Review Article

Role of phage therapy in acute gastroenteritis

Somaieh Sabzali[1], Setareh Pazhouhnia[2], Kiana Shahzamani[3], Peyman Adibi Sedeh[4]

[1]Department of Biology, Faculty of Basic Sciences, Lorestan University, Khorramabad, Iran, [2]Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran, [3]Hepatitis Research Center, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran, [4]Gastroenterology and Hepatology Research Center, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

The gut ecosystem, comprising the gut microbiota and its interactions, plays a crucial role in human health and disease. This complex ecosystem involves a diverse array of microorganisms such as viruses, fungi, and bacteria, collectively known as the gut microbiota. These microorganisms contribute to various functions, including nutrient metabolism and immune modulation, thereby impacting human health. Dysbiosis, or an imbalance in the gut microbiota, has been associated with the pathogenesis of several diseases, ranging from intestinal disorders such as inflammatory bowel disease to extra-intestinal conditions such as metabolic and neurological disorders. The implications of dysbiosis in the gut ecosystem are far-reaching, affecting not only gastrointestinal health but also contributing to the development and progression of conditions such as autoimmune gastritis and gastric cancer. Furthermore, the burden of antimicrobial use and subsequent side effects, including antibiotic resistance, poses additional challenges in managing gastrointestinal diseases. In light of these complexities, investigating the role of bacteriophages as regulators of the gut ecosystem and their potential clinical applications presents a promising opportunity to tackle antibiotic resistance and fight infectious diseases.

Key words: Bacteria, bacteriophage, gastroenteritis, gastrointestinal diseases

How to cite this article: Sabzali S, Pazhouhnia S, Shahzamani K, Sedeh PA. Role of phage therapy in acute gastroenteritis. J Res Med Sci 2025;30:2.

INTRODUCTION

The human gut is home to a complex community of microorganisms, often referred to as the gut microbiota, which plays a vital role in keeping us healthy. Over the years, we have learned that the gut microbiome is not just responsible for digesting food – it also affects our immune system, metabolism, and even our brain health. When the balance of these microorganisms gets disrupted, a condition called dysbiosis, it can lead to a variety of health issues.[1] These range from digestive disorders such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) to metabolic conditions and even cancer. As scientists continue to uncover the many ways our gut microbiota influences our well-being, new treatments are emerging that target this ecosystem. One of the most exciting developments in this area involves bacteriophages (viruses that infect bacteria).[2]

Phages are incredibly common in the human gut, but their role has been somewhat overlooked until recently. Historically, they have been studied as potential alternatives to antibiotics, especially in the fight against antibiotic-resistant bacteria. However, it turns out that phages do a lot more than just attack harmful bacteria. They can shape the entire gut microbiome by targeting specific bacteria, which in turn influences the balance of other microorganisms. Phages may also play a role in transferring genes between bacteria and even help regulate our immune responses. Because of these capabilities, phages hold great promise as a way to treat and prevent various gut-related diseases.[2]

One of the biggest advantages of phage therapy, especially compared to traditional antibiotics, is its precision. Antibiotics tend to wipe out a broad range of bacteria, including many of the beneficial ones that

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jrms

DOI: 10.4103/jrms.jrms_464_24

Address for correspondence: Dr. Kiana Shahzamani, Hepatitis Research Center, Faculty of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran.
E-mail: shahzamani.k@lums.ac.ir

Submitted: 21-Aug-2024; **Revised:** 30-Oct-2024; **Accepted:** 25-Nov-2024; **Published:** 30-Jan-2025

help maintain a healthy gut. This can lead to unwanted side effects and long-term imbalances in the microbiome. Phages, on the other hand, target only specific bacteria, allowing the rest of the gut ecosystem to remain intact. This could not only make treatments more effective but also reduce the risk of side effects. Plus, because phages evolve along with the bacteria they infect, they might be a more sustainable solution in the fight against antibiotic resistance.

This review aims to dive deep into the emerging role of phages in gut health and how they might be used as therapeutic tools. We'll explore how phages affect the makeup of gut bacteria, their involvement in gene transfer between microbes, and how they interact with the immune system. We'll also look at some of the latest research into phage therapy as a treatment for gut-related conditions such as IBD, infections, and antibiotic-resistant bacteria.[3]

As our understanding of the gut microbiome and its interactions with phages grows, so does the potential for innovative treatments. This review highlights the exciting possibilities that phages bring to the table, especially in tackling the challenges of gut dysbiosis and antibiotic resistance. Ultimately, these advances could open up new ways to keep our guts healthy and fight bacterial infections in future.

Gut ecosystem

Gut microbiota factors can be transmitted in early life stages, i.e., from the beginning of the uterus during the fetal period. The intestinal ecosystem of a human is influenced by the early life environment to which they are exposed at the time of birth. Intestinal flora lives in the form of biofilms and contains viruses, fungi, and bacteria. Each individual has a unique profile of gut microbiota which performs some specific functions in the host's nutrient metabolism to modulate immunity against pathogens in the host's body. The gut microbiome plays an important role in human health and diseases, including cardiovascular and digestive diseases.

Dysbiosis of the gut microbiota causes systemic inflammation, hyperammonemia, and endotoxemia, which lead to neuro-inflammation in the brain through communication with the gut, brain, and liver. Changes in the microbial structure and function of the intestine (dysbiosis) are associated with the pathogenesis of various diseases such as IBD. Observations show that microbial dysbiosis is a significant risk factor for common intestinal diseases such as IBD, IBS, and colon cancer.

Understanding this dysbiosis is controversial because the gut ecosystem is extremely complex and there is an enormous diversity of species that make up the human microbiome in healthy individuals. Dysbiosis of the gut microbiota is associated not only with intestinal diseases but also with many extra-intestinal diseases such as metabolic and neurological disorders. The processes associated with the dysbiosis of the intestinal microbiota play a very important role in the pathogenesis of multiple hereditary exostoses.

The gut microbiota has played an important role in human health and disease occurrence in recent years. It is necessary to understand the possibilities of influencing and optimizing the microbial pattern by exploiting the potential of the gut microbiota for human health. The gut microbiota plays a crucial key and very important role in human health and disease. One way the gut microbiota modulates anti-tumor immunity is through metabolites.[1]

Metabolites are the small molecules that diffuse from their original site in the intestine and counteract the local and systemic antitumor immune response. There are many phages in the human biome and they have been mentioned as the potential modulators of the ecosystem. Phages contribute significantly to the dynamics of the intestinal ecosystem.

Regarding gastrointestinal cancers, such as esophageal cancer, colorectal cancer, pancreatic cancer, and hepatocellular carcinoma, we review microbiota data in general. The solutions to the underlying mechanisms of the gut microbiota play a role in the development, prevention, and treatment of cancer. The microflora of the microbial habitat represent different body population patterns and are closely related to the development of diseases.[2]

One of the most important causative agents of stomach cancer is *Helicobacter pylori*. The presence of Helicobacter in the stomach destroys the natural environment of the stomach and makes the stomach a habitat for microbes.

