Jurnal Sains Farmasi & Klinis
p-ISSN: 2407-7062 | e-ISSN: 2442-5435
homepage: http://jsfk.ffarmasi.unand.ac.id
DOI : 10.25077/jsfk.10.3.368-373.2023

ORIGINAL ARTICLE J Sains Farm Klin 10(3):368–373 (Desember 2023) | DOI: 10.25077/jsfk.10.3.368-373.2023

Isolation of *Escherichia coli* bacteriophages from Domestic Wastewater in Tangerang Selatan

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ABSTRACT: In recent years, there has been a continuous increase in the prevalence of antibiotic resistance among bacteria responsible for infectious diseases. Several studies have shown that the occurrence of the COVID-19 pandemic is a major factor contributing to this rapid increase. To overcome this challenge, one promising approach is the use of bacteriophages as a treatment option. Therefore, this study aims to isolate *E. coli* lytic bacteriophages as an alternative antibiotic for treating secondary bacterial infections of COVID-19. Isolation of bacteriophages was carried out using the plaque assay method with double layer agar technique. The isolate obtained was purified and tested for effectiveness in lysing *E. coli* ATCC 25922 bacterial cells. The results showed that *E. coli* had resistance to antibiotics, such as Amoxicillin, Clindamycin, Ciprofloxacin, Azithromycin, and Trimethoprim/sulfamethoxazole. BEB1 isolate obtained from domestic waste had activity in inhibiting the growth of the test bacteria with an effectiveness of 72% at the 25th hour. Based on these findings, it had the potential to be developed as an alternative antibiotic agent to overcome infectious diseases caused by *E. coli* ATCC 25922.

Keywords: **antibiotic; bacteriophage; E. coli; COVID-19.**

Introduction

The Corona Virus Disease 2019 (COVID-19) first appeared in December 2019 in Wuhan, China, and swiftly extended its impact worldwide, severely affecting public health. This viral disease often manifests along with other comorbidities, such as hypertension, diabetes, obesity, and advanced age, intensifying its consequences. COVID-19 is caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) virus, which only affects the respiratory system as the point of entry into the host. However, it can lead to complications in various organs, including gastrointestinal infection symptoms, such as vomiting or diarrhea during the early stage of the disease [1].

In 2019, the COVID-19 epidemic rapidly swept across the globe, and a significant surge in cases of diarrhea was observed [2]. Diarrhea is a gastrointestinal condition, characterized by frequent urination and watery or loose feces. Furthermore, several studies have shown that it is the most prevalent symptom of coronavirus infection. The prevalence of diarrhea in COVID-19 patients reached 30% in MERS-CoV and 10.6% in SARS-CoV [3]. The high mortality rate among affected individuals can be attributed in part to antibiotic-resistant bacterial infections and the occurrence of multi-drug-resistant (MDR) strains. In terms of treatment, people hospitalized with the condition are typically given antibiotics, which can contribute to the development of bacterial resistance [4,5].

Diarrhea is often caused by bacterial infection, particularly *Escherichia coli*. According to previous studies, there has been an increase in antibiotic resistance in *E. coli,* especially toward betalactam, and quinolone. This introduction of new drugs is likely not to provide a lasting solution, as bacteria tend to develop resistance to alternative options. Bacteriophages are antibacterial agents with the potential to be a very promising therapeutic option for infections, either alone or in combination with antibiotics [6]. The agents attack bacteria without affecting the host eukaryotic cells [7]. The use of bacteriophages carries several potential advantages, as these viruses are highly specific, targeting a single type or strain of bacteria while causing minimal damage to the regular flora [6].

