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Review Article

Phage Therapy- AN ALTERNATIVE THERAPEUTICS

Navroop Kaur[1]*, Kirti Jaiswal[2], Rishi Rath[3], Sarvajeet Singh Arora[4] and Ashish Behl[5]

[1]*Reader, Department of Microbiology, ITS Dental College, Hospital & Research Centre, Ghaziabad, Uttar Pradesh, India.*

[2]*Galgotias University, Gautam Budh Nagar, Uttar Pradesh, India.*

[3]*Professor and Head, Department of Pharmacology, ITS Dental College, Hospital & Research Centre, Ghaziabad, Uttar Pradesh, India.*

[4]*Professor and Head, Department of Pathology, ITS Dental College, Hospital & Research Centre, Ghaziabad, Uttar Pradesh, India.*

[5]*Professor and Head, Department of Physiology, ITS Dental College, Hospital & Research Centre, Ghaziabad, Uttar Pradesh, India.*

***Corresponding Authors:** *Dr. Navroop Kaur, Reader, Deptartment of Microbiology, ITS Dental Hospital and Research Center, Uttar Pradesh, India.*

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Abstract

Bacteriophage refers to viruses that can transmit infection to bacteria without harming human or animal cells, since human and animal cells do not have specific receptors for bacteriophages. Because of that, it is expected that they can be used to treat bacterial infections. The aim of this review is to analyse potential use of bacteriophage found in water from Ganga river against bacterial pathogens. The concept of viral phage therapy has gained support in the present era of increasing antimicrobial resistance. With the progress in phage therapy, bacteriophages are used as a novel therapeutic agent in treating pathogenic bacteria. This paper presents an outline of the topic of phage therapy in Ganga water and the benefits and limitations of bacteriophages for use in humans and animals along with problems arising in Ganga River due to pollution.

Keywords: *Water from Ganga river, Bacteriophage, Antimicrobial resistance, Antibiotics, MDR, Covid-19.*

Introduction

Before the antibiotics era, phage therapy was widely applied to combat bacterial infections. However, the revelation of penicillin and some antibiotics substituted phage therapy, and are currently being utilised as the first line of defence against infectious agents. Factors such as adaptation resulted in bacteria becoming insensitive to one or multiple antibiotics, frequently leading to restricted treatment options. This induces a re-development of attentiveness to the phage therapy that remains dubious due to its downsides such as host specificity and the evolution of bacterial resistance against phages. Development of bacterial genomes let bacteria to acquire extensive mechanisms impeding with phage infection [1] such as blocking phage adsorption, preventing phage entry, super infection exclusion, restriction-modification and abortive infection. It is interesting to note that phages have thrived varied counterstrategies to evade bacterial anti-phage mechanisms with digging for receptors, adjusting to new receptors and masking and altering restriction sites. Understanding the intricate dynamics of bacteria-phage interaction is a lead-in step towards forming synthetic phages that can overwhelm limitations of phage therapy and potentially lead to vanquishing MDR bacteria [2]. Phage therapy has many benefits, primarily because phages are very specific (usually restricted to one species) and easy to attain as they are widespread in locations colonized by bacterial hosts including soil and seawater, and they do not have any toxic side effect like antimicrobials [3].

