Jurnal Biota Prodi Biologi Universitas Islam Negeri Raden Fatah Palembang

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To cite this article before publication: Sunarti. R.N. *et al* (2025) Potential of Bacteriophages as Non-Alcoholic Antiseptic Hand Sanitizer. Jurnal Biota. In press
http://jurnal.radenfatah.ac.id/index.php/biota/article/view/25232

Potential of Bacteriophages as Non-Alcoholic Antiseptic Hand Sanitizer

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ABSTRACT

Bacteriophages, or phages, are viruses that can infect and replicate within bacterial cells, such as *Escherichia coli*. Phages demonstrate a strong ability to lyse host bacteria and exhibit high survivability, making them a promising innovation for use in non-alcoholic antiseptic products, such as hand sanitizer sprays and bacteriophage gels. This study aims to evaluate the effectiveness of bacteriophage-based hand sanitizer sprays and gels in reducing E. coli growth and total microbial colonies on palms, compared to commercial alcohol-based hand sanitizers. The method used in this study is a descriptive quantitative approach using an experimental method, specifically the Hand Sanitizer Spray and Bacteriophage Gel Test as Non-Alcohol Antiseptics. The average total bacterial colonies on male palms for the control treatment, phage gel sanitizer and commercial gel hand sanitizer were 1.95 x 10^4 CFU/mL; 1.15 x 10^3 CFU/mL; 2.55 x 10^3 CFU/mL, respectively, while on female palms, the values were 2.35 x 10^4 CFU/mL; 3.05 x 10^3 CFU/mL; 1.65 x 10^3 CFU/mL. The average total bacterial colonies on male palms for control treatment, phage sanitizer spray and commercial sanitizer spray were 1.30 x 10^5 CFU/mL; 2.05 x 10^3 CFU/mL; 9, 04 x 10^4 CFU/mL, respectively, while on female palms, the value was 1.58 x 10^5 CFU/mL; 8.36 x 10^3 CFU/mL; 8.79 x 10^4 CFU/mL. The results demonstrated that both bacteriophage hand sanitizer gel and spray significantly reduce bacterial colonies on palms, with phage-based hand sanitizer showing greater efficacy than commercial alcohol-based hand sanitizer.

Keywords: *Antimicrobial; Bacteriophage; Escherichia coli; Hand Sanitizer.*

Introduction

Bacterial infections can cause disease and become a health problem that develops over time. This condition is caused by the rapid growth and spread of bacteria, which can transfer

from one human to another, from animals to humans, from the air and public spaces or facilities, and even through food consumption [1]. One such bacterium that causes disease is *Escherichia coli* (*E. coli*) [2]. Pathogenic *E. coli* strains can cause meningitis, urinary tract infections, and watery diarrhea [3], as well as mild to severe bloody diarrhea that can develop into hemolytic uremic syndrome, potentially leading to kidney failure [4]. These bacteria can easily transfer to the hands through physical contact with the contaminated surfaces [5].

A commonly used antiseptic for easy hand washing in spray and gel form is hand sanitizer, especially during the COVID-19 pandemic [6]. Hand sanitizers are more efficient and effective than soap and water, making them a popular choice [7]. Hand sanitizers (in spray or gel form) typically contain alcohol (a synthetic antiseptic) that can prevent, inhibit the growth, and even kill disease-causing germs quickly. However, their repeated use can cause dry hand skin, irritation, and allergies [8].

Bacteriophages, or phages, offer a potential solution to the spread of resistant bacteria [9]. Bacteriophages are viruses that infect and multiply inside bacterial cells, and they were discovered in the 1900s [10]. Phages can be isolated from freshwater, seawater, soil, the digestive tract of animals and humans, as well as the genitourinary tract, skin, and milk [11].

Phages infect and lyse bacterial cells by releasing their genetic material into the bacterial cytoplasm [12]. Phages are target-specific for certain bacterial strains or even several bacterial strains simultaneously, making them a promising solution to the problem of bacterial resistance to antimicrobial drugs [13]. According to research [14], phages consist of a nucleic acid molecule surrounded by a protein shell called a capsid. Unlike other viruses that multiply in multicellular organisms, bacteriophages survive and multiply in cellular organisms. The specific properties of phages allow for accurate, fast, efficient, and inexpensive results.

Phages can be used as an alternative main ingredient in hand sanitizers. The use of phages is considered more effective than alcohol for treating pathogenic bacterial infections. Pathogenic bacteria, which cause various diseases, can be controlled using environmentally friendly phages [15]. Phages have the potential to serve as bio-sanitizers in industries, food processing, and daily life, such as controlling *E. coli* growth in cherry tomatoes [16]. Bacteriophage products like Listex P100™ and Eco Shield™ are successfully used in dairy, meat, farm, and marine products [19].

