animal populations has been the subject of many investigations [2]. Bacterial diseases in domestic animals can cause detrimental effects generating direct and indirect economic losses in production systems and, in the case of pets, involves affective cost which is difficult to quantify. Furthermore, there are bacterial pathogens that cause food-borne diseases with high public health impact [3].

On the basis of their unique characteristics and anti-bacterial property, phages are being freshly evaluated taxonomically. Phages replicate inside the host either by lytic or lysogenic mode after infecting and using the cellular machinery of a bacterium. Since their discovery by Twort and d'Herelle in the early 1900s, phage became an important agent for combating pathogenic bacteria in clinical treatments and its related research gained momentum. However, due to recent emergence of bacterial resistance on antibiotics, applications of phage (phage therapy) become an inevitable option of research. Phage particles become popular as a biotechnological tool and treatment of pathogenic bacteria in a range of applied areas [4].

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Phages are considered to be the most diverse and abundant entity on earth and are thought to exist in every ecosystem [5] ranging from extremely hot environments like hot springs, the Sahara, to extremely cold environments like polar inland waters [6]. It is also reported that to carry out important ecological functions, phages express a variety of auxiliary metabolic genes [7]. A single phage particle can hunt for a specific bacterium species or a subset of the same species. After infecting a bacterium, phages replicate inside it. Once a bacterial cell is infected, phages multiply exponentially using the cellular machinery of the bacteria including protein-synthesizing and energy-generating systems [5].

However, the method of propagation may either be lytic or lysogenic in nature. The lytic phages cause lysis of the host bacterial cells to release progeny viruses [8]. On the other hand, lysogenic or the temperate phages integrate their nucleic acid (genome) within the host bacterial cell and replicate along with the host, conferring new properties to the host bacteria [9].

Infectious diseases of animals can be subdivided into those which have mainly economic impacts and those which are zoonotic, i.e., transmissible from animals to humans. The most common bacterial pathogens associated with food animals are *Campylobacter*, *Salmonella* and *Escherichia coli*. Respiratory infections are prevalent in young animals where levels of innate immunity are lower than in adults. Gastrointestinal infections result in enteritis, usually with diarrhea, affecting neonatal animals and those in the immediate post weaning period. The pathogens most frequently implicated in such infections include *Escherichia coli*, *Salmonella* and *Campylobacter*. However, a range of parasites and enteric viruses also may be involved, either individually or as part of a mixed infection. Mixed infections are common, with viral infections predisposing to secondary bacterial infections; the latter frequently caused by members of the *Actinobacillus* and others such as, *Haemophilus*, *Streptococcus* and *Staphylococcus*. Septicemias often arise from respiratory infections and generally involve the same organisms. Added to this list are the mycobacteria, which produce tuberculosis in a variety of livestock species. Many of these infections are currently addressed through a combination of biosecurity measures, vaccination and chemotherapy [10].

The use of antimicrobial chemotherapy in food animals is becoming increasingly difficult to justify in the West. This is due partly to the increase in antimicrobial resistance and also pressure from consumers and regulatory bodies [11]. Antimicrobial resistance is becoming a major issue, which international organizations including the World Health Organization have recognized requires a multifactorial approach, including the use of bacteriophages [11, 12].

Since the discovery of penicillin by Alexander Fleming in 1928 and sulfas by Gerhard Domagk in 1932, numerous drugs with antimicrobial effects have been produced and used as therapy for the treatment of bacterial infections, with great success in controlling these pathogens. Their use has reduced the impact of disease in both people and different animal populations and there is a wide range of substances being available with different mechanisms of action and therapeutic indications. Since their discovery, antimicrobials have been the first line of action against bacterial diseases. However, several years ago the presence and increase of bacterial resistance to many antimicrobials has been observed, becoming a subject of global concern in human and veterinary medicine, which has stimulated the introduction of new drugs [13].

Despite the speed at which new antimicrobial agents are being introduced into the market, bacteria have shown a remarkable and rapid ability to evolve multi-resistance to these drugs. Currently, the emergence of antimicrobial resistant pathogens has led to an interest in discovering new therapeutic tools that allow replacing or complementing antimicrobials when combating bacterial diseases, particularly those associated with food-borne diseases [13, 14].

