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Bacteriophage Therapy in GIT Infections – A Clinical Review

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Abstract

Antimicrobial Resistance (AMR) has become a significant problem in the world. One alternative strategy to fight against AMR is bacteriophage therapy, which utilizes bacteria-specific viruses to kill them. Gastrointestinal tract (GIT) infections are prevalent worldwide, affecting a significant portion of the population. AMR has developed several antibiotics used against GIT infections. Hence, bacteriophage therapy is a potential alternative. Phages attack the bacteria stepwise, including adsorption, penetration, genome injection, replication, assembly, and release from the host cell. Numerous studies on phage therapy in animals and humans for GIT infections have been conducted, and its effectiveness has been established. This article will review the use of phages in treating GIT infections and would cover clinical studies, safety and regulatory requirements, and future perspectives of phage therapy.

Keywords: Phage therapy, Clinical cases, GIT, Microbiology, Antimicrobial Resistance.

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Introduction

The word antibiotic means "against bacteria". Natural, semi-synthetic, and synthetic chemicals are used to kill bacteria and treat infections [45]. Before the discovery of these lifesaving molecules, thousands of patients died due to a lack of proper medication for the management of infectious conditions such as septicemia, cholera, and tuberculosis [58]. Between 1930 and 1960, many antibiotics were developed, but the pace of developing new formulations declined significantly as bacteria developed resistance. This phenomenon is known as "Antimicrobial Resistance (AMR)," which means bacteria can resist and no longer respond to the antimicrobial effects of antibiotics. Irrational and high consumption of antibiotics for humans as well as animals is one of the biggest causes of AMR. Poor wastewater treatment and antibiotics use in pest control also contributed to the development of AMR [5].

According to research, bacterial AMR caused 1.27 million deaths in 2019, with lower respiratory tract infections accounting for most of the deaths [60]. This number can go up to 10 million annually by 2050 if immediate measures are not implemented. In addition to many mortalities, AMR causes economic loss for instance the cost of AMR in the United

States of America alone is $55 billion per year, significantly impacting the economy and quality of treatment given. Due to AMR, on average $1100 is added to each patient's bill who is fighting bacterial infections. These high costs are due to expensive treatments and the resources used to treat patients due to resistant microbes. Additionally, resistant forms make treatment approaches complex and increase the chances of mortality by three times [15].

AMR can be classified into intrinsic, acquired and adaptive resistance. Intrinsic resistance is due to a microbe's inherent capability to resist antibiotics. All gram-positive bacteria show intrinsic resistance against aztreonam and other monobactams. Contrarily, acquired resistance is developed due to the transfer of genetic material between microbes or genetic mutations that allow them to combat antibiotics even at higher concentrations. Three ways can be used to transfer genetic information; Transformation – a process by which fragments of DNA of a dead bacteria enter the cytoplasm of a recipient bacteria and modify its genetic information.

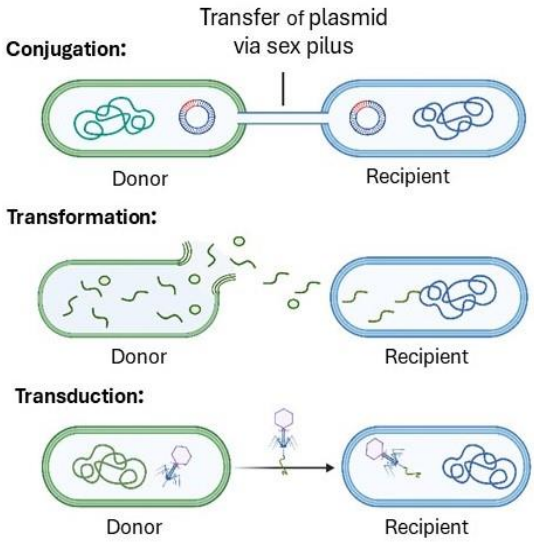

Figure 1: The mechanisms of gene transfer among bacteria resulting in the development of antimicrobial resistance.

Transduction involves the bacteriophages for the transfer of genetic information between a donor and recipient bacteria, and conjugation is the physical process in which a donor bacterium physically contacts a recipient bacterium through a sex pilus. The pilus transfers plasmid on which single or multiple resistant genes can be present. Figure 1 represents these three mechanisms of acquired resistance. In adaptive resistance, bacteria develop single or multi-drug adaptive resistance due to epigenetic gene modifications. Factors such as pH, ionic concentrations, and antibiotic levels can trigger adaptive resistance development [14,75,78].