Hand sanitizers made from phages have many advantages, including being suitable for individuals who are allergic or sensitive to chemicals. The materials are easy to obtain, cost-effective, and the manufacturing process is relatively quick [17]. Bacteriophages can only infect bacteria and can remain viable for long periods, preventing bacterial growth. Reports show that

phages have low toxicity, are environmentally friendly, non-corrosive, and have no harmful or pungent odors [18]. Bacteriophages do not have harmful or toxic effects on eukaryotic cells, do not affect the sensory properties of food, and can be applied during food processing and packaging to reduce pathogen contamination. The specific nature of Bacteriophages, which only infect and lyse certain bacterial species due to their specificity and narrow antibacterial spectrum, makes them safe for eukaryotic cells. Several bacteriophage products have been approved by the FDA and the United States Environmental Protection Agency (EPA) as commercial products such as List Shield™ and Salmo Fresh™ [19].

Phages can also be used to kill biofilm-producing pathogenic bacteria on equipment surfaces. The potential of phages to control pathogenic bacteria underscores the importance of this study, which aims to assess the effectiveness of bacteriophages as a non-alcoholic antiseptic agent in spray and gel hand sanitizers as an alternative solution to replace alcohol in commercial hand sanitizers.

Material and Method

This research was conducted from September to October 2024. The study used a quantitative descriptive research design, which involved conducting tests in the integrated laboratory of UIN Raden Fatah Palembang. The study used the PCA method to test hand sanitizer (HS) on the palm. Testing was done by growing cultures on PCA media from sterile cotton swabs samples taken from palms that had not been treated with phage cocktail HS (control), palms treated with phage cocktail HS in spray and gel form, and palms treated with commercial HS containing alcohol. The results were compared to determine whether bacteriophage-based HS spray and gel were effective as a substitute for alcohol-based commercial HS.

The results of the study are presented in the form of data tables to see an overview of the application test of bacteriophage spray hand sanitizer and non-alcoholic antiseptic gel. Data on the total number of microbial colonies are presented in averages with standard deviations.

1. Equipment and Materials

The tools used were an autoclave, vortex, centrifuge, oven, hotplate, incubator, spectrophotometer, shaker, and other laboratory equipment. The materials required were LB media (13 gr/L), NA (20 gr/L), MHA (38.0 gr/L), PCA (23.5 gr/L), SM buffer (5.8 g NaCl, 2.0 g $MgSO_4$-7 H_2O, 50 ml 1 M Tris-HCl pH 7.4 in 1 L H_2O), and other necessary reagents.

2. *Working Procedure*

a. Bacterial Culture Rejuvenation

Purified *Escherichia coli* isolates were rejuvenated in 50 mL of LB medium, then incubated for 24 hours at 37^{o}C in a shaker incubator set to 100 rpm [20].

b. Phage enrichment

Phage enrichment was performed using the double-layer method consisting of NA and soft agar media. *Escherichia coli* cultures, which had been incubated for 24 hours in 50 mL of liquid LB, were sampled (100 µL) and mixed with 100 µL of filtered supernatant in a sterile test tube. This mixture was then incubated at 37°C for 30 minutes. Following incubation, 5 mL of soft agar at 47°C was added, and the mixture was vortexed to ensure homogeneity. The homogeneous suspension was then poured into a Petri dish containing NA media, gently rotated to distribute the mixture evenly, and allowed to solidify. Incubation was carried out at 37°C for 24 hours [20].

c. Bacteriophage Purification

Single plaques with their own characteristics, obtained from the plaque assay, were transferred using a Pasteur pipette into a tube, and then mixed with 5 mL of SM buffer. The phage suspension was homogenized and left at room temperature for 5-10 minutes. The suspension was then centrifuged at 2500 rpm for 20 minutes, and this process was repeated 3 times. The resulting supernatant was filtered through a 0.22 µm pore filter and stored as phage stock [20].

d. Bacteriophage Quantification

Single plaques with individual features obtained from the plaque assay were transferred using a Pasteur pipette into a tube and then mixed with 5 ml of buffered SM solvent. The phage suspension was homogenized and left at room temperature for 5-10 minutes. The suspension was then centrifuged at 2500 rpm for 20 minutes and repeated 3 times. The supernatant formed was then filtered using a 0.22 µm porous filter and stored as phage stock [20].

$$\text{Virus Titer}\left(\frac{\text{PFU}}{\text{mL}}\right) = \frac{\text{Number of plaques (pfu)}}{\text{Inoculum Volume}}\, x\ Dilution\ Factor \quad \text{...}(1)$$

e. Test of Effectiveness of Bacteriophage in Lysing Escherichia coli

100 mL of sterile Lactose Broth (LB) was inoculated with 500µL of *Escherichia coli* bacteria and incubated for 30 minutes. After that, 500 µl of bacteriophage was added and

incubated for an additional 30 minutes. The absorbance value at λ 600 nm was measured every hour. The absorbance results were compared to those of the control (without the addition of bacteriophage).