It is clear that antibiotics also play a role, that these drugs rapidly and sometimes permanently alter the taxonomic capacity, genomics, and functions of the human gut microbiota according to their action. A change in the microbial ecosystems in the intestines of animals and humans is associated with increased metabolic and immune disorders. Obesity and metabolic diseases can also have an impact on the intestinal flora. In metabolic inflammation, therapeutic approaches aim to model the gut microbial ecosystem to regulate obesity and its associated pathologies.[2,3]

Burden of antimicrobial use (prevalence)

In most cases, gastroenteritis is a self-limiting disease. Antibiotic therapy is not necessary for treatment; supportive

therapies that restore water and ions are the sole medications used. The WHO recommends the use of antibiotic treatment only if *Vibrio cholerae* and *Shigella* spp. bacteria are the cause of the disease, gastroenteritis in children (1–5 years old) after identifying the cause of infection.[4-6]

There are the reports of indiscriminate drug use by patients before hospitalization worldwide. The study by Ahmed *et al*. from Bangladesh reported that 76% of children under 5 years of age in the urban areas and 51% in the rural areas with gastroenteritis received antibiotics before going to the hospital.[5] The study by Ferdoosian *et al*. reported that 19% of patients received antibiotics before going to the hospital.[7] In the study by Abasi *et al*., the rate of indiscriminate use of antibiotics in 104 sick children referred to a hospital in Iran showed that 79.8% of these patients had received ceftriaxone, 5.8% had received ceftizoxime, and only 14.4% of children had not received antibiotics. These results suggest that most antibiotics are prescribed arbitrarily and without consideration of the laboratory results.[8] The use of antibiotics in the outpatient treatment of patients with gastroenteritis who are referred to American hospitals was investigated by Jennifer *et al*. According to their findings, roughly 13.3% of 10,210 individuals who were referred to American hospitals between 2006 and 2015 were given antibiotics. The most often prescribed medications were fluoroquinolones (28.7%), metronidazole (20.2%), and penicillin (18.9%).[9] In a study by Bruzzese *et al*., the most commonly used antibiotics were metronidazole and cotrimoxazole for mild diarrhea and ciprofloxacin and ceftriaxone for severe diarrhea.[10]

Khakshour *et al*. reported that antibiotics should be prescribed based on the laboratory findings (fecal culture and analysis) to prevent microbial resistance in patients with diarrhea.[11] Another problem with treating gastroenteritis is choosing the wrong antibiotic even after hospitalization. The result of a study conducted in Iran showed that 37% of antibiotics used to treat gastroenteritis in children referred to hospitals were incorrectly selected. Furthermore, the pattern of antibiotic consumption in this study was not consistent with the standard treatment protocol.[12]

Subsequent side effects and adverse drug reactions

Indiscriminate and irregular use of antibiotics leads to side effects such as antibiotic resistance. The World Health Organization has recommended avoiding antibiotics as much as possible when treating bacterial infections. Improper use of antibiotics can cause allergic reactions as well as *Clostridium difficile* infection and antibiotic resistance.[13] Chromosomal mutations, the presence of efflux pumps reduction in penetration of antibiotics into the cell wall, alteration of the antibiotic target site from the cell wall alteration of the antibiotic target site, and enzymatic inactivation of antibiotics are among the methods that cause drug resistance in bacteria.[14] The primary mechanism of the spread of multidrug-resistant (MDR) strains in *V. cholerae* can be attributed to spontaneous mutation or horizontal transfer of resistance genes between intestinal coliforms or other microflora present in *V. cholera*.[15] As already mentioned, in some cases, the use of antibiotics is necessary to treat this disease. Some of these cases are listed in the Table 1. To prevent traveler's diarrhea, the use of fluoroquinolone or azithromycin antibiotics is recommended in people with weakened immune systems or taking immunosuppressants. For bloody and mucous diarrhea and fever suggestive of *Shigella* infection, fluoroquinolone, ciprofloxacin, and levofloxacin medications may be used. It can also be used for traveler's diarrhea in people with a fever above 38.5°C.

Rifaximin is also a suitable option if a bacterial infection is suspected. It is recommended to conduct diagnostic tests and identify the bacterial pathogen causing the disease and specifically prescribe antibiotics. Azithromycin is recommended for the treatment of *Campylobacter* infection. Alternative drugs such as macrolides are used in areas where *Campylobacter* is resistant to fluoroquinolones such as azithromycin.[16] The resistance rate to Ciprofloxacin in *Campylobacter* has reached more than 44% in some parts of Europe,[17] while resistance to erythromycin was reported to be < 5%. The resistance rate to this antibiotic was 56% in Mexico and over 92% in Thailand.[18] This resistance rate in Southeast Asia and South Korea was reported to be 29%.[19] Given what was stated, the use of azithromycin is recommended in areas where gastroenteritis caused by fluoroquinolone-resistant *Campylobacter* is prevalent. Although it is unreasonable in European countries to recommend fluoroquinolones as the first-line empirical therapy for community-acquired diarrhea in the United States, this class of antibiotics may be effective for nontravel-related cases.

The use of azithromycin, ciprofloxacin, or ceftriaxone is recommended for the treatment of shigellosis.[20,21] The rate of *Shigella* antibiotic resistance to ampicillin, nalidixic acid, ciprofloxacin, and trimethoprim/sulfamethoxazole antibiotics in South Korea is 49%, 50%, <1%, and 8%, respectively. In another study in South Korea, the resistance of *Shigella* to the antibiotics trimethoprim, sulfonamide, nalidixic acid, and ampicillin was reported to be 100%, 99%, 70%, and 49%, respectively, while no resistance to cefotaxime or ciprofloxacin was observed.[22] The level of resistance to sulfamethoxazole-trimethoprim, ampicillin, cefotaxime, and nalidixic acid was reported to be 79.72%, 85%, 63%, and 47%, respectively.[23]

Doxycycline is recommended for the treatment of *V. cholerae* infection. The antibiotics ciprofloxacin, azithromycin, and

Table 1: Proposed antibiotics for each of the bacterial agents that cause the disease along with the side effects of each of them