The mechanisms by which bacteria exhibit the phenomena of AMR vary between gram-positive and gram-negative species. Some of these mechanisms have been detailed under: -

Increased drug efflux or decreased influx

Gram-negative bacteria have a double lipopolysaccharide membrane that protects the integrity of its structure and is associated with the movement of materials in and out of the bacterium. This membrane has a particular type of outer membrane protein known as 'porin' that forms tiny channels involved in the movement of hydrophilic molecules across the membrane. Bacteria can change the permeability and selectivity of porins, decreasing antibiotic uptake [103]. Another mechanism by which bacteria can limit the uptake of antibiotics is through the formation of biofilms. These are clusters of bacterial colonies along with buildup of polysaccharides and proteins. Glycocalyx is one of the significant components in biofilms, increasing weight, providing adhesion, and boosting the stability of biofilms, which ultimately helps bacteria survive in extreme conditions. Thus, biofilm formation provides a physical barrier against

antibiotics, reducing drug efficacy [85]. Efflux pumps transport proteins present in bacteria. Mutations in these pumps, which cause overexpression, can lead to increased efflux of antibiotics through active transport [19]. Acinetobacter baumannii is a common gram-negative bacterium in skin, soft tissues, and lung infections. The World Health Organization (WHO) has labeled A. baumannii a red alert pathogen due to its resistance against several antibiotics. One of the mechanisms by which this bacterium exhibits AMR is efflux pump inhibition of nodulation division (RND) systems. This system shows resistance against antibiotics and blocks the activity of antiseptics, biocides, and other drugs [1,38].

