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Review Article

BACTERIOPHAGE AND THEIR APPLICATIONS: A REVIEW

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Abstract: Bacteriophages have received renewed attention as possible agent against infecting bacteria. Bacteriophages are a diverse group of viruses which are easily manipulated, and therefore have potential use in biotechnology, research and therapeutics. The application of phages range from the diagnosis of the disease, through phage typing and its prevention (phage vaccine), to treatment (phage therapy). By making a cocktail of phages, it would become easy to treat a wide variety of MDR bacterial infection that are otherwise resistant to latest generations of antibiotics.

Key words: Bacteriophage (phages)

INTRODUCTION

One of the possible replacement options for antibiotics is the use of bacteriophages (commonly called "phages") as antimicrobial agents. The emergence of multiple-drug-resistant (MDR) bacteria has prompted interest in alternatives to conventional antimicrobials. Phage therapy is an important alternative to antibiotics in the current era of MDR pathogens [1]. In this review, we describe in brief about phages and their use in bacterial disease prophylaxis and therapy along with their advantages and disadvantages as therapeutic agent.

Bacteriophages and life cycle: Bacterial viruses, or bacteriophages, appear to be ubiquitous, there being examples in most bacterial species with sensitivity to one or more phages. Most of these phages have double-stranded DNA; all the known RNA viruses are single-stranded. Except for the filamentous phages, all of the phage groups have a polyhedral capsid which contains the phage genome. This capsid is usually joined to a tail, which is a helical protein structure required for adsorption of the virion to the bacterial cell [2].

Bacteriophages undergo two possible life cycles. These are the lytic (or virulent) and lysogenic. Lytic phages multiply vegetatively and kill the host cell at the end of the growth cycle. Temperate phages which undergo the lysogenic cycle as well as multiplying vegetatively can also persist in a lysogenic state, whereby the phage genome can exist indefinitely by being inserted in the bacterial chromosome which is known as the prophage state [3].

History of Bacteriophage therapy (Phage therapy): Bacteriophage therapy for bacterial infections is a concept with an extensive but controversial history [4]. The discovery of bacteriophage particles that seemed to 'eat bacteria' is generally attributed to Twort [5] and d'Herelle [6] in the early 20th century. The therapeutic potential of 'phages' – members of the kingdom of viruses and obligate predators of bacteria – was recognized soon thereafter and applied for several decades before the discovery and widespread adoption of antibiotics [7].

Phages are viruses that infect bacteria and were recognized as early as 1896 as natural killers of

bacteria. Phages take over the host's protein-making machinery, directing the host bacteria to make viral proteins of their own. Therapeutically, phages were used as a prophylaxis against cholera, typhoid fever, and dysentery from the 1920s to the early 1940s. The practice was abruptly stopped when synthetic antibiotics were introduced after World War II. Now there is a plethora of MDR bacteria, so phage therapy once again has become of keen interest.

Antibiotics resistance: Antibiotic-resistant (MDR) pathogens constitute a worsening global health problem exacerbated by interconnected travel, antibiotic overuse, horizontal gene transfer, and bacterial evolution. New classes of antimicrobials are needed to treat these pathogens but the drug development pipeline is dry [8]. For more than half a century, the doctors and clinicians have been relying primarily on antibiotics to treat infectious diseases caused by pathogenic bacteria. However, the emergence of bacterial resistance to antibiotics following widespread clinical, veterinary, and animal or agricultural usage has made antibiotics less and less effective [9, 10].

These days' scientists are now facing the threat of superbugs, *i.e*. pathogenic bacteria resistant to most or all available antibiotics [11,12]. One of the possible replacements for antibiotics is the use of phages as antimicrobial agents [13,14]. In general, there are two major types of phages, lytic and lysogenic. Only the lytic phages (also known as virulent phages) are a good choice for developing therapeutic phage preparations [15,16].