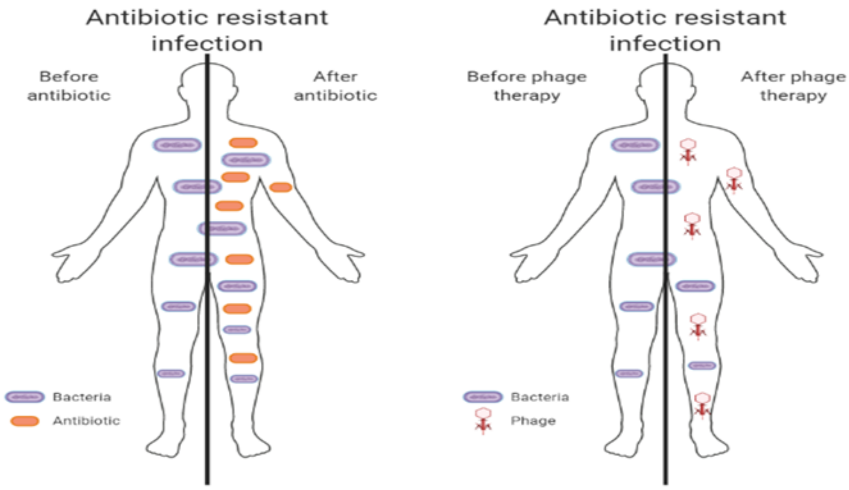

Fig.1: *Use of Bacteriophage against antibiotic resistance*

Speciality of Ganga River

Ganga is known to be a lifeline used for emphasizing mass of people in India and is known to be the heartbeat of the spiritual life of the Hindu culture. The Ganges River is always known for its self-cleaning and healing power. Even scientific research has also supported this claim. The Ganga water has illustrated an interesting antibacterial activity which seems to be correlated to bacteriophage. These bacteriophages have played a part in regulating bacterial growth and thus disallowing putrefaction of Ganga water [4]. They act like soldiers that eliminate bacteria by damaging or contaminating them and this favours that phage therapy is a very gainful substitution of antibiotics. Additionally, the plenty of trace elements in Ganga water might also be a cause for the quelling of bacterial growth. Besides, some biomolecules are present naturally and may provide antiseptic, medicinal and healing strength in Ganga water [5].

Ganga water exhibited specific well-defined properties in terms of higher alkalinity and pH in comparison with other rivers. These attributes may have a place in self-purification and non-corrosive features of river Ganga by elevating the growth of bacteriophages. It has long been reported that phages require divalent metal ions, such as Ca, Mg, Zn, Fe, and Co, for their growth. The divalent metals may be associated with phage development by diverse manners, for example, some phages need metal ions for firmness, while others may need adhering to the host cell for the entrance into the nucleic acid and multiplication [6]. Thus, the higher level of several divalent metal ions observed in the current study have taken part in bacteriophage growth and inhibition of bacterial population in Ganga water [7]. Thus, it can be estimated that Ganga has distinctive water texture in terms of Physico-chemical characteristics and microbial assortment which proffer self-cleansing attribute of Ganga and makes it pure and potable.

An important element related to these phages is that they are basically benign to humans as they are highly strain-specific. For example, if a bacteriophage is specific to bacteria causing cholera '*Vibrio cholerae'*, then the phage will kill only those bacteria rather than killing other species or cells and one interesting fact about phage is that it only kills those bacteria that cause deadly disease. The river has been shown to have antibacterial features and one of the best proofs is that it can hold abundant of dissolved oxygen in immense polluted conditions as well.

In the river Ganga, the bacteriophage turns up about 3 times more in proportion than bacterial isolates. A recent report by National Environmental Engineering Research Institute (NEERI2017) demonstrated that Ganga contained around 1100 types of bacteriophage, in comparison with less than 200 species exposed in Yamuna and Narmada [4]. Even so, antibacterial effects differ considerably along the extent of the river.

Bacteriophage

Bacteriophage, or ninja virus, are a form of viruses that infects or kills bacteria. These phages require a bacterial host so as to make copies of them. Bacterial viruses consists of proteins that acts like a coat to protect central inner of nucleic acid – either DNA (deoxyribonucleic acid) or RNA (ribonucleic acid). Phages structure can vary from the basic to the more intricate. Bacterial viruses are definite to one or finite number of bacteria; thus, named after the bacteria group, strain, or species that they infect. Like, the phages that contaminate the bacterium *Escherichia coli* are called coliphages.

Félix d'Herelle, a French-Canadian microbiologist, known as the father of bacteriophage (or phage) therapy [8] brought an evolutionary discovery of phages as therapeutics for various infections and conditions.