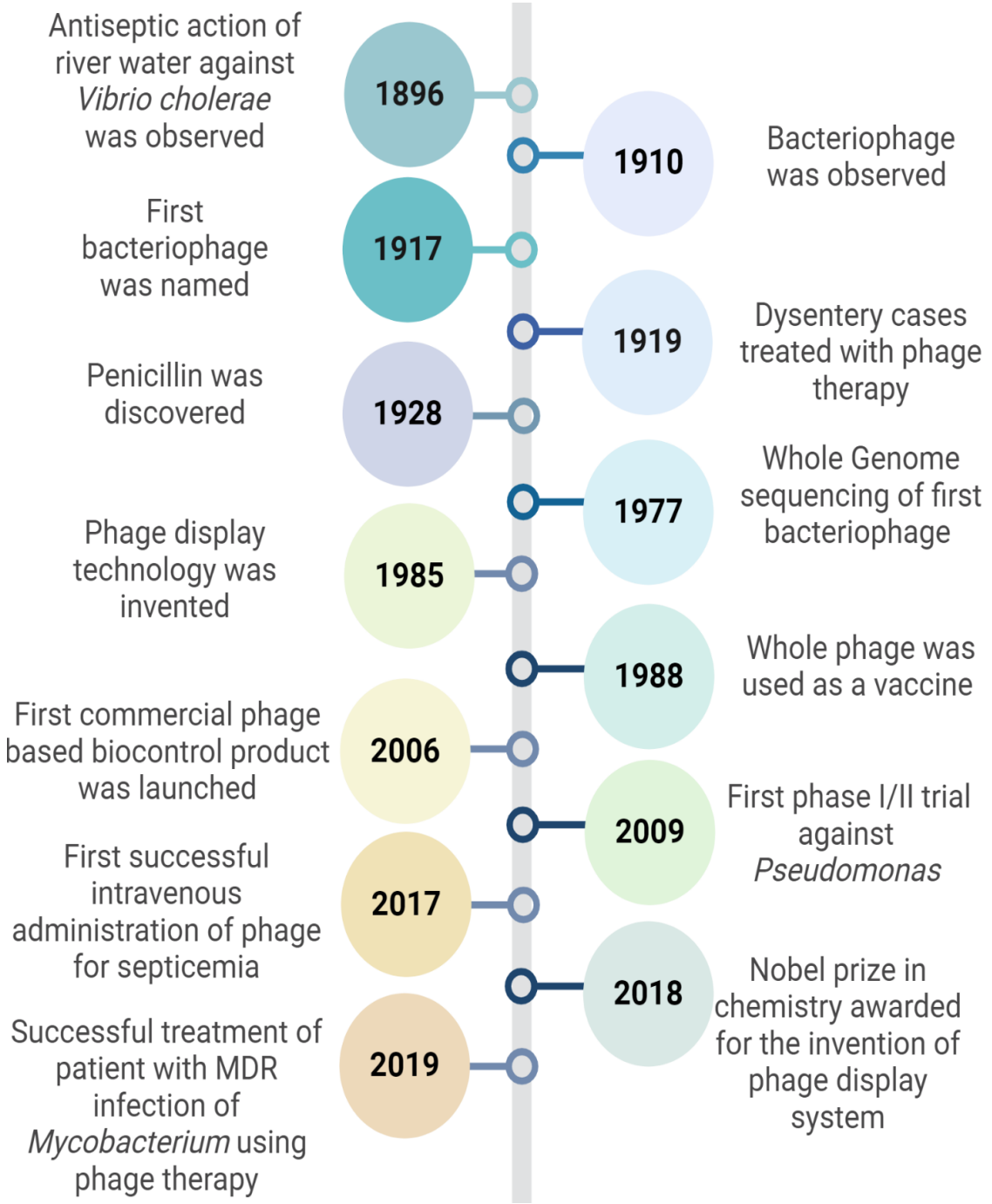

Figure 2: A timeline of phage development for addressing bacterial infections of gastrointestinal tract

Enzymatic degradation of antibiotics

Resistant forms of bacteria can produce antibiotic-degrading enzymes such as beta-lactamases that degrade the 4-membered beta-lactam ring in the antibiotics such as monobactams, penicillin, and cephalosporins [37]. Similarly, bacteria produce transferase enzymes that can transfer certain groups such as phosphate, acyl/acetyl, or hydroxyl to antibiotic structure, which can decrease the overall effectiveness.

Other enzymes that can degrade antibiotics include erythrocyte esterases that degrade macrolides or aminoglycoside-modifying enzymes that break aminoglycosides [100,104].

Modification of target site of antibiotics

Bacteria can prevent drug-target interactions by modifying the target sites. This can be achieved through altering the target site, enzymatic degradation of antibiotic binding regions, or genetic mutations. Glycopeptide resistance is seen in enterococci, where a genetic mutation causes the replacement of D-alanine precursors on glycopeptide targets with D-alanine D-lactate moieties. This structural modification hinders the binding ability of antibiotics to their target, leading to reduced efficacy [14].

The discovery of the very 1st antibiotic, penicillin, was a milestone achieved in the history of medical sciences, but AMR is now posing a significant threat to humanity. Therefore, researchers are continuously working to develop alternate therapies that can kill bacteria accurately and effectively. One of these alternatives is phage therapy, which was developed in the 1900s but recently got a lot of attention. Figure 2 represents the timeline of the development of phage therapy against bacteria causing GI infections. Phages (bacteriophages) are viruses that are obligate parasites with intrinsic ability to kill bacteria. Compared to antibiotics, using phages as antimicrobial/antibacterial agents offers many benefits such as high specificity, low toxicity, various formulation options, preventing biofilm formation, killing multi-drug resistant bacteria, and cost-effective manufacturing processes [37,53]. Gastrointestinal tract infections lead to some of the most common infectious and inflammatory diseases. Phages can be used to treat such infections effectively. This article will review the use of phages in treating gastrointestinal tract infections. Clinical studies, safety and regulatory requirements, and future perspectives on phage therapy would also be covered.

Bacteriophage Basics

Phages are the one the most abundant organisms on the Earth that are not only used as antimicrobial agents but also can be used in diagnosis and decontamination [62]. The structure of a bacteriophage consists of a head (capsid) with a tail, as represented in Figure 3. The capsid protects the genome, while the bacteriophage's tail is involved in transferring genetic information into the host cell. At the end of the tail, spike extensions help recognize and bind to the host [42,89].