Bacteria	Antibiotic	Sid effect
Campylobacter	Azithromycin, ciprofloxacin	Azithromycin: Diarrhea, nausea, abdominal pain, vomiting, nervousness, skin reactions, anaphylaxis, *C. difficile* infection Ciprofloxacin: Nausea, vomiting, and diarrhea, Severe side effects: Increased risk of tendon rupture, hallucinations, and nerve damage
Nontyphoidal salmonella	Usually not indicated	
Salmonella enterica Typhi or Paratyphi	Ceftriaxone or ciprofloxacin, ampicillin, trimethoprim-sulfamethoxazole, or azithromycin	Ampicillin: Angioedema, anaphylaxis, and *C. difficile* infection. nausea, vomiting, itching, and blood dyscrasias Trimethoprim-sulfamethoxazole: Fever, nausea, vomiting, diarrhea, weight loss, rash, muscle aches, joint pain, itch, sore mouth, hyperkalaemia (high blood potassium), thrombocytopenia (low number of platelets in the blood) Azithromycin: Diarrhea, nausea, abdominal pain, vomiting, nervousness, skin reactions, anaphylaxis, *C. difficile* infection
Shigella	Azithromycin, ciprofloxacin, or ceftriaxone, trimethoprim-sulfamethoxazole, ampicillin	Ceftriaxone: Allergic reactions, *C. difficile*-associated diarrhea, hemolytic anemia, gall bladder disease, and seizures
V. cholerae	Doxycycline, ciprofloxacin, azithromycin, or ceftriaxone	Doxycycline: Diarrhea, nausea, vomiting, abdominal pain, and an increased risk of sunburn Ceftriaxone: Allergic reactions, *C. difficile*-associated diarrhea, hemolytic anemia, gall bladder disease, and seizures
Noncholeraic Vibrio	Noninvasive disease: Usually not indicated Invasive disease: Ceftriaxone doxycycline, trimethoprim-sulfamethoxazole, aminoglycoside	Aminoglycoside: Sensorineural hearing loss, vestibular ototoxicity, frequent use of aminoglycosides could result in kidney damage
E. coli	Usually not indicated	

C. difficile=Clostridium difficile; V. cholerae=Vibrio cholerae; E. coli=Escherichia coli

ceftriaxone antibiotics can also be used in this context.[24] In recent years, the prevalence of MDR cholera strains has been reported worldwide, and simultaneous resistance to several antibiotics has been observed, including nalidixic acid, trimethoprim, sulfamethoxazole, erythromycin, and ampicillin.[25]

ANTIBIOTIC RESISTANCE IN *SALMONELLA*

It is estimated that 16 million cases of typhoid fever and 1.3 million cases of gastroenteritis worldwide are caused by *Salmonella*. There are 600,000 deaths from typhus.[26] Pan *et al*. reported that 35%–55% of isolates had high levels of resistance to antibiotics such as ampicillin, tetracycline, streptomycin, carbenicillin, sulfamethoxazole, and trimethoprim. Among them, the highest resistance was tetracycline, streptomycin, and ampicillin.[27] A study conducted in Iran found that 14.8% of isolates were resistant to two or three antibiotics. In the study conducted by Mahmoudi *et al*. in Iran, the resistance rate to ciprofloxacin and cefuroxime among *Salmonella* isolates was reported to be 48.2% and 74.1%, respectively. Another Iranian report also showed the resistance rate to cefotaxime, trimethoprim, and sulfamethoxazole in *Salmonella enteritidis* to be 57% and 23%, respectively.[23]

ANTIBIOTIC RESISTANCE IN *ESCHERICHIA COLI*

Escherichia coli (E. coli) are Gram-negative, facultative anaerobic bacteria that live naturally in the human digestive system. It causes many diseases such as wound infections, pneumonia, urinary tract infections, and gastroenteritis.[28] In recent years, the antibiotic resistance rate of this bacterium has increased, so diseases caused by MDR. In a study conducted in Iran, the resistance rates to ampicillin, sulfamethoxazole, and trimethoprim were reported to be 99%, 87%, and 78%, respectively.[29,30] The results of the studies revealed that the antibiotic resistance rates of amikacin and tobramycin were reported to be 91% and 59%, respectively.[31,32] The resistance rate to fluoroquinolones was 89% in India and 41.3% in Egypt.[33]

DYSBIOSIS

The prevalence of many chronic diseases has increased in the recent decades. Rates of many chronic diseases, including IBD are increasing.[34] Dysbiosis is a disease in which the balance in the composition and metabolic capacity of the natural intestinal flora is disturbed.[34] In this condition, the number of beneficial bacteria is very limited and bad bacteria multiply. Therefore, there are a variety of symptoms of indigestion disorders, including diarrhea, muscle cramps, constipation, bloating, and indigestion. The gut microbiome influences how we deal with problems. The symptoms of dysbiosis include chronic fatigue, digestive problems, difficulty urinating, acid reflux or heartburn, vaginal or rectal infection or itching, food intolerance, gas bloating, joint inflammation and pain, acne, rashes, and psoriasis. If the intestine is in dysbiosis for a long time,

greater anxiety or depressive concentration problems may occur. There are three types of dysbiosis: In the first type, the good and protective bacteria of the intestine are lost or suppressed, leading to the onset of a disease called loss of function dysbiosis. The second type involves an overgrowth of opportunistic pathogens in the stomach, called increased dysbiosis function. In the third type, the overall diversity of the gut microbiome, including good and bad bacteria in the stomach, is lost.[34]

AUTOIMMUNE GASTRITIS

Autoimmune gastritis (AIG) is a chronic inflammatory disease associated with the destruction of the body's parietal cells and gastric fundus. This autoimmune disease is chronic and usually non-erosive. It is more common in people with other autoimmune diseases such as Hashimoto's disease and type I diabetes. The well-known consequence is a Vitamin B_{12} deficiency, which leads to dangerous anemia. The loss of parietal cells reduces the secretion of stomach acid, which is also necessary for the absorption of the mineral iron. Therefore, patients with AIG usually have an iron deficiency. Unlike gastritis caused by *H. pylori*, stress, or medications, persistent inflammation, and atrophy in AIG are limited to the body and fundus. The autoimmune reaction in AIG is directed against the parietal cells. Parietal cells are the epithelial cells located in the body's glands and fundus but not the antrum, that produce hydrochloric acid and intrinsic factors. Gastric acidification is primarily controlled by the gastric H^+/K^+ ATPase, a proton pump, that is the causative autoantigen and is recognized by $CD4^+$ T cells. As the disease progresses, chronic inflammation leads to atrophy of the mucous membrane and ultimately to the loss of parietal cells. This problem leads to an increase in stomach pH and loss of intrinsic factors produced by parietal cells. Intrinsic factor is required for the absorption of Vitamin B12, and Vitamin B12 deficiency (pernicious anemia) is a known consequence of AIG. There is no risk of stomach or duodenal ulcers in AIG patients. The primary clinical manifestations of AIG are known to be pernicious anemia. Iron deficiency symptoms occur independently of and before symptoms associated with anemia and include fatigue, restless legs syndrome, brittle nails, hair loss, impaired immune function, and impaired wound healing. Anemia (regardless of its cause) results in shortness of breath, dizziness, tachycardia, drowsiness, and reduced cognitive and physical performance.[35]