Among these therapeutic tools are the bacteriophages, viruses described as biological agents that lyse bacteria, used before the advent of antibiotics and currently attracting the interest of the international scientific community [14]. Therefore, the objective of this paper is:

- To review the discovery of bacteriophages and its characteristics,
- To review bacteriophages as a combat of zoonotic bacterial pathogens has become increasingly important, due to its promising results regarding the use of bacteriophages as therapeutic and prophylactic agents in food producing animals.

History of Bacteriophage

Brief History of Bacteriophage: British bacteriologist Ernest Hanbury Hankin, in 1896, first reported antibacterial activity in the waters of the river Ganga and the river Yamuna in India. However, how the implications of Hankin's findings related to the existence of bacteriophages remain dubious in the scientific society [15].

In 1915, Frederick William Twort FRS, an English bacteriologist, superintendent of the Brown Institute for Animals and professor of bacteriology at the University of London, while growing viruses in a laboratory condition, found zones of clearance in the form of Transmissible glassy transformation with micrococcus bacteria. He also concluded that this agent multiplied itself in the process of killing the bacteria. On the other hand, Félix d'Herelle, a French-Canadian microbiologist working at the Pasteur Institute in Paris, observed the bacteriophage phenomenon (in the year 1917) on severe hemorrhagic dysentery among French troops stationed at Maisons-Laffitte [16]. He had observed the same phenomenon in 1910 when he was studying microbiology due to an epizootic locust infection in Mexico.

D'Herelle prepared bacteria-free filtrates from fecal samples of patients and incubated the filtrate with the bacterium *Shigella*, isolated from those patients. His primary goal was to develop a vaccine against bacterial dysentery. For observing the growth of the bacteria, an aliquot of the filtrate-bacteria mixture was spread on agar medium and incubated. d'Herelle found the appearance of small, clear zones, which he primarily termed taches, then taches vierges and later, plaques.

D'Herelle also proposed that the phenomenon was caused by a bacteria parasitizing virus and named B bacteriophage phages implying to Beat or B devourbacteria. As per the recollection of d'Herelle, the name B bacteriophage was decided together with his wife Marie and the word came into existence on 18 October 1916 the day before their youngest daughter's birthday. Thus, the credit of discovery of bacteriophages goes independently to two scientists: F.W. Twort and Félix d'Herelle. Primarily, the scientific society first called it B Twort-d'Herelle phenomenon and later, the B bacteriophage phenomenon [16].

History of Bacteriophages in Veterinary Use: Bacteriophages were discovered independently by Twort (1915) and d'Herelle (1917) [17]. The first example of veterinary phage use was by d'Herelle himself, in the experimental treatment of fowl typhoid (*Salmonella gallinarum*) in Eastern France; initially in a pilot study with six chickens, then in a much larger trial with 100 chickens. Both trials were successful, with 95-100% of phage-treated animals surviving compared with 0-25% of untreated control birds [17, 18]. Moreover, in clear contrast to d'Herelle's earlier studies, Pyle (1926) reported phage therapy to be ineffective in treating fowl typhoid, even though he used phage with excellent in vitro activity against the infecting bacterium. On the other hand, injecting bacteriophages into the carotid artery has been claimed to significantly reduce the mortality of rabbits with experimental streptococcal meningitis (Kolmer and Rule, 1933) [19, 20].

Phage treatment appeared to delay rather than prevent death of the birds, regardless of the administration route (oral or intramuscular) and despite clear evidence that the phage used had a broad host range and lysed *in vitro* cultures of S. *pullorum*. Perhaps unsurprisingly, veterinary phage therapy experiments were often used as a surrogate to determine the efficacy of this approach for human infections [21]. Such work included [22] who treated systemic Salmonella infections in mice with oral and intraperitoneal phage administration, largely unsuccessfully. Also, E. coli cystitis in rabbits and guinea pigs has been reported (Marcuse, 1924; Larkum, 1926) to be cured or markedly alleviated by phage treatment. Furthermore, excellent results were reported by [23] in 1943 who used intraperitoneal phage to treat cerebrally injected S. dysenteries infections in mice. Also, many attempts to treat experimental plague in rabbits, guinea pigs, rats and mice failed to influence the course of the disease (Naidu and Avari, 1932; Compton, 1930; 1928; Colvin, 1932). However, bacteriophage could not be recovered from phage-treated animals [18]. Moreover, a similar percentage of mice in the phage-treated group could be protected by injection of formalin-inactivated preparations, suggesting active phage replication was not responsible for this outcome and that protection may have been mediated by immune modulatory levels of bacterial lysis components in the preparation [21].