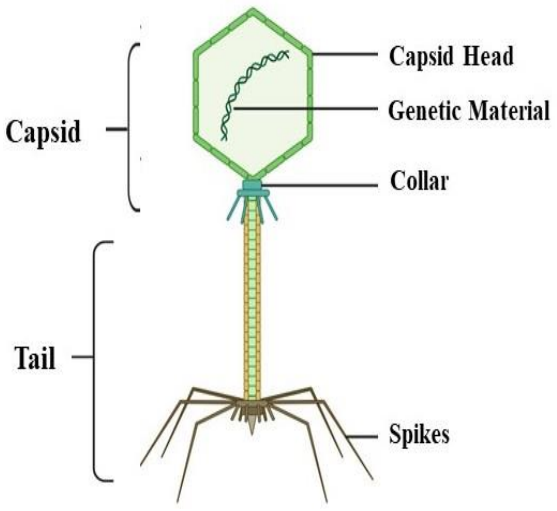

Figure 3: The basic morphology of a bacteriophage

After its attachment to the host, bacteriophage can follow either a lytic cycle or a lysogenic cycle. During a lytic cycle, phage transfers its genome to the host via tail and utilizes its replication machinery to make new copies. Capsid proteins and genetic material are synthesized separately and combined to form new phages, which leads to immediate lysis of the host cell. Alternatively, during a lysogenic cycle, phage's genetic material is passed into a host cell and replicated with the host daughter cells without killing them. Such phages are prophages, and bacteria with prophages are known as lysogens. Prophages can enter the lytic cycle spontaneously or due to various physicochemical and environmental factors [42,65].

GIT Infections - Overview and Challenges

Gastrointestinal Tract (GIT) infections are a global health problem that most often affects the stomach and intestines [47]. Bacteria, viruses, and parasites are the leading causes of GIT Infections [81]. Bacteria cause GIT Infections by producing toxins in the food, colonizing the gut, or invading the intestinal wall [57]. These infections make up a significant number of acute and chronic diseases worldwide. Helicobacter pylori, a bacterium that attacks the stomach lining, infects approximately 50 percent of the world's population. [11]. Diarrhea, defined as the passage of three or more stools per day, is a condition that affects four billion people yearly [8]. There are 200 million cases of Acute Bacterial Gastroenteritis in the United States alone [25]. GIT Infections can be acute, lasting a short time, and chronic or long-lasting 86]. Individuals with diarrhea experience a loss of water and salts, risking dehydration [83]. The manifestations of H. pylori infection include symptoms such as vomiting, nausea, diarrhea, and abdominal pain [12]. Inflammatory Bowel Disease (IBD) is a label that represents Crohn's Disease and Ulcerative Colitis. Both conditions are marked by long-standing (chronic) inflammation of the GIT. Its symptoms include diarrhea, abdominal pain and cramping, blood in stools, unintended weight loss, and fatigue [71]. Table 1 summarizes the clinical microbiology aspects of some of the common bacterial infections affecting GIT.

GIT infections are a global health burden. They account for substantial healthcare utilization around the world. In 2019, 88.99 disability-adjusted life years (DALYs) were due to GIT diseases (3.51% of the global DALYs). DALYs related to GIT diseases were dominant in regions within the middle socio-demographic index (SDI) [102]. Globally, there were 783.95 million reported cases of gastroesophageal reflux disease (GERD) from 1990 to 2019. The prevalent cases, incident cases, and years of healthy lives lost due to disability (YLDs) rose by 77.53%, 74.79%, and 77.19% between 1990 and 2019 (Zhang et al., 2022). For peptic ulcer disease (PUD), there were approximately 8.09 million cases in 2019. Over 30 years, South Asia showed the highest age-standardized prevalence rate of PUD among all global burden of disease (GBD) super regions [96]. GIT diseases account for millions of healthcare encounters and result in hundreds of thousands of fatalities in the United States annually. The economic burden associated with healthcare due to these GIT diseases amounts to billions of dollars. GIT health expenditure was $119.6 billion in 2018 in the United States alone [65]. Diagnosing and managing GIT diseases poses challenges that require various specialists to collaborate. Laboratory, imaging, endoscopic, and both non-invasive and invasive investigations aid the diagnosis of GIT diseases [39]. Enteric fever results from a systemic infection attributed to Salmonella enterica serovars: typhi and Para typhi. It exemplifies the diagnostic challenges associated with GIT infections. The overlap of clinical features with other