Gastric cancer (GC) is one of the most common malignancies and the third leading cause of cancer-related deaths worldwide.[36] *H. pylori* can alter the stomach microbiome and lead to diseases related to GC. In addition, there is evidence that other bacteria besides *H. pylori* are also involved in the development of GC. The initial stage of GC is asymptomatic or has nonspecific symptoms. Most patients are diagnosed at an advanced stage.[37] *H. pylori* infection leads to persistent inflammation of the gastric mucosa, leading to the changes in the cell cycle of gastric epithelial cells ultimately leading to glandular atrophy, intestinal metaplasia, and GC.[38] Studies suggest that microbial diversity is significantly reduced in inflammatory diseases and cancers, including GC.[39] However, some studies have suggested that the diversity of the gastric microbiome is increased in GC tissues compared to control tissues.[40] For example, the bacteria *Prevotella copri* and *Bacteroides uniformis* decreased, while *Prevotella melaninogenica*, *Streptococcus anginosus*, and *Propionibacterium acnes* increased in tumor tissue compared to normal and surrounding tissues.[41] Oral microbiome dysbiosis is associated with IBD, colorectal cancer, and pancreatic cancer. A study by Yang *et al*. showed that the abundance of oral microbiota, including *Peptostreptococcus*, *Streptococcus*, and *Fusobacterium*, is higher in GC samples than in neighboring nontumor samples.[42] The 16s rRNA gene of the gastric microbiome was also sequenced in cases of superficial gastritis, atrophic gastritis, intestinal metaplasia, and GC. This revealed that oral bacteria, including Peptostreptococcus stomatis, *S. anginosus*, Parvimonas micra, and Slackia exerigua, can alter the acidic environment of the stomach in the GC in comparison to the tissue samples from precancerous stages, which in turn can lead to oral bacterial colonization. However, further studies are needed to elucidate the role of the oral microbiome in gastric carcinogenesis.[42]

One significant genus in the intestine is lactobacilli. It has several important biological functions, including immunomodulation, anti-inflammatory, and anti-cancer effects through the production of lactic acid but plays a role in the development of gastric carcinoma.[43]

A study by Castano found that the frequency of *Lactobacillus* increased in patients with GC compared to patients with gastritis or intestinal metaplasia.[44] Another study reported that lactic acid-producing bacteria such as *Lactobacillus lactis* and *Lactobacillus brevis* increased in adjacent nontumorous tissues.[40]

Mechanism of phage effect

Phages that specifically infect bacteria are referred to as bacteriophages. They are very common on the planet and can be found everywhere. Extensive studies have been carried out on the use of phages to prevent, control, and even treat diseases with the phenomenon of antibiotic resistance and increasing strains resistant to multiple antibiotics emerging. Most of the researchers' efforts have been based on replacing bacteriophages with antibiotics. Phages are intracellular parasites that can invade the bacterial cell system [Figure 1]. They reduce the growth of

bacteria and ultimately limit bacterial infections by taking over the bacterial replication system and other cellular elements.[6] Phage therapy proposed in recent decades is based on the use of lytic phages and the fight against bacterial infections. Bacteriophages may have several structural and genetic advantages to replace antibiotics. One of these advantages is their specific functionality. Their further advantage is that they do not infect eukaryotic cells and have a direct effect on the target cells. In contrast to antibiotics, they work without changing or damaging the body's natural flora. They eliminate the bacteria from the body. This treatment method has advantages over antibiotic treatment, such as high specificity for the bacterial host, no side effects for the patient, stability dependent on the presence of the bacterial host, and no need to adjust the dose during treatment.[45] The replication of phages looks similar to that of other viruses. The phage genome enters the cell after binding to the specific receptor in the bacterial cell wall. The phage capsid coat remains mainly on the bacterial cell wall and does penetrate the bacteria. The phage genome can be destroyed and decomposed after the phage enters the host cell. Pathogenic phages do not enter their genetic materials into the host's genetic system. However, they reproduce independently and destroy the host's cell. Such an infection cycle is called the lytic cycle. Therefore, these phages could be used to destroy bacteria and treat bacterial infectious diseases in phage therapy.[46-48] The presence of viruses, particularly phages, in the human digestive system has been known for a century. However, their role in the gut microbiota has not been extensively studied [Figure 2].

BIOLOGIC THEORY AND MECHANISTIC EVIDENCE

It is estimated that the number of active phage species in a healthy human is between 35 and 2022, and more than 52% of them are unique to each human.[49] Gastrointestinal diseases are among the most common inflammatory diseases in health care. Crohn's disease and colitis are the two important inflammatory diseases in this regard. Phages are a promising alternative for altering the gut microbiota

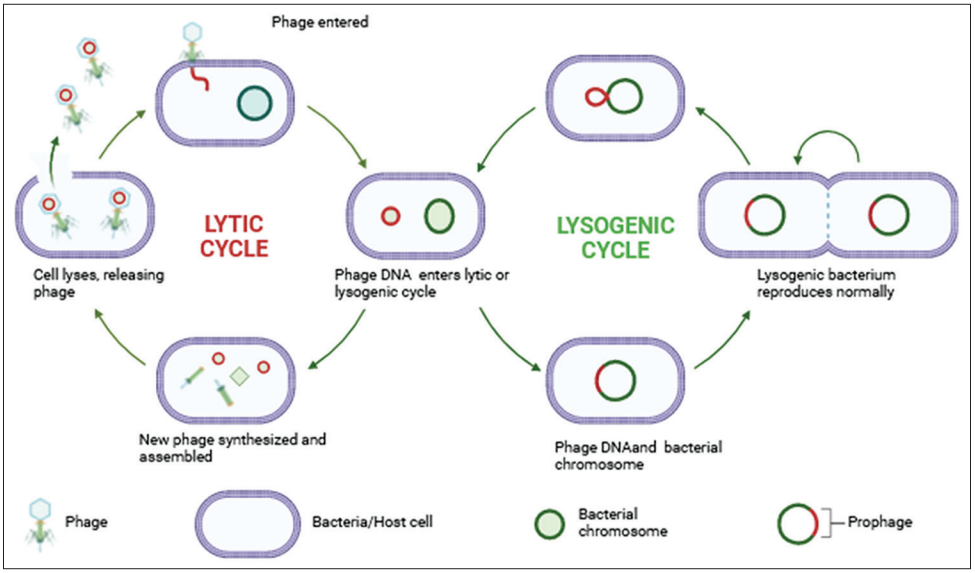

Figure 1: Schematic review of lytic and lysogenic cycle of bacteriophages in bacteria