The COVID-19 pandemic has witnessed a concerning increase in antibiotic resistance among *E. coli* bacteria necessitating further investigations to address

Article history
Received: 01 Des 2023
Accepted: 05 Des 2023
Published: 30 Des 2023
Access this article

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this critical issue. Bacteriophages offer a promising avenue for combating bacterial infections, both alone or in combination with antibiotics, paving the way for a future strategy to combat COVID-19 and its associated pathogens. This indicates that it is important to isolate *E. coli* lytic bacteriophages to overcome this condition [8–10]. The results of this study are expected to provide information on the effectiveness of bacteriophages as a bacterial control agent, especially in secondary infections of *E. coli* bacteria among COVID-19 patients.

Methods

Bacterial Stock Preparation

E. coli ATCC 25922 bacteria were collected from the microbiology laboratory of the Faculty of Medicine, University of Indonesia. Furthermore, the bacteria were grown on Nutrient Agar (NA) media (Merck, Millipore Corp, USA) as bacterial stock.

Resistance test of *E. coli* to Several Antibiotics

E. coli ATCC 25922 bacteria were tested for resistance to several antibiotics, such as Amoxicillin, Clindamycin, Ciprofloxacin, Azithromycin, and Trimethoprim/sulfamethoxazole. Furthermore, the antibiotics were diluted to several concentrations and tested for antibacterial activity using the disc diffusion method. A total of 50 µL of antibiotic solution was dripped onto the disc and placed in NA media, which had been inoculated with *E. coli* ATCC 25922 bacteria. The culture was then incubated at 37℃ for 24 hours, and the antibacterial activity could be observed by measuring the diameter of the clear zone formed. Resistance could occur when the impacted clear zone was very small or did not form [11].

Isolation of Bacteriophage

Based on a modified study method [12], bacteriophage samples were taken from several sources of domestic wastewater in South Tangerang. The first stage was the enrichment of bacteriophage isolates. A total of 14 mL of samples were taken and centrifuged at 5000 rpm for 5 minutes, followed by filtration using a 0.22 µm membrane filter. The filtrate (4.5 mL) obtained was then added with 0.5 mL Triptose soy broth (TSB), and 0.5 mL *E. coli* culture , followed by incubationfor 24 hours at 37°C using a shaker incubator. After 24 hours, the enriched sample was centrifuged for 25 minutes at 5000 rpm and filtered through a 0.22 m membrane filter. The filtrate was diluted to a concentration of 10-7 using physiological saline. The last three dilutions were made in 100 L (10-5, 10-6, and 10-7) and incubated for 30 minutes at 37°C with 100 L of *E. coli* bacterial culture. Furthermore, the mixture was cultivated on double-layer media after incubation. At 37°C, the samples were incubated, and the presence of bacteriophage on the medium indicated the proliferation of plaque 24 hours later, followed by counting the plaques.

Purification of Bacteriophage

A colony counter was used to count the plaques that grew on the double-layer media. Plaques were classified according to their size and clarity, and samples with various properties were purified. Bacteriophage was taken from one clear plaque 1cm and placed in an Eppendorf tube containing PBS (Phosphate Buffer Salin), followed by homogenization and incubation for 30 minutes at room temperature. The process was then continued with centrifugation at 5000 rpm for 20 minutes, and filtered using a membrane filter. The purified bacteriophages were then stored at 4℃ [12].

Effectiveness of Bacteriophages in Lying Bacterial Populations in Vitro

A modified approach was used to assess the effectiveness of bacteriophages in lysing *E. coli* bacteria. The treated culture consisted of 100 µL of *E. coli* culture combined with 100 µL of pure bacteriophage. As a control, 100 µL of *E. coli* culture was grown without bacteriophage therapy. Controlled and treated cultures were then cultured in NA media at room temperature for 25 hours, and the microbe population was observed throughout this time. During the incubation period (0, 5, 10, 15, 20, and 25 hours), the population was monitored by counting the number of plaques created every predefined hour [12].

Statistical Analysis

All data in this study were determined by the mean value of replicate in each analysis.