Table 1: Clinical Microbiology of Bacterial GIT Infections

Pathogen	Symptoms	Transmission	Diagnostic Methods	References
Salmonella typhi and other species	Fever, diarrhea, abdominal cramps	Contaminated food and water, contact with an infected animal	Stool Culture, blood tests, polymerase chain reaction (PCR)	[27,101,44]
Escherichia coli	Bloody diarrhea, abdominal pain, vomiting, nausea	Contaminated food and water, person-to-person contact	Stool culture, serotyping, PCR	[3,88,59]
Shigella dysenteriae	Shigellosis, diarrhea, abdominal pain, fever	Person-to-person, contaminated food and water	Stool culture, serological tests, PCR	[7,4]
Campylobacter jejuni	Diarrhea, fever, stomach cramps	Contaminated food, undercooked meat, unpasteurized milk	Stool culture, Blood sample culture	[23,21]
Clostridium difficile	Watery diarrhea, colitis (inflammation of the colon), stomach cramps	Hospital settings, antibiotic use, severe underlying illness	Nucleic acid amplification test (NAAT), toxigenic culture, glutamate dehydrogenase (GDH)	[74,48]
Vibrio cholerae	Cholera, watery diarrhea, vomiting, thirst, leg cramps	Contamination of food and water	Stool culture, rectal swabs	[13,72]
Yersinia enterocolitica and pestis	Fever, abdominal pain, diarrhea	Contamination of food and water, undercooked pork, unpasteurized milk	Stool culture, blood tests	[84,68,34]
Helicobacter pylori	Chronic gastritis, peptic ulcer disease, abdominal pain	Person-to-person, oral-oral, or fecal-oral routes	A urea breath test, stool antigen test (SAT), monoclonal antibody tests	[24]
Listeria monocytogenes	Listeriosis, fever, muscle aches, diarrhea	Contaminated food, unpasteurized dairy, deli meats (lunch meat)	Culture-based methods, antibody-based tests, enzyme-linked immunosorbent assay	[92,7]

febrile illnesses complicate the diagnosis of enteric fever. The limited sensitivity of conventional tests, such as blood culture, and the invasive nature of bone marrow tests also constrain the diagnosis of enteric fever [75]. In a 2020 study by Fatima Bachir Halimeh et al., several discrepancies were found in identifying Escherichia coli and Shigella spp. Traditional methods like Api 20E showed inconsistencies in identifying bacteria, highlighting the need for accurate diagnosis and effective treatment [36]. Campylobacter bacterium is difficult to isolate in laboratories. The misidentification of species and lack of specific strains in identification leads to problems identifying these bacteria, which pose diagnosis challenges [20], Clostridium difficile can be diagnosed with the current methods, but it is challenging to diagnose it as clinical correlation is needed. Susceptible molecular tests may lead to over-diagnosis of CDI and amplified facility CDI rates [47].

GIT diseases present longterm complications for those affected. In a study conducted [82], it was found that GIT diseases severely impacted health-related quality of life (HR QoL) [82]. In a separate study involving 422 outpatients diagnosed with fecal incontinence (FI), chronic constipation (CC), or a combination of both (mixed FI-CC), the impact of these conditions on the quality of life (QoL) was assessed. The findings indicated that both conditions significantly contributed to the impairment of QoL [64]. These findings significantly impact the patients' lives due to GIT infections.

Drug Resistance is a major problem in treating GIT Infections. The effectiveness of antibiotics to treat GIT Infections, notably those caused by Salmonella, Campylobacter, and Helicobacter pylori, has diminished in a significant proportion of isolates [46]. In Lahore, Pakistan, a study on raw meat samples revealed a high prevalence of Salmonella (51.35%), varying rates across poultry, buffalo, cow, and goat meats. Salmonella strains exhibited extensive antibiotic resistance, with notable resistance to Erythromycin (100%), Cefepime (98.24%), and Colistin (94.73%) [22]. Another study [95] was conducted in which 100 strains of E. coli were isolated from various samples. The study revealed the highest resistance rates against amoxicillin (85%), cefuroxime (65%), and ceftriaxone (60%). Ceftazidime, gentamicin, ciprofloxacin, and sulfonamides exhibited resistance rates of 31%, 20%, 33%, and 47%, respectively, revealing that the multi-drug-resistant E. coli has become a significant and complicated problem in clinical treatment [95]. Shigella spp. has become resistant to nearly all antimicrobial classes, showing a rising global prevalence and increasing dominance. Within the GIT, Shigella shows proficiency at surviving and replicating, acquiring antimicrobial resistance genes from other bacteria. The administration of specific antimicrobials may quicken the emergence of resistance against additional drug classes [6]. These studies all emphasize the challenge posed by drug-resistant bacteria in GIT Infections.

Mechanism of Bacteriophage Therapy in GIT Infections

The utilization of bacteriophages as therapeutic agents commenced two decades before the first clinical use of antibiotic drugs. It saw a decline after the rise in the use of broad-spectrum antibiotics. Recent global concerns about multidrug-resistant infections made scientific communities reengage with phage therapy to address antibiotic resistance [62]. Bacteriophages are being recognized, purified, and formulated as pharmaceutical drugs in line with the industrial standards. Phages can be applied topically, inhaled, administered orally, or delivered parenterally [18]. Bacteriophage therapy has been established using in vitro and in vivo studies, clinical trials, and documented clinical cases

involving patients. This alternative treatment has enhanced efficacy when administered with antibiotics, particularly in infections involving biofilm formation [10]. Bacteriophages can potentially treat GIT infections as they can also modulate the gut-liver axis, which plays a role in GIT diseases [16].

