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Revolutionizing Infectious Disease Management in Animals Through Advanced Phage Therapy: A Comprehensive Strategy for the Eradication of Multidrug-Resistant Bacterial Pathogens

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Abstract | In this study, the researcher's goal is to find out if the use of phages can eradicate *E. coli* infections in cattle or not. Four such farms, positive to *E. coli*, were randomly chosen out of the sampled feces of the calves. This is best explained by the method of oral gavage; where the phages were added to milk and then the calves fed on the mixture on a daily basis. All the calves in the study were treated with an oral decoquinate that would help prevent coccidiosis in the calves, and vaccination was also conducted against respiratory as well as gastrointestinal infections. Some of the microbiological techniques employed in counting the *E. coli* O157:H7 bacteria involve centrifugation and culturing and latex agglutination among others. Finally, regarding evaluation of the experiment, the phage group had 14 days less diarrhea in average than the control group. Thus, on the basis of this investigation, phage treatment has an efficacy to reduce the bacterial count of *E. coli* O157:H7 in stool samples. It had not been very effective, but the results were still positive enough and for Er. PDi still became a stubborn germ. Future work may extend from this work to establish the potential causes of suboptimal *P. multocida* clearance, methods of optimizing the dosage dose and regimen used in the therapy, the possibility of using a combination therapy involving phage therapy with other treatments. With regard to the above, this study may provide shed light on the possible application of phage treatment in agricultural context and how the presented method may help to combat bacterial resistance.

Keywords | Phage therapy, Multidrug-resistant bacterial pathogens, Infectious disease, *E. coli*, Claves

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INTRODUCTION

Bacteriophages or just phages are the most abundant form of life on the planet and may be present wherever

bacteria are present. Food products, sewers, sources of water, soils, forests and undergrowth and so many other possibilities are homes to the bacteriophages. By doing so for the purpose of combating bacterial diseases, it has been

so thanks to their various environs (Suttle, 2005). It is also present in commercial sera, human vaccines, human oral cavity especially on the dental plaque and in saliva, and in intestines of animals and humans. Taking into account that bacteria have existed on the planet for millions of years the role of bacteriophages in regulation of bacterial numbers has always been critical (Batinovic et al., 2019). It is estimated that there are about 1032 virions in Earth, that is ten times more the number of bacteria that exist and are described. The abundance of phage in soil is approximately 109 virions g⁻¹ as stated in Karn et al. (2023) and Gopikrishnan et al. (2024) while in water body it is between 104 and 108 virions mL⁻¹. The INSDC databases currently contain over 25,000 bacteriophages' nucleotide sequences (Adriaenssens and Brister, 2017). Another interesting attribute of the bacterial viruses, or bacteriophages, is their capacity to clear such infections. The concept of phage treatment has been in use for some time, more so it was used in the late 1800s that was when bacteriophages were discovered also during the development of antibiotics (Sahoo et al., 2024). Pointing out the fact that the waters of the Ganges and Jumna Rivers could neutralize cholera bacteria, the English scientist Ernest Hankin described the presence of bacteriophages for the first time toward the close of the nineteenth century (Saeed et al., 2023). This means that though Felix d'Hérelle is revered today as the father of bacteriophages and phage treatment, and while indeed d'Hérelle was the first to experiment with bacteriophages and phage treatment, Hankin (1896) did offer some account of the phenomena. It took around twenty years for a prominent discovery of Frederick Twort, a discovery that stated that the plates workers used were unhealthy because the microbes found on it could actually kill germs. However, for the fact that Grashof initially began to speculate over bacteriophages he penned them down under theory and without concrete evidence. When Félix d'Hérelle 'banged on the door of the elite' Comptes Rendus de l'Académie des Sciences" in 1917 by publishing a brief note, which said that it was a new kind of microbe that can exist only inside the bacteria, in the فنشر (thin) of an obligate intracellular parasite, he was making one of the most tremendous innovations. As D'Hérelle pointed out in his studies, the virus particles could indeed reproduce themselves, it required host cells but still was capable of repeating the cycle. In Tbilisi, Georgia, in the year 1923, Giorgi Eliava and Félix d'Hérelle established. The Eliava Institute of Bacteriophages, Microbiology, and Virology. This renowned institute has made significant advancements in the development of various phage cocktails. Notably, d'Hérelle formulated the most famous ones, which have been effectively utilized in the treatment of purulent wounds and Enterobacteriaceae infections. In 1926, Pyle conducted an experiment to employ phage therapy for the treatment of salmonellosis in hens, specifically Salmonella enterica serotype pullorum. Unfortunately, the outcome of this

