



## Experimental therapeutic challenge of two bacteriophages isolates against *E. coli* serogroup causing diarrhea

Nidham M. Jamalludeen<sup>1\*</sup>, Dania M. Shakir<sup>2</sup>, Mariem N Mohammed-Ali<sup>3</sup>

<sup>1,2</sup>University of Basrah, College of Medicine, Department of Microbiology, Basrah, Iraq.

<sup>3</sup>University of Makeel, College of Pharmacy, Department of Microbiology, Basrah, Iraq.

\*Corresponding author: Nidham M. Jamalludeen, University of Basrah, College of Medicine, Department of Microbiology, Basrah, Iraq, Email: njovc@yahoo.com

Submitted: 05 February 2023; Accepted: 18 March 2023; Published: 17 April 2023

### ABSTRACT

The study aimed to determine the efficacy of selected bacteriophages in the treatment of experimental diarrhea in a group of laboratory animals infected with *Escherichia coli* used as challenge bacteria. Bacteriophage was administered orally, up to three times, 24 hours after administration of challenge bacteria, and following diarrhea. Weight change, diarrhea duration, diarrhea severity, diarrhea degree score, and the rate of challenged *E. coli* bacteria shedding over the course of six days were the parameters utilized to determine if changes had occurred. The bacteriophages that were tested for treatment were successful in making a qualitative change in these parameters. These bacteriophages produced specific changes in all parameters without affecting the normal flora shed in the feces. These results indicate that the selected bacteriophages had an effective effect in treating experimental diarrhea in laboratory animals after giving a challenge bacterium orally.

**Keywords:** *phage therapy, E. coli, experimental trails, Guinea pigs*

### 1. INTRODUCTION

The rise of antibiotics resistant bacteria has become a factor that has a qualitative impact on human and animal health. Concern has been raised about this issue recently and especially with multidrug-resistant bacteria among the pathogenic microorganisms. However, there is a lot of pressure to limit and reduce the use of antibiotics in animal production. Similarly, drug-resistant *E. coli* has been on the rise in recent years (1, 2, 3). In addition, the concern that bacteria that consistently select for resistance and are compatible with the use of antibiotics as animal treatments might be harmful to human health by transferring drug-resistant genes to other pathogenic bacteria.

Therefore, there is an urgent need to find and obtain a practical and safe alternative to the use of antibiotics in the technology of the animal production industry, and it can be used as prevention and treatment. Bacteriophages are viruses that have the excellent ability to kill pathogenic bacteria, and they are considered as a distinctive alternative because they have the characteristic of non-toxicity and they multiply when injected into the host bacteria and their number increases because they can destroy the bacterial population (4, 5, 6, 7, 8). In 1915 (8) and 1917 (9), respectively, Twort and D'Herelle separately discovered a phage, a virus consisting of a protein closure covering a nucleic acid that could be either DNA or RNA.

They are either virulent or temperate and differ in quality-of-life cycle. Only virulent bacteriophages are used for bacteriophage therapy. (6, 10).

There are several studies describing how bacteriophages may eliminate bacteria that infect or injure people and other animals, and their ability to treat pathogens that are resistant to antibiotics has been proven (11, 12, 13, 14, 15). D'Herelle was the first scientist to use bacteriophages as a treatment since the beginning of the year 1926 and before the period of antibiotics. He used them to treat the causes of avian typhosis in birds and in the laboratory to treat dysentery causes in rabbits (16). After that, the bacteriophages were successfully used to treat human plague, cholera, and wound infections. As well, several researchers used bacteriophages for treatment and prevention (3, 6, 12, 14, 15,17).

There had been a reduction or change in the use of bacteriophages in treatment, after the discovery of antibiotics, although they were widely used for treatment in some countries (4). Then, bacteriophage therapy was re-discovered after the bacteria formed severe resistance against antibiotics and as an alternative strategy to antibiotics in the treatment of resistant bacteria. The researcher Smith and his group in the year 1980 conducted important and encouraging research using bacteriophage therapy. These researchers have experimented with the effect of bacteriophages and their control on E. coli, which causes septicemia. They note that a mixture of two or just one bacteriophage was able to control the E. coli causing infection in the animals used in the experiments (18, 19). After that, successful studies have been done using bacteriophages as a treatment for infections in humans and animals (12, 20, 21, 22, 23). The aim of this study was to investigate the susceptibility of bacteriophage isolates in the treatment of E. coli induced diarrheal infections in guinea pigs.