Bacteriophages are precise tools that can engineer gut microbiota and are also being explored as a potential solution in combating bacterial pathogens resistant to antimicrobials. These two applications require the use of bacteriophages to kill bacterial pathogens in the gut microbiota [41]. Phages show specificity towards their hosts and typically affect a single bacterial species or even specific strains within that species [42]. They can selectively target and kill pathogenic bacteria without upsetting the gut microbiota. This ability of phages maintains the balance of gut microbiota, which is crucial for gut health and immune function. This approach also preserves the benefits provided by gut microbiota [73].

The life cycle of phages is a multi-step process that starts with recognizing and attaching phages to the bacterial host, through adsorption. This adsorption process relies on the interaction between bacterial receptor proteins and viral proteins known as receptor-binding proteins (RBP) [49]. It generally has two stages: reversible and irreversible. This initial interaction is pivotal in determining the infection process, so studying bacteriophages' molecular mechanisms is significant. Adsorption of tailed phages is generally the reversible or irreversible attachment of RBPs to bacterial receptors [28]. These receptors need to be specific for the phages to initiate adsorption while the process relies on environmental variables and physicochemical elements such as temperature, pH, nutrients, and ion availability. Furthermore, the condition of the bacterial cell plays a critical role in shaping this interaction [33]. This specificity of phages is significant in targeting GIT pathogens [55]. Penetration and genome injection is the next step in the life cycle. Bacteriophages introduce their genetic materials into the host cell's cytoplasm while an emptied capsid remains outside the bacterial membrane [97]. Phages use various mechanisms to insert the genome into the bacterial cell. One mechanism is the "contractile tail" mechanism, where bacteriophages contract their tail sheath. It pushes the tail tube through the bacterial cell envelope and injects the genome into the cytoplasm [90]. Some phages use the "non-contractile tail" mechanism, where non-contractile tails are crucial in penetrating the cell's envelope. These tails do not contract but still serve as a conduit for the phage genome to enter the bacterial cell [63]."Direct Penetration" is another mechanism where phages directly penetrate the bacterial host cell surface. This mechanism is prevalent in higher organisms [81]. After replication, novel phages are assembled in the bacterial cell. The assembly of infectious bacteriophage particles involves four main events: nucleation, DNA packaging, the removal of scaffolding, and structural maturation, as well as the binding of tail proteins [70].

Phages have garnered attention as immunomodulators of the GIT ecosystem. These phages can affect bacterial communities, influencing the host immune system. They substantially shape the gut ecosystem through an interconnected system between gut bacteria and the host's immune system [105]. Phage-delivered CRISPR-Cas systems are being developed to target multi-drug resistant (MDR) pathogens in the GIT. The combinatorial approach of using phages as a delivery system of the CRISPR-Cas gene is proposed to target specific bacterial communities in the GIT and enhance drug sensitivity [61]. Phages' immunomodulatory properties can induce a bacterial-induced inflammatory response or lysis [35]. In a 2018 study [32] the potential

use of "endogenous phages" in some biofluids and other specimens was discussed. They hypothesized that endogenous phages, particularly those in the gut, may exert immunomodulating properties, playing a role in immunological homeostasis in the intestines [32].

Bacterial biofilms are commonly characterized as communities of bacteria attached to surfaces [79]. In a study [94] phages with antibiofilm activity against Bacillus subtilis were extracted from the intestines of chicken tripe of beef sourced from traditional Indonesian markets. They demonstrated the capacity to inhibit B. subtilis as a biofilm-forming spoilage bacterium, affirming that the isolated phages exhibit considerable promise as biocontrol agents targeting biofilm-forming bacteria [94]. In another study, comparisons were made among in vitro, ex vivo, and in vivo models using phages and phage-derived lysins. The synergistic use of phages and antibiotics showed promise in efficiently removing biofilms [54]. Bacteriophages are, hence, emerging as safe alternatives, effectively eliminating biofilms. The dynamics between phages and biofilms are such that they either control biofilm formation or eradicate bacteria totally [91]. All these studies demonstrate that bacteriophages are a promising tool for fighting and eliminating biofilms in GIT. Figure 4 summarizes the mechanisms adapted by bacteriophages for the elimination of biofilms. As presented in Table 2, several studies demonstrate that bacteriophages are a promising tool to eliminate and fight biofilms in GIT.