f. Percentage of OD_{600} Value Decrease

In the bacteriophage effectiveness test, in addition to measuring the OD_{600} value per hour, the percentage decrease in the OD_{600} value per hour was calculated using the following formula:

$$\% \text{ OD value decrease} = \frac{B}{A} x\ 100\% \quad \text{.....(2)}$$

Description:

A: OD count of the control (without phage)

B: OD value with phage treatment

g. Preparation of Hand Sanitizer

To prepare the gel, 0.4 grams of carbopol base was weighed and placed into a previously calibrated beaker. Then, 100 µL of TEA, 0.2 grams of sodium metabisulfite 10 mL of glycerin and 10 mL of distilled water were added. Bacteriophage was also added, and the mixture was homogenized until a hand sanitizer gel was formed [4].

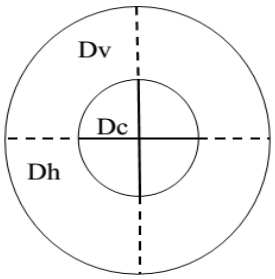
The formulation of the bacteriophage spray hand sanitizer was modified from research by [20]. A beaker was prepared, and 20 mL of glycerin, 0.2 grams of sodium metabisulfite, and 100 µL of TEA were added. Then, 100 mL of distilled water, and bacteriophage were added. The preparation was transferred into a spray bottle.

h. Zone of Inhibition Test (anti-microbial)

Tests were carried out on bacteriophage gel and spray hand sanitizers using the agar diffusion method, specifically the disc diffusion method. Sterile Mueller Hinton Agar (MHA) media (20 mL) was placed into a sterile petri dish. Then 1 mL of *Escherichia coli* O157:H7 bacterial suspension was pipetted into the center and spread evenly to allow solidification. The turbidity of the bacterial suspension was adjusted to Mc. Farland 0.5, which is equivalent to 1.5x108 bacterial cells.

Next, the gel or bacteriophage hand sanitizer spray was applied to a sterile paper disc, which was then placed into the Petri dish. The dish was left for a while to allow the diffusion process take place. The dish was incubated for 24 hours at 37^{o}C. After incubation, the diameter

of the inhibition zone was measured using a caliper. The treatment was performed in triplicate [21]. The inhibition zone was calculated using the following formula:



$$\frac{(Dy - Dc) + (Dh - Dc)}{2}$$

Dv: Vertical Diameter

Dh: Horizontal Diameter

Dc: Disc/Well diameter

Calculation formula of inhibition zone diameter [22]. The determination of antibacterial activity of the test sample's inhibition zone diameter is based on the following equation [23].

Weak: $\frac{A}{B}$ x 100 % < 50%, …… *(3)*

Currently : 50% < $\frac{A}{B}$ x 100 % < 70%, ……..…….…. *(4)*

Strong : $\frac{A}{B}$ x 100 % > 70%, …….*(5)*

Description:

A: Inhibition zone (mm) of the test sample

B: Inhibition zone (mm) of standard antibiotics

i. Test Hand Sanitizer on the Palm

Tests were conducted as follows: (1) Control: The palm was swabbed using a cotton swab and sterile distilled water. The cotton swab was squeezed in a test tube, and 1 mL of the squeezed water was taken for dilution. The sample was then planted on PCA media in a Petri dish. The media was incubated at 37ºC for 24 hours. After incubation, the number of bacterial colonies was observed and counted. (2) Test preparation: the palms were treated with bacteriophage hand sanitizer spray and gel and then allowed to dry. After drying, the palms were swabbed using a cotton swab and sterile distilled water. The cotton swab was squeezed in a test tube, and 1 mL of the squeezed water was taken for dilution. The sample was plated on PCA media in a Petri dish. The media was incubated at 37ºC for 24 hours. After incubation, the number of bacterial colonies was observed and counted [21]. The cup or Total Plate Count (TPC) method was used for calculation based on SNI 01.2332.3-2006 Using the ALT formula (CFU/ml) [21].

$$\text{ALT} = \frac{\text{Number of Colonies}}{\text{Volume Planted x Dilution Factor}} \quad \ldots\ldots(6)$$

3. Data Analysis

The results of the study are presented in the form of data tables to see an overview of the application test of bacteriophage spray hand sanitizer and non-alcoholic antiseptic gel. Data on the total number of microbial colonies are presented in averages with standard deviations.

Result and Discussion

Escherichia coli colonies growing on EMBA media appear metallic green (Figure 1). The metallic green color indicates that the bacteria can ferment lactose as stated by [24]. *Escherichia coli* grown on EMBA media typically appears metallic green or black [24]. This is because EMBA media contains lactose, which allows bacteria capable of fermenting lactose to produce acids, resulting in the formation of metallic green colonies.