Progress in phage therapy was hampered in the subsequent decades due to a poor understanding of bacterial pathogenesis and phage-host interactions and was compounded by the absence of good animal models of disease. Regrettably, this led to a succession of poorly designed and executed experiments and field trials. Such mistakes included selecting poor phage canddates, which failed to lyse cultures fully in vitro and the simultaneous administration of the bacterial inoculum with the phage, which essentially replicated an *in vitro* interaction between phage and host. After the advent of antibiotic therapy in the West in the 1940s-1950s, the idea of using phages largely became discredited. It was only during the 1980s that rigorously controlled phage therapy experiments were performed [18, 21].

General Characteristics of Bacteriophages: Bacteriophages (or phages) are viruses that infect only prokaryotes (bacteria and archaea) and produce their lysis, being this activity the cornerstone supporting the idea of using them as therapeutic agents [7].

Bacteriophages are highly ubiquitous, occupying all those world ecosystems where bacteria develop successfully and may be isolated from surface and deep water ecosystems, soils, oral cavity and blood and guts of healthy humans and animals [22].

They have been isolated from aquatic systems in quantities ranging from 10^4 plaque forming units (PFU) to more than 10^8 PFU/ mL, in fresh water sediments in ranges of 0.65 PFU to $3x10^9$ PFU/g and in marine environments in quantities over $12x10^9$ PFU/ml. In soils, concentrations of 0.7 to $2.7x10^8$ PFU/g have been found. Some bacteriophages are highly specific attacking only certain bacterial strains, while others are quite broad in their host range [22, 24].

Nucleic acids present in these viruses can be DNA or RNA, single or double stranded with most phages containing double stranded DNA. Phages transfer their genome from one susceptible bacterium to another, wherein they direct the production of viral progeny. A specific group of bacteria is host to each phage: this group is often only one bacterial species, but several related species can sometimes be infected with the same phage. The phage infection cycle follows a number of programmed steps, where efficiency and coordination depend strongly on the metabolic state of the host cell regarding the molecular mechanisms of the infection of their hosts, bacteriophages can follow two different destinations and they are lytic and Lysogenic cycles [24].

Lytic Cycle: Lytic or virulent phages are phages, which multiply in bacteria and kill the cell by lysis at the end of the life cycle. In the lytic life cycle, the virus breaks open or lyse the host cell in which it results in the death of the host. The host cell undergoes lysis and dies, simultaneously liberating a large number of progeny phages, which are each then ready to start another cycle by infecting new neighboring bacterial cells. The lytic cycle initiates with the attachment of the phage on the bacteria with the aid of a complex of proteins [25, 26].

The so called "lytic or virulent bacteriophages" follow the lytic infection cycle, wherein the phage genome is injected into the bacteria and multiplies in the bacterial cell altering its metabolism, resulting in the lysis at the end of the cycle due to the action of a viral lysozyme, which allows the release of the viral progeny formed. This phenomenon, which occurs within minutes or hours, shows that the viral particles are auto replicative entities at least as long as a bacterial population in sufficient numbers to support this event exists. Their lytic mechanism from an ecological viewpoint constitutes a predator/prey system and from an epidemiological viewpoint a host-parasite model. The phenomenon of transduction (transfer of bacterial DNA via phage) is rare in lytic phages [27].