Result and Discussion

The resistance rate of *E. coli* bacteria to most antibiotics has been reported to be on an increasing annual trend [13]. F Furthermore, most antibiotic resistance factor genes were found in Enterobacteriaceae, and plasmids containing these genes could be transferred across bacteria and even between species. This suggested that great resistance to new antibiotics could occur [14]. The COVID-19 pandemic is one of the causes of increased antibiotic resistance in *E. coli* bacteria [15]. In this study, the test pathogenic bacteria used were *E.*

Table 1. Resistance pattern of *Escherichia coli* ATCC 25922

No	Antibiotics	Disk content (ug/ml)	Interpretation of diameter criteria zone (mm)*			Diameter zone (mm)	Results
			Sensiti-ve (S)	Intermedi-ate (I)	Resista-nce (R)		
1	Amoxicillin	1	≥ 18	14- 17	≤13	0	R
2	Azithromycin	15	≥ 13	-	≤12	0	R
3	Clindamycin	0,2	≥ 21	15- 20	≤14	14	R
4	Ciprofloxacin	1,5	≥ 21	16- 20	≤15	8	R
5	Trimethopr m/sulfamet oxazole	0,125	≥ 16	16- 15	≤10	8	R

*Base on Clinical and Laboratory Standards Institut (CLSI 2019); R: resistant, I: intermediate

coli bacteria. The microbe was tested for its resistivity to determine the extent of its ability. Table 1 shows the results of resistance testing of several antibiotics against *E. coli* bacteria. The results showed that the microbe was resistant to Amoxicillin, Clindamycin, Ciprofloxacin, Azithromycin, and Trimethoprim/sulfamethoxazole antibiotics.

E. coli played an essential role in life because it could cause infections and comprised the microbiota of the human digestive system. Multidrug resistance had become an alarming problem in the health field. Furthermore, the microbe was intrinsically susceptible to almost all antimicrobial agents. This bacterial species also had a large capacity to accumulate resistance genes, mostly through horizontal gene transfer [16]. The species used in this study showed resistance to several antibiotics, including Amoxicillin, Clindamycin, Ciprofloxacin, Azithromycin, and Trimethoprim/sulfamethoxazole. This threatened the treatment of infectious diseases caused by bacteria, particularly *E. coli* because fewer drug options were previously curable. According to Poirel et al., (2018), *E. coli* was resistant to antibiotics, such as tetracyclines, phenicols, sulfonamides, trimethoprim, and fosfomycin [17].

Antibiotic resistance is a global health issue comprising gene transfer among bacteria, people, animals, and the environment. This condition could occur due to mutations in pre-existing bacterial genomes or foreign DNA transformation. Mutations were more likely to occur and persist in patients treated with antibiotics [18,19]. Pathogenic bacteria constantly acquired new resistance elements from different species, leading to the presence of numerous resistant microbes compared to new treatments [20]. During the COVID-19 pandemic, antibiotic resistance in Gram-negative bacteria, such as *Acinetobacter baumannii, Klebsiella pneumonia, E. coli,* and *Pseudomonas aeruginosa* was documented [15].

Bacteriophage was one of the solutions to the increasing antibiotic resistance. Several waste samples were used in the investigation to isolate bacteriophages. However, only one sample, namely household liquid waste 2, contained *E. coli* lytic bacteriophage based on the results of isolation from six samples. These complexes were one solution to reduce the rate of bacterial resistance to antibiotics. According to previous studies, they were infectious viruses that replicated inside certain bacterial cells. The life cycle of bacteriophages depends on the presence of host bacteria. Bacteriophages could be found in nature in relation to the availability of their bacterial hosts. The most common habitats to live and thrive included soil, oceans, deserts, wastewater, sewage, animal intestines, and activated sludge [21-23]. Table 2 displayed

Table 2. Source of sample bacteriophage

Source of phage	Amount of sample	Phage isolation result
Situgintung river	25 ml	-
Livestock waste, Ciputat	10 g	-
Hospital waste tangerang selatan	25 ml	-
Landfills, Ciputat	25 ml	-
Domestic waste 1	25 ml	-
Domestic waste 2	25 ml	+