Based on the results of bacteriophage isolation, the clear plaque containing bacteriophage is indicated by the formation of double-layer media, as shown in Figure 2. These bacteriophages form plaque due to their ability to lyse bacterial cells, while areas without plaque formation appear cloudy because bacterial cells grow well and are not infected by the phages. This bacteriophage can also directly kill bacterial cells by infecting them [25].

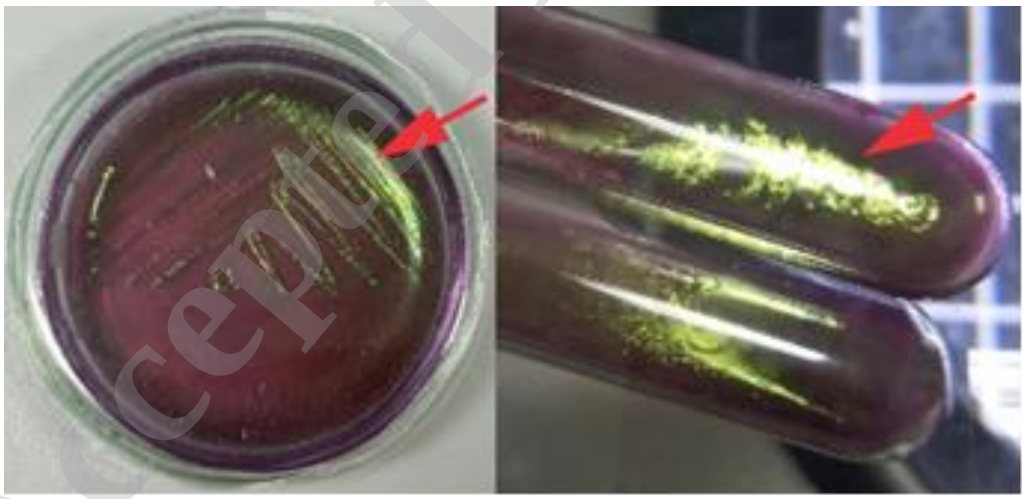


Figure 1. Isolation on EMBA Media, metallic green culture.

Plaque formation occurs as a result of the bacteriophage's ability to lyse *E. coli.* The turbid surface layer, which does not form plaques, occurs because *E. coli* grows well there, and the bacterial cells are not infected by the bacteriophage in each sample. In contrast, plaques are formed when the bacteriophage successfully infects and lyses *E. coli.* Therefore, *E. coli* bacteriophages can be used to detect the presence of polluted water. The presence of *E. coli* will lead to direct infection and lysis by the bacteriophage.

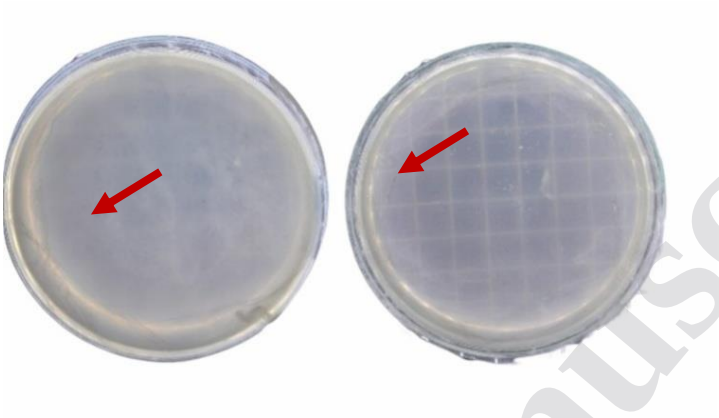


Figure 2. Plaque forms clear.

Test Results of Bacteriophage Effectiveness in Lysing *Escherichia coli*

The effectiveness test was conducted to assess the ability of phages to lyse the host bacteria, *E. coli*. The results of observations taken from 0 hours to 10 hours can be seen in (Figure 3).