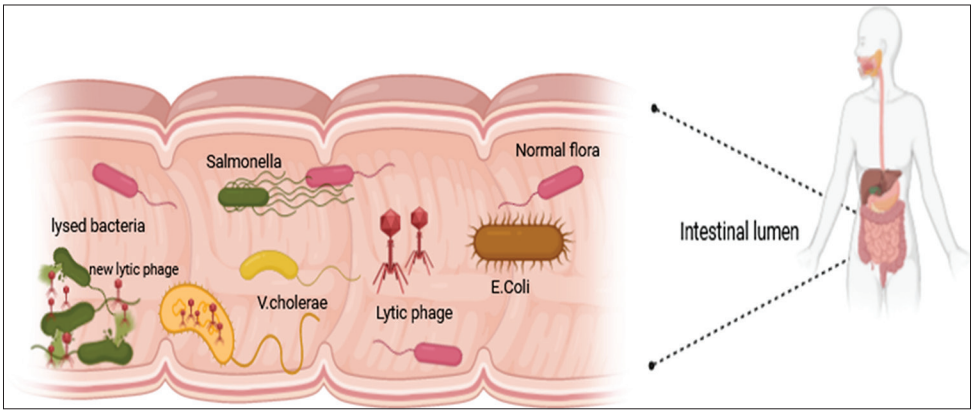

Figure 2: Lytic bacteriophage in lumen can eliminate host bacteria without having a negative effect on normal flora

by eliminating pathogenic bacteria. Currently, lytic phage therapy is being studied primarily in the digestive tract of the body to combat Crohn's disease and eradicate *E. coli* in Crohn's disease.[45] Evaluating the safety and effectiveness of the phages used is the most important issue in the therapeutic development of phages. The majority of phages are moderately abundant in the human gut, suggesting that the gut microbiome is relatively stable in the gastrointestinal tract. This idea has led to the creation of global intestinal phages, indicating the connection between phages and health status as well as their role in maintaining the structure and function of the intestinal microbiome.[49] Resistance systems of bacteria and mutual defense strategies of phages were investigated. More specific and novel systems have recently been discovered through large-scale data mining and screening, in addition to extensive mechanisms, such as the changes in receptors and restriction-modification systems. It is assumed that many more cases remain undetected. The flexibility of genetic information forms the cornerstone of all these systems. Therefore, to integrate the evolution of phage and bacterial interactions in intestinal tissue, different levels of information must be taken into account, ranging from small viral genomes to the behavior of large organs.[50] One of the first studies of phage community richness and diversity associated with gut microbiota changes was conducted on stool samples from patients with Crohn's disease and ulcerative colitis. Surprisingly, the richness and diversity of phages were greater in these patients than in healthy people. However, the richness and diversity of bacteria were lower.[51] Phages can be useful as vectors for horizontal gene transfer. The high induction of prophages during inflammation supports the mechanism of horizontal gene transfer between its bacterial hosts, which increases the recombination rate and genetic diversity. This process actively shapes the development of virulence-modifying bacteria and antibiotic-resistance factors. In addition, phage genes can indirectly increase the production of bacterial toxins and have effects on adhesion, colonization, and invasion of the immune response.[52]

Biologic theory and mechanistic evidence

Another fascinating property of phages is their potential to regulate immune responses. The immune system interacts with the microbiota by maintaining noninflammatory homeostasis based on multiple mechanisms, such as the physical barrier of the mucosa and the secretion of antimicrobial compounds. Intestinal phages can actively eliminate the invading bacteria. They can also reduce local immune and inflammatory responses and maintain immune homeostasis.[52] The most immediate effect of phages on the immune system may occur during sepsis where the lytic activity of phages can reduce the bacterial load. In contrast, bacterial residues caused by phages can also lead to sepsis. The immunomodulatory properties of phages could lead to a partial attenuating of the inflammatory response caused by bacteria or bacterial lysis. It appearss that cell phage-mediated lysis is involved in the production of pathogen-associated molecular patterns (PAMP). As intestinal permeability increases, PAMPs are translocated and activated immune responses. Phages can stimulate bacterial phagocytosis by macrophages through opsonization.[53] The intestinal mucosa forms an interaction between phages and their bacterial hosts. Phage communities contact the mucosal barrier and generate phage immunity. In this model, innate immunity protects common microorganisms in the upper layers of the mucosa during the lysogenic cycle, and acquired immunity destroys invading pathogens in the deepest mucosa through lysis.[54]

Adhesive phages should reduce bacterial colonization of the mucosa and thus act as an effective antimicrobial agent for the host. Several phages express proteins that have C-type lectin folds and immunoglobulin-type domains and interact with glycosylated mucin-O MUC2 in the colon. For example, the outer capsid protein of phage T4 binds preferentially to O-glucan chains in mucins and increases the proportion of phages in the mucosal layer. Accordingly, it plays a protective role against bacteria penetrating the mucous membrane. Therefore, alteration of mucosal glycosylation may influence the abundance of specific phages and have effects on specific bacterial groups. In addition, the pathogen that disrupts the innate immune response is fought by the acquired immune system. The Ig-type fold of bacteriophages is found in antibodies and T-cell receptors.[55]

In addition, phage-neutralizing antibodies have been identified in the sera of various species, suggesting that phage antibodies may be common in the human population. Interestingly, the production of specific immunoglobulin A (IgA) is the limiting factor of phages in the gut. It was shown that when IgA levels are low, phages are found in the feces. However, due to the increase in IgA levels, there are no active phages in the feces.[56] Therapeutic phages act as a bactericide and lead to the elimination of pathogenic bacteria. Unlike antibiotics, the process of identifying and isolating bacteriophages from the environment is rapid and the cost of isolation is low.[57] It is possible to produce various dosage forms such as creams, solutions, ointments, tablets, etc. from the bacteriophages. The phage is currently approved as a drug by the Food and Drug Administration. Several phage products have been produced.[58]

Phages can be used in combination with antibiotics to increase the effectiveness of antibiotics against resistant bacteria. For example, studies have shown that the simultaneous use of the phage OMK01 with antibiotics led

to the inhibition of resistant *P. aeruginosa*.[59] Furthermore, it is possible to treat resistant infections by using phage cocktails with simultaneous action on multiple sites and phage receptors.[60] In addition, the use of phages increases the penetration rate of antibiotics into bacterial biofilms.[61,62] Bacteria use specific and nonspecific mechanisms to prevent bacteriophage activity, inhibit bacteriophage binding to surface receptors, develop resistance to infection by secondary phages, prevent phage genome entry into the host cell, or obtain CRISPR sequences.[63,64] However, because bacteriophages are intelligent systems, they have developed various ways to evade the bacteria's defense systems. One of these mechanisms is the use of alternative receptors for entry into the host bacteria, for example, the use of the OMPF receptor instead of the LamB receptor for entry into the *E. coli* bacterium in bacteriophage lamda (λ).[60] Phages also use enzyme systems such as depolymerase and hydrolases to destroy exopolysaccharides on the surface of bacterial cells.[65] At the end of the spike of some bacteriophages such as CJR-Pm-Pmis (specific to proteus bacteria), there is a pectate enzyme that helps to degrade the biofilm matrix of Proteus bacteria.[66] Bacteria recognize the foreign genome and eliminate the foreign genome through enzymatic cleavage. Phage produce enzymes such as MTase that protect the phage genome from being cut by bacterial endonuclease.[67] Bacteria use CRISPR systems to fight phages bacteriophages employ CRISPR-anti systems to counteract this resistance mechanism. Recent research utilizing animal models has investigated the efficacy of phage therapy against several clinically significant pathogens. When challenged by intestinal sepsis caused by *S. aeruginosa*, oral administration of phages saved the lives of 66.7% of mice.[68]