The infectious cycle of a lytic bacteriophage comprises the following steps. Bacteriophage adsorption to the bacterial cell through is recognizing specific bacterial cell structures by means of their fibers or tail spicules [24]. For this purpose, the phage may use bacterial capsules, different parts of the LPS, flagella, fimbriae and some other surface proteins, oligosaccharides and lipopolysaccharides. Injection of the phage genome into the host bacterium: this is facilitated by an enzyme present in the phage tail tip which degrades the peptidoglycan. This introduction of the viral genome is energy-dependent, obtained from available ATP or the membrane potential of the bacterium [2]. Early phage gene expression and synthesis of early proteins, involved in the intervention of the bacterial enzyme systems and viral genome replication [2, 24].

Phage genome replication, Expression of late phage proteins, involved in the formation of new viral particles, viral capsid formation and the lysis of the host bacteria, Assembly of phage heads and tails and viral genome compaction, Lysis of the host bacteria and release of new phage progeny [2] This lysis is produced by the action of two enzymes which degrade the cell wall and the inner membrane, an endolysin and holin, respectively [27]. Their action makes the cell lyse due to the structural inability to resist internal osmotic pressure. This rupture of the cell wall and membranes allows the release of the viral progeny previously formed, thereby enabling a subsequent infection of other bacteria [2, 24].

Lysogenic Cycle: In comparison to the lytic cycle, the "lysogenic or tempered bacteriophages" use the lysogenic pathway, where the phage genome, after being injected in the bacterial cytoplasm, is integrated (prophage) and replicates as part of the host genome, remaining latent for extended periods; if the host bacterium faces adverse environmental conditions this prophage may activate and return to the lytic cycle and later the newly formed phage particles are released after bacterial lysis [27]. The integration of the viral genome into the bacterium's is the reason why they are not used in phage therapy since they could incorporate, carry and transfer genes coding for undesirable elements such as the Shiga toxin from Escherichia coli [24].

Taxonomy of Phages: As mentioned earlier, there are billions and billions of phages. Deciphering taxonomic characterization is a challenging task, especially for such nano-sized phage particles. In 1967, phages were first classified by Bradley and were subsequently approved by the International Committee on Taxonomy of Viruses (ICTV) and total 111 phag`es were listed in classification. Bradley's classification projected six basic morphological types of tailed phages that further, categorized on the basis of morphotypes (contractile tails, long and non-contractile tails and short tails), small isometric ssDNA viruses, filamentous phages and small ssRNA phages. The regulating body for the viral taxonomic system (ICTV) characterizes phage particles taking into consideration numerous parameters like host range, physical characteristics (such as structure, capsid size and shape), type of genomic material (single or double-stranded DNA or RNA), genome size and resistance to organic solvents. More than 96 % of phages are tailed and carries dsDNA as genetic material; however, they may vary in shape like cubic, filamentous and pleomorphic. Polyphasic taxonomy was revised and emphasis was given on genomic relationship [28].

Earlier in 2008, only 18 genera and 36 species were listed among three caudoviral families, Myoviridae, Podoviridae and Siphoviridae. Subsequently, the order caudovirales expanded and an updated and detailed bacteriophage classification was presented in ICTV Release 2015 [4]. Some alterations have also been suggested by committee, like replacement of word Bphage^ with Bvirus^ in prokaryotic virus taxon names, omission of infix Blike^ from prokaryotic virus genus names, discouragement of use of BPhi^ and other Greek letters in prokaryotic virus genera, exclusion of hyphens and encouragement to use of host genus name in replacement of taxon names is being encouraged to avoid confusion related to phage action on specific bacterial host [4].

Mechanism of Proliferation: Specific receptors (like lipopolysaccharides, teichoic acids, proteins and flagella) on the surface of the host bacteria are required for the phage to infect the bacteria. Due to this specificity of the receptor present on the bacterial cell surface, phages can infect specific hosts only. However, in solution, this interaction with the host is a random phenomenon for the phages. Bacterial type (Gram-negative and Gram-positive), growth conditions and virulence also influence the phage to attach on the host's surface [29]. The outer membrane of Gram-negative bacteria has an external lipopolysaccharide (LPS) layer and embedded outer membrane proteins (OMPs) for transport and diffusion of nutrients. These act as phage receptors and in some infection strategies, they are essential for adsorption of phage particles as well [30]. In comparison, teichoic acids (peptidoglycan interspersed with acid polysaccharides) present in the Gram-positive bacteria cell wall act as receptors for their corresponding phages [31].