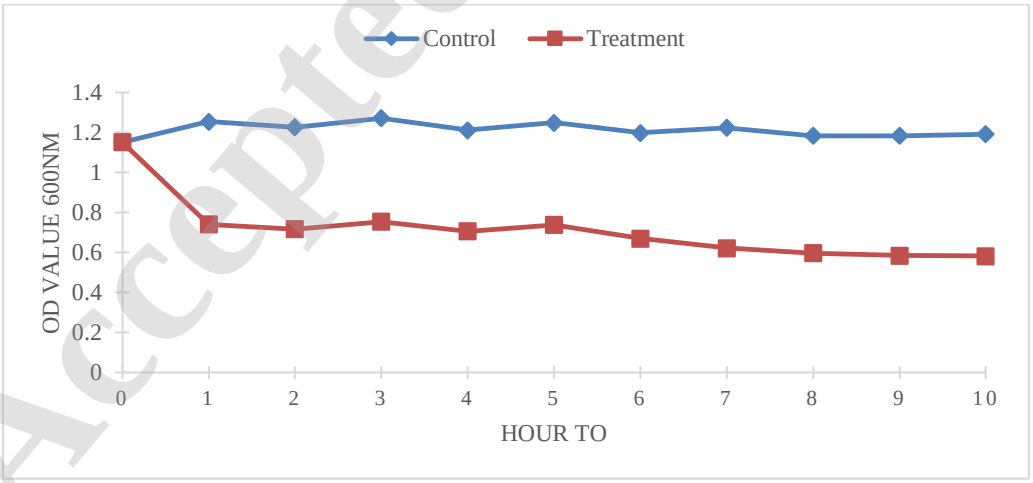


Figure 3. Graph of Phage Effectiveness Test in Lying *E. coli* Bacteria.

Based on the results of the phage effectiveness test in Figure 3, the phage treatment sample actively lyses the host bacteria, *E. coli* from hours 0 to 10. If the treatment graph is

lower than the control, it indicates that the phage can lyse *E. coli*. The greater the difference between the treatment and control, the more effective the phage is in lysing *E. coli*. The comparison of the absorbance values, reflecting the phage's ability to lyse *E. coli,* can be observed from the control and treatment values. The OD_{600} (Optical Density) value in the treatment sample is lower than the control (without phage). Figure 3 shows that phages have inhibitory activity *against E. coli.* This inhibition is indicated by the decrease in absorbance value (OD_{600}), which is caused by the lysis of *E. coli*. The lysis of *E. coli* bacteria occurs because pathogenic bacteria have receptors that are compatible with the phage receptor. This compatibility allows the phage to adsorb to the bacteria and insert its genetic material, using the bacterial machinery for reproduction. Bacteriophages can only enter the bacterial cell membrane if the natural receptor of the bacterial cell is compatible with the bacteriophage receptor.

Table 1. OD Value (600nm) Test of Bacteriophage Effectiveness in Lying *Escherichia coli*

OD600 Value Hour To	Control (Without Phage Addition)			Treatment (Addition of Phage			Percentage of Decrease in Number of *E. coli* (%)
0	1.000	±	0	1.000	±	0	0
1	1.253	±	0.0005	0.74	±	11.102	40
2	1.225	±	0	0.716	±	0.0005	42
3	1.271	±	0.0005	0.754	±	0	41
4	1.120	±	0.0005	0.705	±	0	37
5	1.248	±	0	0.738	±	0.0005	41
6	1.197	±	0.0008	0.67	±	0.0005	44
7	1.223	±	0.0005	0.622	±	0	49
8	1.183	±	0	0.596	±	0.0008	50
9	1.182	±	0	0.584	±	0	51
10	1.190	±	0.0005	0.582	±	0	52

Based on Figure 3, the OD_{600} value of the control (no bacteriophage added) continues to increase, indicating that *E. coli* has grown and multiplied. The OD_{600} measurement for the control showed a consistent increase throughout the observation period, suggesting that the host cell is in the normal growth phase, specifically the log phase. The logarithmic phase begins with an increase in the number of bacteria at a regular growth rate over time. In this phase, one bacterial cell is divided into two. The logarithmic phase typically lasts 3 to 10 hours.

In the bacteriophage treatment, the OD_{600} value continuously decreased. This decrease indicates that many *E. coli* cells were lysed by the bacteriophage. Bacteriophage secretes the

enzyme lysozyme when penetrating the bacterial cell wall, creating holes so that DNA can enter and lyse *E. coli*. The lysozyme enzyme breaks the β-1,4-glycosidic bond with N-acetylglucosamine, causing holes in the bacterial cell wall. The β-1,4-glycosidic bond with N-acetylglucosamine is a bond found in bacterial cell walls. This bond is an important bond that provides strength and stability to the bacterial cell wall. The β-1,4-glycosidic bond with N-acetylglucosamine consists of a polysaccharide chain called peptidoglycan.

The reduction in OD value results from the lysis of the host bacteria, which decreases the total number of bacteria used at the beginning of the treatment [15]. In measuring bacterial density, optical density is used to estimate the density of bacterial cells in a solution. The OD (optical density) value is used to estimate the cell density in liquid culture: the more bacterial cells in the solution, the higher the optical density value produced [26]. Bacterial division can occur every 15 minutes to several days, depending on the species of bacteria [27]. Each bacteriophage infecting a bacterium can produce 200-300 new bacteriophages, causing the infected bacteria to be lysed. The measurement of host cell density, expressed in (OD) value, is a method used to measure live cells in liquid culture [15].