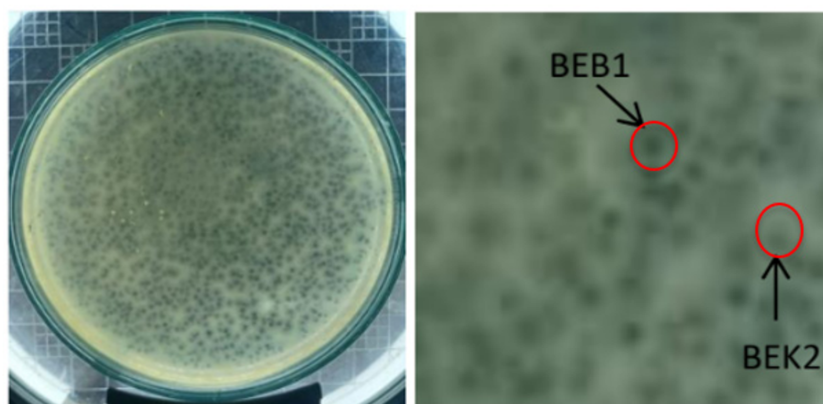


Figure 1. Cultures of *E. coli* ATCC 25922 infected with bacteriophages on double-layer media were incubated at 37 °C for 24 hours

the findings of bacteriophage isolation from various samples. The samples utilized had a significant impact on the success or failure of the procedures. The isolate must contain a host for the bacteriophage to reproduce.

In this study, two morphologically distinct bacteriophages were successfully isolated from residential sewage, namely BEB 1 and BEK 2. BEB 1 had a greater plaque diameter and was clearer, hence, it was chosen to test its efficacy. Plaque diameter and clarity could be used to calculate the time required for the virus to replicate and produce new progeny. The new samples lysed the cells and were ready to infect other host cells, thereby increasing their population. Furthermore, the diffusion ability in the growing media influenced plaque diameter. The time required for the bacteriophage to engage with the host was affected by the molecular density of the agar employed [24].

The presence of growing bacteriophages was characterized by the presence of clear, round-shaped plaques between bacteria that grew on a double layer of media in a petri dish or lysis. Plaques occur due to the infection of harmful bacteria by these viruses. From one bacteriophage that infected bacteria, 200-300 additional species were created, leading to the lysis of infected bacteria.

Based on the results of this study, lytic bacteriophage *E. coli* isolates were successfully isolated from domestic waste. From the isolation results, 2 isolates with different macroscopic morphology were obtained, namely BEB 1 and BEK 2 (Figure 1). Existing samples were selected based on morphological differences in terms of size, clarity, and plaque shape, as shown in Table 3. BEB1 formed plaques with a size of 1 mm and a clear large regular shape, while BEK2 formed clear plaques with a smaller size of 0.8 mm.

The diameter of the clear zone formed by isolate BEB1 was clearer and larger compared to BEK2. This indicated that the replication ability of BEB1 was faster than BEK2, leading to a higher bacteriophage titer. Furthermore, the isolate was chosen to test the ability of bacteriophages to reduce the population of *E. coli* bacteria. The ability of BEB1 to lyse *E. coli* bacteria was categorized as very good, which had an impact on reducing the number of host bacterial populations. Measurement of the effectiveness of the samples was carried out by comparing the number of living cells between control (*E. coli* without BEB1) and treatment cultures (*E. coli* infected with BEB1) every 5 hours of incubation.

The effectiveness of bacteriophages BEB1 in lowering the population of *E. coli* bacteria can be determined by comparing the population growth of control and treatment cultures. Every 5 hours, the number of cells was counted using the Total Plate Count method, which could directly

Table 3. Characteristics of bacteriophage plaque

Phage isolates	Characteristic of plaque	Diameter of plaque (mm)
BEB1	Translucent plaque	1
BEK2	Translucent plaque	0,8

BE Bacteriophage *E. coli*.