## 2. MATERIALS AND METHODS

### 2.1. Media and bacterial strain

Different culture media were used to grow and search for bacteria and to obtain bacteriophages.

This media has been prepared as directed by the manufacturer. Luria-Bertani (LB broth, agar, agarose, and top agarose) was purchased from (Oxoid, UK) and prepared as reported before (24). As well as MacConkey's agar, blood agar and Brain Heart Infusion broth. Phosphate buffer solution (PBS) was also used.

A strain of E. coli, previously isolated in local laboratories and a cause of diarrhea (25), was used as the bacterium for laboratory challenge experiments on guinea pigs. This strain was purified, microbiologically determined, and then frozen at  $-70^{\circ}\text{C}$  in a freezing solution (26, 27, 28).

### 2.2. Bacteriophages

A mixture of two bacteriophages (EC-BSR1 and EC-BSR2, Figure 1), previously isolated by (25) was used to assess their susceptibility in the treatment of experimental diarrhea in guinea pigs subjected to laboratory infection with E. coli.

### 2.3. Titration of phages in feces

Feces were collected from each experimental animal and kept in tubes and placed on ice. Then 9 ml of sterile phosphate-buffered saline (PBS), pH 7.1 and 0.2 ml of chloroform were added to 1 gram of feces. The mixture was titrated according to the procedure mentioned in (12). Plagues observable at the highest dilution for the feces was considered to determine the titration.

### 2.4. Experimental Animals

A total of 36 guinea pigs were used to conduct an experimental trial to find out the susceptibility of a mixture of two bacteriophages (EC-BSR1 and EC-BSR2) in the treatment of E. coli infection, which causes diarrhea, given to these laboratory animals after induction of the disease. Guinea pigs used for the trials were transported from their purchase site and isolated in the Laboratory Animal Isolation Unit, Basrah University, and left for two days to adapt to the place. An ethical approval was obtained from the Board of the Iraqi Health and Higher Education Committee. Before the challenge, feces were collected from experimental animals, then the samples were

diluted ten-fold to 10<sup>-8</sup> and cultured on culture media such as blood agars and McConkey's agar to see if they were infected with the bacteria used for the challenge for the purpose of excluding the infected one. Feces samples were examined to ensure the presence of bacteriophages that may cause decomposition of challenge bacteria, using the spot test (24). All experimental animals were re-examined for the purpose of verification.

### 3. Experimental clinical trials

Twenty guinea pigs were used for the first experiment and were divided into two groups of ten animals. The first group is for control and the second is for examining the mixture of bacteriophages (EC-BSR1 and EC-BSR2) (Trial 1). Each guinea pig was administered with 10<sup>10</sup> colony-forming units of the challenge bacteria E. coli. The severity of the diarrhea was assessed 24 hours after the challenge by recording the score system consisting of zero, one, two, and three” (Briefly, Similar to what Jensen et al. (29) described, a scoring system was used to determine how severe the diarrhea was. When the feces were solid and normally formed, a score of 0 was given; when they were soft but could maintain some shape, a score of 1; when they were brown and liquid, a score of 2; and when they often passed watery feces, a score of 3 was provided). Fecal samples were taken to determine the extent and quantity of challenged bacteria and the total level of E. coli presence in the feces after challenge. The concentration of E. coli challenge bacteria was determined by making decimal dilutions feces and culturing the bacteria on blood agars and McConkey agars. After that, the guinea pigs randomly separated into two groups, one of the groups was given a treatment with bacteriophage and the other was left without treatment. Guinea pigs in the treatment group were given a mixture of bacteriophages (EC-BSR1 and EC-BSR2) at three times at a dose of 10<sup>8</sup> pfu for each bacteriophage.

