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A UNIQUE APPROACH TO TREAT RESISTANT BACTERIAL STRAINS WITH COST EFFECTIVE *COLIPHAGES*

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Abstract

Bacteriophages are the viruses which cause the lysis of bacterial cell that are safe for human health. This property of safe viruses can be used as an alternative of antibiotics to treat life threatening bacterial infections. Phage therapy is safe, cost effective and effective against resistant bacterial strains. In this article the bacteriophages against *Escherichia coli* (coliphages) sources, isolation methods and their beneficial properties are discussed. They will act as a new therapeutic entity in near future to treat resistant bacterial strains.

Keywords: Resistant bacterial strain, *Coliphages* , unique future approach of treatment

Introduction

Bacteriophages are unique type of viruses that infect bacteria and use machinery of bacteria for their multiplication, in some cases it causes lysis of bacterial cells and released of new progeny of viruses which will infect further more bacterial strains. These characteristics of bacteria can be used to treat bacterial infection. Bacteriophages were first isolated in 1917 and Felix d'Herelle use these agents to treat bacterial dysentery first time in oral preparation [1]. After the successful treatment most of industries started to formulate the phage preparations in Germany and France [2,3,4]. Bacteriophages are so discriminatory in their nature they infect only specific bacteria which possess receptor for binding bacteriophages. Phages do not infect all type of bacteria but if they infect they may causes lysis of bacterial progeny and they are helpful in treating bacterial infections caused by bacteria which are vulnerable to such bacteriophages. In this article I will discuss the bacteriophages specifically infect the Escherichia coli specie. Escherichia coli are present in intestine of human and animal as normal flora and are beneficial for then but certain pathogenic E. coli can cause many infections such asgastroenteritis, urinary tract infections, neonatal meningitis.

Certain rare E. coli can case life threatening infections as hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and Gram-negative pneumonia. The phage therapy will be very cost effective in treatment of bacterial infections; in past we find so many evidences when these entities were used to kill bacteria. Significant attention was not given to phage therapy in past in western world but these were studied and a lot of scientific research was continued in Eastern Europe and Poland. Now a days, the bacterial resistance to antibiotics bending the attention of western world toward phage therapy. As it is considered that we have entered into pre antibiotic era with emergence of bacterial resistance to existing antibiotics [5]. Bacteriophages can be isolated from many cheap resources such as from sewage water, river water, hospital sewage water and poultry and animal houses abortories. These bacteriophages, collected from such humble resources further purified by spot test and plaque assay. So many animal studies up till conducted which provides the strong evidence that bacteriophages are very effective against pathogenic bacteria, in some cases are more effective than conventionally used antibiotics. These are more beneficial as compare to antibiotics in case of self-replication at site of infection as this unique property is not present in the case of antibiotics. Therefore these are helping tool to overcome antibiotic resistant as a promising future. A lot of studies are needed on purification and formulation of bacteriophage preparation. The lack of knowledge of specificity of bacteriophage receptors and purification techniques are main obstruction in phage therapy.

The developing countries face many endemics, diarrhea is one of them. According to WHO 5 million children die each year due to acute diarrhea [6]. Diarrhea caused by various strains of bacteria, However, *Escherichia coli* and rotavirus causing childhood diarrhea up to 50% in underdeveloped countries. Enterotoxigenic, *Escherichia coli* also a causative agent of traveller diarrhea [7].

There are various strains of *Escherichia coli* associated with many infections of respiratory tract and gastrointestinal tract. *Escherichia coli* cause diarrhea in infants and in severe infection lead to kidney failure. *Escherichia coli* infections range from self-limited watery diarrhea and hemorrhagic colitis to life-threatening manifestations such as the haemolytic uremic syndrome and thrombotic thrombocytopenic purpura. It is a food born pathogen spread through contact with domestic animals. [8]. *Escherichia coli* producing illness through food [9]. T4 phages are typical example of lytic phages. T4 are best and suitable candidates against *Escherichia coli* [10]. *Escherichia coli* strain is susceptible to many phages obtained from sewage water, stools of calves and from some other sources as well Such type of phages can be easily isolated as phages are widely distributed in our environment and present in our daily foods as well. Coliphages can be isolated from highly polluted river water, sewage water, stools of diarrhea patient, hospital sewage water. *Coliphages,* after isolation was purified by using various techniques and indicators, so the required phage against specific strain is used to rationale phage therapy. Each phage is specific for each host but *Escherichia coli* strain susceptible to wide range of phages [10]. Bacteriophage activity against bacteria can be observed by agar plates and test tube methods. The outer cell surface of *Escherichia coli* bacteria have many proteins attached from 10,000 to 40,000, these proteins pose as receptors at which specific phage binds and cause lysis of bacteria [11]. Many studies are conducted on implementation of phage therapy against *Escherichia coli* Phages can be given through oral, rectal, intraperitoneal and local routes of administration and no complications were observed .The phages also cross blood brain barrier. Sandra – Chibani-