The penetration processes in bacterial cell also vary in different phage groups. In general, myoviridae phage inserts its genetic material into the bacterial cell by using a syringe-like movement of its tail. After receptor recognition, in a reversible binding mode, the phage particle attaches its base plate with the bacterial surface utilizing the flexing activity of tail fibers. The phage takes sufficient time to make its surface binding irreversible. Thereafter, with the help of ATP, the contraction of its tail takes place, along with insertion of its genetic material. On the contrary, podoviridae phage, which is devoid of the tail part of the myoviridae phage, inserts its genetic material after enzymatically degrading a portion of the bacterial cell membrane using its small, tooth-like tail fibers [31].

Biosensor Development: A biosensor is typically composed of bio-based recognition and transducer components and electronic systems (signal amplification, processing and display). Potential applications of biosensors are diverse and numerous, from defense security to pharmaceutical science to environmental monitoring and assessment. Biosensors have a number of advantages like sensitivity, specificity, speed and accuracy of detection, easy sample preparation, cost-effectiveness, etc [32]. Like that of many other biomaterials, unique biological, geometrical and mechanical characters of bacteriophages can be exploited for bacterial identification, pathogen detection and biocontrol [33].

It is simpler to develop biosensor surfaces by surface adsorption of phages, though it may give inconsistent results due to unstable immobilization densities. However, chemically anchoring (cysteamine-modified and glutaraldehyde-activated gold substrate) phages on a detection platform display a consistent improvement in the phage density and detection.

For chemically functionalizing phage-based biosensors, selection (biopanning) purity of the phage suspension is an important criterion which should be of various other bio-contaminating agents like other carbohydrates, proteins and lipids. However, genetically modified or engineered phages are more appropriate to develop bio-probes than intact wild-type phages. Being biologically active, wild-type phages upon infection lyse the host bacterium that may lead to reduction of signal on a biosensor platform [34].

Recently, utilizing unique ability of phages to display peptides or proteins on their surface, called Bphage display, is becoming a powerful tool to screen diversity of targets like proteins, carbohydrates, small molecules, or an entire cell. For the phage display technology, lambda, f1, M13, fd, T4 and T7 phages have most widely been used. This emerging technology can revolutionize diagnostics by creating molecules that are otherwise unavailable via conventional approaches. Researchers have successfully expressed cellular proteins and peptides, antibody (or its fragments) and antigen molecule on the surface of phages for developing pathogen detection biosensors, molecular imaging and gene delivery [35].

Bacteriophages for Control of Pathogens: The most common bacteria inducing foodborne infections in humans include bacteria of the genera *Salmonella* and *Campylobacter* and *E. coli* [36]. According to the 2015 EFSA report on resistance to antibacterial agents in selected zoonotic bacteria (*Salmonella* and *Campylobacter*), indicator bacteria (*E. coli* and *Enterococcus* spp.) and other bacteria isolated from poultry and from food, a considerable percentage of the isolates posing a threat to humans and animals are resistant to available antibiotics, partially as a result of their widespread use in treatment of disease in humans and animals [37]. The use of bacteriophages to eliminate pathogens seems quite promising, especially as they are present in every ecosystem and number 1031, which is more than 10 times the number of characterized bacteria [36, 38].

The effectiveness and safety of phage therapy in comparison to antibiotics is partially due to the specificity of bacteriophages for particular bacteria, manifested as the ability to infect only one species, serotype or strain. This mechanism of action does not cause destruction of the commensal intestinal flora. Self-replication of bacteriophages takes place during treatment, which eliminates the need to apply them repeatedly. Another advantage of phages is that they cannot bind to and replicate in eukaryotic cells, which causes a decrease in their titer, correlated with a marked reduction in the number of pathogenic bacteria inducing a given infection in the organism. An equally important advantage is that phages are not toxic, because most of them are composed mainly of proteins and nucleic acids [37].

