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Bacteriophages- a natural active cleanser proposed for Ganga clean up

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ABSTRACT

With increasing population river pollution is becoming very serious day by day. Developing countries do not have much facility for sewage treatment and hence most of the raw sewage falls into the rivers directly. This activity loads pathogens to the river and thus the public health is severely affected. However, rivers like Ganga have bacteriophages which naturally kept the water clean from time immemorial. This nature's gift can be employed to bring out an eco friendly technique which can disinfect water without any adverse side effects as seen in the case of chemical disinfection. Also, there is a natural check of life and death cycle which controls the phage population and hence makes this biological technique an easily controllable process. Also such natural tools are cheaper comparatively and can be harnessed to keep rivers clean.

Introduction

The disease burden is high in India due to the contaminated drinking water supply. Other reasons are poor sanitation, lack of access to freshwater, poor hygiene etc. responsible for intestinal tract diseases among the population. Water pollution is a serious problem in India as almost 70% of its surface water resources and a growing number of its ground water reserves are already contaminated by biological, toxic, organic and inorganic pollutants. All of India's 14 major river systems are heavily polluted, mostly from the 50 million cubic meters of untreated sewage discharged into them each year. It is estimated that 1.1 billion of the world's population does not have access to safe clean water. Transmission of waterborne disease occurs by drinking contaminated water. This has resulted in outbreaks of cholera and typhoid. Water washed diseases occur due to lack of sufficient quantity of water for washing and personal hygiene. Skin and eye infections are easily spread in such situations (World Health Organisation, 2000). On a global basis, around two million deaths per year are attributed to waterborne diseases and especially diarrhea in children. It has been estimated that around 37.7 million Indians are affected annually by waterborne diseases such as viral Hepatitis, Cholera, Jaundice and Typhoid. 1.5 million Children are estimated to die of diarrhoea alone and 73 million working days are lost due to waterborne diseases each year.

Ernest Hankin, a British bacteriologist, reported in 1896 on the presence of marked antibacterial activity against *Vibrio cholerae*, which he observed in the waters of the Ganga and Yamuna in India. Western scientists have now re-discovered this natural bacteriophage therapy as a potent weapon against antibiotic-resistant bacteria. Phages have been used as therapeutic agents against various bacterial infections (Sivera Mirza et al. 2006).

This natural antibacterial action can be used in treating rivers like Ganga which face pollution problems mainly because of untreated sewage disposal. The natural predators have huge potential in water treatment by simply completing their life cycles. The authors are interested in highlighting this property of the bacteriophages so that an eco-friendly, cost effective technique can be developed to solve developing countries' water pollution problems.

Sanctity of Ganga

Ganga was famous for its self purifying process. For this clarity and sanctity it is worshiped in India till date. It is ancient knowledge that Ganges water does not putrefy, even after years of storage, thus water from the Ganges has for millennia been regarded as incorruptible since ages.

Going back to 7500 BC, Ganga is mentioned in Rigveda (Nautilya, 2009). Hippocrates, in about 500 BC, wrote about the healing of disease with this water. Bathing held a prominent place in the law that was prepared by Moses under divine instruction for the government of the Hebrew nation. The role of the bath in the treatment of leprosy also would lead one to believe that water was used for curative effects. Though invisible, it was possible to show that this principle was valid. This was justified by the coinage of the term ''bacteriophage'' by D'Herelle (1922). Thus in a way the world owes the discovery of bacteriophages to the Ganges water (Singh et al., 2011). In spite of this the river faces sanctity crisis.

Every system has a limited homeostasis and if the damage occurs above its resilience then the system begins to collapse, as in case of river Ganga. So it is not only important to revive the river again but also use natural means which were used by the natural river ecosystem. Since Ganga harbours coliphages the pathogenic bacteria can be degraded of very economically and quickly.

Bacteriophages

Bacteriophages are viruses which invade bacteria, as natural parasites. The term bacteriophage has been derived from 'bacteria' and Greek *phagein*, 'to eat' (Reddy, 2013). Typically, bacteriophages consist of an outer protein head enclosing genetic material. The genetic material can be double stranded (ds) RNA, single stranded (ss) DNA, or double stranded DNA between 5 and 500 kilo base pairs long with either circular or linear arrangement. Bacteriophages are much smaller than the bacteria they destroy - usually between 20 and 200 nm in size. The dsDNA tailed phages, or *Caudovirales*, account for 95% of all the phages reported in the scientific literature, and possibly make up the bulk of phages on the planet. Bacteriophages get