To infect a host cell, the bacteriophage bounds itself to the bacteria's cell wall, specifically on a receptor found on the bacteria's surface. To hydrolyse and degrade the bacterial cell wall, phages possess lysins. The range of effectiveness of natural lysins is generally restricted to Gram +ve bacteria; however, recombinant lysins have potential to disrupt the outer membrane of Gram-negative bacteria and eventually lead to rapid death of the target bacteria. Once it firmly attach to the cell, the bacterial virus inserts its genetic material into the host cell. Depending on the type of phage, one of two cycles will occur – the lytic or the lysogenic cycle.

ADVANTAGES OF PHAGE THERAPY

Phage therapy was practiced against bacterial diseases but become unpopular after the finding and development of penicillin and other antibiotics in 1928. But with the seemingly unstoppable march of antibiotic resistant bacteria, the method of using bacteriophage has emergedagain.

This method helps in the treatment against gram-negative bacteria carrying the enzyme NDM-1 (New Delhi Metallo-beta-lactamase-1) that makes bacteria resistant to a wide range of beta-lactam antibiotics. The enzyme disintegrates the cell wall in minutes so there is very little time for the bacterium to build up resistance. Antibiotics however can take days to have an effect, allowing the bacteria to develop defenses. Phages are like moon-landers; act on a cell wall; locate the right receptors and adhere to them by lowering their base plate. Highly specific enzymes present on base plate of phage first breaks down the bacterial cell wall, then inject their DNA and replicates to cause bactericide.

There are as such no bacteria that can't be lysed by minimal one bacteriophage. In this regard, bacteriophages are significantly more effective as compared to antibiotics, though some antimicrobial drugs have a broad spectrum of activity, an antibiotic capable to eliminate all the bacterial species does not exist. However, the most attractive characteristic of bacteriophages is their specificity of action, i.e., their ability to kill only the pathogen that they can recognize [9]. They have a very small spectrum of activity, which evades the main issue linked with antibiotic administration [10].

Bacteriophage is significantly harmless and better tolerated and accepted, as they are specific and replicate only in the bacterium which is targeted [11]. Moreover, as compared to antibiotics, administration is much easier in phage therapy, i.e., uncomplicated and unproblematic, because they do not require continuous administrations soon after one another as they can survive in the human body for extremely long period of time, i.e., up till several days [12,13]. Along with this, bacteriophage genetic modifications can help to fight bacteria that are even resistance to antibiotics [14]. Eventually, the phage therapy is cheaper than antibiotics whose objectives are multidrug-resistant pathogens [15]. Bacteriophage can also be used to kill bacteria found in food.

Phage use a method known as TRANSCYTOSIS that transverse the epithelial cell layers. Around 10^9 phages are transcytosed frequently from the gut into other tissues making an intrabody phageome contributing to human wellness and immunity [16]. Phages may modulate the action of the immune system contributing to the maintenance of immune homeostasis [17]. The existing phage impression seems to be anti-inflammatory and immune protective, some phages (e.g. filamentous phages) may also aggravate prevailing disease [18].

Bacteriophages are further used to study its effect in animal and human:

In Animals

- Use to treat acute diarrhea against *E.coli*.
- Administration of bacteriophage prevents the damaging of skin grafts by *P. aeruginosa* [19].
- Bacteriophages treatment of food producing animals is related with a deduction of the chance of contamination of the resulting food products during processing [20].
- Multiple attempts to use bacteriophage to cure respiratory infections of animals have been made. Furthermore, providing bacteriophages with an aerosol spray decreased the death rate of broiler chickens with *E. coli* respiratory infection [21].

In Humans

- Number of studies has looked over the use of bacteriophage for topical medication of skin bacterial infections [18].
- Positive effects of bacteriophage for healing of localized ailment in wounds, burns, and trophic ulcers, including diabetic foot ulcers is observed [22].

- Oral administration of bacteriophage is effective in prevention and treatment of cholera [23].
- Deals with respiratory tract infections [24].
- Various in vitro studies have suggested that bacteriophages could be effective in treating cystic fibrosis (CF) patients [25].

Although resistance to phage(s) may develop during treatment, this may be overcome by using several phages with different bacterial receptors in combination, genetically modifying phages to overcome various bacterial resistance mechanisms, and utilizing phages serially in a personalized treatment strategy [26].