Chennoufi 2004 reported that four type of phages were obtained from sewage water and stool samples of diarrhea patients have in vivo activity against *Escherichia coli* K 803 strain in adult mice, these phages were also effective against ampicillin resistant colonies of Escherichia *coli*, when given orally by drinking water of mice [11]. Anne Bruttin and Harald Brussow 2005 conduct the safety studies of phage therapy in 15 human volunteers in which low dose, high dose of phages and placebo given orally,show no decrease of *Escherichia coli* in feces but they reveal that serum transaminases level remain normal and no antibodies were produced against coliphages, this study act as base to rationale phage therapy against *Escherichia coli* associated diarrhea [12]. In Soviet Union and Poland human phage therapy studies were conducted and successful results were obtained as31 patients of suparative skin infections caused by *pseudomonas, staphylococcus, klebsiella* and *Escherichia coli* were treated by phage therapy and 74% successful results were obtained [13] reported that septicemia and meningitis caused by K^+ strains of *Escherichia coli* prevented by implementation of phages which were isolated from sewage water. *Coliphages* are effective *in-vitro* and *in-vivo* against *Escherichia coli* except K^-strains of bacteria reported that *Escherichia coli* strains causing respiratory tract infections of broiler chicken can be prevented by inoculating phages in air sac but not prevented if phage therapy given orally [14]. But uptill now no enough research is conducted to rationale bacteriophage therapy against diarrhea caused by *Escherichia coli* strain.

Escherichia coli

These are gram negative, short rod shaped, anaerobic facultative bacteria. *Escherichia coli* are a part of normal flora specially residing at large intestine and producing vitamin K for the host [15]. These can live outside body for a short period of time therefore act as an indicator for determination of faecal contamination of environment, as where poor sanitary conditions are prevailing. *Escherichia coli* usually cause diarrhea in immunosuppressed, malnourished host when the protective, defensive system of gastrointestinal tract is disturbed. *Escherichia coli* can cause sepsis, diarrhea and urinary tract infections. There are certain strains of *Escherichia coli* which get plasmid DNA or bacteriophages which are encoded by enterotoxin genes such strains become virulent and cause dysentery, watery or plain diarrhea accompanied by symptoms of abdominal pain, fever, vomiting and nausea. Disease is contracted by intake of contaminated water or contaminated food, in developing countries where sanitation is very poor. Contaminated water is a major cause of diarrhea and a cause of death in infants. There are certain strains which can cause bloody diarrhea such as serotype O157:H7, O26 etc. In the plasmid of virulent strains certain genes which are encoded for BFP (bundle forming pilus) are present. Such strains due to presence of BFP can attach at the intestinal cells as BFP act as antigenic adherence factor for them. Such type of *Escherichia coli* called enterotoxin producing *Escherichia coli* these toxins are cytotoxic will exert their effect by attaching at mucosal cells and causing destruction of these cells.

Escherichia coli can be subdivided on the bases of surface antigen into various serotypes as K antigen: capsule, H antigen: flagellin, O antigen: as a part of lipopolysacchride layer [16].

There are certain pathogenic strains of *Escherichia coli* which can cause Haemolytic-uremic syndrome, septicaemia, gram negative pneumonia, urinary tract infections, and gastroenteritis [17]. Among other gastroenteritis disorders diarrhea also a leading cause of illness and death in developing countries. The pathogenic strains of *Escherichia coli* contain specialized fimibrial antigens due to which their attachment at intestinal mucosa is increased [18, 19]. *Shigella* like *Escherichia coli* are more virulent a very small quantity of such bacteria can cause severe illness in the host they attack at colon after attachment to cells cause destruction of epithelial cells, epithelial tissues and causing acute inflammation which resulted in decreased excretion of water with large volume excretion of blood with mucus sheet Diarrhea-genic *Escherichia coli* can be classified on the bases of mechanism which they follow for causing diarrhea in their host. ETEC (enterotoxigenic *E .coli*) acts via attaching enterocytes of small bowel via pilli. They produce two type of enterotoxins ST, LT[20] certain strains produce only LT and some produces only STs but there are some strains of ETEC producing both LT and ST [21, 22, 23, 24, 25, 26].