endeavor did not yield successful results. Bacteriophages, found ubiquitously in the environment, have emerged as a highly eco-friendly antibacterial treatment in veterinary care, as well as in the production of plants and animals. Despite phages being biologically active, they do not pollute the environment like the other chemotherapeutics such as antibiotics. They have the potential to be very of interest to be incorporated into various segments of the cattle business. Clinical bacteriophage therapy has been used successfully in a number of infection cases including, Salmonella and *E. coli* in mice, poultry, calves, piglets, and lambs, Clostridium spp. and Pseudomonas aeruginosa in mice, hamsters, and rats, and Staphylococcus aureus in mice, cows, and other livestock (Hatem et al., 2024). Diarrhea in the new born calves is a severe disease that affects calves with the first few weeks of their lives. The disease is mainly caused by the pathogenic *Escherichia coli* strains. This disease has a severe economic effect to the facts are that because of fecal contamination a large number of *E. coli* is found in the agricultural environment. First encounter with pathogenic *E. coli* occurs in contaminated sources, but specific climate factors, lack of adequate transmission of immunity, or other illnesses, normally, are needed for a bacterial outbreak. ETEC strains of *E. coli* are the major cause of *E. coli* infection mainly in young stock through a noninvasive manner involving the secretion of intestinal toxins. These pathotypes are also responsible for diarrhea in children and tourists in developing countries (Farooq et al., 2022). *E. coli*, diarrheal pathotypes in calves are enterotoxigenic (ETEC) pathotype and enteropathogenic (EPEC) pathotype, both being highly prevalent and have pin point mobility and mortality among the affected animals. Duration of treatment and the right drugs to use in treating diarrhea caused by *E. coli* is one of the main problems. A number of known antibiotics like β -lactams, aminoglycosides, fluoroquinolones or tetracyclines cause immunosuppressive effect that favors bacterial resistance in calves thereby complicating the disease. Government's regulations on the use of antibiotics make substitutes, including natural products like garlic, aloe, lactoferrin, and probiotic more relevant. This has happened due to abuse of antibiotics in the treatment of various human and animal infections as well as in feeding foods.

The last couple of years has seen the use of the repeat, phages or bacteriophages, as the cure to chronic or difficult to contain infections and diseases. Phages are known to be bacteria viruses selective in their action that involve infecting and killing of the host bacteria only. Specifically, phage treatment refers to the introduction of intensively hostile phages into a body of a patient with the aim to destroy the bacteria pathogen responsible for a clinically significant infection in the respective patient. Phage therapy has also emerged as a better option as a replacement for antibiotics in the post antibiotic era

due to several advantages that it has over the traditional antibiotics. Phage therapy, which can be described as the use of bioengineered bacteriophages, which provide specific methods of bacteria elimination without affecting the useful microbiota, is considered to be one of the more innovative means of combating the diseases are infections. This novel approach is focused on the modification of the genetic makeup of bacteriophages themselves in an effort to optimize such desired variables that include the stability, host range and bacteriolytic activity of therapeutic bacteriophages (Oechslin, 2018). Some of the viruses are called 'bacteriophages' that could directly bind to bacteria and kill them in different manner. These microbes are useful when they do not have a receptor on their surface that binds to eukaryotic cell and they are used to treat many infections and because there is resistance to antibiotics, they are given more attention. First, bacteriophages are found in water, oil and fecal material which are natural environments; second, bacteriophages' function is not interfered by antibiotic resistance mechanisms and since bacteriophages replicate the site of infection, they do not require many doses as antibiotics. Considering the report done in the recent past, bacteriophages can be used as antibiotics because of their high immunity, minimum interference with eukaryotic cells, stability at different conditions and effectiveness in targeting various types of MDR bacteria (Romero-Calle et al., 2019).

The effects of phage therapy have been investigated in animal models that revealed that it reduces bacterial loads and improves clinical result (Gordillo and Barr, 2019). In experiments on animals, valuable data have been obtained about phage therapy in human diseases, proving the applicability of the method when using various infection models and bacterial species and non-toxicity. To enhance the translation of phage therapy into routine clinical practices, these studies have underlined the need to optimize routines, enhance the pharmacokinetic modelling and develop models to mimic human bacterial infection time courses (Górski et al., 2020). Further, phages could be a potential weapon against MDR bacterial infections owing to the fact that they selectively target bacteria, they can reproduce in bacterial infected hosts, and can also transverse biofilms. Phage therapy as an approach towards the management of the infectious diseases has not developed as far as antibiotics have but research is still underway to carry out obstacles such or immune responses to the phages or phage inactivation. It is due to the use of animal models, therefore, that were possible to establish the mechanisms of phage action, to discover which dose regimens work best, and to evaluate the phage therapy's positive impacts in the battle against antibiotic-resistant bacteria (Kortright et al., 2019). In general, the phage therapy is a new concept in controlling infections, a

successful targeted approach to addressing the ever-rising concern of drug-resistant bacteria in both humans and animals. In this study, it is the goal of the researcher to determine if phage therapy holds potential in managing the MDR bacterial pathogen problem.

