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# Exploration of bacteriophages from waters in Palembang, Indonesia as biocontrol of antibiotic-resistant *Escherichia coli*

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**Abstract.** *Sunarti RN, Hariani PL, Budiarti S. 2023. Exploration of bacteriophages from waters in Palembang, Indonesia as biocontrol of antibiotic-resistant* Escherichia coli. *Biodiversitas 24: 6069-6081.* The emergence and dissemination of antibiotic-resistant bacteria in the aquatic environment demand for utilization of bacteriophage (phage) therapy as an ecologically conscious alternative to mitigate the consequences of drug resistance. The presence of antibiotic-resistant *Escherichia coli* (*E. coli*) in the river of Palembang City is a matter of concern for public health. It is imperative to explore the potential of phages as biocontrol agents against antibiotic-resistant *E. coli* sourced from the rivers of Palembang City. The effectiveness of the phage was then evaluated in managing antibiotic-resistant *E. coli* in contaminated water. The exploration resulted in the acquisition of 12 phage isolates from various water sources. Specifically, three phage isolates were obtained from Kedukan River (FgSK11.2, FgSK22.2, FgSK31.3); three phage isolates were obtained from Buah River (FgSB13.2, FgSB13.3, FgSB33.1); and six phage isolates were obtained from PU River (FgPU11.2, FgPU11.3, FgPU31.3, FgPU33.1, FgPU33.2, FgPU33.3). The isolates have demonstrated the ability to lyse antibiotic-resistant *E. coli* bacteria. The findings indicate that bacteriophages can mitigate pollution in collected water samples. The phage cocktail utilized effectively inhibited the growth of antibiotic-resistant *E. coli*, reducing their population. Furthermore, the treatment resulted in satisfactory water quality improvements by reducing the Fe and Pb levels. The results of this study show a significant advancement in the use of phages as a first step in addressing the problem of *E. coli* pathogen-caused water pollution in Palembang City.

**Keywords:** Antibiotic-resistant, bacteriophages, *Escherichia coli*, natural biocontrol, phage cocktail

## INTRODUCTION

The proliferation of antibiotic-resistant bacteria has emerged as a significant global issue in the realm of public health, while the prevalence of tolerance towards disinfectants has also become increasingly prevalent (Prestinaci et al. 2015; Leptihn 2019; Kauppinen et al. 2021; Garvey 2022). According to the World Health Organization (WHO 2021), antibiotic resistance is currently recognized as one of the ten pressing challenges to human health. Based on Tacconelli et al. (2018) and Mulani et al. (2019), the categorization of antibiotic-resistant pathogens is classified into three distinct groups: critical, high, and medium, which are determined based on the urgency and priority of developing new antibiotics to combat them. One of the foremost antibiotic-resistant pathogens is *Escherichia coli* (*E. coli*), which belongs to the crucial category of Enterobacteriaceae (WHO 2017). The prevalence and dissemination of antibiotic-resistant bacteria demonstrate a rising trajectory within enteric bacteria, notably *E. coli*. The transmission of antibiotic-resistant bacteria to pathogenic bacteria poses a significant peril to the ecological system. The role of the environment as a significant contributing factor in the dissemination of antibiotic-resistant bacteria has been widely acknowledged, with water being identified as a prevalent vector (Srivastava 2022). The emergence of antibiotic-resistant bacteria in aquatic environments can be attributed to genetic mutation or antibiotic resistance genes' acquisition via horizontal gene transfer. Von Wintersdorff (2016) emphasizes that horizontal gene transfer is primarily responsible for the spread of antibiotic resistance genes from environmental and commensal species to pathogenic species.

In their study, Verawaty et al. (2020) successfully isolated multidrug-resistant *E. coli* MDR strains from aquatic ecosystems in Palembang City. Overall, the findings indicated that a majority of the *E. coli* isolates exhibited resistance to ampicillin (82%), tobramycin (57%), and tetracycline (71%). The isolates exhibited an intermediate category for kanamycin at a rate of 50%, co-trimoxazole at a rate of 57%, cefixime at a rate of 50%, and gentamicin at a rate of 54%. The *E. coli* isolates that were studied exhibited a high susceptibility to ciprofloxacin (86%) and chloramphenicol (61%). In addition, Sunarti et al. (2022) documented the isolation of diverse strains of antibiotic-resistant *E. coli* from three rivers in Palembang

City, namely the Kedukan, Buah, and PU Rivers. The identified strains exhibited resistance to multiple antibiotics, including tobramycin (95%), ampicillin and gentamicin (73%), chloramphenicol (55%), kanamycin and co-trimoxazole (36%), and tetracycline and ciprofloxacin (18%). It calls for prompt attention to the issue of antibiotic-resistant *E. coli* bacteria contamination in water sources. Certain strains of *E. coli*, a species of bacteria, have been identified as human pathogens capable of causing a range of diseases (Kim 2016; Poolman and Wacker 2016; Christine et al. 2018; Lee et al. 2018; Liu et al. 2021). Therefore, employing a methodology that ensures safety and minimizes environmental contamination, specifically by utilizing eco-friendly biocontrol measures, is imperative.

Bacteriophage therapy emerges as an up-and-coming alternative. Bacteriophages, which possess the inherent ability to infect particular bacteria exclusively, represent a plentiful and varied reservoir of biological entities (Dalmasso et al. 2016; Liao et al. 2019). Bacteriophages offer several advantages that make them promising candidates for treating bacterial infections. Phages possess high host specificity, which allows them to target specific bacteria without harming normal flora or infecting eukaryotic cells. Besides that, bacteriophages also can be administered in low therapeutic doses and multiply rapidly within the host bacteria (Domingo-Calap and Delgado-Martnez 2018; Pirnay et al. 2018; Yulinery et al. 2019; Necel et al. 2020). Furthermore, it is essential to note that phages and antibiotics differ in their mechanisms of action. Phages possess the unique capability to adapt and acquire novel infectious properties, enabling them to combat mutated strains of bacteria effectively (Romero-Calle et al. 2019). Using bacteriophages for biocontrol purposes exhibits promising prospects in various aquatic contexts, including antibiotic-resistant strains responsible for causing diseases.

Numerous studies have proposed employing host-specific bacteriophages as an environmentally sustainable strategy. The utilization of bacteriophages for biocontrol in mitigating environmental pollution exhibits significant promise. However, phages are ubiquitous in the environment, particularly those that inhabit their respective host bacteria. Therefore, it is necessary to isolate specific phages and evaluate their effectiveness in treating antibiotic-resistant bacteria in the contaminated river of Palembang City.