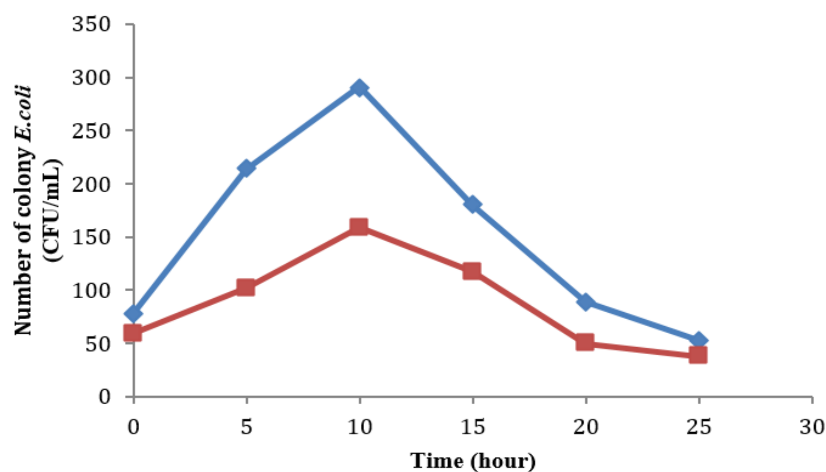


Figure 2. Curve of effectiveness bacteriophage lying *E. coli*

■ : Control
■ : Infected by bacteriophages

count live bacterial colonies. The growth line pattern increased after the fifth hour, indicating that *E. coli* bacteria had reached the log phase. However, differences could be seen between the control and treatment populations. The population of *E. coli* infected with BEB 1 decreased by 48%. Furthermore, the reduction continued until it reached 72% of the total control population at the 25th hour. The results showed that it took time for the bacteriophage virus to infect bacterial cells [25,26].

The reduction in bacterial cell population caused by bacteriophage isolates BEB1 indicated that the virus was efficient against *E. coli* and could be used as a control agent for infection. Since the first 5 hours of incubation, the population of *E. coli* cultures infected with BEB1 decreased, reaching 48% lower compared to the control. The decline continued until the 25th hour of incubation when the number of *E. coli* infected with BEB1 isolate was 38 x109 CFU/mL, and those in the control without administration of BEB1 isolate was 53 x109 CFU/mL. This indicated a 72% decrease in the number of bacteria, as shown in Figure 2.

Bacteriophages attached to host cells randomly to prevent the attachment of all cells. The infection process was also affected by the low recognition of bacteriophage receptors in the host. Furthermore, the low density of the cells in the culture also reduced the potential for viral attachment. Abiotic factors, such as temperature, pH, salinity, and ions affect the interaction between host and bacteriophage [25]. The decrease in the bacterial population treated with isolates BEB 1 indicated that the virus significantly reduced the growth of *E. coli*.

Conclusion

In conclusion, lytic bacteriophages of *E. coli* bacteria from household waste samples were successfully isolated in this study. The isolation results obtained 2 different isolates based on the morphology of the plaque, namely BEB1 and BEK2. *E. coli* bacteria used in this study had high resistance to several antibiotics, including Amoxicillin, Clindamycin, Ciprofloxacin, Azithromycin, and Trimethoprim/sulfamethoxazole. The results showed that BEB1 could lyse *E. coli* bacterial cells up to 72% at the 25th hour compared to the control. This indicated that the bacteriophage had the potential to be developed as a therapeutic agent for infectious diseases caused by *E. coli*

Acknowledgement

The authors are grateful to Puslitpen UIN Syarif Hidayatullah Jakarta for the research grant number B -302/ LP2M-PUSLITPEN/ T L .0 3 /2022.

References

[1]. Villapol S. Gastrointestinal symptoms associated with COVID-19: impact on the gut microbiome. Transl Res. 2020;226:57–69. https://doi.org/10.1016/j.trsl.2020.08.004.