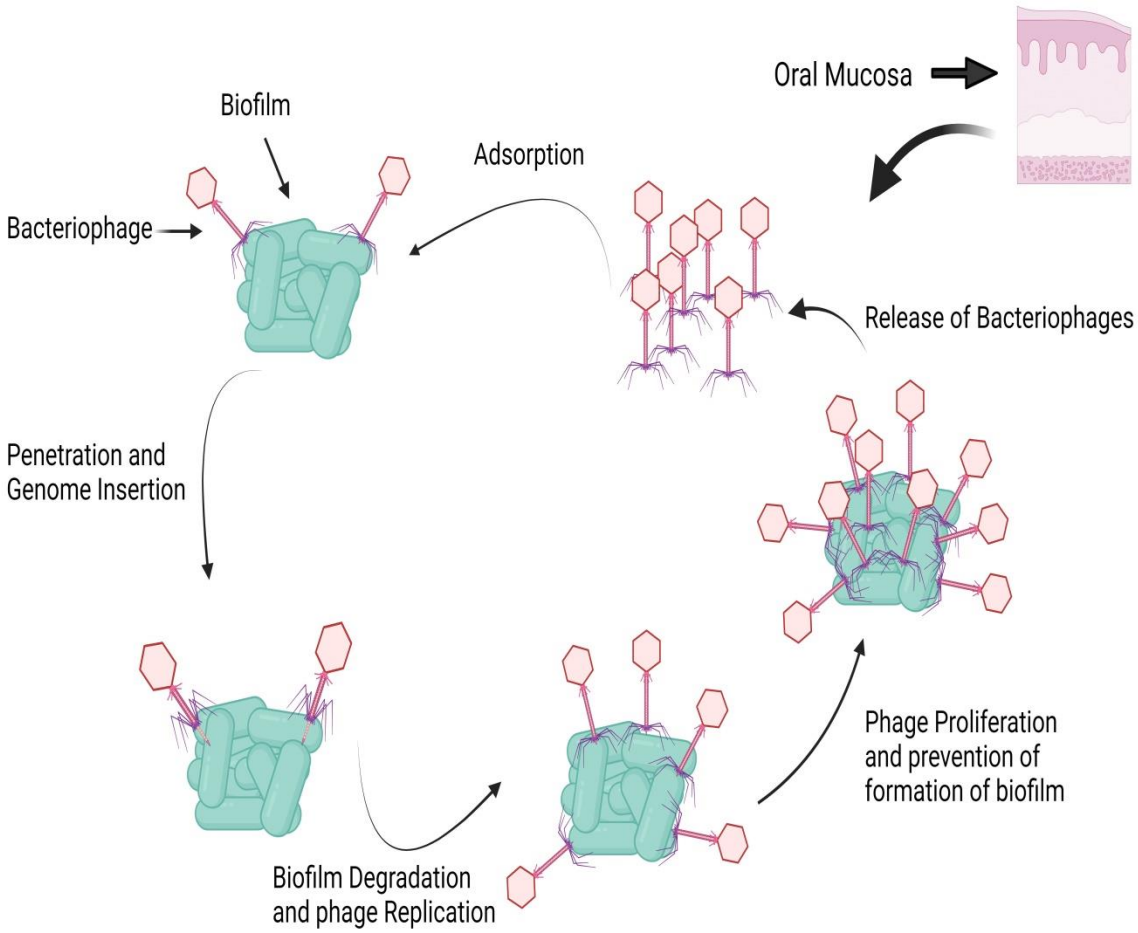

Figure 4: Steps for biofilm degradation and prevention of biofilm formation using bacteriophages on the epithelial layer of oral mucosa

Table 2: Applications of bacteriophages in treating infections

Study (Year)	Target Pathogen(s)	Infection Model	Intervention	Key Findings	Reference
Phage Therapy in Gastrointestinal Diseases (2022)	C. difficile, E. coli	Humans with moderate GIT inflammation symptoms	Oral phage therapy	Safe and tolerable, potential for future treatment	[35]
Advancements in bacteriophage therapies and delivery for bacterial infection (2023)	P. aeruginosa	Animal models	Phage cocktails	Enhanced efficacy, exploring delivery routes	[17]
Phage therapy: An alternative to antibiotics in the age of multi-drug resistance (2016)	P. aeruginosa, C. difficile	Animal models	Phage cocktails	Effective in treating gut-derived sepsis	[50]
Efficacy of cocktail phage therapy in treating Vibrio cholerae infection in rabbit model (2013)	Vibrio cholerae	Animal model	Oral phage therapy	Highly effective in reducing infection	[40]