Advantage of phage therapy over antibiotics; Phages, a promising way of fighting bacterial infectious diseases by using viruses are considered potential substitutes for conventional antibiotics [17]. Phages are very specific to their hosts, so this minimizes the chance of secondary infections, but antibiotics do target both pathogens and normal flora of patients, which can cause the secondary infections or sometimes super-infections. Also, phages replicate at the site of infection where they are mostly needed to lyse the pathogens, but antibiotics travel throughout the body and do not concentrate at the site of infection. No side effects have been reported during or after phage application, but resistant bacteria, allergies (sometimes even fatal anaphylactic reaction), and secondary infections are the common side effects of antibiotics treatment [18]. Lastly, phages are environmentally friendly and are based on natural selection, isolating and identifying bacteria in a very rapid process compared to new antibiotic development, which may take several years, may cost millions of dollars for clinical trials, and may also not be very cost effective [19]. Moreover, although bacteria can become resistant to phages, phage resistance is not nearly as worrisome as drug resistance. Like bacteria, phages mutate and therefore can evolve to counter phage-resistant bacteria [20].

Safety of the therapeutic phage preparation: During the long history of using phages as therapeutic agents, there has been no report of serious complications associated with their use [21]. Phages are extremely common in environment and regularly consumed in foods [22]. They have been commonly found in human gastrointestinal tract, skin and mouth, where they are harboured in saliva and dental plaques [23]. Phages are also abundant in environment including saltwater, freshwater, soil, plants and animals.

From a clinical standpoint, phage therapy appears to be very safe. Efficacy of natural phages against antibiotic-resistant *Streptococci, Escherichia, Pseudomonas, Proteus, Salmonella, Shigella, Serratia, Klebsiella* [24], *Enterobacter, Campylobacter, Yersinia, Acinetobacter* and *Brucella* are being evaluated by researchers [2]. Phages can be modified to be an excellent therapeutic agent by directed mutation of the phage genome, recombination of phage genomes, artificial selection of phages *in vivo*, chimeric phages and other rational designs which confer new properties on the phages. These new modified phages have been shown to successfully overcome challenges to earlier phage therapy [25].

Production and usage of phages: Production and usage of phages for therapy and prophylaxis continued on a small scale. Several companies had small-scale productions of phage preparations for various purposes [26]. Human phage therapy has been practiced in France since 1919, when d'Herelle, by using the phage, first successfully treated several children who were suffering from severe dysentery at the Hospital des Enfants Malades in Paris [27]. Since then, the Pasteur Institute in France produced

phage preparations against various pathogens (*Pseudomonas, Staphylococcus, Escherichia coli,* and *Serratia*) until 1974. These phages were used mainly against skin infections, septicemia, osteomyelitis, wound infections, urinary tract infections, and middle ear and sinus infections. There continued to be regular scientific reports on phage therapy in France until at least 1979. The reason that phage therapy was terminated, we believe, was that antibiotics were thought to "cure" infection without ever having to test for the real causative agents and thus became an easy way to treat patients [1]. In developing nations, this practice is so common that one can purchase a range of antibiotics at a pharmacy window without a prescription.

Phage application as biocontrol: Effective elimination of pathogenetic bacteria from gastrointestinal diseases using phage preparation has been demonstrated in multiple experiments that focused on the therapeutic use of phages [28]. The therapeutic effect of the phages can be limited to a decrease in the pathogen's population down to a point at which the immune system can effectively control its reproduction. Several current strategies to combat livestock-associated pathogens such as toxinogenic *E. coli, Campylobacter*, and *Salmonella* are direct extensions of "classical" phage therapy approaches in that they focus on targeting the bacteria.