MATERIALS AND METHODS

SAMPLING METHODOLOGY

In selecting the particular farm for the investigation we used a random sample technique; selected from a group of existing dairy farms positively identified with *E. coli* infections. The study farm was chosen because there were calves which were infected with *E. coli* to varying degrees. Two attempts were made to collect fecal samples at each farm: one sample was collected randomly from each farm and the other sample was taken at our discretion from farms where infected calves were observed (++ , ++ , +++ , + +++). It was in this regard that it was believed that the method would help in making sure that the selected farm was a good sample of all dairy farmers who had been infected by *E. coli*.

HOUSING AND BEDDING PROTOCOL

From birth until they were weaned, calves on the chosen farm were kept in separate cages. Twice a day, they were fed. After every cycle, the bedding, which was a sand and mud mixture, was changed over rather than refilled? The purpose of this housing and bedding procedure was to mimic actual circumstances frequently observed on dairy farms.

PHAGE ADMINISTRATION

The phage-treated group received 5 grams (5×10^6 PFU) of bacteriophages daily (Zagaliotis et al., 2022). *Salmonella typhimurium*, *Salmonella enteritidis*, *Salmonella dublin*, *Salmonella derby*, *Staphylococcus aureus*, *Escherichia coli* k99 and f41, and *Clostridium perfringens* types A and C were among the bacterial pathogens that the particular phages that were employed to target were. The phages were given orally by mixing them into the milk that each calf was fed on a daily basis.

CONTROL MEASURES

The control measures in the study involved splitting the calves into two groups: The control group, the phage group. The subjects were randomly assigned to the groups at birth in order to be able to provide an equal distribution. The particular control group did not undergo the administration of any phage while the phage group was given administration of bacteriophages launched against certain bacterial pathogens. Therefore, comparing the results of the Control group with that of the Phage group, it sought to evaluate the efficacy of the Phage therapy in

either eliminating bacteria or averting their infections. For that reason, as a preventive action all the calves in the experiment were administered 1.5 mg/Kg of decoquinate in its oral form. This medication was probably used to treat or prevent coccidiosis which is a disease afflicting birds and mammals with protozoa of the genus *Eimeria*. A sample was collected randomly at different stages of the study to check for the protozoa and to also check the efficacy of the preventative treatment. Vaccinations administered were for gastrointestinal diseases including rotavirus and coronavirus and respiratory diseases including bovine infectious rhinotracheitis, bovine viral diarrhea type I and II, bovine respiratory syncytial virus, and parainfluenza 3 in calves in the study. The dams, however, were not offered any vaccinations so that they could concentrate on shielding the calves from certain types of sicknesses that plagued the gastrointestinal systems. In terms of controlling or preventing the occurrence as well as the extent of epidemic diseases in animals, vaccinations are often used.

MICROBIOLOGICAL METHODS

Centrifugation was used to separate the solid particles and cells from the specimens that were initially obtained from the calves. This was done in order to prevent any contamination. The concentration of *E. coli* was achieved by resuspending the specimens in the suitable enrichment or direct processing medium. This was done in order to achieve the level of concentration. Latex agglutination and culture techniques were used in order to successfully confirm the presence of *E. coli* O157:H7.

The diagnostics conducted in the investigation include the use of Ziehl Neelsen technique to test for *E. coli*. Live cultures were quantified using culture methods that included direct plating on SMAC-CTVM plates and enrichment in TSB. Thus, topographical differentiation of the *E. coli* O157:H7 samples was done and latex agglutination backed the observations. In the context of the assessment of the quantity of *E. coli* O157:H7 bacteria this investigation employed particular culture conditions and plates. As was said, before the experiments the SMAC-CTVM plates were incubated with potassium tellurite, vancomycin, MUG (4-methylumbelliferyl-beta-D-glucuronide), and sevixim. The latter were used for direct culturing of the fecal samples on these plates. Before bacterial enumeration, uncoated solid cells and other debris that may interfere with counts were pelleted by centrifugation of the specimens at 10,000 × g for 5 minutes at 4 °C. This step was further intended in order to enrich the bacterial cells from the fecal samples, more so, there is a mild filtration method was described which involved step ascendancy of suspension particles. In the study, *E. coli* O157:H7 was undertaken using an enrichment method. The fecal samples were cultured for 18 hrs at 37 °C using TSB (Tryptic Soy Broth) with a provision for ventilation.

For the purpose of detection and enumeration of *E. coli* O157:H7 if it is present at low levels this step is designed to enhance growth of the bacteria.