Every day, for a period of five days, guinea pigs were monitored for clinical signs and diarrhea were also observed. Fecal samples were taken every day to examine them for the presence of challenge bacteria and the number of phages and bacteria shed with the feces after a decimal dilution of the feces and cultured on blood agars

and MacConkey agar. Recovery of challenge E. coli and the criteria used to evaluate the efficacy of phage treatment were adopted from the previous publication by Jamalludeen et al., (12). However, the experiment was repeated using 16 new guinea pigs that were divided into a group of 8 as a control group and a group of treatment with mixture of two phages (EC-BSR1 and EC-BSR2) (Trial 2).

### 4. Statistical analysis

ANOVA test is the statistical test that was used to analyze the results of the two trials in this research and to compare the results of the association between guinea pigs that took bacteriophages from the group of guinea pigs that did not take treatment and the result was considered significant with a statistical standard of less or equal to 0.05.

## 5. RESULTS

All 20 guinea pigs in the first experiment developed diarrhea after being given the E. coli bacteria used in the challenge for this experiment, and 24 hours after the start of the experiment, they were divided into two groups, with 10 guinea pigs in each group, and the same thing happened in the second experiment, after giving challenge bacteria. Figures 2 & 4 show that treatment with the mixture of phages was effective in improvement of mean weight change, mean diarrhea duration, mean diarrhea severity (total daily diarrhea score/number of days of diarrhea), mean degree of diarrhea score (the average score times duration of diarrhea), and mean shedding of the challenge E. coli as compared with the control untreated Guinea pigs for the 5 days following day 1.

Figures 3 & 5 For the guinea pigs that were treated with the mixture of phages (EC-BSR1 and EC-BSR2), the mean total E. coli that were shed increased on days 1, 2 after challenge then returned to the day 0 level. The mean titre of all E. coli shed in the feces for the guinea pigs in the control group was consistently greater than the titre on day 0 throughout the whole six days following the challenge.

## 6. DISCUSSION

The aim of this study was to determine if there was any effect after administration of a mixture of two phages (EC-BSR1 and EC-BSR2) as a treatment protocol to guinea pigs that developed diarrhea due to administration of *E. coli* bacterium, that causes diarrhea. These two phages were chosen for this assay due to their high efficiency in destroying *E. coli* bacteria in vitro, which was known from a previous study (25).

The apparent results from the first and second experiments showed a significant difference between the incidence of diarrhea and the rate of weight gain between the group of guinea pigs treated with phages compared to the group of guinea pigs prepared for control. It is also evident that there was a clear difference in the shedding rate of *E. coli* used in the challenge between the group of laboratory animals treated with the phage and the group of animals used as control. These findings using phages selected for the treatment of *E. coli* diarrhea were identical and similar to those previously conducted by Smith and Huggins (19) researchers who examined the effect of phages on diarrhea in calves, lambs and newborn piglets. These researchers found that a mixture of bacteriophages cured newborn lambs, calves, and pigs from the diarrhea they experienced in their experiment due to Enterotoxigenic *E. coli* (ETEC) infection. The authors also found that numbers of *E. coli* shed used in the challenge were more in the control group than in the group of animals treated with the phage, where the number of shedding bacteria was clearly significant (19).

The dose of phages given in the trials is an important factor in the outcome of the experiment in terms of time, quantity, and number of times of administration. The phages in this study were given to treat immediately after the onset of diarrhea as a result of challenged *E. coli* bacteria and for the bacteriophages to come into contact with the *E. coli* bacteria, which may be present in the intestine or on the intestinal wall at the early stage of giving of experimental guinea pigs. However, the chance of contact with treated bacteriophages may be limited after diarrhea has occurred, since the majority of ingested *E. coli* may associate with the intestinal wall and

intestinal epithelial lining (30, 31, 32) and the Components of the small intestine tube may help flush out the treated bacteriophages. Therefore, it was preferable to give doses of treated phages in the form of three times, separating them from 6 hours between one dose and another, up to 18 consecutive hours. The effect of administering different doses of phage to calves at different times was studied by Smith et al. (33). The researchers found that a dose as low as 10<sup>5</sup> pfu of phage given to the calves 6 hours before challenge protected them against development of ETEC diarrhea but a dose of 10<sup>2</sup> pfu of phage given to them 12 or 18 hours after challenge was ineffective.