Despite its numerous advantages, the use of phage therapy is substantially limited, partly because single bacteriophages cannot be used to combat broad-spectrum infections. In many cases complex identification and characterization of the etiological agent is necessary. Moreover, not all bacterial viruses meet the criteria for use in treatment, particularly lysogenic phages, which encode genes of bacterial toxins and thereby transform harmless bacteria into pathogenic ones. They can also be involved in transferring drug-resistance genes among bacteria. Another adverse phenomenon in phage therapy is that phages can be cleared by the reticulo endothelial system, reducing their half-life in the organism and limiting the effectiveness of treatment [38].

The increased use of treatment with bacteriophages is determined by their ability to lyse infected bacteria and mutate resistant bacteria, as well as by the high specificity of phages for particular bacteria. A vast number of infections in humans are induced by multi-drug resistant hospital strains of bacteria and by bacteria which have acquired resistance traits in the natural environment. Phage therapy has found application in treating bacterial infections in dermatology, stomatology, otolaryngology, ophthalmology, gynaecology, paediatrics, gastroenterology, urology and pulmonology. The use of bacteriophages in treating infections in humans has had a high success rate (about 85%), particularly in the case of mixed infections induced mainly by *Staphylococcus aureus*, *Klebsiella*, *Escherichia coli*, *Proteus*, *Pseudomonas*, *Enterobacter* and vancomycin-resistant *Enterococci* [36].

Application of phages in bio-control and therapeutic design Phage therapies are also an effective tool in eliminating bacterial infections in various species of animals. Bacteriophages have also proven successful in treating diseases in different food producing animals [39]. One of the objectives of phage therapy in animals is to assess the suitability of bacterial viruses for control of pathogens having an important influence on animal productivity and health as well as protecting zoonotic

bacterial diseases. Phages used in treatment have been effective in preventing infections and in treatment of colibacteriosis in poultry. Positive results, with a high success rate in eliminating pathogens have also been obtained in combating infections induced by various Salmonella serotypes in game fowl, such as Enteritidis and *Typhimurium*, as well as campylobacteriosis in poultry, particularly infections induced by *Campylobacter jejuni* and *E. coli*. The effectiveness of phage therapy has also been confirmed in infections of broiler chickens by anaerobic *Clostridium perfringens* during the course of necrotic enteritis [2].

Application of Phages

Medicine and Clinical Application: About a century ago, in 1917, the bacteriophage era was started when Félix d'Herelle published a paper demonstrating Bun bactériophage obligatoire [40]. Bruynoghe and Maisin, in 1921 initiated bacteriophage therapy by treating patients having staphylococcal infections. BPhage therapy has become a potential therapeutic. Immense research has been initiated to employ phage therapy as an alternative [41]. Treatment of various pathogens and antibiotic-resistance bacteria (like *Salmonella* spp. *Clostridium difficile* and diarrheagenic *E. coli*) has been tried using phage. Phage-based products were first developed in the commercial laboratory of d'Herelle in Paris [42].

However, due to exponential growth of antibiotic-based drug companies and pharmaceutical giant companies, phage products became less popular. In favor of therapeutic uses of phages, recent reports on the application of phage cocktails for open septic wounds and burn injuries have contributed encouraging results. Phage cocktail containing 82 phages against *Pseudomonas aeruginosa* and 8 phages against *Staphylococcus aureus* was successfully applied on eight patients (43). Recently, Pherecydes Pharma, a biotechnology company in France, have announced the phase I/II single-blind multicenter clinical trials of its bacteriophage based product 'Phagoburn'. Phagoburn contains bacteriophage cocktail targeting pathogenic strain of *Escherichia coli* and *Pseudomonas aeruginosa* and its infection in serious burn patients [42, 43].

Phage therapy is important alternative to overcome critical limitations of antibiotic therapy due to emergence of bacterial resistance, like, in the case of Clostridium difficile infection (CDI). CDI is responsible for inducing the dysbiosis, which has extremely high recurrence rates. As per reports, treatment of CDI using current antibiotics has become more and more ineffective. Further, prolonged use of antibiotics can also harm beneficial gut flora causing discomfort to the patients. Phage therapy against C. difficile involves specifically targeting the causative agent, sparing the other bacterial organisms of the human gut. Similarly, treatment of nosocomial infections (common hospital-acquired infections) caused by *Pseudomonas aeruginosa* is a huge therapeutic challenge currently [43].