## MATERIALS AND METHODS

### Sampling area

The samples were taken along the Kedukan, PU, and Buah Rivers in Palembang City, South Sumatra, Indonesia. Furthermore, each river consists of 9 sampling points in the lower and middle parts as well as the upstream region, as shown in Figure 1. The sampling coordinates can be seen in Table 1. Surface water samples were collected for microbiological testing from 27 points using the purposive sampling method. They were taken using sterile bottles and then placed in a cool box at 4°C before arriving at the laboratory. The samples were tested for water quality based on physical and chemical. The chemical variables tested include biochemical oxygen demand (BOD) mg/L (SNI 6989.72:2009), chemical oxygen demand (COD) mg/L (SNI 6989.2:2009), total suspended solids(TSS) mg/L (SNI 06-6989.3:2004), Fe mg/L (SNI 6989.4:2009), Cd mg/L (SNI 06-6989.38:2005), Pb mg/L (SNI 6989.8:2009), and $Cl_2$ mg/L (SNI 6989.19:2009).

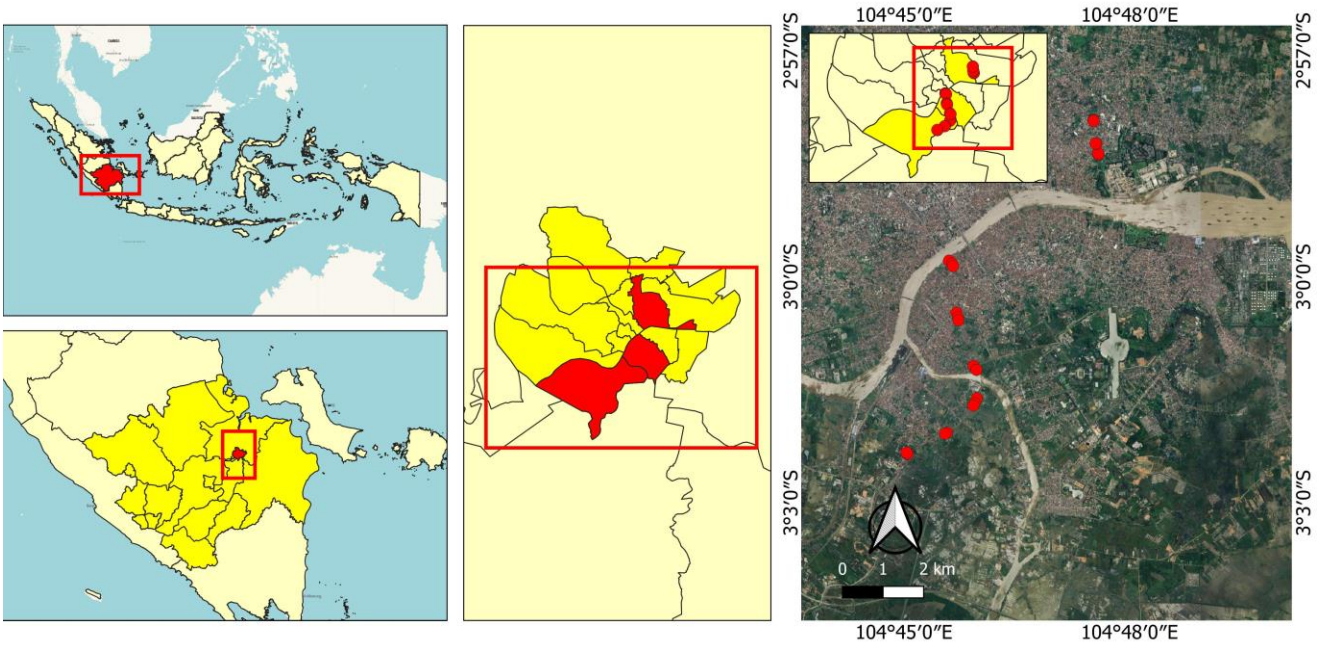


**Figure 1.** Location of river water sampling points in Palembang City, Indonesia

**Table 1**. Water sampling locations for the Kedukan River, PU River and Buah River, Palembang City, Indonesia

| Locations | Coordination point | Sample code | River section |
|---|---|---|---|
| Kedukan | 2°59'46.90" S, 104°45'36.10"E | SK1-1 | Upstream |
| River | 2°59'49.30"S, 104°45'38.70"E | SK1-2 | |
| | 2°59'51.30"S, 104°45'39.30"E | SK1-3 | |
| | 3°00'28.40"S, 104°45'42.01"E | SK2-1 | Middle |
| | 3°00'32.10"S, 104°45'43.20"E | SK2-2 | |
| | 3°00'34.50"S, 104°45'43.60"E | SK2-3 | |
| | 3°01'10.80 S, 104°45'55.70"E | SK3-1 | Downstream |
| | 3°01'13.90"S, 104°45'58.01"E | SK3-2 | |
| | 3°01'16.60"S, 104°46'00.00"E | SK3-3 | |
| PU River | 3º1'36.59"S, 104º45'58.24"E | PU1-1 | Upstream |
| | 3º1'40.65"S, 104º45'56.53"E | PU1-2 | |
| | 3º1'42.31"S, 104º45'55.49"E | PU1-3 | |
| | 3º2'4.02"S, 104º45'34.51"E | PU2-1 | Middle |
| | 3º2'4.80"S, 104º45'32.91"E | PU2-2 | |
| | 3º2'5.41"S, 104º45'31.89"E | PU2-3 | |
| | 3º2'20.98"S, 104º45'2.89"E | PU3-1 | Downstream |
| | 3º2'20.82"S, 104º45'3.25"E | PU3-2 | |
| | 3º2'19.82"S, 104º45'2.20"E | PU3-3 | |
| Buah | 2°58'22.82''S, 104°47'35.46"E | SB1-1 | Upstream |
| River | 2°58'22.38''S, 104°47'35.50"E | SB1-2 | |
| | 2°58'21.37''S, 104°47'34.99"E | SB1-3 | |
| | 2°58'13.69''S, 104°47'33.12"E | SB2-1 | Middle |
| | 2°58'13.90''S, 104°47'33.74"E | SB2-2 | |
| | 2◦58'13.63''S, 104°47'32.98"E | SB2-3 | |
| | 2°57'54.78''S, 104°47'31.31"E | SB3-1 | Downstream |
| | 2°57'55.42''S, 104°47'31.34"E | SB3-2 | |
| | 2°57'55.67''S, 104°47'31.51"E | SB3-3 | |

**Water physical and chemical quality assessment**

Following the implementation of bacteriophages as biocontrol, water quality analysis was conducted on the samples. The examination encompassed the assessment of physical parameters, specifically total suspended solids (TSS), as well as chemical parameters, including chemical oxygen demand (COD), biochemical oxygen demand (BOD), iron (Fe), lead (Pb), and chlorine ($Cl_2$) levels. Table 1 presents the coordinates of the water sampling locations.