The total number of *E. coli* shed with feces was monitored in all guinea pigs used in these two experiments, and studied for the effect of using a mixture of phages (EC-BSR1 and EC-BSR2) on *E. coli* flora present in the intestines of these animals used in the two experiments. From examining the data available in the results, Figures 3 and 5, it was observed that, on day 0 and before the administration of challenge bacteria, the numbers of *E. coli* in the feces were similar in all guinea pigs used in treatment or used in control. It was also observed that the numbers of *E. coli* in the feces increased in both groups of laboratory animals after the first day of the challenge. This increase in numbers has been attributed to an increase in bacteria given for the purpose of challenge. It was observed that this increase, Figures 3 and 5, remained elevated in the group of guinea pigs used as a control in these two experiments under study and for six days after the pre-challenge (zero day), possibly due to the presence of the challenge *E. coli* bacteria in this untreated group. On the contrary, in the group of guinea pigs treated with the mixture of phages, the total number of *E. coli* bacteria began to decrease after the second day of challenge, reaching a number similar to what it was on the previous day 0 of the challenge. This information indicates that treatment with this mixture of phages did not harm fecal numbers of *E. coli* flora. It is worth noting that Chibani-Chennoufi et al. (31) note that the numbers of normal commensal *E. coli* bacteria in the mice gut used in their experiment did not decrease after exposing them to a mixture of orally administered phages. They

concluded that E. coli bacteria in the gut are resistant or protected from damage. At the same direction, Bruttin and Brussow., (34) found in an experiment on a group of humans who were given experimental bacteriophages orally that the population of commensal E. coli was not affected by exposure to bacteriophages T4. The specific efficacy of bacteriophages is considered one of the limitations of using bacteriophages for treatment, as it determines the broad effect of treating other pathogens. However, this defect is not considered important as long as it eliminates pathogenic bacteria without affecting the harmless flora (4, 35).

In conclusion, the results described in this study indicate a clear significant change in the treatment of experimentally caused diarrhea in guinea pigs as a result of ingestion of diarrhea-causing E. coli with a mixture of two phages (EC-BSR1) and (EC-BSR2). These two phages showed a statistically significant change in reducing the number of E. coli bacteria used in the challenge in this study without affecting the number of E. coli flora. Further extensive study on these two phages may demonstrate their broad therapeutic potential.

#### ACKNOWLEDGMENTS

The authors are grateful to the Laboratory Animal Isolation Unit staff for their cooperation. We are also grateful to the Microbiology department, Basrah Medical college staff for kind support.