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adsorbed on to the host cells and phage entry is mediated by specific receptors such as carbohydrates, proteins and lipopolysaccharides present on the surface of host cell (Marks and Sharp 2000). Host range for bacteriophages is influenced by the specificity of interaction between phage attachment structures and host cell surface receptors. Host range for aquatic phages is generally assumed to be narrow (Alonso et al., 2002), however, cyanophages show broad host range (Suttle, 2000). Payne and Jansen (2001) suggested that for successful phage treatment, phage inoculation should coincide with a bacterial population density sufficient to support phage replication. Phages are classified by the International Committee on Taxonomy of Viruses (ICTV) according to morphology and nucleic acid. There are a variety of these viruses, called coliphage, which infect many subspecies of *Escherichia coli*. These phages are of major attention because the fecal nuisance caused in river Ganga is naturally cured by them. These phages are usually present in wastewater in reasonably large numbers as compared to enteric animal viruses. Their source is the feces of humans and animals. A variety of coliphage are called "male specific" because they infect the bacteria via the pili (small appendages on the bacterium's surface) and bacteria with these appendages are called "male". Certain of this male specific phage, MS-2 for instance, have the same shape and size of small enteroviruses (25 nm) and have single stranded RNA. It has been noted that they exhibit the same or greater resistance to environmental factors, including disinfection, as do the most resistant animal enteroviruses. Those coliphage that infect the bacteria via the cell membrane are called somatic coliphage. These are also present in wastewater but because of the similarity of many of the male coliphage with animal enteric viruses the latter are of more interest as surrogates for the presence of animal viruses. Bacteriophages are the most numerous organisms on the earth that play a key role in bacterial gene exchange and bacterial pathogenesis and continue to provide important insights into the basic molecular working of life. Through a combination of their antagonistic but metabolically intimate relationship with their bacterial hosts, lytic phages possess ideal properties to serve as agents of both antibacterial bio-control and bacterial identification.

Water disinfection and its disadvantages

The removal of disease causing agents from water is called water disinfection. This is a must for all drinking water treatment plants. The pathogenic bacteria are killed in this process and is a tertiary treatment process. It has been reported that by decreasing the contamination of source water, (1) the amount of treatment required is reduced, which lowers the probability of the production of by-products and (2) the rate of water-related outbreaks is curbed with the reduction in the source pathogen load (World Health Organisation, 2008). There are different methods that are used as disinfectants: (1) Chemical, (2) Physical, (3) Radiation. Physical agents include heat and light. Radiation includes use of electromagnetic, acoustic and particle radiations. Radiations are expensive and require higher technical knowhow while physical agents like heat can be insufficient in handling bulk quantities.

Chemical disinfectants have side effects too. Chlorine can cause eye, nose, stomach problems and sinus irritation when ingested via drinking water. Inhalation of chlorine leads to asthma. In 1976, the U.S. National Cancer Institute Published results showing that Chloroform, one of the trihalomethanes (THM) that occurs as a by product of drinking water disinfection, was carcinogenic in rodents (U.S. NCI Report, 1976). Since that time, there has been a concern that disinfection against microbial risks could also pose chemically induced cancer risks for humans (Melnick et al. 1994). So if the quantity is not checked disinfectants will further lead to ill health.

Another very costly disinfection process, Ozonation, produce by-products like bromates that are again carcinogenic to rodents (Balmier et al. 1995). Recently, it has been shown that consumption of drinking water with high THM levels is associated with adverse reproductive outcomes (Xie, 2004). Continuous use of disinfectants produce tolerant pathogens which become another problem. Chlorine resistance was seen to increase in *E. coli.* O157:H7 (Cherchi and Gu, 2011). Also microbes differ greatly in their sensitivity to disinfectants. Specific protozoa, viruses and bacteria are known to be highly resistant to chemical disinfectant and pose a unique challenge to the water treatment industry. Micro-organisms produce a gelatinous material known as exopolysaccharide to form biofilms which make the disinfection even arduous. Bacterial biofilm, predominantly made up of an exopolysaccharide, has the capacity to resist many antimicrobial treatments that results in the public health problems (Xavier et al. 2005). Hence they can be relatively resistant to traditional methods of pathogen removal. There are many reports on application of phages to disperse biofilms (Timothy and James 2007; Curtin and Donlan 2006; Bedi et al. 2009). Biofilm associated bacteria have been reported as being up to 3,000 times more resistant to free chlorine (Lechevallier et al., 1998).

Therefore, to overcome all the above mentioned problems associated with the use of chemical disinfectants, an alternative strategy can be bacteriophage as biological disinfectant. Bacteriophages fulfil most of the characteristics of ideal disinfectants. They are specific and precise in their action of predation.

Table: Disinfectant characteristics of bacteriophage (Ahiwale et al. 2012).