**Bacteriophage isolates**

The *E. coli* isolates were cultured in a 50 mL volume of lactose broth (LB) medium using an incubator shaker at 37°C and allowed to grow overnight. The optical density at 600 nm ($OD_{600}$) of the *E. coli* culture was subsequently assessed using spectrophotometry at regular intervals, with measurements taken per hour, until the culture entered the logarithmic growth phase. Subsequently, a volume of 5 mL was extracted for filtration, which was then mixed with 50 mL of lactose broth (LB) medium. Additionally, a 10 mL water sample was introduced into the mixture. The mixture underwent incubation in a shaker incubator set at 37ºC for 24-48 hours. The mixture was then collected through centrifugation with a force of 3000 xg at a temperature of 4ºC for 20 minutes, twice on separate occasions. The obtained liquid portion was filtrated using a 0.22 µm filter to generate phage lysate. A host culture of *E. coli* with $OD_{600}$ = 1 in 50 mL of lactose broth medium was prepared to detect the presence of lytic phages. This culture was incubated for 24 hours at 37ºC with continuous shaking at a speed of 100 rpm. Subsequently, 100 µL of the culture was collected and combined with 100 µL of filtered supernatant in a sterile tube for homogenization. Next, the sample was placed in an incubator and maintained at 37ºC for 30 minutes. A 3 mL of soft agar (molten top agar) with a temperature of 45ºC was introduced to the mixture. The mixture was then placed in a cup containing nutrient agar (NA) medium and subjected to slow rotation to achieve uniform distribution. Subsequently, the mixture was left undisturbed to solidify. After that, the sample was incubated at 37ºC for 24 hours (Chibani-chennou et al. 2004).

Following incubation, the samples were visually inspected for plaque formation. The plaques indicated the presence of phages. The phage purification process involved the transfer of individual plaques from the plaque test into a tube using a Pasteur pipette (Foschino et al. 1995). These plaques were mixed with 2-3 mL of SM buffer. The suspension of phages was subjected to homogenization and then allowed to incubate at room temperature for 5-10 minutes. The suspension was then subjected to centrifugation with a speed of 3000 xg at 4ºC for 25 minutes, with the process being repeated twice. The liquid portion is filtrated using a porous filter with a pore size of 0.22 µm, which is preserved for stock or production materials. The quantification of phages was conducted by assessing the number of plaques formed per milliliter (PFU/mL). The phage stock was diluted to a concentration of $10^7$, after which 100 µL of each dilution was combined with 100 µL of *E. coli* bacterial culture that had been incubated for 3-4 hours on lactose broth (LB) media. The suspension was subjected to incubation at 37ºC for 15 minutes. A volume of 5 mL of soft agar with a temperature of 45ºC, incubated at 37ºC for 24 hours, was served for plaque formation and counted.

**Determination of phage host range**

Phage host ranges were determined using the double agar-plating method, as previously described by Lingga et al. (2020). A volume of 100 µL of *E. coli* bacterial culture, was cultivated in lactose broth (LB) media for 3-4 hours. Each sample was combined with a phage stock solution at a concentration of 100 µL phage stock solution with a titer of $10^9$ pfu/mL and subsequently subjected to incubation at 37°C for 15-30 minutes. A volume of 5 mL of molten top agar at a temperature of 42°C is combined and subsequently poured into the nutrient agar (NA) medium. The samples should be incubated at 37°C for 24 hours. During this incubation period, it is essential to observe the samples for plaque formation. If plaques form, it indicates that the phage can lyse the host cell.

**The effectiveness of phages against antibiotic-resistant *E. coli* in vitro**

The effectiveness of phages in inducing cell lysis in *E. coli* was assessed using the methodology described by Atterbury et al. (2007). A volume of 100 µL of *E. coli* bacterial culture, cultivated lactose broth (LB) media until

reaching $OD_{600}$ = 1, containing approximately $10^8$ CFU/mL, was evenly distributed into two centrifuge tubes, each containing 50 mL. The centrifugation process was performed using a Beckman centrifuge at a speed of 3000 xg, at a temperature of 4°C, for 30 minutes, with three repetitions. The liquid portion of the sample was removed, followed by the implementation of two distinct experimental conditions: the control group, consisting of the pellet without the introduction of phage, and the treatment group, where the pellet was supplemented with 1 mL of phage. Both samples were subjected to incubation at a temperature of 37°C for 30 minutes. Subsequently, successive additions of 50 mL of lactose broth (LB) were made and then transferred into separate Erlenmeyer flasks. These flasks were then placed in a shaker incubator operating at 37°C. The $OD_{600}$ value of each culture was assessed at hourly intervals, commencing at 0 hours and continuing until a decrease in the $OD_{600}$ value was observed.

**Effectiveness test of antibiotic-resistant *E. coli* as water pollution biocontrol**

The effectiveness of phages in river water was assessed using the methodology described by Lingga et al. (2020). Effectiveness assessment is carried out in two ways, namely unsterilized river water and sterilized river water. Test the effectiveness of phage lysis against *E. coli* on sterilized river water samples by centrifuging the collected river water and undergoing partial sterilization via autoclaving. After cooling, river water samples were inoculated with 100 µL of antibiotic-resistant *E. coli* culture for 24 hours, characterized by an $OD_{600}$ value of 1. The *E. coli* used was a mixture of all phage hosts (EcSK1-1, EcSK2-2, EcSK3-1, EcSB1-3, EcSB3-3, EcPU1-1, EcPU3-1, EcPU3-3). Next, the sample was infected with 100 µL of phage cocktail stock which had a titer of $10^8$ PFU/mL. The phages used were a mixture of phages that were successfully isolated (FgSK11.2, FgSK22.2, FgSK31.3, FgSB13.2, FgSB13.3, FgSB33.1, FgPU11.2, FgPU11.3, FgPU31.3, FgPU33.1, FgPU33.2, FgPU33.3). Quantification of bacterial populations in river water is determined by measuring the $OD_{600}$ value.

## RESULTS AND DISCUSSION

**Physical and chemical water quality**

Following the implementation of bacteriophages, water quality analysis is conducted to ascertain the appropriateness of water quality. The quality of water is influenced by the presence of various constituents, including organic substances, inorganic substances, and heavy metals. The physical and chemical parameters of the examined water pollution were compared with the river quality standards specified by Regulation No. 32 of 2017, issued by the Minister of Health of the Republic of Indonesia (Table 2). The parameter included is total suspended solids (TSS) (max 50 mg/L), biological oxygen demand (BOD) (max 2 mg/L), chemical oxygen demand (COD) (max 10 mg/L), iron (Fe) (max 0.3 mg/L), cadmium (Cd) (max of 0.1 mg/L), lead (Pb) (max 0.05 mg/L), and chlorine ($Cl_2$) (max 0.03 mg/L).

The investigation compared the aforementioned parameters to assess the pollution decline between the control and treatment groups. The river water that absence of phage serves as the control. The quality of river water only met the standard by two parameters, specifically Cd and Pb, along with the TSS value of the Kedukan River. The surrounding ecosystem impacts the quality of river water. According to Boyd (2015), the process of soil erosion can result in the contamination of natural water bodies. It occurs primarily due to the lack of vegetation, such as trees or plants, which would otherwise serve as a means of retaining soil and preventing its displacement by flowing water. Consequently, the absence of vegetation leads to increased turbidity in the water bodies, primarily caused by suspended sediment.