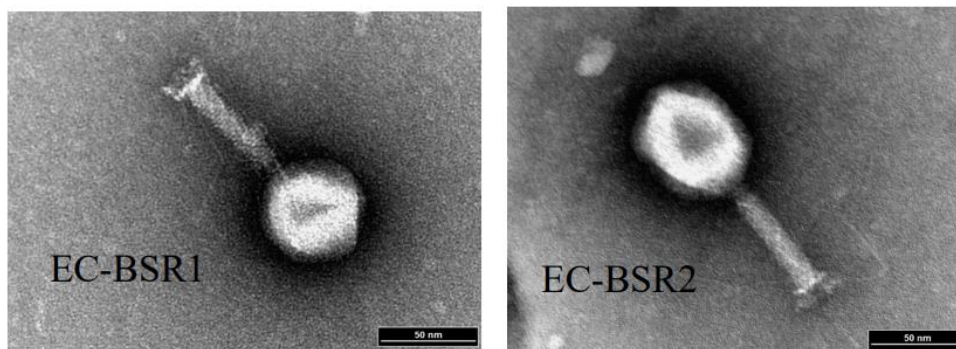
#### CONFLICTS OF INTEREST

There are no conflicts of interest

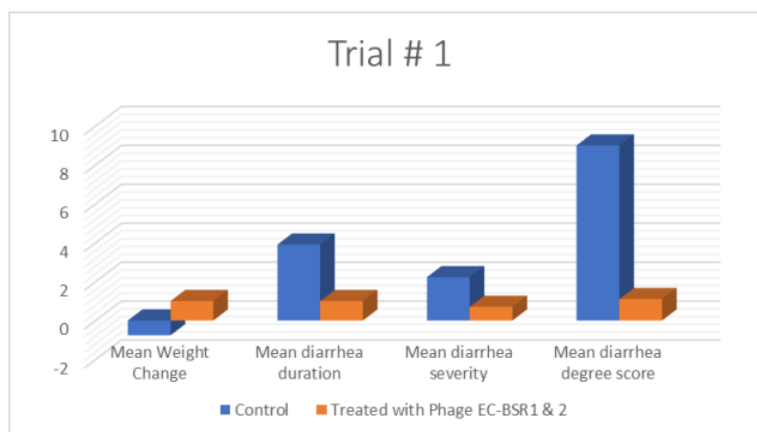
#### REFERENCES

1. Noamani, B., J. M. Fairbrother, and C. L. Gyles. 2003. Virulence genes of O149 enterotoxigenic Escherichia coli of postweaning diarrhea in pigs. *Vet. Microbiol.* 97: 87-101.
2. Allocati, N., M. Masulli., M.F. Alexeyev., and C. Di Ilio. 2013. Escherichia coli in Europe: An Overview. *Int. J. Environ. Res. Public Health.* 10, 6235-6254; doi:10.3390/ijerph10126235.
3. Kim, J., H. Park., S. Ryu1., and B. Jeon. 2021. Inhibition of Antimicrobial-Resistant Escherichia coli Using a Broad Host Range Phage Cocktail Targeting Various Bacterial Phylogenetic Groups. *Frontiers in Microbiology.* 12; 1-10. www.frontiersin.org.
4. Sulakvelidze, A., Z. Alavidze, and J. Glenn Morris, JR. 2001. Bacteriophage therapy. *Antimicrobial Agents and Chemotherapy.* 45. 649-659.
5. Huff, W. E., G. R. Huff, N. C. Rath, J. M. Balog, and A. M. Donoghue. 2005. Alternatives to antibiotics: utilization of bacteriophage to treat colibacillosis and prevent foodborne pathogens. *Poult. Sci.* 84: 655-659.
6. Loc-Carrillo, C., and S. T. Abedon. 2011. Pros and cons of phage therapy. *Bacteriophage* 1:2, 111-114; March/April 2011; © 2011 Landes Bioscience
7. Chegini, Z., A. Khoshbayan., S.Vesal., A. Moradabadi., A. Hashemi., and A. Shariati. 2021. Bacteriophage therapy for inhibition of multi drug-resistant uropathogenic bacteria: a narrative review. *Ann Clin Microbiol Antimicrob* (2021) 20:30. <https://doi.org/10.1186/s12941-021-00433-y>.
8. Twort, F. W. 1915. An investigation on the nature of ultramicroscopic viruses. *Lancet.* 16:1241-1243.
9. D'Herelle, F. 1917. Sur un microbe invisible antagoniste des bacilles dysenteriques. *C. R. Acad. Sci (Paris).* 165: 373-375.
10. Campbell, A. 2003. The future of bacteriophage biology. *Nature Rev.* 4: 471-477.
11. Summers, W. C. 2001. Bacteriophage therapy. *Annu. Rev. Microbiol.* 55: 437-451.
12. Jamalludeen, N., R. P. Johnson., P. E. Shewen., and C. L. Gyles.2009. Evaluation of bacteriophages for prevention and treatment of diarrhea due to experimental enterotoxigenic Escherichia coli O149 infection of pigs. *Veterinary Microbiology* 136 (2009) 135–141. <https://doi.org/10.1016/j.vetmic.2008.10.021>.
13. Jassim, S. A. A., and R. G. Limoges. 2014. Natural solution to antibiotic resistance: bacteriophages 'The Living Drugs'. *World J Microbiol Biotechnol.* 30:2153–2170.
14. Jamalludeen N. M., H. K. Atea., and H. T. Thwiny. 2021. Coliphages Lytic; as a potential biological control agent against E. coli (O157:H7) in a mouse model. *European Journal of Molecular & Clinical Medicine.*Vol. 08, Issue 03. ISSN 2515-8260.
15. Hussain, H. E., A. S. Abdulmir., I. G. Farhood., A. R. Ali., M. A. Al-azzawy. 2023. In vitro and in vivo study for antibacterial activity of endolysin-HEC gel and mixture gel on acne vulgaris caused by multidrug-resistant Staphylococcus aureus