Sr. No.	Characteristics	Lytic bacteriophages
1	Cost of production	Cost effective production liquid and powder formulations can be possible
2	Homogeneity	Homogenous
3	Availability	They are replicate at the site of its host and are thus available where they are most needed
4	Non corrosive	They are non-corrosive
5	Safety concern	They are highly safe, no serious side effects have been found
6	Non toxic to higher forms of life	They are highly specific in their action
7	Effect on pathogenic bacteria	Lytic phages are found to be highly effective in killing pathogenic bacteria. Phage resistant bacteria remain susceptible to other lytic phages having similar target range

Advantages of Bacteriophages

Apart from the above mentioned properties some more detailed goodness of the phages are enlisted below.

- The phages have been used as more precise indicators of fecal pollution. F-RNA coliphages provide a more specific index for faecal pollution (Brion et al., 2002). Bacteriophages have the potential to reduce competition between useless bacteria and functionally important microbial populations.
- For every type of bacteria known in nature, there is at least one complementary bacteriophage that specifically infects a single bacterial species. So bacteriophage therapy is possible in all bacterial infections. Since selection of active phages is a

natural process, therefore active phage can be selected against every resistant bacterium, by an ever ongoing process of natural selection. Some bacteriophages infect several related species of bacteria. However they do not infect antigenically unrelated bacteria.

- If a suitable bacteriophage is introduced onto an infected wound, it will continue to increase in numbers as long as there are bacteria to infect and destroy. However, as soon as all the bacteria have been destroyed, the action of the phage will cease and the dormant phage particles will disperse harmlessly. So there is a self check.
- Because phages are so specific to the bacteria they infect, they will not harm other beneficial bacteria present in the intestine and other parts of the body and will not affect the microbial community in the body. There is no chance of super infection with other bacteria. That may be because phages are omnipresent on earth, found in soil, water, plants and humans.
- An important feature of phage therapy is that bacteriophages do not infect human or animal cells. Phages do not cause allergies or affect the human body's natural immune system.
- Production is simple and relatively inexpensive and can be readily cultured and enumerated in the laboratory (Tyagi et al., 2006). The methods to recover coliphages from environmental waters is relatively simple and within the capability and resources of most water quality laboratories. So the treatment costs of bacterial infections will be reduced. Phage therapy is also found to be effective in *Klebsiella* infections (Sandeep, 2006).
- Phage therapy is consistent with "green-natural-alternative" ideology, and its production is environment-friendly.
- Chemical reactions are pH dependant so their fluctuation may lead to disruption but coliphages have wide range of pH for survival. Some coliphages thrive well in acidic pH while others in basic pH. So it is expected that the water is always therapeutically active even if pH fluctuates.
- Pathogens are found to have strong ability to persistently adapt to surrounding conditions for survival. Hence they can be relatively resistant to traditional methods of pathogen removal. It is known that most of the *Salmonella spp.* are resistant to amphicillin, chloramphenicol, and other classes of antibiotics (Salehi et al., 2005). It has also been found that *Salmonella typhimurium* can grow at pH 4.0. Similar observations are noted with *Shigella spp.* and *E. coli spp.* (Lin et al. 1995). It shows that, contaminated water associated bacteria have an efficient adoptive behavior against environmental conditions. *Vibrio spp.* is one of the most notorious and highly pathogenic bacterium found in the contaminated water. *Vibrio cholera* responsible for cholera is highly prevalent in estuarine conditions and is related to cholera outbreaks in developing countries, most notably in Bangladesh (Alam et al., 2006).These strains of *Vibrio* are frequently mutate to give rise to new antibiotic resistant and toxic strains. In one study, it has been found that cholera epidemics are self limiting in nature due to phage mediated biocontrol; which can be said to be related to amplification of *Vibrio cholerae* specific bacteriophages due to host (Shah et al. 2005).

Success of bacteriophage in water treatment

Some experiments have already successfully established the role of bacteriophage in disinfection of water in lab scale experiments. Phages can be used as potential disinfectant in the natural water bodies alone or in combination with physical and chemical process (Ahiwale et al, 2012). There are reports in which phages have been used to control pathogens in aqueous environment, *in vitro*. EPA (Environmental Protection Agency) worst case water (WCW) microcosm studies were carried out for testing biocontrol of *Salmonella* species with the help of bacteriophages. These treatments showed high inactivation rate of *Salmonella* group (McLaughlin and Brooks 2008). In another study river water microcosms were used in plates for testing potential of coliphages and phages specific for *Staphylococcus aureus* against *E. coli* and *Staphylococcus aureus* (Bahadoor 2005). According to Withey et al. (2005), phages have the ability to control environmental waste water process problems such as foaming in activated sludge plant, sludge dewaterability and digestibility.