Solid substrate culture of Latex agglutination was employed by the Pro-Lab Diagnostics Toronto Canada confirm the identity of *E. coli* O157:H7 colonies. Latex agglutination: It is a fast procedure of identifying and detecting bacterial antigens which utilizes latex beads that are coated with specific types of antibodies. Direct culturing and check colonist morphologies of *E. coli* O157:H7 grown on bovine fecal samples were also observed. Adherence to colony morphology was assured and clearly atypical colonies was sub cultured onto SMAC-CTMV plates and subjected to colony morphology examination once again.

STATISTICAL ANALYSIS

The statistical analysis that used in the study was t-test to determine if there is any difference between the group which was treated with phage and the group that was not treated. The number of the bacteria in individual samples was calculated based on the counts of *E. coli* O157:H7 obtained in the above steps and the bacterial colonies were analyzed statistically.

ETHICAL CONSIDERATIONS

The experimental methods and the animals' handling were assessed as well as accredited by the Committee of Animal Research Ethics. Some of the factors that were considered to balance with the ethical practice included that of the welfare of the animals used in the study. Some of the ethical concerns entailed the right environmental conditions for the animals, animal health checks, the level of discomfort caused to the animals, and/ or the legal provisions governing animal use.

RESULTS AND DISCUSSION

In comparison to the control group, the phage group had fewer days overall (4.69±2.29 and 13±4.88, respectively, p= 0.04) with diarrhea. There was a statistically significant difference (p= 0.03) in the beginning of diarrhea between the groups. Despite the high frequency of *E. coli* O157:H7, the phage group showed the best health, showing fewer days with diarrhea and delaying their arrival. The bacteriophages group (14 d) had a larger range of appearance than the control group (Figure 1), but they appeared in the control group more quickly (14d).

Phage therapy appeared to be a viable treatment option for cattle when phage treatment effectively removed intestinal *E. coli* O157:H7 from calves as in this study. Day 0, fecal samples contained 4- to 5-log values of *E. coli* O157:H7, which all treated calves tested positive for in their cultures. Throughout the trial, *E. coli* O157 was present in all

untreated control animals. On days 0, 1, 2, and 4, phages were applied to the animals in the phage therapy group. The average number of *E. coli* O157:H7 CFU in fecal samples from calves subjected to phage treatment dropped dramatically to 2.4 logs on the first day after phage treatment, while the control group's average remained at 3.9 logs. The amount of *E. coli* O157 CFU dropped further seven days after phage therapy, to an average of 1.4 logs, compared to 2.7 logs in the control group. From the first posttreatment day to the tenth day, the calves treated with phage had significantly less *E. coli* O157 CFU/swab ($P < 0.05$) than the control group.

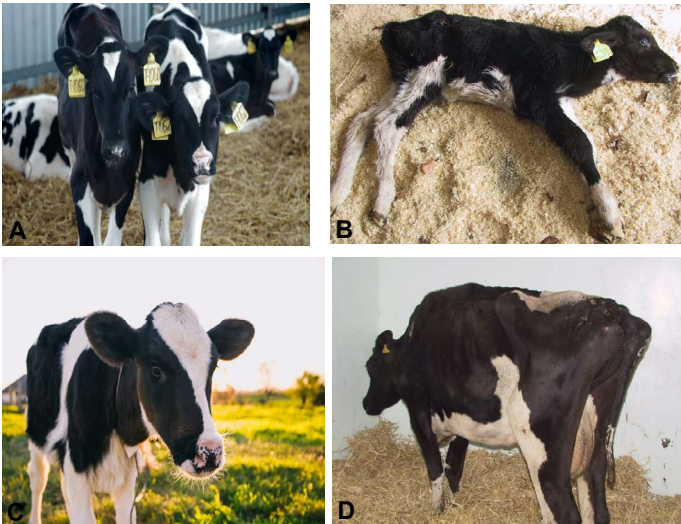


Figure 1: Comparison of case of control (neonatal calf diarrhea) and phage-therapeutic calves.

Table 1: Comparison of the phage-therapeutic effect in calves.

Parameter time point (Days)	Control (group 1) n = 6	Phage-treated group (Average <i>E. coli</i> O157:H7 CFU) (group 2) n = 6
0	4.5 logs	4.5 logs
day 1	3.9 logs $\pm$ 0.2	2.4 logs $\pm$ 0.3
day 2	3.9 logs $\pm$ 0.3	2.2 logs $\pm$ 0.2
day 3	3.7 logs $\pm$ 0.4	2.1 logs $\pm$ 0.3
day 4	3.4 logs $\pm$ 0.3	1.6 logs $\pm$ 0.2
day 5	3.2 logs $\pm$ 0.5	1.4 logs $\pm$ 0.1
day 6	3.2 logs $\pm$ 0.4	1.3 logs $\pm$ 0.2
day 7	2.7 logs $\pm$ 0.3	1.4 logs $\pm$ 0.2
day 8	2.6 logs $\pm$ 0.2	1.2 logs $\pm$ 0.3
day 9	2.6 logs $\pm$ 0.3	1.1 logs $\pm$ 0.2
day 10	2.5 logs $\pm$ 0.4	1.2 logs $\pm$ 0.2
day 11	2.4 logs $\pm$ 0.2	1.1 logs $\pm$ 0.1
day 12	2.4 logs $\pm$ 0.2	1.1 logs $\pm$ 0.1
day 13	2.7 logs $\pm$ 0.3	1.0 logs $\pm$ 0.2
day 14	2.3 logs $\pm$ 0.2	1.0 logs $\pm$ 0.1