Bacteriophages take over the metabolism of the bacterial cell to replicate themselves, using the host cell's biosynthetic machinery for reproduction. During the lytic cycle, bacteriophage nucleic acid takes control of the host's biosynthetic machinery and bacteriophage-specific m-RNA to synthesize protein. Virulent phages cause host cell death through lysis at the end of their life cycle. The stages of the lytic cycle include 1) adsorption, and 2) penetration, where the phage injects its nucleic acid into the host cell cytoplasm, passing through the cell wall and cytoplasm. After the nucleic acid is injected into the cell, the bacteriophage cycle enters the eclipse period. During the eclipse phase, no bacteriophage particles are found either inside or outside the bacterial cell. The eclipse phase is the interval between the entry of the bacteriophage nucleic acid into the bacterial cell and the release of the mature bacteriophage from the infected cell. [28]. The next stages during the eclipse period are 3) replication, where the phage components (capsomeres, protein envelopes, base plates, tail fibers, and phage enzymes) are multiplied, and 4) maturation, where bacteriophage components are assembled into mature particles, which are then released by destroying the host cell wall using phage proteins such as holing or lysozyme. The process of phage release from the host cell is known as lysis [15].

The most common mechanism of resistance to phage infection is the lack of bacterial receptors, which prevents phage adsorption on the bacterial surface, blocking the ability to produce viral progeny. The lack of receptors can be due to structural modification or target

masking, as seen in *Escherichia coli,* where the outer membrane protein TraT modifies the conformation of outer membrane protein A (OmpA), the receptor for T-like phages. Similarly, in *Staphylococcus aureus,* protein A masks the phage receptor. Loss of receptors can also occur through host phase variation, where changes in cell surface composition occur [29].

Phage populations usually generally require the presence of a bacterial host, and environmental factors can influence host-virus interactions. Bacterial conditions and physiological states can alter these interactions. On one hand, compromised bacterial conditions may reduce phage attachment to bacteria and host susceptibility. Changes in bacterial physiology can affect the structure of the bacterial cell wall, which serves as a receptor for phages. Modifications to phage receptors can prevent phage binding to the host. Upon phage attachment to the host, the phage genome is injected into the host cell, and replication of phage particles begins. However, inadequate nutrition, poor environmental conditions, and switching to a stationary growth phase can reduce phage infection productivity and lytic activity, as phage replication depends on host cell growth [30].

Zone of Inhibition Test Results (Anti-Microbial) Bacteriophage Hand Sanitizer

The anti-microbial test of phage hand sanitizer against *E. coli* bacteria involved five treatments: a control treatment using sterile distilled water, test treatment using gel and spray hand sanitizers, and a comparison using commercial hand sanitizers (gel and spray) sold in the market (Table 1). The method used in this study was Kirby Bauer disc diffusion method to assess the antibacterial activity of the bacteriophage hand sanitizer against *E. coli* bacteria. Antibacterial activity was observed by the presence or absence of an inhibition zone around the disc**.**

Table 2. Results of Vertical and Horizontal Inhibition Zone Diameter Measurement Tests

Treatment	Inhibition Zone Diameter (mm)				
	P1	P2	Amount	Average±stdev	Criteria Diameter (%)
Control	0	0	0	0	0
HS phage Gel	3.4	2.8	6.2	3.1 ± 0.3	97 (Strong)
HS Commercial Gel	3.03	3.4	6.4	3.2 ± 0.18	
HS phage Spray	2.4	3.6	6	3 ± 0.6	92 (Strong)
Commercial HS phage Spray	3.9	2.6	6.5	3.25±0.65	

Notes: *HS (Hand Sanitizer), P1: Repeat 1, P2: Repeat 2, Diameter criteria: Antibacterial activity +++ Strong (Inhibition ≥70%); (inhibition 50-70%); + Weak (inhibition <50%); no inhibition zone (TM)*

Based on the inhibition test experiment of phage hand sanitizer against *E. coli* bacteria, Table 2 presents data on the average presence of inhibition zones from the treatment with repetitions. In the distilled water control, no inhibition zone was observed. In the first treatment (phage gel hand sanitizer), an inhibition zone was found with an average of 3.1 mm. In the comparison treatment (commercial gel hand sanitizer), an inhibition zone was observed with an average of 3.2 mm. In the second treatment (phage spray hand sanitizer), an inhibition zone was observed with an average of 3 mm. In the comparison treatment (commercial spray hand sanitizer), an inhibition zone was found with an average of 3.25 mm. The highest zone of inhibition was obtained with the phage gel hand sanitizer treatment, while the lowest was observed with the commercial gel hand sanitizer treatment. Bacteriophages have antibacterial properties, as evidenced by the formation of an inhibition zone on the growth of *E. coli*.