[2]. D'Amico F, Baumgart DC, Danese S, Peyrin-Biroulet L. Diarrhea During COVID-19 Infection: Pathogenesis, Epidemiology, Prevention, and Management. Clin Gastroenterol Hepatol. 2020;18(8):1663–72. https://doi.org/10.1016/j.cgh.2020.04.001.

[3]. Chan JFW, Yuan S, Kok KH, To KKW, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 2020;395(10223):514–23. https://doi.org/10.1016/S0140-6736(20)30154-9.

[4]. Chaudhry R, Sreenath K, Vinayaraj E V., Sahoo B, Vishnu Narayanan MR, Kiran KVPS, et al. Mycoplasma pneumoniae co-infection with SARS-CoV-2: A case report. Access Microbiol. 2021;3(3):1–4. https://doi.org/10.1099/acmi.0.000212.

[5]. Bhargava S, Negbenebor N, Sadoughifar R, Ahmad S, Kroumpouzos G. Virtual conferences and e-learning in dermatology during COVID-19 pandemic: Results of a web-based, global survey. Clin Dermatol. 2021;39(3):19–20. https://doi.org/https://doi.org/10.1016/j.clindermatol.2021.06.002.

[6]. Alomari MMM, Dec M, Nowaczek A, Puchalski A, Wernicki A, Kowalski C, et al. Therapeutic and Prophylactic Effect of the Experimental Bacteriophage Treatment to Control Diarrhea Caused by E. coli in Newborn Calves. ACS Infect Dis. 2021;7(8):2093–101. https://doi.org/10.1021/acsinfecdis.1c00010.

[7]. Abdul Choliq F, Martosudiro M, Istiqomah I, Fanhash Nijami M. ISOLASI DAN UJI KEMAMPUAN BAKTERIOFAG SEBAGAI AGENS PENGENDALI PENYAKIT LAYU BAKTERI (Ralstonia solanacearum) PADA TANAMAN TOMAT. VIABEL J Ilm Ilmu-Ilmu Pertan. 2020;14(1):8–20. https://doi.org/10.35457/viabel.v14i1.996.

[8]. Amelia P, Elya B, Hanafi M. Antioxidative activity of xanthone from Garcinia benthami Pierre leaves. Int J PharmTech Res. 2014;7(2):254–7.

[9]. Amelia P, Nugroho AE, Hirasawa Y, Kaneda T, Tougan T, Horii T, et al. Two new bisindole alkaloids from Tabernaemontana macrocarpa Jack. J Nat Med. 2021;75(3):633–42. https://doi.org/10.1007/s11418-021-01510-4.

[10]. Amelia P, Nugroho AEAE, Hirasawa Y, Kaneda T, Tougan T, Horii T, et al. Two new sarpagine-type indole alkaloids and antimalarial activity of 16-demethoxycarbonylvoacamine from Tabernaemontana macrocarpa Jack. J Nat Med. 2019;73(4):820–5. https://doi.org/10.1007/s11418-019-01317-4.

[11]. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition. Vol. 40, Clsi. 2020. 50–51 p.

[12]. Sufa HI, Budiarti SRI, Rusmana I. Diversity of uropathogenic Escherichia coli lytic phage from Cisadane river, west Java, Indonesia based on morphology and protein molecular weight characteristics. Biodiversitas. 2018;19(6):2359–64. https://doi.org/10.13057/biodiv/d190646.

[13]. Pormohammad A, Nasiri MJ, Azimi T. Prevalence of antibiotic resistance in escherichia coli strains simultaneously isolated from humans, animals, food, and the environment: A systematic review and meta-analysis. Infect Drug Resist. 2019;12:1181–97. https://doi.org/10.2147/IDR.S201324.

[14]. Critchley IA, Nicole Cotroneo, Pucci MJ, Rodrigo Mendes. The burden of antimicrobial resistance among urinary tract isolates of Escherichia coli in the United States in 2017. PLoS One. 2019;14(12):1–11. https://doi.org/10.1371/journal.pone.0220265.