A single dose of phage was administered concurrently with *C. difficile* was sufficient prevention of ileocolitis caused by *C. difficile* in a hamster model. Phage therapy after infection saved the lives of 11 of 12 mice. However, control animals given *C. difficile* and clindamycin died within 96 h.[69] The combination of phages also significantly reduced *C. difficile* growth *in vitro* and limited *in vivo* proliferation using a hamster model.[70] Intraperitoneal administration of a phage strain was sufficient to rescue 122% of mice in bacteremia models with vancomycin-resistant, beta-lactamase-producing, and imipenem-resistant *P. aeruginosa* and *E. coli*.[71-73] Fecal microbiota transfer (FMT) for the treatment of recurrent *C. difficile* infections is a recent development that supports the active role of phages in forming the gut microbial community. Donor viruses were found to be transmitted to the recipient after 6 weeks of FMT treatment. All transmitted viruses were phages, providing additional arguments for the safety of FMT and the suspected role of phages in the success of this treatment.[74] A 12-month follow-up study recently revealed that donor phages were still detectable in recipients, indicating long-term invasion of the phages into the primary microbiota and consequently their differential ability to adapt to the environment.[49] In general, these data highlight the important role of phages in manipulating the gut microbial population. However, it is not clear which phages apply these effects alone and by what mechanisms they develop. Phage cocktails have also been used in animal models to treat *P. aeruginosa*-resistant skin, lung, and gastrointestinal infections. Further animal studies provided similarly promising results for *E. coli* O-25: H4-ST131, *Vibrio parahaemolyticus*, *A. baumanii*, and *S. aureus*, which are resistant to multiple drugs.[68] The use of bacteriophages as new targets to limit the growth of bacteria causing infectious diseases may open new perspectives in the development of new drugs to reduce the rate of bacterial infections.

CONCLUSION

This review explores the fascinating relationship between our gut microbiota and overall health, shining a spotlight on a relatively underexplored but highly promising area: Bacteriophages, or simply "phages." These are viruses that specifically infect bacteria, and as the review points out, they might be the key to unlocking new treatments for a variety of diseases.

What makes this review stand out is how it delves into the role of phages in the gut ecosystem. While much has been written about gut bacteria and how they affect everything from digestion to immune responses, the role of phages is still emerging. This review brings phages into the spotlight, showing how they could be game-changers in managing our gut health and even treating diseases.

One of the most exciting aspects of the review is the way it highlights phages as a potential alternative to antibiotics. We know that antibiotic resistance is a growing problem worldwide, making it harder to treat bacterial infections. Phages, with their ability to target specific bacteria without harming the beneficial microbes in our body, offer a very precise and effective solution. Unlike antibiotics, phages do not wipe out the good bacteria that help keep our systems in balance. This specificity makes phage therapy an appealing option, especially as we continue to battle antibiotic-resistant bacteria.

Beyond fighting infections, phages also seem to have a surprising ability to interact with our immune system. The review discusses how phages can help reduce inflammation and maintain immune balance in the gut. This could have huge implications for treating inflammatory diseases like Crohn's disease or ulcerative colitis, where the immune

system goes haywire. Phages may help to calm things down and restore a healthier environment in the gut.

Another novel point the review raises is how phages can influence bacterial evolution. They do this through a process called horizontal gene transfer, where phages help bacteria exchange genetic material. This can lead to changes in bacterial behavior, such as increasing their ability to resist antibiotics or becoming more virulent. While this sounds alarming, it also means that phages are key players in shaping the microbiome and understanding them better could lead to new ways to control harmful bacteria before they become problematic.

What makes this review especially engaging is how it pulls together findings from both laboratory and clinical studies. It paints a picture of phages as active participants in our health, not just passive agents floating around in our bodies. This more holistic view of the gut microbiome, which includes phages as crucial players, opens up exciting possibilities for future treatments – especially in tackling diseases linked to gut dysbiosis (imbalances in the microbiota) and antibiotic resistance.

In essence, this review brings new insights into the potential of phages to transform how we manage gut health and treat infections. It suggests that phages could soon be an important part of our medical toolkit, offering targeted, efficient solutions to some of the most challenging health issues we face today. By putting phages in the spotlight, the review invites us to rethink how we approach gut health and the fight against bacterial diseases.

Authors' contributions

SS, KS, and PA conceived and designed this study; SS and SP performed the experiments and study; SS, SP, and KS wrote the manuscript; KS and PA improved and revised the manuscript. All authors read and approved the final manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Martín R, Miquel S, Ulmer J, Langella P, Bermúdez-Humarán LG. Gut ecosystem: How microbes help us. Benef Microbes 2014;5:219-33.
2. Zuppi M, Hendrickson HL, O'Sullivan JM, Vatanen T. Phages in the gut ecosystem. Front Cell Infect Microbiol 2021;11:822562.
3. Tasnim N, Abulizi N, Pither J, Hart MM, Gibson DL. Linking the gut microbial ecosystem with the environment: Does gut health depend on where we live? Front Microbiol 2017;8:1935.
4. Ahmed S, Korpe P, Ahmed T, Chisti MJ, Faruque AS. Burden and risk factors of antimicrobial use in children less than 5 years of age with diarrheal illness in rural Bangladesh. Am J Trop Med Hyg 2018;98:1571-6.
5. Ahmed S, Farzana FD, Ferdous F, Chisti MJ, Malek MA, Faruque AS, *et al.* Urban-rural differentials in using antimicrobials at home among under-5 children with diarrhea. Sci J Clin Med 2013;2:81-6.
6. Qadir MI, Ali M, Saleem M, Hanif M. Hepatoprotective activity of aqueous methanolic extract of *Viola odorata* against paracetamol-induced liver injury in mice. Bangladesh J Pharmacol 2014;9:198-202.
7. Ferdoosian F, Zare A, Aflatoonian M. Evaluation of Optimal Antibiotic Use in Children with Gastroenteritis Abstract. JSSU 2021;29:3514-23.
8. Abasi E, Nasimfar A, Karamiyar M, Nikibakhsh A, Mahmoudzadeh H, Nowrouzi M, *et al.* Evaluation of antibiotic utilization pattern for nonbacterial gastroenteritis in patients hospitalized at Motahari hospital: A descriptive cross-sectional study. Urmia Med J 2019;29:881-6.
9. Collins JP, King LM, Collier SA, Person J, Gerdes ME, Crim SM, *et al.* Antibiotic prescribing for acute gastroenteritis during ambulatory care visits-United States, 2006-2015. Infect Control Hosp Epidemiol 2022;43:1880-9.
10. Bruzzese E, Giannattasio A, Guarino A. Antibiotic treatment of acute gastroenteritis in children. F1000Res 2018;7:193.
11. Khakshour A, Sheikhi Z, Saeidi M. Study of inappropriate prescribing of antibiotics in pediatric gastroenteritis in Imam Reza hospital-Bojnurd. Int J Pediatr 2014;2:76-82.
12. Nikbakht G, Paymard A, Saeedinejad S. Ecological effects of antibiotic use and antibiotic resistance on natural environment: Case of Yasuj Shahid Beheshti hospital in 2016. J Zanjan Univ Med Sci 2016;1:48-54.
13. Zhen X, Stålsby Lundborg C, Sun X, Zhu N, Gu S, Dong H. Economic burden of antibiotic resistance in China: A national level estimate for inpatients. Antimicrob Resist Infect Control 2021;10:5.
14. Krauland MG, Marsh JW, Paterson DL, Harrison LH. Integron-mediated multidrug resistance in a global collection of nontyphoidal *Salmonella enterica* isolates. Emerg Infect Dis 2009;15:388-96.
15. Thapa Shrestha U, Adhikari N, Maharjan R, Banjara MR, Rijal KR, Basnyat SR, *et al.* Multidrug resistant *Vibrio cholerae* O1 from clinical and environmental samples in Kathmandu city. BMC Infect Dis 2015;15:104.
16. Bartels C, Beaute J, Fraser G, de Jong B, Urtaza J, Nicols G, *et al.* Annual Epidemiological Report 2014: Food-and Waterborne Diseases and Zoonoses. Vol. 1. Stockholm: ECDC; 2014. p. 1-4.
17. Authority EF, Prevention EC. Control. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2011. EFSA J 2013;11:3196.
18. Zaidi MB, McDermott PF, Campos FD, Chim R, Leon M, Vazquez G, *et al.* Antimicrobial-resistant *Campylobacter* in the food chain in Mexico. Foodborne Pathog Dis 2012;9:841-7.
19. Hakanen A, Jousimies-Somer H, Siitonen A, Huovinen P, Kotilainen P. Fluoroquinolone resistance in *Campylobacter jejuni* isolates in travelers returning to Finland: Association of ciprofloxacin resistance to travel destination. Emerg Infect Dis 2003;9:267-70.