On days 5 and 7 of the trial, cultures of two calves produced negative results for *E. coli* O157, while the cultures of one calf showed no signs of the pathogen at all. However, four of the five treated calves still had evidence of *E. coli* O157 in them even after getting therapy. During the first seven days following medication, the phage concentration in the medicated calf samples varied between 4 and 6 log/swab; it subsequently steadied at 2 log/swab until the completion of the trial. Depending on the day of the sample and the calf, the ratio of phages to *E. coli* O157:H7 in fecal samples varied from 1 to 104. We postulated that the large size of the bovine intestine, potential differences in the digestive environments of monogastric and ruminant species, and changes in the number of *E. coli* O157:H7 CFU organisms in the intestinal tract after oral administration and the bacteria/phage ratios below 102 could all be contributing variables to the insufficient elimination of enteric *E. coli* O157 in ruminants following the use of phage therapy.

All the *E. coli* O157 isolates reacted to the phage; some of them were of atypical colony morphology and were from the affected calves that were infected by the phage and had administer it. The management of diarrhea caused by *E. coli* is associated with number of challenges, which include the duration of the treatment and use of antibiotics during the process. Some of the drugs like β -lactams, aminoglycosides, fluoroquinolones and tetracyclines have the potential of immunosuppression in the immune system of the calves and also the potential to enhance the ability of the bacteria to develop resistance mechanisms effectively causing the elimination of the infections. Due to the legal measures that have been made and enacted to limit the use of antibiotics, some of the legal substitutes include. The misuse of antibiotics in fighting human and animal diseases resulted to the emergence of antibiotic resistant bacteria. This resistance has been as a result of overuse of antibiotics. Still there were a number of calves with positive results for *E. coli* O157 at all given time even after the phage therapy was introduced in the calves. This has led to a situation where more studies need to be done on the treatment regimen making it possible to enhance the efficiency of the protocol in the elimination of the pathogen. The use of kits, on the other hand, has to be followed with research that enhances the effectiveness of eradicating *E. coli* O157 within the accordance to the set guidelines for treatment. Because of this, it might be necessary to increase or decrease the concentration of the phage, the interval between applications or the duration of the therapy. In addition, concerning the so-called phage cocktails, that is many phages simultaneously, or the treatment of the patients with many phages in sequence, it could be relevant to consider the possibility of such use.

Phage treatment is offered as a possible option to the problem of antibiotic resistance, and it is the main focus of

this work. The frequencies of diarrheal events in the animals of the phage group were significantly lower in the first half of the working time in comparison with the animals of the control group. Also, the phage group appeared healthier and thus, held off bacteriophages' penetration for a longer time. Hence phage therapy was considered to be one of the methods that could be used to treat cattle since it was illustrated to have the ability of eliminating intestinal *E. coli* O157:H7 from calves. The control group, in this research, had 3 logs. 9 logs but the mean value of *E. coli* O157:H7 count for the feces of the calves that were given phage was reduced to 2.4 logs. This was in comparison with the amount of log that was detected in the controlled group. Thus, seven days after administering phage treatment to *E. coli* O157 positive patients, the average number of CFU decreased to 1 and the level of bacteremia, as well as the general health of the patients, improved considerably. 4 logs. In general, the number of *E. coli* O157 CFU/swab of the steers which were administered with phage were comparably lesser compared to the control group. Using the results of the study, it can be concluded that four out of the five calves which were treated with the phage therapy did not undergo the efficient elimination of *E. coli* O157. It was expected that the inability to eliminate intestinal *E. coli* O157 in ruminants may have been due to phage treatment which would have been one of the factors that brought the failure. Several factor can help explain this; CFUs of the *E. coli* O157:H7 strain that survived oral treatment, bacterium to phage ratio being lower than 102, the extensive capacity of the bovine gut and inter species differences in GI tract of monogastric animals and ruminant animals. Administration of bacteriophages in experimental diarrhea due to *Escherichia coli* in calves has been try within certain limit. It has been witnessed that certain area specific phage can be used in the treatment of *E. coli* diarrhea in calves, pigs and lambs. It was also ascertained that even after the progress from infection, and the ability to use the phages that have been administered in the solution that works on the calves, the calves will expel the phages in their feces (Penziner and Schooley, 2021). Further, phage therapy has been demonstrated to decrease bacterial loads in calf clinical diarrhoea due to *E. coli* strains, indicating the possibility of this natural antibiotic as a substitute to conventional antibiotics for the management of bacterial diseases in animals (Alomari et al., 2021). In one of the studies using animal models, phage therapy has been observed to be effective in lowering the mortality levels and managing bacterial infections compared to the use of antibiotic drugs, and that phage therapy can be a specific and reliable method of dealing with multidrug resistant bacteria in veterinary practices (Sachithanandan et al., 2024). *Escherichia coli* bacteria are a common human infective agent causing UTI, sepsis, Enteritis, Neonatal meningitis among other diseases in other organs. Uro-