**Table 2.** Physical and chemical quality of river water in Palembang City, Indonesia

| Sample code | TSS (mg/L) | | BOD (mg/L) | | COD (mg/L) | | Fe (mg/L) | | Cd (mg/L) | | Pb (mg/L) | | $Cl_2$(mg/L) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | T | C | T | C | T | C | T | C | T | C | T | C | T | C |
| SKI | 78.0 | 48.6 | 6.98 | 5.81 | 51.000 | 45.000 | 1.36 | 0.93 | <0.0025 | <0.0025 | 0.10 | 0.08 | 134.0 | 54.6 |
| SKT | 28.4 | 31.4 | 7.76 | 4.85 | 67.000 | 38.000 | 0.55 | 0.48 | <0.0025 | <0.0025 | 0.04 | 0.11 | 69.5 | 45.0 |
| SKU | 39.6 | 37.8 | 7.68 | 1.58 | 44.000 | 23.000 | 1.00 | 1.02 | <0.0025 | <0.0025 | 0.05 | 0.13 | 83.4 | 39.7 |
| SPI | 255.6 | 280.5 | 16.8 | 17.6 | 34.000 | 62.000 | 0.27 | 0.64 | <0.0025 | <0.0025 | 0.11 | 0.11 | 59.6 | 29.8 |
| SPT | 284.6 | 26.0 | 17.8 | 8.75 | 62.000 | 45.000 | 0.39 | 0.88 | <0.0025 | <0.0025 | 0.08 | 0.10 | 59.6 | 29.8 |
| SPU | 205.7 | 295.2 | 15.7 | 12.6 | 17.000 | 29.000 | 0.59 | 1.37 | <0.0025 | <0.0025 | 0.06 | 0.09 | 69.5 | 24.8 |
| SBI | 65.2 | 52.2 | 9.41 | 6.68 | 35.000 | 14.000 | 0.15 | 0.40 | <0.0025 | <0.0025 | 0.09 | 0.07 | 99.3 | 79.4 |
| SBT | 63.4 | 51.7 | 12.8 | 8.62 | 41.000 | 34.000 | 0.52 | 0.26 | <0.0025 | <0.0025 | 0.05 | 0.11 | 79.4 | 59.5 |
| SBU | 79.8 | 55.8 | 10.6 | 6.63 | 58.000 | 70.000 | 0.11 | 0.40 | <0.0025 | <0.0025 | 0.11 | 0.11 | 50.6 | 51.1 |

Note: Lower Kedukan River (SKI), Middle Kedukan River (SKT), Upper Kedukan River (SKU), Lower PU River (SPI), Middle PU River (SPT), Upper PU River (SPU), Lower Buah River (SBI), Middle Tengah River (SBT), Upper Buah River (SBU), Treatment Group (T), Control Group (C)

The human settlements along riverbanks give rise to detrimental anthropogenic activities that result in domestic waste, encompassing organic, inorganic, and chemical waste. The presence of agricultural and livestock farming communities, alongside industrial infrastructure such as factories, contributes to the introduction of diverse forms of waste into the river. Various substances such as pesticides, synthetic organic chemicals, industrial heavy metals, pharmaceutical compounds, and their decomposition products can potentially be harmful to aquatic organisms (Boyd 2015).

The presence of organic waste impacts the oxygen demand associated with BOD and COD. Elevated levels of BOD can lead to mortality as the available oxygen necessary for the sustenance of aquatic organisms becomes fully consumed by bacteria during the process of decomposition. The COD parameter serves as a measure of the concentration of organic substances, encompassing both biodegradable and non-biodegradable components (Koda et al. 2017). This phenomenon arises due to the chemical oxidation of numerous organic substances, which cannot undergo biological oxidation. Moreover, a significant quantity of organic compounds in wastewater contributes to an elevation in the suspended solid. The suspension of microorganisms in the water also takes part in elevated levels of TSS, particularly in downstream areas (Razif 2022).

Another parameter that needs to be concerned with is the increase of heavy metals in aquatic environments which is frequently attributed to the introduction of industrial, mining, agricultural, and domestic effluents. While aquatic organisms require elevated levels of certain substances for various metabolic processes, it is essential to note that excessive concentrations of these substances can have toxic effects on animals. Heavy metals introduced into the human body tend to accumulate and persist throughout the various levels of the food chain.

Fe has been identified as a mutagenic agent capable of inducing mutations and causing damage to DNA (Payus et al. 2016). The $Cl_2$ level in all rivers is below the required standards, which poses a significant health risk. Multiple studies have documented the mutagenic and carcinogenic properties of $Cl_2$. Moreover, it has the potential to induce asthma, provoke irritation of the esophagus, elicit burning sensations in the oral cavity and pharynx, and trigger episodes of spontaneous vomiting. Additionally, it has been observed that $Cl_2$ can lead to detrimental effects on the liver and bladder, along with the development of colon cancer, atherosclerosis, high blood pressure, and allergies (Mohsen et al. 2019).

The study utilized mixed phage for the treatment of river water samples. Three samples from each river were tested. The Buah River results indicated that the treatment increased the water quality, as the Cd, Pb, and average Fe levels met the quality standards. Regarding suspended solids, the TSS value of the Kedukan River generally declined toward standards, although it exhibited an increase in samples that originated from downstream. Notably, TSS levels in the PU and Buah Rivers met the standard after treatment. The Kedukan and PU Rivers samples met the established criteria in BOD, COD, and Fe concentration. As for $Cl_2$ levels, all treated samples were found to exceed the threshold.

During the procedure, the incubation process positively impacted the microbial biomass levels. The occurrence is believed to be associated with the rise in TSS, BOD, and COD levels. Introducing organic matter into aquatic environments leads to a decrease in dissolved oxygen concentrations and contributes to the levels of TSS. This phenomenon remains inherent to nature and can be accommodated through natural biodegradation. Subsequently, there was a notable decline in Fe concentrations observed in both the PU and Buah rivers after treatment.

Regarding the Pb parameter, the concentration has decreased in all treated samples. It was suggested that the bio-sorption mechanism occurred during the incubation period of the treatment process. Various types of biomasses exhibit different levels of affinity for adsorbing metals. The biomass comprises various functional groups that engage with metals via ionic interactions, polar interactions, or a combination of both. Heavy metal ion binding on the cell surface has been observed in deceased and viable cells within biomass (Abiodun et al. 2023). Overall, while minimal impact was observed in mitigating the levels of physical and chemical contamination, the utilization of bacteriophages demonstrates potential in controlling antibiotic-resistant *E. coli* pathogens.

**Isolation of host-specific bacteriophages from *E. coli***

In this study, a collection of 12 phage isolates was obtained from three rivers, namely Kedukan, Buah, and PU, located in Palembang City. These phage isolates were successfully obtained using antibiotic-resistant *E. coli* strains as the host, which were also sourced from the same rivers. Phage isolates were acquired from distinct sampling locations, exhibiting unique plaque characteristics. The assessment findings of phage isolation are displayed in Table 3, while Figure 2 depicts the sample plate illustrating the presence of phages.