High rate of morbidity and mortality is connected with the infection, along with a greater possibility of drug resistance in the bacteria during the course of therapy. With such limited scope for developing new drugs, scientists have also found considerable success in alternative treatment options including phage-based approaches [44]. In 2009, phase I clinical trial of bacteriophage cocktail (Biophage-PA) targeting three pathogenic strains of *S. aureus*, *P. aeruginosa* and *E. coli* targeting venous ulcers was approved US FDA [29]. Similarly, a randomized, double-blind, placebo-controlled phase I/II clinical trial approved by UK Medicines and Healthcare products Regulatory Agency (MHRA) and the Central Office for Research Ethics Committees (COREC) ethical review process was performed to treat chronic otitis against multidrug resistant bacteria *P. aeruginosa* and found lower bacterial count and significant improvement with a single input dose of 600,000 bacteriophages [44, 45].

Bacteriophage application trials have shown that PFU count of 10^2-10^3 plaque forming unit (PFU) is adequate to counter 10^6-10^9 CFU per milliliter proliferation threshold of bacteria in vivo. However, the reported trials with bacteriophages have been performed with 10^5 and 10^9 PFU of individual bacteriophages. Development of phage bioderm is another important area of application in clinical sector. It is a therapeutic auto degrading, non-toxic, biopolymer complex containing phages that help in healing of wounds and burns, osteomyelitis and periodontal diseases. Studies reported 20-95 % lysis of clinical isolates of *Serratia marcescens* using specific phage strains and phage type. However, there are certain limitations on the application of phage bioderm, like reduction in phage activity due to development of antibodies against phages, induction of toxin genes and fast discharge of bacterial endotoxins due to the lytic effect of phage [45].

Phage Therapy in Veterinary Medicine: Phage therapy has been used in veterinary science since the beginning of the 20th century. In 1919 phages were first used in France against bird typhoid fever. *Salmonella gallinarum*, as well as their bacteriophages, were isolated from the infected chicken and when tested, phages proved to be effective against *Salmonella gallinarum*. The first trial model for testing phage therapy was mice salmonellosis, mainly caused by *Salmonella typhimurium*. Phages were both administered intraperitoneally and orally, where the latter did not give a positive bactericidal result and the former only decreased microbial spread insignificantly. This trial failed most probably because the right type of phage was not used, since later scientists managed to infect typhoid bacteria in vitro successfully with a well-chosen anti-typhoid phage. Many subsequent experiments in treating rabbits, Guinee pigs, mice and rats, infected with streptococci and staphylococci have not shown to be effective. Nevertheless, there are multiple evidence of successful phage treatment of streptococcal meningitis in rabbits, *E. coli* cystitis in rabbits and Guinee pigs [46].

Today phages are used in both human and veterinary medicine. Among their advantageous characteristics, such as their specificity on bacteria, qualifies them for use in phage therapy. Modern phage medicine is based upon virulent phages of a broad range of action that are active against antibiotic resistant bacteria. The latter phenomenon (antibiotic resistance development) has in recent decades led to less efficient bacterial disease treatment, as well as to an increase of persistent infections and latency. Antibiotics usage today is narrowed down as much as possible, in order to avoid, or at least, decelerate resistance growth, which provides more importance of the ecologically safe phage therapy as a modern anti-epizootic treatment, which is to be opted for [46, 47].

Phage Applications in Food-producing Animals: The presence of phages throughout the human food chain has been widely documented. Some of these phages have the potential to be beneficial (for example as bio-control agents) while others can be detrimental, e.g. by infecting starter cultures used for yoghurt manufacture [47]. Most phage therapy (PT) trials in food producing animals have been directed against important zoonotic pathogens, principally *E. coli*, *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp [48]. Antibiotic resistance among some of these bacteria is a major and growing concern [49]. With regulatory bodies in the European Union (EU) banning the routine use of antibiotics in livestock and restricting chemical treatments of carcasses during processing, alternative interventions are urgently required [50, 51].