CHALLENGES FACING PHAGE THERAPY

Though the phage therapy utilization proves to be an effective and successful treatment against bacterial infections but there are some factors considered as a challenge to it as a mainstream antimicrobial. One drawback is that phage-host relation and struggle for survival bring about the adaptation of bacterial and viral genomes and, thus, to the evolution of resistance mechanisms. Bacteria are constantly developing many molecular mechanisms, driven by gene expression to prevent phage infection. Such advanced phage-resistance mechanisms in bacteria persuade the parallel co-evolution of phage diversity and adaptability [27, 28]. Bacteria have also evolved varied non-adaptive resistance (non-specific) and adaptive resistance linked with Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) along with CRISPR -associated (Cas) proteins [29]. Also, bacteria defend themselves from exotic DNA by chemically modifying their own DNA, and destroying any DNA that is not modified. The enzyme that destroys foreign DNA is called restriction enzymes.

Another issue is that bacteriophages and their products are non-self-antigens and can be identified by the immune system which then induces responses and reduces the benefits of bacteriophage administration. Natural resistance to bacteriophages has been illustrated in both experimental animals and humans. In animals, bacteriophage was preoccupied with phagocytic cells a few minutes after the administration and might be injured by these cells within 2 hours [30].

Furthermore, phages are intrinsically narrow range agents. Not only to a one bacterial species, but they are also further restricted to a subset of strains within that species. Phages are natural components of all ecosystems. However, they could create an imbalance if they are introduced into the environment at higher concentrations than normal, which would ultimately impact ecological communities [31].

PROBLEMS ARISING IN GANGA WATER

Though Ganga water is considered to be the most sacred and Holy River but there are some factors which are diminishing its value like pollution, the religious and ritualistic activities. The amount of various toxic elements (As, Cd, Cr and Pb) has been increased in Ganga water from anthropogenic sources. Such anthropogenic activities in the Ganga are eroding water quality and raising a serious question about purity of the Ganges river water and its suitability to be used for drinking and other purposes. One of the root causes of the impurity of Ganga River is faecal coli forms bacteria and organic pollution which is caused by open defecation and emission of wastewater directly through small drains into the river [32].

Though the river Ganga supports a wide diversity due to its self-cleaning and regeneration properties but according to one study, the river Ganges have an unusual amount of bacteriophage, i.e., it has now less count of bacteriophages due to increase in pollution. The increased load of pollution has reduced its self cleaning properties. So, it is understandable that there is barely any effect of bacteriophages in Ganges water.

CAN GANGA USE TO TREAT CORONAVIRUS?

In light of the novel coronavirus, scientists and researchers from all over the globe have been looking at traditional and non-traditional methods to develop a vaccine. Although no research has been done so far, it is believed that Ganga River can help to sort out the COVID-19 outbreak since it is medicinal water. The bacteriophages exist in the holy river could curtail the growth of the infection via the soil, air, and water. With intensify diffusion of bacteriophages in water and soil, an extend of coronavirus will be impacted and reduced.

Many proposals have been made regarding Ganga water. Since Ganga water have “ninja virus” it is estimated that pure Ganga water boosts immunity which helps fight the virus. Although the bacteriophage virus kills bacteria, the Coronavirus is a virus, not bacteria, so it is unlikely that the water containing a higher amount of bacteriophage will be beneficial in treating Coronavirus infection. But it may be beneficial in eliminating some harmful bacteria; therefore, studies in this regard may be conducted in future when situation returns to normalcy.

Conclusion

Phage therapy is an exciting rediscovered method that will definitely provide numberless benefits to every field. Bacteriophages have several aspects that make them capable curative agents i.e., they are particular and very effectual in lysing targeted pathogenic bacteria, safe, and have potential to rapid adaptation to stand against exposure of newly arrived bacterial menace. Considering the efficacy and diverse applications of phage therapy, this area of research is requisite and thus we should try not to pollute the Ganga River as pollution results in decrease in count of bacteriophages. This review brings together a diverse range of ideas regarding phage therapy and its potential utility.

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