The identification of phages can be determined by observing distinct, clear plaques on double-layer media. The characteristics of these plaques, including their quantity and size, are subject to various factors, such as the specific type of phage, environmental conditions, e.g., temperature, pH, presence of ions, and exposure to light (Jatmiko et al. 2018). It was observed that phages exhibited significant effectiveness in vitro, as evidenced by their capacity to generate distinct circular plaques, effectively eliminating antibiotic-resistant strains of *E. coli*, which is in agreement with similar results obtained by Atterbury et al. (2007).

**Table 3.** Bacteriophage isolates from *Escherichia coli*

| Phage host *E. coli* isolates | Isolated phages | Sample origin | Plague diameter (mm) | Plaque characteristics | Phage quantification (PFU/mL) |
|---|---|---|---|---|---|
| Ecsk1-1 | FgSK11.2 | Kedukan river | 2 | Clear | $6.3 \times 10^{10}$ |
| Ecsk2-2 | FgSK22.2 | Kedukan river | 1 | Unclear | $6.9 \times 10^{10}$ |
| Ecsk3-1 | FgSK31.3 | Kedukan river | 1-2 | Mostly clear, few plaques unclear | $2.6 \times 10^{10}$ |
| Ecsb1-3 | FgSB13.2 | Buah river | 1 | Clear | $9.2 \times 10^{10}$ |
| | FgSB13.3 | Buah river | 1-2 | Mostly clear, few plaques unclear | $1.6 \times 10^{10}$ |
| Ecsb3-3 | FgSB33.1 | Buah river | 1 | Clear | $7.9 \times 10^{10}$ |
| Ecpu1-1 | FgPU11.2 | PU river | 1 | Clear | $6.9 \times 10^{10}$ |
| | FgPU11.3 | PU river | 1 | Unclear | $2.1 \times 10^{10}$ |
| Ecpu3-1 | FgPU31.3 | PU river | 1 | Unclear | $1.7 \times 10^{10}$ |
| Ecpu3-3 | FgPU33.1 | PU river | 1 | Clear | $6.3 \times 10^{10}$ |
| | FgPU33.2 | PU river | 1 | Clear | $3.0 \times 10^{10}$ |
| | FgPU33.3 | PU river | 1 | Clear | $8.2 \times 10^{10}$ |

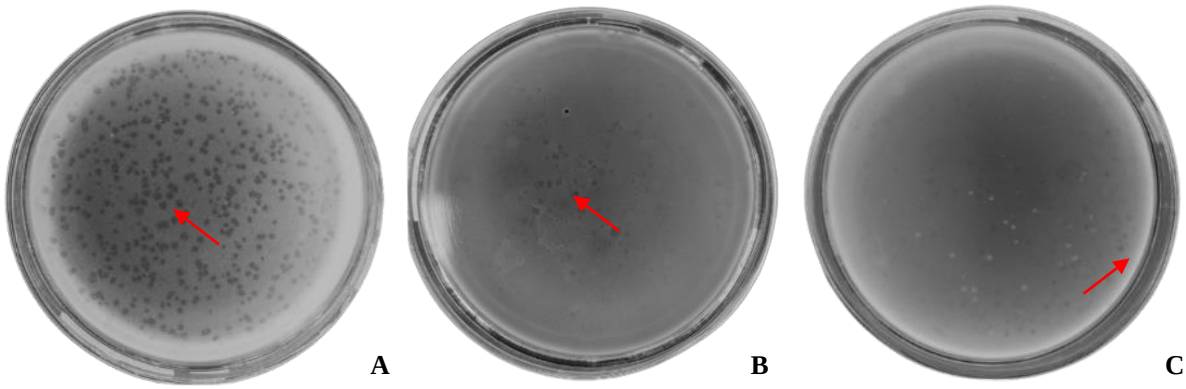


**Figure 2.** Arrows indicate plaques formed due to lysis of *Escherichia coli* cells by phages. Isolated plaque phage: (A) FgSK11.2, (B) FgSB13.3, and (C) FgPU33.1

The isolated phages exhibited the ability to form plaque on the outer layer of the bacterial culture of *E. coli*. During infection, cells susceptible to phages undergo lysis upon releasing new phage progeny. This event amplifies the local infection process in the neighboring cells, forming a distinct circular clearance region (plaque). Hence, plaque formation clearly indicates that the phages in question are of the lytic type. The average diameter of the plaques is approximately 1 mm, with the most prominent plaque measuring around 2 mm.

Each sample exhibits distinct characteristics. The quantification of phages revealed a range of 158-924 x $10^8$ PFU/mL across the samples. The disparity in quantification results is hypothesized to be associated with the degree of phage distribution within their environment. According to Zhang et al. (2015), *E. coli* phages are predominantly influenced by environmental factors such as temperature and humidity levels.

The examined phages exhibited diverse concentrations, sizes, and plaque distribution patterns. The plaque morphology provides insights into the multiplicity of infection (MOI) mechanism employed by phages to target bacterial cells (Zou et al. 2022). The factors influencing the determination of plaque morphology include phage, host, and growth conditions (Li et al. 2020). According to Storms et al. (2020), an effective phage growth medium facilitates the diffusion of phages, thereby promoting favorable outcomes in adsorption, latent phase duration, and plaque burst size. The observed lysis pattern suggests the existence of an inherent biological equilibrium between the phage and the bacterial host. The uneven distribution of bacterial growth in terms of cell density can have implications for the infectivity of phages as they move from one cell to another (Li et al. 2020).

Conversely, the exponential proliferation of bacteria will impede the effectiveness of phage infection. Bacterial cells exhibiting a substantial population density possess the capability to stimulate the synthesis of quorum, which serves as a means of intercellular communication among bacteria (Hoyland-Kroghsbo et al. 2013). Consequently, the presence of quorum leads to a decrease in the abundance of phage receptors, thereby reducing phage production (Li et al. 2020).

The lytic activity of specific phages isolated from the river of Palembang exhibited clear plaque when they were introduced to antibiotic-resistant *E. coli* as host organisms. The clear plaque exhibits distinct characteristics that indicate the isolated phage infects and lyse the host

effectively. The isolated phages possess the capacity to serve as a biologically-based control mechanism in mitigating the prevalence of antibiotic-resistant *E. coli* bacteria.

**Host range assessment**

A host range assessment was conducted using eight distinct strains of antibiotic-resistant *E. coli* bacteria (EcSK1-1, EcSK2-2, EcSK3-1, EcSB1-3, EcSB3-3, EcPU1-1, EcPU3-1, and EcPU3-3) to ascertain the host range of isolated phages. The confirmation of susceptibility of the bacterial strain was achieved through the implementation of a two-layer agar overlay test. Table 4 displays the outcomes of the host range test.

The phage exhibits a high degree of specificity to induce lysis in its host. The capacity to cause lysis in their host is attributed to the obligate parasitic nature of phages, which enables them to exploit the metabolic processes of their host to proliferate and subsequently induce lysis in the host cells. Since phages, as obligate parasites of bacteria, must coexist with their host organisms, they are commonly observed close to bacterial species (Naureen et al. 2020). Bacteriophages rely on host organisms possessing the necessary cellular machinery for protein synthesis, as phages lack this capability. Phages exhibit selectivity based on their suitability to the system. They are only suitable if compatibility exists, resulting in the infection of a limited number of bacteria (Galtier et al. 2017).