Phages in the eradication of biofilms: Biofilms are densely packed communities of microorganisms growing on a range of biotic and abiotic surfaces and surround themselves with secreted extracellular polymer (EPS). Many bacterial species form biofilms and it is an important bacterial survival strategy. Biofilm formation is thought to begin when bacteria sense environmental conditions that trigger the transition to life on a surface. A major problem of biofilms is their inherent tolerance to host defences and antibiotic therapies. Therefore there is an urgent need to develop alternative ways to prevent and control biofilm-associated clinical infections [29]. Bacteriophages have been suggested as effective antibiofilm agents [30]. The ability of bacteriophage and their associated polysaccharide depolymerases was investigated to control enteric biofilm formation. It has been reported that phages alone can disrupt biofilm colonies of target organisms, such as *Staphylococcus epidermidis* growing on silicon catheters [31]. Age of biofilm is a decisive factor in determining the outcome of antibiotic/ disinfectant treatment. The action of combined treatments of disinfectant and phage enzyme, as a potentially effective biofilm control strategy was evaluated and the results showed that the combination of phage enzyme and disinfectant was found to be more effective than either of these when used alone [32].

Phage cocktail and recombinant phages: Phage cocktail has great therapeutic potential for MDR bacteria or superbug infection. It has been observed that the phage cocktail has great therapeutic potential for MDR bacterial infection as it is more effective as compared with monophage in reducing bacterial mutation and rescue frequency [33]. In one research, treatment using phage cocktail proves positive recovery from diarrhea in calves after oral administration [34]. Recombinant or genetically modified phages efficiently kill target bacteria while eliminating many of the problems associated with the use of natural phages in phage therapeutic applications. Also labelled phages with toxic molecules are used to destroy the bacterial cell. It has been seen that genetically altered phages remain in the circulation for longer periods than wild-type phages [35].

Limitations of Bacteriophages: There are also some disadvantages with the phage therapy approach. These include:

- This therapy cannot be used for intracellular bacteria as the host is not available for interaction.
- Phages are more difficult to administer than antibiotics. A physician needs special training in order to correctly prescribe and use phages.
- The problem which requires attention is the rapid clearance of phage by the spleen, liver and other filtering organs of reticuloendothelial system [36].
- Theoretically development of neutralizing antibodies against phages could be an obstacle to the use phage therapy in recurrent infections [37].
- The shelf life of phages varies and needs to be tested and monitored.

Bacteriophages have received renewed attention as possible agents against infecting bacteria but phage therapy can be effective only under certain circumstances. Major limitations faced by them are the narrow host range of many phages, the issue of

phage resistance, and the possibility of phage-mediated transfer of genetic material to bacterial hosts. Also emergence of phage-resistant variants was observed rapidly if only one phage strain was used against a particular bacterium [38]. Rigorous regulatory issues and the high cost of producing such alternative antimicrobial agents are also other factors that might prevent application of these agents in the near future [39]. Some concerns about the use of phages include the safety and efficacy issues, as well as immune response to the administered phages. Growth optimization and purification strategies of phages are also some issues needed to be addressed. One of the major criticisms in phage therapy is the need to identify the causal agent before treatment. However, some phages are highly speciûc, while others are extremely broad in their host range which posses serious hidden threats.

CONCLUSIONS

A resurgent interest is emerging in the use of phages or their gene products as alternative therapy to currently utilized antibiotics for MDR bacteria. Further, phage therapy is more specific, accurate, and thus could complement as well as replace current antibiotics by facilitating virus way out from the host. Furthermore, phages can replicate at the site of infection and thus become available in abundance at the desired site [40,41]. Phage cocktail has great therapeutic potential for MDR bacteria infection. Phage therapy will compensate for unavoidable complications of chemotherapy such as the appearance of MDR. The use of bacteriophages to treat and/or prevent bacterial infections is promising yet challenging therapy. There is no doubt that bacteriophage application in biocontrol of pathogens will be beneficial for food safety and public health. It is critically important to notice that there are some concerns about the use of phages that include safety and efficacy issues, as well as immune response to the administered phages. Due to the rapid progress in the fields of biotechnology and molecular biology, it is hoped that phages, which are presently abundant in the biosphere, could answer many questions.

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