LT are the heat labile enterotoxins have two serogroups LT-I, LTII. LT-I is associated with diarrhea in animals and in human beings but LT-II is not associated with diarrhea in both human and animals. When LT –I attached with host cell membrane it will be endocytosed. At cellular level it will cause activation of adenylate cyclase, as A1 peptide will transfer ADP-ribosyl moiety from NAD to Gs (α subunit) which will cause activation of adenylate cyclase that resulted in higher level of cellular cAmp.

It will cause activation of protein kinase A. Protein kinase A will cause phosphorylation of Cl^- channels (CFTR) [26]. These chloride channels will be stimulated and sodium chloride absorption is inhibited. Due to increase ionic contents in lumen it will draw water from Para cellular sites and resulting in osmotic diarrhea. LT-I also act via PGE_1, PGE_2, and platelet activating factors, enteric nervous system, serotonin, vasoactive intestinal peptides and interleukin-6 via inflammation [27].

ST are heat stable toxins produced by ETEC these are of two types STa and STb. STa acts via activation of guanylcyclase C [28, 29] Which results in increased level of cGMP which will cause activation of chloride channels and inhibition of sodium chloride absorption.

Also via phosphotidyl inositol triphosphate and DAG pathway which cause activation of protein kinase C resulting in increased level of cellular calcium level.STb it will act via damaging the tissues of intestine causing partial atrophy of villi. Its receptors are not so well defined. It stimulates the secretion of bicarbonate ions [26]. It also acts via PGE_2, serotonin, and enteric nervous system is also involved.

EPEC (Enteropathogenic *Escherichia coli*) this bacterium mostly causes infection in infants in developing countries. It will cause serious infection by attaching enterocytes and effacing tissues of small intestine. After attachment at epithelium, it caused effacement of microvilli and condensation of actin [30]. EPEC typical and a-typical their adherence is largely dependent on the presence of EAF (*Escherichia coli* adherence factors). EPEC act by attaching at intestinal cells of large intestine via BFP, these strains will produce a lesion after attachment due to destruction of microvilli, this damage results in malabsorption and osmotic diarrhea. EHEC (Enterohemorrhagic *Escherichia coli*) will cause haemorrhage and edema of lamina propria. *Escherichia coli* O157:H7 included in this group. Other groups of diarrheagenic *Escherichia coli* include DAEC (diffusely adherent *Escherichia coli*), EIEC (Enteroinvasive *Escherichia coli*) and EAEC (Enteroaggregative *Escherichia coli*).

Resistance is developed in strains of Escherichia *coli* via active efflux pumps, inactivation of antibiotic molecule by enzymes and by alteration of targeted enzymes. *Escherichia coli* strains are resistant to fluoroquinolones, cephalosporin, tetracycline, chloramphenicol, ampicillin, nalidixic acid and rifampicin. Due development of resistance in certain strains of *Escherichia coli* and prevalence of resistance phage therapy will be reasonable choice to overcome resistance. In this study we use pathogenic strains of *Escherichia coli* for isolation, purification of bacteriophages, the activity of theses phages is analyzed on these pathogenic strains.

Mechanism of action of bacteriophages/ coliphages

Bacteriophages that infect especially E. coli and multiply within these bacteria, causing lysis of bacterial cells and producing their new progeny are called coliphages. Sewage water is the main source of coliphages. The sewage water of highly populated area is the main reservoir of coliphages. The bacteriophages are of two types mainly lytic and lysogenic in nature. There are very few reports are available on the pharmacokinetics of phage therapy and additional studies are required in case of pharmacology and toxicology of bacteriophages. Some reports are available showing that bacteriophages are present in blood after 2 hrs of administration and the also reach to body vital organs such as liver, kidney and lungs. In Eastern Europe these bacteriophages have been used orally, intravenously, intrapleurally and rectally. These studies showed that they are safe in all such routes of administration. All phages do not interact with bacteria in similar way, some are lytic and some bacteriophages are lysogenic in nature. It was reported that aerosol administration of bacteriophages against *Escherichia coli* which is causative agent of respiratory disease in chicken decreased mortality [14]. Phages, e11/2 and e4/c, pp0, cause reported that e11/2 and e4/c, pp01 phages cause lysis of *Escherichia coli* O157:H7 *in vivo* and *in vitro* [36]. Anti-K1 phages produced protective effect against O18:K1:H7Co1v+ *Escherichia coli* designated as MW as compare to other phages in vivo and in vitro studies [37].

A new bacteriophage reported that a new bacteriophage named CEV1 efficiently infects the *Escherichia coli* O157:H7 aerobically and anaerobically. In vivo studies conducted in sheep and cause 2log –unit reduction within two days [38].

Escherichia coli O157:H7 infected by its specific bacteriophage PP01 at chemostat continuous culture that phage cause effective lysis of *Escherichia coli* O157:H7 [39].