FgSK11.2 demonstrates the wide range of hosts, as evidenced by their ability to lyse not only their host, EcSK1-1 but also EcSK3-1, which were not the natural hosts. Meanwhile, the phage isolates of FgSB13.2 and FgSB13.3 exhibit the capability to lyse both EcSB1-3, which serves as the host and EcSB3-3. The widespread levels of host tolerance may infer that these isolated phages originate from different hosts with identical cell surface receptors. It is also supported by the fact that the corresponding isolate was taken on connected waterways. However, it does not necessarily indicate a problem, as the infected bacteria strain remains consistent regarding the bacterial type. Furthermore, a considerable number of isolated phages possess the ability to recognize multiple surface receptors. Variability of host range was also observed in phages Bp7 (Chen et al. 2020), phage rV5 (Kropinski et al. 2013), phage PA13076 and PC2184 (Bao et al. 2015).

The assessment revealed that the isolated phages exhibited a broad spectrum of infectivity and lytic effectiveness against *E. coli*. The ability of phages to selectively target specific bacteria may be attributed to the presence of phage receptor ligands on the surface of the bacterial host cell. These ligands facilitate recognition for the subsequent fag adsorption (Brüssow 2016). Phages demonstrate a highly selective host range, limited to a subset of the examined *E. coli* strains, while incapable of infecting other *E. coli* strains or strains of different species (Peng and Yuan 2018). Furthermore, the host range is expected to be associated with host receptors and correlated with DNA methylase activity (Jatmiko et al. 2018).

Phages can adhere to bacterial cells, particularly in specific vulnerable regions surrounding the bacterial cell wall (Kudva et al. 1999). Gram-negative bacteria exhibit high sensitivity in their receptor binding protein (RBP), lipopolysaccharide (LPS), and the peptidoglycan layer encompassing the cell wall's external surface. Different strains or groups of phages exhibit specificity in their attachment to receptors on the cellular structure of bacteria, with certain phages binding to specific receptors while others bind to distinct receptors. Several bacterial structures, including flagella, pillus, capsule, teichoic acid, LPS, and outer membrane proteins (OMP), have been identified as specific receptors that play a role in facilitating phage infection (Javed et al. 2013; Santos 2011; Brüssow 2016; Steimle et al. 2016; Washizaki et al. 2016). The surface proteins of *E. coli* encompass the ferrichrome transporter FhuA and TrxA (thioredoxin) (Zou et al. 2022). Phages utilize these receptors for DNA injection. Furthermore, it is believed that phages with a broad spectrum of hosts are capable of identifying R-type LPS, which are prevalent on the bacterial surface and commonly found in various gram-negative bacterial groups (Santos et al. 2011).

**Table 4.** The result of the host range assessment

| Fag isolates code | Host isolates code | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **EcPU1-1** | **EcPU3-1** | **EcPU3-3** | **EcSB1-3** | **EcSB3-3** | **EcSK1-1** | **EcSK2-2** | **EcSK3-1** |
| FgSK11.2 | - | - | - | - | - | + | - | + |
| FgSK22.2 | - | - | - | - | - | - | + | - |
| FgSK31.3 | - | - | - | - | - | - | - | + |
| FgSB13.2 | - | - | - | + | + | - | - | - |
| FgSB13.3 | - | - | - | + | + | - | - | - |
| FgSB33.1 | - | - | - | - | + | - | - | - |
| FgPU11.2 | + | - | - | - | - | - | - | - |
| FgPU11.3 | + | - | - | - | - | - | - | - |
| FgPU31.3 | - | + | - | - | - | - | - | - |
| FgPU33.1 | - | - | + | - | - | - | - | - |
| FgPU33.2 | - | - | + | - | - | - | - | - |
| FgPU33.3 | - | - | + | - | - | - | - | - |

Note: Able to lyse (+), unable to lyse (-)

FgSK11.2 demonstrated the ability to lyse their host (EcSK1-1) and non-host strain (EcSK3-1) due to their affinity towards a specific receptor molecule present in both strains. FgSB13.2 and FgSB13.3, which were obtained from EcSB1-3, exhibited a similar specificity pattern in which they were also capable of lysing EcSB3-3 due to the presence of specific receptors that shared similar properties. The result is comparable to Park et al. (2020), which showed vB_EcoM-ECP26 phage's ability to induce lysis in *E. coli* NCCP 13893 (O-negative antigen) and exhibited a wide range of host specificity. Therefore, a total of 12 isolated phages showed potential as biocontrol agents (FgSK11.2, FgSK22.2, FgSK31.3, FgSB13.2, FgSB13.3, FgSB33.1, FgPU11.2, FgPU11.3, FgPU31.3, FgPU33.1, FgPU33.2, and FgPU33.3).

**Effectiveness of bacteriophage**

The efficiency of the phage in lysing antibiotic-resistant *E. coli* was tested after the host range assessment. The correlation between the growth of control cultures and infected cultures (phage treatment) is visualized using bacterial growth curves. Figure 3 illustrates how the presence of phages alters the number of bacteria cultures in the treatment and control. The lytic activity caused by phages can destroy cells within a set amount of time, resulting in a decrease in the number of bacteria cells in the culture.

Bacterial growth curves are employed to assess the effectiveness of bacteriophages in inducing the reduction of bacterial populations through their virulent properties. The effectiveness of phage infection in combating antibiotic-resistant *E. coli* was demonstrated by the reduction in the population of *E. coli* upon the administration of phage (Figure 3). Phage infections have the potential to cause substantial reductions in populations. Introducing bacteriophages can reduce the population of *E. coli* due to phage-induced lysis within a specific timeframe. Compared to the control culture, the phages SK31.3, SB13.3, and PU11.3 exhibited the most significant activity, as evidenced by the slightest decrease in $OD_{600}$ of *E. coli* cells after a one-hour incubation period.