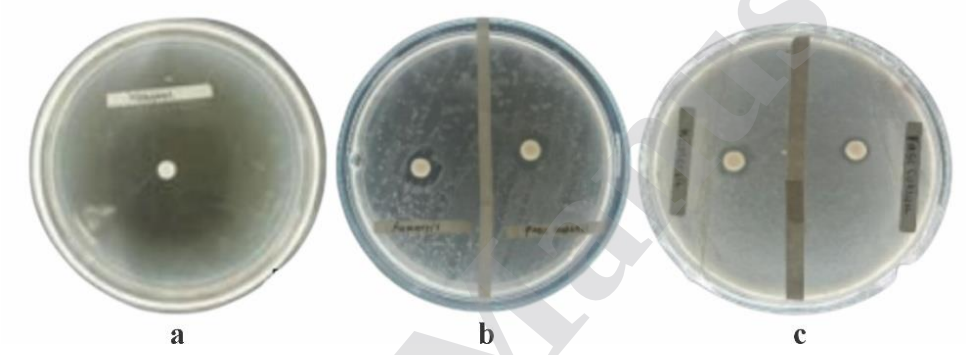


Figure 4. Results of the antibacterial inhibition zone test for phage hand sanitizer: (a) Aquadest control, (b) Phage gel hand sanitizer treatment, (c) Phage spray hand sanitizer treatment.

The results of the antimicrobial inhibition zone test can be seen in Figure 4 phage gel and spray hand sanitizers were shown to inhibit the growth of the host bacteria, *E. coli*. Viral phages infect bacteria by releasing their genetic material into the cytoplasm of bacterial cells. Phages are specific to certain strains of bacteria, or even several strains simultaneously. This specificity is why phages are considered one of the solutions to overcoming the problem of bacterial resistance to antimicrobials, which is an increasing issue worldwide. Phage therapy has been identified as a potential treatment for infections caused by antibiotic-resistant bacteria [12].

Understanding phage specificity is important for assessing the success or potential side effects of phage therapy. Phages typically infect only certain strains, species, or even genera of bacteria. However, with a wide host range of bacteriophages within the same species,

bacteriophage preparations have a high possibility of infecting multi-drug resistant *E. coli*, offering an alternative to antibiotics in combating antibiotic resistance [31].

Phages are useful for reducing multi-drug-resistant *E. coli* contamination, both in liquid suspension and on hard surfaces. Phages can also be inoculated into solutions for use in antiseptic hand washes. However, factors such as phage concentration and incubation time (duration of phage contact with bacteria) should be considered when reducing the risk of multi-drug-resistant *E. coli* contamination.

Bacteriophages specifically targeting *E. coli* were successfully isolated and identified from Palembang City waters. These bacteriophages were used to prevent the development of phage-resistant *E. coli* mutants. Preliminary effectiveness tests verified the ability of bacteriophages to combat *E. coli*, highlighting their potential as antimicrobial agents. Research previously reported by [32], showed that phage mixtures could survive in 100 ppm free chlorine and 100 ppm peroxyacetic acid. In studies of the survival ability of coliphage-specific RNA in 50 ppm free chlorine concentration at various temperatures (4°C, 25°C, and 37°C) over 28 days, F-RNA coliphages showed a higher survival rate (7-14 days) at all temperatures. These findings suggest that coliphages, due to their resistance to chlorine, could serve as indicators for high concentrations of chlorine-based cleaning products [32]. Given the phages' strong survival capabilities, this represents an innovative approach for utilizing phages as cleaning agents, potentially revolutionizing industrial cleaning practices worldwide.

Test of Bacteriophage Gel and Spray Hand Sanitizer on the Palm

Based on the results of research conducted on the test of bacteriophage gel and spray hand sanitizer, three treatments were used: a control treatment with sterile distilled water, and a test treatment with gel and spray hand sanitizer. The results of the observations can be seen in Table 3. The method used in this study is Total Plate Count (TPC), which involves growing live microbial cells on an agar medium, to assess the effectiveness of the tested hand sanitizer.

The Total Plate Count (TPC) method is commonly used to measure the number of live microbial cells on an agar medium [33], under set temperature and incubation time conditions. After the TPC test, each treatment showed a different total plate count as shown in Table 4. In the HS Gel control for men, the average total microbes were 1.95 $x10^4$ and in the HS Gel control for women, the total average was 1.68 $x10^4$. In the HS Phage Gel treatment for men, the average total microbes were 1.15 $x10^3$ and in the HS Phage Gel treatment for women, the average total microbes were 3.05 $x10^3$. For the HS Phage Spray control in men, the average total microbes were 1.25 $x10^4$, and in women, the HS Phage Spray control showed an average

of 1.35 x10^4. In the HS Phage Spray treatment for men, the average total microbes were 2.05 x10^3 while for women, the average was 8.36 x10^3. The difference in numbers is most likely due to the varying daily activities of the palms tested.