20. Ochoa TJ, Chen J, Walker CM, Gonzales E, Cleary TG. Rifaximin does not induce toxin production or phage-mediated lysis of Shiga toxin-producing *Escherichia coli*. Antimicrob Agents Chemother 2007;51:2837-41.
21. Ohara T, Kojio S, Taneike I, Nakagawa S, Gondaira F, Tamura Y, *et al.* Effects of azithromycin on Shiga toxin production by *Escherichia coli* and subsequent host inflammatory response. Antimicrob Agents Chemother 2002;46:3478-83.
22. Oh JY, Yu HS, Kim SK, Seol SY, Cho DT, Lee JC. Changes in patterns of antimicrobial susceptibility and integron carriage among *Shigella sonnei* isolates from Southwestern Korea during epidemic periods. J Clin Microbiol 2003;41:421-3.
23. Mahmoudi S, Pourakbari B, Moradzadeh M, Eshaghi H, Ramezani A, Haghi Ashtiani MT, *et al.* Prevalence and antimicrobial susceptibility of *Salmonella* and *Shigella* spp. among children with gastroenteritis in an Iranian referral hospital. Microb Pathog 2017;109:45-8.
24. Kim YJ, Park KH, Park DA, Park J, Bang BW, Lee SS, *et al.* Guideline for the antibiotic use in acute gastroenteritis. Infect Chemother 2019;51:217-43.
25. Saboorian R, Rahbar M, Rahnamaye Farzami M, Saffarian P. Evaluation of antimicrobial susceptibility pattern changes and antibiotic resistance genes of *Vibrio cholerae* O1 strains isolated in 2012-2015 outbreaks in Iran referred to reference laboratory using phenotypic and molecular methods. J Ardabil Univ Med Sci 2019;19:172-90.
26. Sabzali S, Bouzari M. Isolation, identification and some characteristics of two lytic bacteriophages against *Salmonella enterica* serovar paratyphi B and *S. Enterica* serovar typhimurium from various food sources. FEMS Microbiol Lett 2021;368:fnab037.
27. Pan Z, Wang X, Zhang X, Geng S, Chen X, Pan W, *et al.* Changes in antimicrobial resistance among *Salmonella enterica* subspecies enterica serovar pullorum isolates in China from 1962 to 2007. Vet Microbiol 2009;136:387-92.
28. Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JD. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. Clin Microbiol Rev 2019;32:e00135-18.
29. Soleimani N, Aganj M, Ali L, Shokoohizadeh L, Sakinc T. Frequency distribution of genes encoding aminoglycoside modifying enzymes in uropathogenic *E. coli* isolated from Iranian hospital. BMC Res Notes 2014;7:842.
30. Sohail M, Khurshid M, Saleem HG, Javed H, Khan AA. Characteristics and antibiotic resistance of urinary tract pathogens isolated from Punjab, Pakistan. Jundishapur J Microbiol 2015;8:e19272.
31. Varughese LR, Rajpoot M, Goyal S, Mehra R, Chhokar V, Beniwal V. Analytical profiling of mutations in quinolone resistance determining region of gyrA gene among UPEC. PLoS One 2018;13:e0190729.
32. Mirzaii M, Jamshidi S, Zamanzadeh M, Marashifard M, Malek Hosseini SA, Haeili M, *et al.* Determination of gyrA and parC mutations and prevalence of plasmid-mediated quinolone resistance genes in *Escherichia coli* and *Klebsiella pneumoniae* isolated from patients with urinary tract infection in Iran. J Glob Antimicrob Resist 2018;13:197-200.
33. Eltai NO, Al Thani AA, Al Hadidi SH, Al Ansari K, Yassine HM. Antibiotic resistance and virulence patterns of pathogenic *Escherichia coli* strains associated with acute gastroenteritis among children in Qatar. BMC Microbiol 2020;20:54.
34. Wilkins LJ, Monga M, Miller AW. Defining dysbiosis for a cluster of chronic diseases. Sci Rep 2019;9:12918.
35. Kulnigg-Dabsch S. Autoimmune gastritis. Wien Med Wochenschr 2016;166:424-30.
36. Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A. Erratum: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca Cancer J Clin 202068:394-424. [doi: 10.3322/caac. 21492].
37. Luan F, Li X, Cheng X, Huangfu L, Han J, Guo T, *et al.* TNFRSF11B activates Wnt/β-catenin signaling and promotes gastric cancer progression. Int J Biol Sci 2020;16:1956-71.
38. Correa P. Human gastric carcinogenesis: A multistep and multifactorial process – First American Cancer Society award lecture on cancer epidemiology and prevention. Cancer Res 1992;52:6735-40.
39. Gong D, Gong X, Wang L, Yu X, Dong Q. Involvement of Reduced Microbial Diversity in Inflammatory Bowel Disease. Gastroenterol Res Pract 2016;2016:6951091-7. doi: 10.1155/2016/6951091.
40. Chen XH, Wang A, Chu AN, Gong YH, Yuan Y. Mucosa-associated microbiota in gastric cancer tissues compared with non-cancer tissues. Front Microbiol 2019;10:1261.
41. Liu X, Shao L, Liu X, Ji F, Mei Y, Cheng Y, *et al.* Alterations of gastric mucosal microbiota across different stomach microhabitats in a cohort of 276 patients with gastric cancer. EBioMedicine 2019;40:336-48.
42. Yang J, Zhou X, Liu X, Ling Z, Ji F. Role of the gastric microbiome in gastric cancer: From carcinogenesis to treatment. Front Microbiol 2021;12:641322.
43. Ghosh T, Beniwal A, Semwal A, Navani NK. Mechanistic insights into probiotic properties of lactic acid bacteria associated with ethnic fermented dairy products. Front Microbiol 2019;10:502.
44. Hsieh YY, Tung SY, Pan HY, Yen CW, Xu HW, Lin YJ, *et al.* Increased abundance of *Clostridium* and *Fusobacterium* in gastric microbiota of patients with gastric cancer in Taiwan. Sci Rep 2018;8:158.
45. Fernebro J. Fighting bacterial infections-future treatment options. Drug Resist Updat 2011;14:125-39.
46. Qadir MI, Khan TJ, Abbas G, Ahmad B, Janbaz KH, Ali M. Antibacterial activity of vacuum liquid chromatography (VLC) isolated fractions of chloroform extracts of seeds of *Achyranthes aspera*. J Chem Soc Pak 2012;34:589-92.
47. Qadir MI, Malik S. Effect of Eugenia jambolana leaves extracts on blood glucose levels of experimental diabetic rabbits. Pharmacologyonline 2009;3:829-35.
48. Mallhi TH, Qadir MI, Khan YH, Ali M. Hepatoprotective activity of aqueous methanolic extract of Morus nigra against paracetamol-induced hepatotoxicity in mice. Bangladesh J Pharmacol 2014;60-6.
49. Manrique P, Bolduc B, Walk ST, van der Oost J, de Vos WM, Young MJ. Healthy human gut phageome. Proc Natl Acad Sci U S A 2016;113:10400-5.
50. Doron S, Melamed S, Ofir G, Leavitt A, Lopatina A, Keren M, *et al.* Systematic discovery of antiphage defense systems in the microbial pangenome. Science 2018;359:eaar4120.
51. Norman JM, Handley SA, Baldridge MT, Droit L, Liu CY, Keller BC, *et al.* Disease-specific alterations in the enteric virome in inflammatory bowel disease. Cell 2015;160:447-60.
52. Weiss GA, Hennet T. Mechanisms and consequences of intestinal dysbiosis. Cell Mol Life Sci 2017;74:2959-77.
53. Van Belleghem JD, Dąbrowska K, Vaneechoutte M, Barr JJ, Bollyky PL. Interactions between bacteriophage, bacteria, and the mammalian immune system. Viruses 2018;11:10.
54. Barr JJ, Youle M, Rohwer F. Innate and acquired bacteriophage-mediated immunity. Bacteriophage 2013;3:e25857.
55. Gogokhia L, Buhrke K, Bell R, Hoffman B, Brown DG, Hanke-Gogokhia C, *et al.* Expansion of bacteriophages is linked to aggravated intestinal inflammation and colitis. Cell Host Microbe 2019;25:285-99.e8.