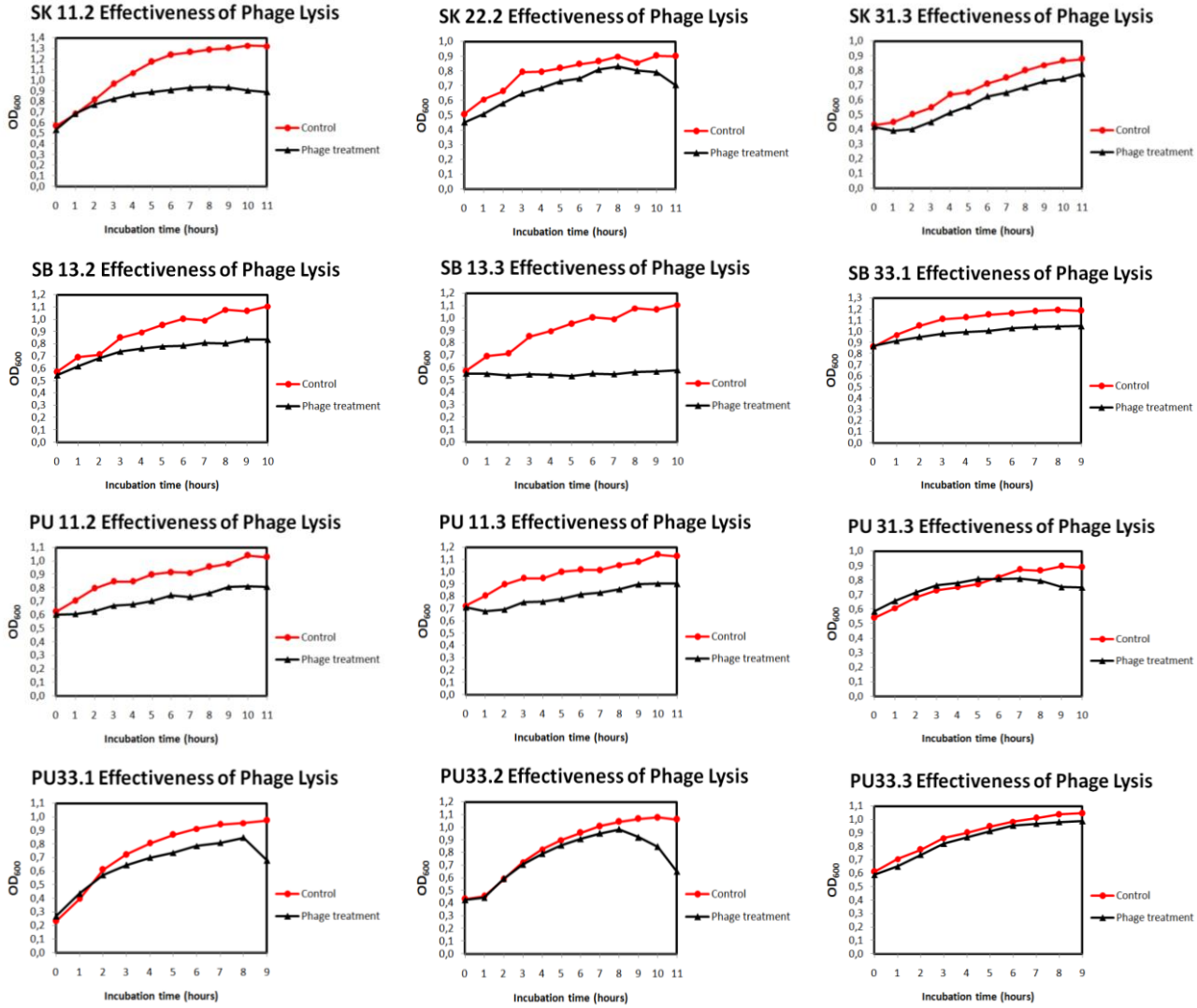


**Figure 3.** Phage effectiveness in lysing *Escherichia coli*

This is in agreement with similar results obtained by Storms et al. (2020), the T7 phage demonstrated high virulence, as proven by its ability to cause extensive lysis of *E. coli* ATCC 11303 cultures within an hour. Similar behavior was observed at various MOI ranging from 1 to $10^{-3}$. In comparison, namely T4 and T5, the T7 phage exhibited the highest degree of malignancy.

The study recorded a prolonged period of decline in PU11.2 at the seventh hour, SB13.2 and PU31.3 at the eighth hour, SK11.2, SK22.2, PU33.1, and PU33.2 at the ninth hour. Multiple phage treatments, including SK11.2, SK22.2, SB13.3, PU31.3, and PU33.2, consistently exhibited a reduction in bacterial cell count immediately after administration. This is highly commendable as the effectiveness of phage performance is remarkably notable. Following a decrease in *E. coli* cells, the remaining treatments gradually increased. It is widely believed that certain bacteria employ various adaptations, such as the concealment or obstruction of phage receptors on their cellular surface, to evade phage attacks. However, this defence mechanism is temporary as phages possess the ability to identify alternative receptors, rendering this strategy ineffective (Zou et al. 2022).

The *E. coli* cells exhibited a pattern of gradual increase in each phage treatment after a notable reduction of cells. This trend persisted until the final observation period, encompassing the $10^{th}$ and $11^{th}$ hours. Nevertheless, the quantity of *E. coli* cells in the treatment group remained below the control group. The observed pattern of lysis and phage propagation is associated with inherent characteristics that facilitate the proliferation and dissemination of infection. The observed proliferation of *E. coli* cells may be attributed to the comparatively slower rate of viral replication, resulting in a substantial increase in the cell population. Consistent with Abedon et al. (2011), the bacterial host's population density increased after 24 hours. The observed rise in population levels could be attributed to the preferential survival of bacterial strains that are not susceptible to phage infection. In turn, it will lead to an overabundance of phenotypes that exhibit resistance or the emergence of mutated strains within the bacterial population.

Phages will replicate as long as the host is available. Nucleic acids and capsomeres can be produced in the host if the restriction-modification (RM) system does not recognize the phage genome matrix (Asakura and Kobayashi 2009). When the phage component has finished replicating, the RM gene complex is removed by interfering with bacterial DNA methylation, the impact is restriction damage in the genome, and post-segregation cell killing occurs (Mruk and Kobayashi 2014). Phages, with their ability to increase virulence, will be better because they can quickly reduce the number of bacteria and inhibit bacterial growth (Storms et al. 2020). The level of virulence is supported by environmental and physiological parameters described in Lindberg et al. (2014) and Vandersteegen et al. (2011). The composition of the media, pH, aeration, and temperature can all affect the host bacteria, which is directly related to the proliferation of phage growth (absorption, latent length, blast size) in the host infection process.

The quantification of infected bacterial cells is frequently accomplished through the utilization of latency period, burst period, and phase burst sizes (Bao et al. 2015; Park et al. 2020). Park et al. (2020) observed that the latency period of the *E. coli* phage vB_EcoM-ECP26 was 55 minutes, with a burst size of 1914 PFU per cell. In another case, Manohar et al. (2018) reported that the *E. coli* phage my PSH1131 shows a latency period of 20 min and a burst size of 130 PFU/cell. These findings indicate that the latency period and burst size can vary across bacteriophages. Hence, it is imperative to consider all concurrent factors to assess the phage capabilities effectively. The parameter was estimated by monitoring the formation of visible plaque. The formation of each plaque, which comprises a maximum of $10^9$ virions, is initiated by a single virus particle (Bao et al. 2015). Upon infection by a bacteriophage, the formation of any plaque initiates the lytic reproductive cycle, gradually disseminating to adjacent cells. Various factors impacting plaque formation encompass the optimization of incubation and coating conditions. The condition is imperative in facilitating the adsorption process of bacteriophages onto their respective host bacteria. The latency period of isolated phages is seemingly extended, lasting 12 hours. This interval denotes the period starting from the adsorption of phages onto the bacterial cell and ending with the release of an adequate number of phage progeny, forming a visible plaque. A decreased latency period suggests successful host infection and robust phage lytic activity. Previous studies have documented extended latency periods in various phages, such as adenoviruses (Elshayeb et al. 2011). This phenomenon can be attributed to the sluggish adsorption of phages onto the lining and their subsequent gradual release into the surrounding medium.