KH1 and SH1 phages were characterized *Escherichia coli* O157:H7 strains SH1 have broader host range as compare to KH1 on lawn of *Escherichia coli* O157:H7 in vivo given to infected mouse and cause lysis of *Escherichia coli* O157:H7 [8]. A mouse infected with *Escherichia coli* O157:H7 was treated with a bacteriophage that was isolated from bovine manure. The phage (10^8 PFU / mouse) cause

clearance of *Escherichia coli* O157:H7 within 48 hrs [41]. Multidrug resistant uropathogenic *Escherichia coli* cause urinary tract infection. Experimentally urinary tract infection was developed in mice show the decrease in mortality of mice by inoculation in peritoneal cavity of mice by KEP10 [42] (table 1).

Isolation and purification techniques for bacteriophages

Phage JS4, JS94, JSD.1, JSL.6 Type of bacteriophages was isolated from paediatric patients with undifferentiated diarrhea, their stool samples, Environmental water and sewage water. The stool sample was diluted with water containing sodium chloride and peptone up to 30 ml. This mixture was centrifuged at 14,500 × g for 15 minutes and filtered through 0.45 µm minisart filters. 50 ml of water sample water were centrifuged at 10,000 rpm and through 0.45 µm pore size filter and tested by spot test by using K803 strains. Plaque assay was performed to further purify. A Six-week-old C3H male mouse was used as animal model [10]. Bruttin and Brussow, 2005 reported that *Escherichia coli* phage T4 were obtained from C. Georgopoulos, Geneva University, Geneva, Switzerland. T4 bacteriophages from Geneva University were obtained and added to Hershey broth medium which was inoculated by strain K803. This mixture was centrifuged at 4,000×g for 15 minutes. The supernatant was filtered by 0.22 µm. The phage pellets were centrifuged at 35,000× g and diluted with water. That experiment was performed on human volunteers [12].

Coliphages as tool to treat diarrhea

It was reported that *Escherichia coli* strain K803 was used to check the activity of bacteriophages which have the morphology like T4phages and possessing 170-kb genome. These phages were isolated from stool samples of 140 children hospitalized in hospital due to diarrhea in Bangladesh. These phages show lytic activity confirmed by spot test on upto 27 types of *Escherichia coli* strains out of 40 types. This study showed that 19% of acute diarrhea yielded the T4 like phages. These types of bacteriophages were isolated from sewage water but these yielded low titter as compare to stool samples. In this study it is revealed that these phages isolated from different patients show similarities with each other and with T4 like phages and showing that patients living in same climate and epidemiological link with each other [10]. Jamalludeen *et al*, 2009 evaluated that EC-Nid1 and EC-Nid2 phages were isolated from waste water of different poultry houses drainage and were evaluated to show lytic activity against different bacterial strain such as *Escherichia coli* O1, O2, O78 serotypes causing infection in poultry such as avian colibacillosis. In this study it was concluded that these EC-Nid1 and EC-Nid2 phages are highly active against the bacteria causing avian colibacillosis. Thes phages were subjected to further characterization which revealed that are belonging to *Myoviridae* class as they possess contractile tail and icosahedral head. These phages can tolerate the 5 to 9 pH [43]. Phage activity against bacterial strains such as different serotypes of *Escherichia coli* was evaluated both *in vitro* and *in vivo* Bacteriophages T4 like against *Escherichia coli* were isolated from stool samples of diarrhea patients. The sewage water and different sources of water drainage were used for isolation of bacteriophages by using *Escherichia coli* as host strain. The ampicillin resistant *Escherichia coli* was inoculated to conventional mice the phages were used to treat infection by using mininmum dose 10^3 PFU(plaque forming units). These phages were recovered from feces of animals and lowering the bacterial shed in the feces. In this study phages show significant activity in vivo but very minute lytic activity against *Escherichia coli* in vitro [10].

Escherichia coli is a causative agent of diarrhea which is responsible for a large number of mortality and morbidity in developing Asian countries [44]. Enteropathogenic (EPEC) and enterotoxigenic (ETEC) *E. coli* are usually the main cause of diarrhea [45, 46]. Diarrhea treated by oral rehydration solutions remarkably decrease the mortality and morbidity in developing countries but these solutions have no inhibitory effect on the growth of pathogens like *E. coli* strains [47]. So many antibiotics are in conventional use to treat diarrhea but now a day's their use is limited [48]. Felix d' He´relle almost hundred years ago discovered the viruses cause killing of bacterial cells named as bacteriophages, he concluded that these viruses can be used against the bacterial infections [49].

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Table 1. Mode of action of bacteriophage