Poultry have been the most commonly used models for PT in food-producing animals. The large scale, high throughput and mechanization of poultry production lend itself to the benefits of PT in a way that large animal meat production does not. The high population density of chickens in conventional rearing systems, which can reach hundreds of thousands on a single farm, increases the risk of a rapid spread of disease and concomitant economic losses. However, the same high population density also favors the spread of phages through a flock, facilitating and reducing the cost of treating a large number of animals [50]. One poultry disease which has been targeted by PT is colibacillosis, an *E. coli* infection which causes airsacculitis, pericarditis, perihepatitis in chickens and is a significant cause of morbidity and mortality in the industry [52, 53].

Following their early trials with mice, used cocktails of phages to treat enteritis in calves, piglets and lambs [21]. The calves were either fed or deprived of colostrum and then challenged with enterotoxigenic *E. coli* strain O9:K30.99. None of the nine colostrum-fed calves treated with a high titre (1011 PFU) cocktail of two different phages became ill (compared with 93% in the control group). Two out of the 13 colostrum-deprived calves treated with phages at the onset of diarrhea died (compared with 100% in the control group). This study demonstrated that phages could greatly reduce morbidity and mortality even when used at the onset of clinical symptoms. Similar successes were recorded with enterotoxigenic *E. coli* strains used to challenge piglets and lambs. Callaway and colleagues (2008) used a cocktail of phages to significantly reduce numbers of *E. coli* O157:H7 in the intestinal tract of sheep [52].

Problems Associated With the Use of Phages: Although phage therapy has many advantages, previous research suggests that the use of phages exhibit some disadvantages. Firstly, phages have a narrow range of hosts resulting in a limitation of their use for broad-spectrum protection. In addition, it is possible to have an immune response to the administered phages in the animal body [56]. Finally, bacteria resistance to the virulent phage can be caused by phage and bacteria co-evolution. However, due to rapid developments in the field of phage therapy, it is hoped that all limitations which currently exist will soon be resolved [18].

Table 1: Some lists of bacteriophage and their derivative against selected zoonotic pathogens

Name of bacteriophage	Zoonotic bacteria they act on	Reference
cocktail named BPT2	*Salmonella*	[54]
Intestiphage	*Enterococcus*	[46]
Staphage, pyophage, intestiphage	*Staphylococcus*	[46]
ShigActive™	*Shigella*	[55]
Phage k,	*Staphylococcus aureus*	[54]
Colo-jel, Ento-jel	*Escherichia coli*	[22]
Staphylo-jel	*Staphylococci*, *Streptococci*	[22]
Biophage-PA	*S. aureus*, *P. aeruginosa* and *E. coli*	[29]
cocktail of phages (CNPSA1,CNPSA3 and CNPSA4)	*Salmonella enteritidis* strains, *S. gallinarum*,	[38]
phage R	Colibacillosis, *Escherichia coli* ($K1^+$ strain)	[2]
suspension of bacteriophage CP220	*Campylobacter jejuni* and *Campylobacter coli*	[2]
Intestiphage	*Enterococcus*	[54]

CONCLUSION AND RECOMMENDATION

The increasingly observed acquisition of antibiotic resistance by bacteria necessitates new strategies for combating drug-resistant bacteria. In the continuous search for new therapies, there is a renewed interest on the application of bacteriophages, viruses that kill bacteria, as potential antimicrobial agents. The results of research on bacteriophages, indicating that they can be an alternative means of eliminating pathogens posing a threat to humans and animals, justify its continuation, particularly in view of increasing drug-resistance in bacteria and restrictions on the use of antibiotics. Many of the reasons why some early PT trials were unsuccessful are now understood. However, it seems clear that the subtleties of different phage-host interactions alongside issues such as immunity, viral replication dynamics and the presence of decoys and the physiology of the host bacterium may all need to be considered when developing new phage treatments. The development of adequate phage preparations may in the future prove to be one of the most effective methods for fighting bacteria that are pathogenic for humans and animals and will also make it possible to obtain products that are safe and free of antibiotics. Based on the above conclusion, the following recommendations are proposed:

Further investigation will be needed to continue phage research to open new horizons to protect antibiotic resistance by bacteria by developing new strategies for combating drug-resistant bacteria.

Promote bacteriophage studies on phage therapy and bio-control in different disciplines of the higher education system, especially in medical and veterinary Medicine.

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