- bacteria. *J Popul Ther Clin Pharmacol* Vol 30(1): e242–e257; 11 January 2023. DOI: 10.47750/jptcp.2023.1057.
16. D’Herelle, F. 1926. The bacteriophage and its behavior. The Williams & Wilkins Company. USA. pp. 490-497 & pp. 540-549.
  17. Mohammed-Ali, M. N., and N. M. Jamalludeen. 2015. Isolation and Characterization of Bacteriophage against Methicillin Resistant *Staphylococcus aureus*. *J Med Microb Diagn* 2015, 5:1 <http://dx.doi.org/10.4172/2161-0703.1000213>.
  18. Smith, H. W., and M. B. Huggins. 1982. Successful treatment of experimental *Escherichia coli* infections in mice using phage: its general superiority over antibiotics. *J. Gen. Microbiol.* 128: 307-318.
  19. Smith, H. W., and M. B. Huggins. 1983. Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. 129: 2659-2675.
  20. Slopek, S., B. Weber-Dabrowska., M. Dabrowski., and A. Kucharewicz-Krukowska. 1987. Results of bacteriophage treatment of suppurative bacterial infections in the years 1981-1986. *Arch. Immunol. Ther. Exp.* 35: 569-583.
  21. Weber-Dabrowska, B., M. Dabrowski., and S. Slopek. 1987. Studies on phage penetration in patients subjected to phage therapy. *Ar ISSN 2515-8260ch. Immunol.Ther.Exp. (Warsz).* 35:563-568.
  22. Biswas, B., S. Adhya., P. Washart., B. Paul., A. N. Trostel., B. Powell., R. Carlton., and C. R. Merrill. 2002. Phage therapy rescues mice bacteremic from a clinical isolate of vancomycin-resistant *Enterococcus faecium*. *Infect.Immun.*70:204-210.
  23. Huff, W. E., G. R. Huff, N. C. Rath, J. M. Balog, and A. M. Donoghue. 2003. Bacteriophage treatment of a sever *Escherichia coli* respiratory infection in broiler chickens. *Avian Dis.* 47: 1399-1405.
  24. Jamalludeen, N., R. P. Johnson., R. Friendship., A. M. Kropinski., E. J. Lingohr., and C.L. Gyles. 2007. Isolation and characterization of nine bacteriophages that lyse O149 enterotoxigenic *Escherichia coli*. *Veterinary Microbiology* 124; 47–57.
  25. Jamalludeen, N.M., and D. M. Shakir. 2022. In vitro effect of newly bacteriophages isolates against *Escherichia coli* serogroups collected from the local hospital laboratory: their specific characterization and identification. *J Microbiol Biotech Food Sci* : 11 (6) e5478. <https://doi.org/10.55251/jmbfs.5478>.
  26. Harris, R. J. C. 1954. Biological Application of Freezing and Drying. Academic Press Inc. New York. PP.215-252.
  27. Toval, F., CD. Köhler., U. Vogel., F.Wagenlehner., A. Mellmann., A. Fruth., A. Schmidt., H. Karch., M. Bielaszewska., and U. Dobrindta. 2014. Characterization of *Escherichia coli* Isolates from Hospital Inpatients or Outpatients with Urinary Tract Infection. *Journal of Clinical Microbiology.* Vol. 52 Number 2. p. 407– 418.
  28. Son, M. S., and R. K. Taylor. 2021. Growth and Maintenance of *Escherichia coli* Laboratory Strains. *Curr Protoc.*1(1): e20. doi:10.1002/cpz1.20.
  29. Jensen, G. M. K. Frydendahl, O. Svendsen, C. B. Jorgensen, S. Cirera, M. Fredholm, JP. Nielsen, and K. Moller. 2006. Experimental infection with *Escherichia coli* O149: F4ac in weaned piglets. *Vet. Microbiol.* 115, Issues 1–3, 15 June 2006, Pages 243-249. <https://doi.org/10.1016/j.vetmic.2006.01.002>.
  30. Moon, H. W., B. Nagy., and R. E. Isaacson. 1977. Intestinal colonization and adhesion by enterotoxigenic *Escherichia coli*: ultrastructural observations on adherence to ileal epithelium of the pig. *J.Infect.Dis.* 136 Suppl: 124-129.
  31. Chibani-Chennoufi, S., J. Sidoti., A. Bruttin., E. Kutter., S. Sarker., and H. Bru’ssow. 2004. In Vitro and In Vivo Bacteriolytic Activities of *Escherichia coli* Phages: Implications for Phage Therapy. *Antimicrob. Agents Chemother.* Vol. 48, p. 2558–2569 .No. 7 0066-4804/04/\$08.00 0 DOI: 10.1128/AAC.48.7.2558–2569.2004.
  32. Kasman, L. M. 2005. Barriers to coliphage infection of commensal intestinal flora of laboratory mice. *Viol. J.* 2:24.
  33. Smith, H. W., M. B. Huggins., and K. M. Shaw. 1987. The control of experimental *Escherichia coli* diarrhea in calves by means of bacteriophages. *J. Gen. Microbiol.* 133: 1111-1126.
  34. Bruttin, A., and H. Brussow. 2005. Human volunteer receiving *Escherichia coli* phage T4 orally: a safety test of phage therapy. *Antimicrob. Agents Chemother.* 49:2874-2878.
  35. Projan, S. 2004. Phage -inspired antibiotics? *Nature Biotechnol.* 22:167-168.