pathogenic *E. coli* (UPEC) is the most common infective agent in UTIs and due to increased incidence, this disease has become more challenging, costly, and need longer hospitalization. The cure for UPEC resistance is believed to be addressed by phage therapy and an entirely different treatment modality named VB_ecoS-Golestan. Among the isolates under study, 78.8 % MDR and 56 % of the isolates exhibited susceptibility to this phage lytic action. The phage is non-host specific with multiple drug resistant isolates and there is no presence of any lysogenic mediated gene in the genome of the phage (Abreu et al., 2019). VB_EcoP-EG1 have larger burst size, no hazardous protein production, short lytic cycle, and is host wide on UPEC strains. It also has the ability to infect 10 out of 21 MDR UPEC, causes lysis of MDR UPEC and reduces biomass of the biofilm in both MDR and sensitive isolates. And the phage may be able to treat patients with biofilm related UTI. In healthcare settings, this biofilm increases the virulence of both the acute and chronic infections such as the Catheter-associated urinary tract infection (CAUTI) due to UPEC to a catastrophic level. There is, however, several major challenges that are associated with phage therapy like the narrow host specificity of phages and the emergence of phage-resistant biofilm (Kaur et al., 2021). It has been shown that phage and antibiotic combined therapy reduces bacterial numbers and manages phage-resistant bacterial populations. To explore the combined efficaciousness of phage and ampicillin in treating drug-resistant *E. coli* O157-associated urinary tract infections, Moradpour et al. discovered that phage application inhibited bacterial growth in general, maintained ampicillin and amoxicillin clavulanic acid free zones, and showed the susceptibility of resistance phenotype in the inhibition zones. Furthermore, because the *E. coli* strain ATCC 13,706 is resistant to multiple medications, current research has focused on the effects of co-administration of antibiotics and phages. The effectiveness, therefore, of the combination therapy using phage and antibiotics depends on; the bacteria under study's resistance to the used antibiotic and the nature of the applied antibiotic whether it is bacteriostatic or bactericide (Easwaran et al., 2020). Specific diseases that have been studied in relation to phage therapy include enteritis inflammation of the small intestine or colitis inflammation of the large intestine. These investigations perform the task of making phage therapy administration easy by allowing the exploration of oral phage while conducting experiments because diarrhoeal illnesses mainly affect the lumens of the gastrointestinal tract. Using research essay sources, *E. coli*, that is known to be one of the main culprits responsible for diarrheal diseases among humans across the globe, has been studied severally (Leung et al., 2019). Smith and Huggins (1983) studied the husbandry of *E. coli* diarrhea used eight lambs for experiment purpose and they orally infected with *E. coli* strain O157:H7. Two days post

eviction all eight sheep were euthanized and four of them were treated with an oral phage with a MOI of 10. It was also found that Cutler's sheep's colons were 2-3 log₁₀ less colonized with bacteria when compared with untreated sheep. An oral dose of 10¹⁰ CFU of enterotoxigenic *E. coli* (ETEC) were administered orally to pigs by Jamalludeen et al. (2009). After 24 h three injections with a 6 h interval were administered of cultured phage at a concentration of 10⁸ PFU of a mixture of two phages. Oral phage therapy reduced ETEC load in the pig feces and diarrhea incidence as compared to the animals which did not undergo phage treatment (Bhat and Rashid, 2023). Ruminants such as cattle have been established as source hosts and reservoirs for these diseases hence exhaustive studies of the use of phage therapy to eliminate pathogenic as well as animal strains of *E. coli* inclusive of O157:H7 (Gupta et al., 2019). Research by Johnson and his colleagues has demonstrated effectiveness of the phage therapy for combating infections caused by *E. coli* strains that release the intestinal toxins among neonatal calves (2008). Several conditions determine such effectiveness and they include the nature of the infection during the experiment, method of application of the phage, and the magnitude and quality of the phage dosage. A major problem in ruminants is the relatively short persistence of phages' antibacterial action, which is due their susceptibility to the conditions in the gastrointestinal tract. Hence, it is clear that the ability of phages to survive oral delivery is limited by existing bacteria, the effects of stomach acid, enzymes, and other agents of digestion (Zia and Alkheraije, 2023). Our results are in line with the work of Alomari et al. (2021) who have investigated the effectiveness of suppositories containing selected bacteriophages of pathogenic *E. coli* and probiotic lacto-bacteria. this in young calves affected with diarrhoea. Both prophylactic as well as treatment outcomes based on specific and non-specific immunologic reactions were also measured in the current research. Female HF calves were 24 in number, weighed 35-46 kg and were 2-7 days old at the time of the study. The calves were split up into the following four groups (n= 6): Group 1 and 2 were non-medicated control; Group 3 and 4 were medicated, but having diarrhea; Group 5 was a diseased but well managed group of calves. During the next five days, the animals received suppositories containing bacteriophages of pathogenic *E. coli* and *Lactobacillus* spp. The suppositories were given to the calves twice a day on the first day. This should practice is supposed to be done once in the morning and once in the afternoon. After the first round of suppositories, the health of calves was assessed for 11 days out of which the results have been depicted below. Preventative and protective, was the conclusion as to the nature of the effect that the experimental treatment had in the study. The probiotic-phage suppositories reduced the calves diarrhea duration