[15]. Sulayyim HJ Al, Ismail R, Hamid A Al, Ghafar NA. Antibiotic Resistance during COVID-19: A Systematic Review. Int J Environ Res Public Health. 2022;19(19). https://doi.org/10.3390/ijerph191911931.

[16]. Frazão N, Sousa A, Lässig M, Gordo I. Horizontal gene transfer overrides mutation in Escherichia coli colonizing the mammalian gut. Proc Natl Acad Sci U S A. 2019;116(36):17906–15. https://doi.org/10.1073/pnas.1906958116.

[17]. Poirel L, Madec J-Y, Lupo A, Schink A-K, Kieffer N, Nordmann P, et al. Antimicrobial Resistance in Escherichia coli Sepsis. Pediatr Infect Dis J. 2018;6(4). https://doi.org/10.1097/01.inf.0000141751.73083.7a.

[18]. Tuan VP, Narith D, Tshibangu-Kabamba E, Dung HDQ, Viet PT, Sokomoth S, et al. A next-generation sequencing-based approach to identify genetic determinants of antibiotic resistance in cambodian Helicobacter pylori clinical isolates. J Clin Med. 2019;8(6). https://doi.org/10.3390/jcm8060858.

[19]. Windels EM, Michiels JE, Fauvart M, Wenseleers T, Van den Bergh B, Michiels J. Bacterial persistence promotes the evolution of antibiotic resistance by increasing survival and mutation rates. ISME J. 2019;13(5):1239–51. https://doi.org/10.1038/s41396-019-0344-9.

[20]. Larsson DGJ, Flach CF. Antibiotic resistance in the environment. Nat Rev Microbiol. 2022;20(5):257–69. https://doi.org/10.1038/s41579-021-00649-x

[21]. Mizuno CM, Luong T, Cederstrom R, Krupovic M, Debarbieux L, Roach DR. Isolation and characterization of bacteriophages that infect citrobacter rodentium, a model pathogen for intestinal diseases. Viruses. 2020;12(7). https://doi.org/10.3390/v12070737.

[22]. Akremi I, Merabishvili M, Jlidi M, Haj Brahim A, Ben Ali M, Karoui A, et al. Isolation and Characterization of Lytic Pseudomonas aeruginosa Bacteriophages Isolated from Sewage Samples from Tunisia. Viruses. 2022;14(11):1–15. https://doi.org/10.3390/v14112339.

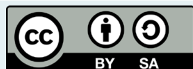
[23]. Skliros D, Kalatzis PG, Kalloniati C, Komaitis F, Papathanasiou S, Kouri ED, et al. The development of bacteriophage resistance in vibrio alginolyticus depends on a complex metabolic adaptation strategy. Viruses. 2021;13(4). https://doi.org/10.3390/v13040656.

[24]. Lingga R, Budiarti S, Rusmana I, Wahyudi AT. Isolation, characterization and efficacy of lytic bacteriophages against pathogenic escherichia coli from hospital liquid waste. Biodiversitas. 2020;21(7):3234–41. https://doi.org/10.13057/biodiv/d210745.

[25]. Li L, Yu M, Yang C, Deng C, Ma L, Liu Y. Effects of abiotic factors on the stability and infectivity of polyvalent coliphage. Water Sci Technol. 2022;85(1):141–51. https://doi.org/10.2166/wst.2021.505.

[26]. Alharbi NM, Ziadi MM. Saudi Journal of Biological Sciences Wastewater as a fertility source for novel bacteriophages against multi- drug resistant bacteria. Saudi J Biol Sci. 2021;28(8):4358–64. https://doi.org/10.1016/j.sjbs.2021.04.025.

[27]. Li L, Yu M, Yang C, Deng C, Ma L, Liu Y. Effects of abiotic factors on the stability and infectivity of polyvalent coliphage. Water Sci Technol. 2022;85(1):141–51. https://doi.org/10.2166/wst.2021.505.



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