Few attempts have been made to use bacteriophages to treat diseases in aquaculture. Wu and Chao (1982) examined the effect of a phage, F ET-1, isolated from pond water in Taiwan, on *Edwardsiella tarda*. In *in-vitro* experiments, phage killed 25 of 27 *E. tarda* strains and reduced the bacterial count to less than 0.1% when a bacterial suspension of 1.2×10^{12} cells/ml was infected with F ET-1 at multiplicity of infection (MOI) of 0.08 after 8 h. The studies of Park et al. (1997) and Nakai et al. (1999) have shown that bacteriophage could be used to control *Lactococcus garvieae* infections of yellowtail and other marine fishes. Polyvalent phages have been isolated from sewage treatment plants which have wide range of hosts (Jensen et al., 1998). Lytic phages are commercially important in terms of their bacteria killing activity. Lysogenic or temperate phages are not much important commercially; but they do posses research importance in terms of their capacity to integrate their genome into host genome and reside in the host genome in the form of prophage.

Constructed wetland system with bacteriophage application offers attractive alternate for storm water management for reducing load of disease causing viruses to the receiving waters (Yousefi et al., 2004). Bacteriophages are used to decrease the bacterial load from sewage water entering the rivers and lakes (Pretorius 1962). Specific bacteriophages such as *Salmonella* spp. phages or *Vibrio* spp. phages can be used to remove these pathogens from waste water (McLaughlin et al. 2006). To understand the scope of phages in waste water treatment processes, it is important to understand the phage and bacterial host relationship.

Diseases incited by bacterial plant pathogens are responsible for major economic losses to agricultural production also. Disease control is challenging for many diseases incited by bacteria. Chemical control with bactericides has been extremely difficult because few effective bactericides are available. Bacteriophages have been found to reduce citrus canker disease (*Xanthomonas axonopodis* pv. *citri*) severity both in greenhouse and field trials (Balogh, 2006). Phage-based integrated management of tomato bacterial spot is now officially recommended to tomato growers in Florida (Momol et al. 2002). In case of Ganga, Nautilya (2009) has reported self check characters as seen in decline of E.coli stains.

Conclusion

Sewage water can be treated by bacteriophage when introduced into it during disinfection. If such treated water is introduced into Ganga then its purifying capacity will be strengthened, treating pathogens as far as the phage life cycle permits. This may retain at least the bathing water quality in some stretches. But at first the phages of Ganga are to be inventorised without which therapeutic properties will not be fully known. On the basis of phage identification the commercialisation of phages can be done. One such example is commercialization of coliphage against *E.coli* O157:H7 which causes blood dysentery. Under the trade mark of Eco Shield (formerly ECP–100), a cocktail of three different bacteriophages is available against food borne bacterium *Escherichia coli* O157:H7. Phages have been applied successfully as disinfectants in pond water, swimming pools, industrial water systems, aquaculture system and in the treatment of waste water. Phages could be applied as disinfectant during disinfection step, along with other physicochemical processes (aeration, coagulation, flocculation, sedimentation and filtration). When such water will be used for irrigation purpose, plant diseases will be curbed too, thus reducing the burden on fertilizers and pesticides. If excess phages are detected then their removal is easy and natural. As the prey population declines the phages growth is also arrested. Coliphages present in waste water are removed during activated sludge treatment. The effectiveness of coagulation, flocculation, sedimentation and filtration processes to remove viruses in water has been effective.

Major problem regarding phage mediated biocontrol of bacteria is the efficiency of the phage production. Loss of phage infectivity needs to be fulfilled reapplying phage preparations constantly. It could constraint the practicality of some phage treatments but this can be dealt with. Also, the phages on viable but non culturable bacteria have not been evaluated. It has been found that *Salmonella* spp. exists in stagnant water in dormant form and not in an active form. Most of the phages cannot attack dormant bacteria due to improper adsorption on bacterial surface. However, unlike chemical therapeutic agents such as antibiotics, phages constantly evolve to circumvent their host's defences and resistant bacteria are often less fit or less virulent than their phage sensitive counter parts.

Instead of using so much of funds in treating sewage waste of Ganga, this effective natural tool can be developed potentially so that all adverse side effects are curbed. This natural remedy is now becoming a necessity as chemical and other treatments have created havoc with their side effects than being rather beneficial. Also, the antimicrobial activity which is naturally present in Ganga water is not observed in any other perennial river anywhere in the world. Development of new antimicrobial agents from Ganga water is a future prospect. Nature has given natural remedies for all problems and so these must be followed to keep the system healthy.

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