and eradicated it 24 to 48 h post-treatment. It made immunologic activity of the calves more heightened thus enabled them to fight not only the specific pathogens but also the other pathogens which made their defense mechanisms gain strength. Callaway et al. (2008) confirms that, administration of phage cocktail of O157:H7 strain 933 (ATCC 43895), which is a standard *E. coli* strain, strongly negated bacterial count in rectum, caecum and rumen in two cases. After the administration of the phage cocktail, the caecal and rectal samples from the animals harbored between 10² and 10³ PFU of pathogenic bacteria per gram of the sample in all the investigated samples except in only two rumen samples. In addition, a more recent human study by Bielke et al. (2012) in which a double-blind, randomized, placebo-controlled approach was used evaluated the effects of an oral bacteria cocktail in relation to fecal *E. coli*-CFU. In total, twenty-two neonates were treated, ten doses of the bacterium were assigned to the ten calves at random while the rest of the calves received placebo. Five milliliter of plaque formation units (PFU) (volume) originating from each of the four groups was administered to the phage therapy group of six persons, while the placebo group of four persons just received brine with phosphate (5 ml). Each calf daily had fresh stool and blood samples collected with the aim of determining kind and quantity of bacteria and phages detected. All the samples contained phages infected with *E. coli* and their density ranged from 10²-10³ PFU/g rectal content. When compare the phage-treated group to the control group, a decline in fecal *E. coli* was observed: In the present study, the average log CFU with the phage treatment group was found to be 9.11 (SE=0.34) in the original study while it was 9 in the present study. 25 (SE=0.42) for the placebo treated group. However, no statistically significant difference was brought out. From the research done it was evident that when adult cattle were orally fed bacteriophages it reduced the *E. coli* O157:H7 in contrast to steers treated with medication through an enema (Rozema et al., 2009). They found out that due to the digestion in both liquid and solid stage, the oral phage's longer duration for circulation in the digestive tract paved way to better interaction of the phage RNA with its targets, the *E. coli* cells, thus enlarging the phage population and the likelihood of infecting more *E. coli* cells. Some of the ways in which the relations between the phage and the bacteria may be disturbed include concentration of the inoculum, time and method of its administration, the presence of anti-phage immune response and the appearance of phage-resistant mutants (Smith et al., 1987). They also talked about the influence of the stomach acidity to the effectiveness of oral administration of phages. As mentioned earlier, an assessment by Smith et al. (1987) indicated that the stomach contents does not harm oral phages even when