Table 3. Results of the Bacteriophage Gel and Spray Hand Sanitizer Test

Treatment	Microbial Count (CFU/mL))
Aquadest Control LK	1.95 x 10^4
HS Gel Fag LK	1.15x 10^3
HS Gel Commercial LK	2.55 x 10^3
Aquadest Control PR	2.35 x 10^4
HS Gel Fag PR	3.05 x 10^3
HS Gel Commercial PR	1.65 x 10^3
Aquadest Control LK	1.30 x 10^5
HS Spray Fag LK	2.05 x 10^3
HS Spray Commercial LK	9.04 x 10^4
Aquadest Control PR	1.58 x 10^5
HS Spray Fag PR	8.36x 10^3
HS Spray Commercial PR	8.79 x 10^4

Notes: HS (Hand sanitizer), LK (Male), PR (Female)

The bacteriophage gel and spray hand sanitizer test results show that hand sanitizers are effective in reducing bacteria on the palms of the hands. The bacteriophage gel hand sanitizer is more effective than the spray hand sanitizer. This is likely because the gel tends to be thicker and stickier, allowing the phages to remain on the skin surface for a longer time. This longer contact time makes the *E. coli* killing process more efficient. In addition, gels can cover the entire surface of the hand evenly, providing better protection [33]. Spray hand sanitizers, on the other hand, may be more easily carried by the wind or evaporate, which could affect their efficiency. The choice between gel and spray hand sanitizers may depend on personal preference, usage situation, and specific needs [34].

Bacteriophages have the potential to be used as hand sanitizers because they naturally possess remarkable bacteriostatic and bacteriolytic activities as part of their natural lytic life cycle. This involves first disrupting bacterial metabolism to produce new virus particles, followed by lysing the host cell to release its progeny [35]. The ability of bacteriophages to reduce the number of microbes is associated with their ability to produce endolysin, a peptidoglycan hydrolase that destroys the peptidoglycan in the host bacterial cell wall. This enzyme works by breaking down the structural components of the bacterial cell wall, allowing the virus to exit the bacterial cell after replication is complete. Due to its specific bacterial destruction mechanism, endolysin holds great potential for medical applications, especially in

combating drug-resistant bacteria [36].

Endolysin is responsible for degrading the peptidoglycan layer of the bacterial cell wall during the final stage of lytic phage replication, causing the cell to rupture and release newly formed virus particles. This event occurs after endolysins accumulate in the cytoplasm, and are translocated through holes formed in the plasma membrane by holins. Since the peptidoglycan layer provides structural integrity and rigidity to the bacterial cell, its degradation leads to cell wall instability and eventual rupture due to differences in cellular and environmental osmotic pressures (osmolysis), especially in Gram-positive bacteria, which lack an outer membrane. The lysis of Gram-negative bacteria is more complex, which catalyzes the fusion of the inner and outer membranes [37].

Total Plate Count Examination aims to quantify microorganisms that grow and form colonies, which can be directly observed and counted [38]. The criteria for microbial colonies that can be calculated are those in the range of 30-300 colonies [39], which are then calculated using the microbial calculation formula. According to [40], the plate count method includes three techniques: pour plate, spread plate, and drop plate. A diluent solution is used in the sample dilution process before the microorganisms are planted in the growth medium [34]. The Multilevel Dilution Technique is employed to reduce the number of microorganisms in the sample. A ratio of 1: 9 is used (1 part sample and 9 parts diluent solution) for the first dilution, and this process is repeated until the microbial cell count is reduces by a factor of 1/10 with each dilution [41].

Phage tolerance to temperature treatment has advantages in phage stability under varying environmental conditions or for storage purposes [42]. Higher storage temperatures (e.g., 25°C) can cause instability in phages, while colder storage temperatures improve phage stability and infectivity. Phages stored in a buffer at 4°C [43] can remain stable for up to 6 months, compared to only 1 month at 20°C [44]. The results showed that phage hand sanitizers were more effective than commercial alcohol-based hand sanitizers. Further research could focus on testing phage hand sanitizers in individuals who are allergic to chemical products or biologically based products.

Conclusions

The phage cocktail hand sanitizer has been proven to reduce the test bacteria, namely *Escherichia coli*. The phage cocktail gel hand sanitizer is more effective than the spray phage cocktail hand sanitizer. This effectiveness is supported by the antibacterial inhibition zone test, which has shown the phage cocktail hand sanitizer to possess significant antibacterial activity.

The results from the hand sanitizer application test on the palms revealed that the phage cocktail hand sanitizer was more effective than commercial alcohol-based hand sanitizers. Therefore, the phage cocktail hand sanitizer can serve as a viable alternative to replace commercial alcohol-based hand sanitizers.

Acknowledgments

The author would like to thank LPPM UIN Raden Fatah for supporting and fostering this research activity with DIPA BLU funding sources for the 2024 budget year.

Conflict of interest

We declare that there is no conflict of interest.

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