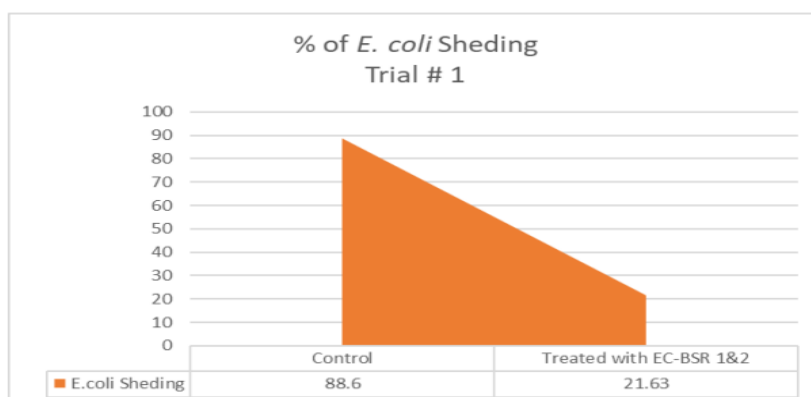


**FIG. 1:** Electron microscope appearance of phages EC-BSR1 and EC-BSR2. The phages have a neck and a contractile tail and icosahedral head. Bar = 50 nm (25).

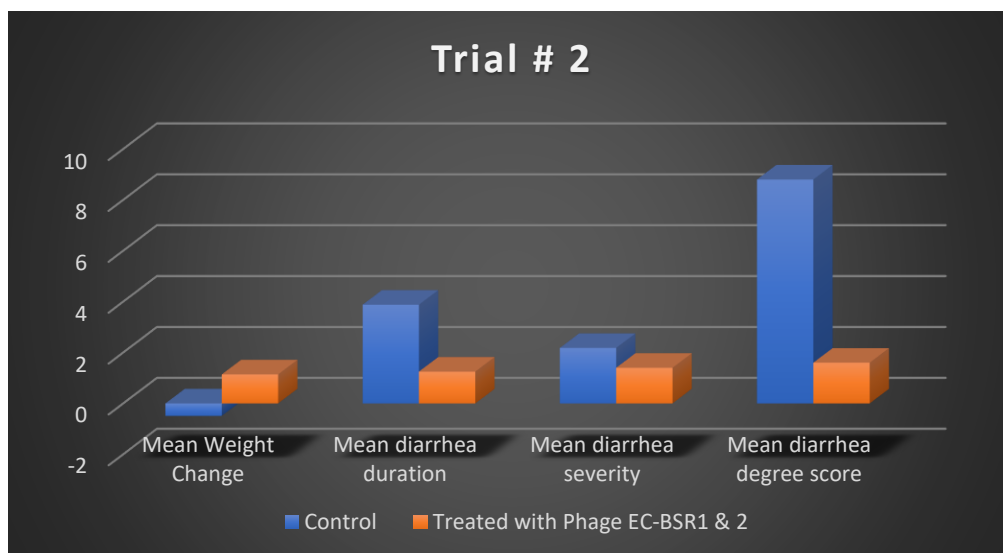


**FIG. 2:** The effects of a combination of phages (EC-BSR1 and EC-BSR2) on weight change and diarrhea, when the phages were administered after experimental diarrhea due to *E. coli* strain had developed (Trial 1).

Statistical analysis (ANOVA) showed significant differences in weight change, duration of diarrhea, diarrhea score, degree of diarrhea score for the pigs that were treated with phages (EC-BSR1 and EC-BSR2) compared with the control group.

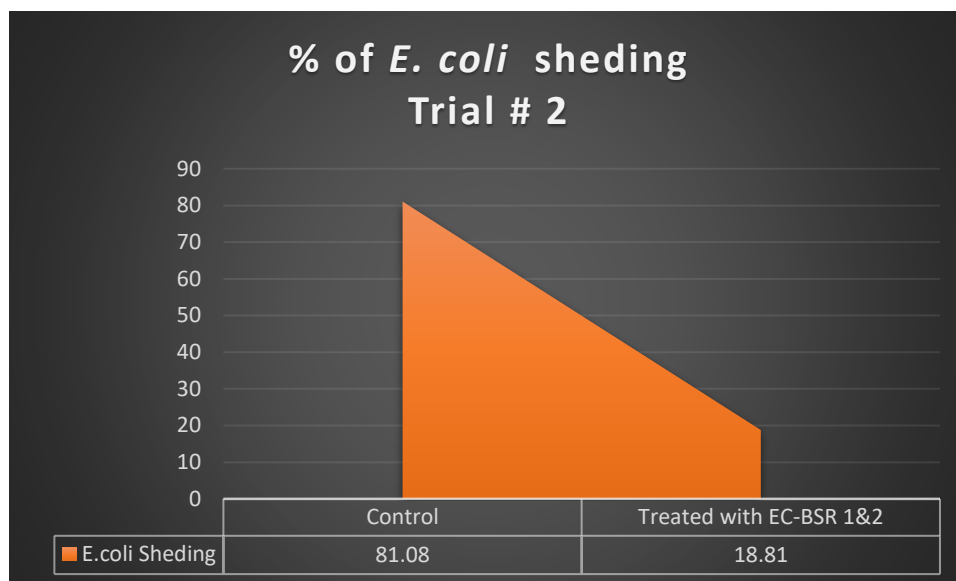


**FIG. 3:** The effects of a combination of phages (EC-BSR1 and EC-BSR2) on shedding of the challenge *E. coli*, when the phages were administered after experimental diarrhea due to *E. coli* strain had developed (Trial 1).



**FIGURE 4:** The effects of a combination of phages (EC-BSR1 and EC-BSR2) on weight change and diarrhea, when the phages were administered after experimental diarrhea due to *E. coli* strain had developed (Trial 2).

Statistical analysis (ANOVA) showed significant differences in weight change, duration of diarrhea, diarrhea score, degree of diarrhea score for the Guinea pigs that were treated with phages (EC-BSR1 and EC-BSR2) compared with the control group.



**FIG. 5:** The effects of a combination of phages (EC-BSR1 and EC-BSR2) on shedding of the challenge *E. coli*, when the phages were administered after experimental diarrhea due to *E. coli* strain had developed (Trial 2).