the pH level of the contents is at a mild acidic level of pH $3 < 4.3$ after milk feeding. On the other hand, when it was tested in an almost empty stomach that has an acidity level of pH $2.3 < 3.0$ the phages died. Adesanya et al. (2020) noted that there was reduction in the concentration of the phage in the large intestine and mice as compared to the amount added in the drinking water. Through science the elimination of the phages was perhaps caused by the low level of pH 3 in the stomach of the mice. However, according to the results obtained by Brutin and Brüssow (2005) it can be concluded that phage stability could be important during the passage through human digestive tract. This is because in a concentration of 10^5 Fu/mL, the researchers were able to identify phages in each of the fecal samples collected from people who ingested phages in water they consumed. While our findings are contrary to the study conducted by Safir et al. (2020) that examined the effectiveness of two newly acquired lytic bacteriophages, KH1 and SH1 which target O157, given individually or in combination on the intestinal *Escherichia coli* O157:H7 in animals. Supplementation of Phage KH1 to the sheep through oral route did not reduce the concentration of *E. coli* O157:H7 that was present in their system. On the other hand, the Pla expression by Phage SH1 was more distinct and significant on the lawns of each of the tested *E. coli* O157:H7 strain. This only compared with Phage KH1 the results for solely O55: H6 and 18 the 120 isolates which were tested on *E. coli* other than O157. Also, Phage SH1 was found to be quite eclectic concerning its hosts. Neither of the two control agents myosin or bovine mucus could prevent the bacterial degradation process caused by Phage SH1 or *in vitro* KH1. Given a mouse model that possessed an intestinal holder of *E. coli* O157:H7 bacteria a phage therapy system was developed. In SH1 treated groups, the stool *E. coli* O157:H7 was eradicated within two to six days after therapy with oral SH1 or a combination of SH1 and KH1 of phage/bacteria ratios ≥ 102 with co-administration of medications. Conversely the percentage culture positive animals in the untreated group remained culture positive for up to 10 days. Seventh day before the challenge Holstein steers were treated with *E. coli* O157:H7 rectally and then they were given 1010 PFU SH1 and KH1 to increase the phage delivery and bacterial shedding in cattle. At phage to bacterium ratios of less than or equal to 102, the recto-anal junction mucosa was directly exposed to phages. Also, the water drunk by the members of the phage therapy group had 106 PFU/ml of phage. Nevertheless, after the administration of phage therapy most steers were still positive for *E. coli* O157:H7 CFU than the control steers although the average number of bacteria present in the steers subjected to phage therapy was lower than those in the control group ($P < 0$). Some may say that our phage cocktail would have yielded even better result if the *E. coli* strains isolated from bovine uteri were not used as hosts instead of fecal *E. coli* isolates

(Bicalho et al., 2010). An example of the hunting act between phage and the bacteria it is hunting may be gathered from Tanji et al. (2004). The increase in population densities of *E. coli* O157: H7 in waste-water streams is followed by the increase in the populations of bacteriophages that target these bacteria. The reason for this is because the identification of particular cell-surface receptors is central to the host specificity of each phage (Considering that *E. coli* strain which was taken from metritis dairy cow's uterus was used as the host for phage enumeration, the result can be explained by the fact that no environmental phages were found in stool samples of the control group.

CONCLUSIONS AND RECOMMENDATIONS

If bacteriophage preparations are used then of course experiments on animals must be carried out. There is the probability of bacterial infections if bacteria gain resistance towards antibiotics. These are meant to assess an efficacy of an experimental treatment for these germs. Since there have been evidenced based changes in the treatment of many disorders, this assumption cannot be ruled out. There has been a lot of procrastination as to taking any step regarding the increasingly emerging issue of antibiotic resistance, which has emerged as a new concern only recently. Phage therapeutic use is still just one of the adaptation strategies that has been employed so as to utilize the available information that is easily accessed now days. This research outcome will assist in the reduction of time taken to opt for phage as an additional to other drugs in the treatment of infections that have resisted to these products. This is important among the myriad factors of the deepening crisis. That there is no single approach to the clinical application of this therapeutic bacteriophage anymore is also a plus to the idea. The following are two of the greatest strengths of the previously proposed notion out of many. Guidelines on the administration of the phage treatments concerning bacterial diseases will require refinement in the following years since the available literature is expanding rapidly. However, it is high time that we determine answers to these questions remaining as follows. There are still some things which are undetermined and that is bad for trade. Last but not the least, on the general consideration of the phage treatment, it would not be appropriate to say that it does not lack the broad brush on its large-scale treatment. But there are so many social, commercial, and economic realities that were much to benefit from the adoption of this treatment.

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NOVELTY STATEMENT

This study offers a novel idea of overcoming the problem of MDR bacterial pathogens in animals through the use of the sophisticated phage therapy. It presents a general plan that enables an effective use of bacteriophages that are inherently specific and efficient in treating infecting diseases in veterinary practices. This sort of innovation presents a viable and effective new treatment to the common attack of antibiotic resistance in animals which is currently one of the biggest concerns in the field of medicine today. Thus, it establishes a promise for future therapeutic approaches directed to manage the MDR infections.

AUTHOR'S CONTRIBUTION

AKM: Design of the research strategy, formulation of perceptions for the study, and general supervision of the work. SEJ: Sample collection, data analysis and interpretation and also writing of the manuscript. TTA: Carried out various experimental process and offered specialized knowledge in isolate of bacteriophage and its use. AMA-E: Offered support in the experimental design and offered technical support during the whole study. QRL: Organization of laboratory activity, preparation of documents, work with manuscripts, communication with reviewers and publishers. MM: Assisted in data analysis, helped revise the paper and made assurance that the data was accurate and the results obtained were valid.

CONFLICT OF INTEREST

We have no conflict of interest to report with regards to the research, authorship or publication of this article. In every study, the investigation was carried out solely and without the support of consignment organise business or monetary gain.

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