Safety and Regulatory Considerations in Bacteriophage Therapy

The current research on phage therapy needs to be more comprehensive (2020). Phages have been used to treat patients in the US under the FDA's emergency investigational new drug (eIND) protocol. Bacteriophage therapy is regulated as a medicinal product in France, and phages must be produced according to Good Manufacturing Practices (GMPs). Phages must additionally show that they are safe and effective in randomized controlled trials (RCTs). However, no marketing authority exists for phages in France, the European Union, or the US [9].
According to a 2016 article titled. Bacteriophage Therapy: A Regulatory Perspective, the regulatory pathway for bacteriophage therapies must be defined as phage therapy products approval varies with countries. In some countries, it falls under existing regulations for biological products, while others require specific phage therapy regulations [67]. More data on the safety and efficacy of bacteriophage therapies needs to be collected. Ensuring the purity and potency of phage preparations is crucial to prevent contamination and guarantee effectiveness. More research is required to develop standardized manufacturing processes for bacteriophage therapies

In a review of the safety of phage therapy during animal and clinical studies, it was found that phage therapy has the potential to cause adverse events, although serious events are infrequent. However, standardized reporting of potential toxicities associated with phage therapy has generally

been lacking. Furthermore, studies given on phage therapy lacked data about the phage preparations and only gave information regarding the phage concentration [51]. Phage therapy may affect the human body, human flora, and immune system, leading to the release of endotoxin due to lysis of bacteria. The presence of bacterial residues and possible chemical components in phage preparations during the purification process can significantly influence the safety of phage therapy. Phages might readily enter the bloodstream and gather in different tissues [86]. Some indications have been that eukaryotic cells sometimes take up phages, which can cause innate immunological responses in the body [31]. A more comprehensive assessment of safety is, hence, required for standardization of safety monitoring. Some evaluation techniques include gastrointestinal questionnaires, Visual Analogue Scales [30] and scoring methods for assessing physical examination findings [29].

The primary regulatory body in charge of investigating phage preparations in the US is the FDA's Center for Biologics Evaluation and Research (CBER), and they require that all current modern phage therapy products must be made to GMP standards [98]. While specific guidelines for phage therapy are lacking, both agencies provide relevant documents outlining data requirements, manufacturing considerations, and clinical trial design. The regulatory landscape for phage therapy is evolving, and both FDA and EMA are actively involved in shaping future frameworks [26,98].

Conclusion and Future Perspective

The current article has discussed bacteriophage therapy in GIT Infections from a clinical perspective. Due to the devastating effect of AMR on public health, an alternative to antimicrobials is needed. The unique pairing of bacteriophage/bacterial host, the inability of phages to harm humans and the non-disruptive nature towards normal beneficial flora in the human body make these credible alternatives [77]. Bacteriophages hold significant promise in understanding gastrointestinal diseases. Studying phages in the gut environment can help us gain valuable insights into how phages maintain balance in the gut environment. The identification of healthy phages in a community in the GIT will develop an exciting approach towards the treatment of GIT Infections [35].

The utilization of phages as therapeutics also has significant challenges. One reason is that only lytic phages can be used in phage therapy, as lysogenic phages can contribute to the spread of AMR. It is difficult to administer and manufacture phages because calculating the correct dose has multiple complexities. There is also a chance that the body will develop bacteriophage resistance. All these challenges, combined with a lack of clinical trials, hinder the development of phage therapy [2,99].

There are many emerging approaches in phage therapy. Combining phages with other agents like antibiotics has shown a synergistic effect. Engineered phages, modified for improved activity, include those co-administered with enzymes like DNAases and depolymerases, which have enhanced activity for biofilm eradication [68]. Phages with desired properties can be acquired through targeted genome modifications of known phage isolates. This alternative strategy offers a potential solution to the challenges associated with obtaining, characterizing, and approving phages for therapy [5]. In conclusion, bacteriophage therapy has excellent potential in treating GIT infections. However, there are still several challenges for its widespread clinical use.

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