56. Gutiérrez B, Domingo-Calap P. Phage therapy in gastrointestinal diseases. Microorganisms 2020;8:1420.
57. Durbas I, Machnik G. Phage therapy: An old concept with new perspectives. J Appl Pharm Sci 2022;027-038.
58. Vandenheuvel D, Lavigne R, Brüssow H. Bacteriophage therapy: Advances in formulation strategies and human clinical trials. Annu Rev Virol 2015;2:599-618.
59. Chan BK, Turner PE, Kim S, Mojibian HR, Elefteriades JA, Narayan D. Phage treatment of an aortic graft infected with *Pseudomonas aeruginosa*. Evol Med Public Health 2018;2018:60-6.
60. Hasan M, Ahn J. Evolutionary dynamics between phages and bacteria as a possible approach for designing effective phage therapies against antibiotic-resistant bacteria. Antibiotics (Basel) 2022;11:915.
61. Bedi MS, Verma V, Chhibber S. Amoxicillin and specific bacteriophage can be used together for eradication of biofilm of *Klebsiella pneumoniae* B5055. World J Microbiol Biotechnol 2009;25:1145-51.
62. Rahman M, Kim S, Kim SM, Seol SY, Kim J. Characterization of induced *Staphylococcus aureus* bacteriophage SAP-26 and its anti-biofilm activity with rifampicin. Biofouling 2011;27:1087-93.
63. Leon LM, Mendoza SD, Bondy-Denomy J. How bacteria control the CRISPR-cas arsenal. Curr Opin Microbiol 2018;42:87-95.
64. Mohanraju P, Makarova KS, Zetsche B, Zhang F, Koonin EV, van der Oost J. Diverse evolutionary roots and mechanistic variations of the CRISPR-cas systems. Science 2016;353:aad5147.
65. Samson JE, Magadán AH, Sabri M, Moineau S. Revenge of the phages: Defeating bacterial defences. Nat Rev Microbiol 2013;11:675-87.
66. Rice CJ, Kelly SA, O'Brien SC, Melaugh EM, Ganacias JC, Chai ZH, *et al.* Novel phage-derived depolymerase with activity against *Proteus mirabilis* biofilms. Microorganisms 2021;9:2172.
67. Dy RL, Richter C, Salmond GP, Fineran PC. Remarkable mechanisms in microbes to resist phage infections. Annu Rev Virol 2014;1:307-31.
68. Watanabe R, Matsumoto T, Sano G, Ishii Y, Tateda K, Sumiyama Y, *et al.* Efficacy of bacteriophage therapy against gut-derived sepsis caused by *Pseudomonas aeruginosa* in mice. Antimicrob Agents Chemother 2007;51:446-52.
69. Draper LA, Ryan FJ, Smith MK, Jalanka J, Mattila E, Arkkila PA, *et al.* Long-term colonisation with donor bacteriophages following successful faecal microbial transplantation. Microbiome 2018;6:220.
70. Nale JY, Spencer J, Hargreaves KR, Buckley AM, Trzepiński P, Douce GR, *et al.* Bacteriophage combinations significantly reduce *Clostridium difficile* growth *in vitro* and proliferation *in vivo*. Antimicrob Agents Chemother 2016;60:968-81.
71. Biswas B, Adhya S, Washart P, Paul B, Trostel AN, Powell B, *et al.* Bacteriophage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. Infect Immun 2002;70:204-10.
72. Wang J, Hu B, Xu M, Yan Q, Liu S, Zhu X, *et al.* Therapeutic effectiveness of bacteriophages in the rescue of mice with extended spectrum beta-lactamase-producing *Escherichia coli* bacteremia. Int J Mol Med 2006;17:347-55.
73. Wang J, Hu B, Xu M, Yan Q, Liu S, Zhu X, *et al.* Use of bacteriophage in the treatment of experimental animal bacteremia from imipenem-resistant *Pseudomonas aeruginosa*. Int J Mol Med 2006;17:309-17.
74. Zuo T, Wong SH, Lam K, Lui R, Cheung K, Tang W, *et al.* Bacteriophage transfer during faecal microbiota transplantation in *Clostridium difficile* infection is associated with treatment outcome. Gut 2018;67:634-43.