On the other hand, the magnitude of the phage burst was assessed by calculating the ratio between the final count of particles and the number of bacterial cells infected during the latency period (Park et al. 2012). Litt and Jaroni (2017) succeeded in isolating a total of seven *E. coli* bacteriophages. These bacteriophages were found to possess relatively short latency periods, ranging from 12 to 30 minutes, and substantial burst sizes, ranging from 89 to 631 virions per infected cell. Park et al. (2020) reported a brief latency period of 25 minutes for the host phage SFP10 in *E. coli* O157:H7. On the other hand, it is noteworthy that the burst size exhibited a remarkably substantial magnitude, amounting to 100 PFU per cell. The latency period was determined to be approximately 55 minutes, while the burst size was measured to be 1914 PFU per cell. The size of the bursts is determined by a multitude of factors, including but not limited to the specific type of host bacteria, metabolic processes, and prevailing environmental conditions (Abedon et al. 2011).

During the procedure, the isolated bacteriophages were subjected to meticulous conditioning, including pH, temperature, and the initial quantities of hosts and phages. The $OD_{600}$ measurements indicated that the isolated phages exhibited favorable characteristics and demonstrated effectiveness in reducing the population of antibiotic-

resistant *E. coli*, which served as their host organisms. The presence of antibiotic-resistant *E. coli* in the rivers of Palembang City poses a potential risk for individuals who rely on the water for their sanitation and hygiene requirements. Phages that were successfully isolated from the KedukanRiver, BuahRiver, and PU River isolates (FgSK11.2, FgSK22.2, FgSK31.3, FgSB13.2, FgSB13.3, FgSB33.1, FgPU11.2, FgPU11.3, FgPU31.3, FgPU33.1, FgPU33.2, and FgPU33.3) exhibit potential as a natural biocontrol agent for mitigating the contamination of antibiotic-resistant *E. coli* bacteria in the river of Palembang City.

**Effectiveness of bacteriophage biocontrol**

The phages have been proven to exhibit lytic activity towards their respective hosts, and certain phages demonstrated a broad host range against isolated strains of antibiotic-resistant *E. coli*. To comply with regulatory requirements, it is imperative to examine cocktail phages within contaminated water samples before their utilization for water treatment. A phage cocktail from a composite of 12 distinct isolated phages was examined. Contaminated water samples were collected from the same rivers, namely Kedukan, Buah, and PU rivers, to conduct in vitro biocontrol experiments on antibiotic-resistant *E. coli* phage. The examination will be conducted on the sterilized and non-sterilized water samples. The antibiotic-resistant *E. coli* bacteria reduction in the water sample was evaluated under laboratory conditions by measuring their $OD_{600}$ (Figure 4).

The study observed the effectiveness of phage cocktails in reducing antibiotic-resistant *E. coli* in sterilized polluted water samples. The results demonstrated that the treatment was highly effective, with the shortest duration of cell reduction observed in the PUI (1 hour) and PUT (2 hours). In contrast, the quantity of cell masses in other samples progressively increases until the end of the observation. The data presented in Figure 4 indicates that cell mass growth in the phage-treated group was comparatively less pronounced compared to the control group, implying the effectiveness of the phage cocktail treatment on host bacterial cells. However, the effectiveness was reduced in certain contamination samples, namely SBT and SPU. This observation was supported by the fact that the cell count in these samples was higher compared to the control group. The bacteria in aquatic environments may acquire resistance to phages, resulting in the ineffectiveness of phage cocktails in reducing bacterial cell populations. Nevertheless, Chan et al. (2016) and Oechslin (2018) implied that phage-resistant bacteria could directly attenuate bacterial virulence, evidenced by the low magnitude and the lack of statistical significance in cell increase. Kauppinen et al. (2021) documented that the resistance of PAO1 to phage V524 resulted in a progressive transformation of the host colonies on the non-standard agar plates into a more spherical shape. Therefore, for treatment in aquatic environments, weak pathogens that have been treated with phages are more vulnerable to disinfectants (Agún et al. 2018).

The phage infection also effectively reduced antibiotic-resistant *E. coli* in the non-sterilized water sample. The shortest duration for cell reduction was observed in the PUU and PUT treatments. Both achieved a significant reduction in host cells within one hour after inoculation. Meanwhile, the SKI group exhibited a longer duration of 5 hours, even more than the SBU group, which is 6 hours. The findings indicated that cocktail outcomes were more favorable in non-sterilized samples. Only particular samples demonstrated a continued increase in the $OD_{600}$ value until the end of the observation period. Generally, the number of cells exhibited a decline compared to the control group, which implies that a reduction in cellular mass occurred, thereby indicating the capacity of the phages to induce lysis in their host.

The difference between sterile and non-sterile samples is not substantial. While it is observed that the cell density in non-sterile media tends to be higher, the majority of samples exhibit favorable outcomes due to the presence of naturally occurring phages that expedite the decline of *E. coli*. Besides that, the presence of appropriate natural bacteria in non-sterile can serve as a source of phage nutrition, leading to phage proliferation and enhancing the infectivity of phages (Kauppinen et al. 2021).

Overall, the examination of the effectiveness of a phage cocktail in targeting antibiotic-resistant *E. coli* in contaminated water samples yielded predominantly positive outcomes. Phages can regulate bacterial growth and population density in aquatic environments. The utilization of phage cocktails, which comprise a blend of phages targeting various species or strains of bacteria, pertains to diverse phages functioning as bacterial sensors (Chan et al. 2013). Moreover, a wide range of host susceptibility in specific phages in this study bolsters the performance of phage cocktails, as recommended by Kauppinen et al. (2021). Furthermore, the examination demonstrates promising potential based on burst size and latency period. Significant burst size and rapid reduction were observed within one hour of inoculation. These attributes are desirable for phages in biocontrol applications (Gill and Hyman 2010).

In conclusion, the study successfully isolated phages from the river of Palembang City, which were contaminated with antibiotic-resistant *E. coli*, specifically, three phage isolates (designated as FgSK11.2, FgSK22.2, and FgSK31.3) were obtained from the Kedukan River, three phage isolates (coded as FgSB13.2, FgSB13.3, and FgSB33.1) were obtained from the Buah River, and six phage isolates (with codes FgPU11.2, FgPU11.3, FgPU31.3, FgPU33.1, FgPU33.2, and FgPU33.3) from the PU River. The isolate demonstrates significant lytic effectiveness in mitigating antibiotic-resistant *E. coli*. The study focused on addressing the issue of bacteriophage contamination in water samples by examining the effectiveness of phage infection in mitigating the presence of antibiotic-resistant *E. coli*. Although the decrease in physical and chemical water pollution levels was not highly significant, the parameters for Fe and Pb showed satisfactory results following phage treatment. A further understanding is required to enhance the effectiveness of these phages as biocontrol agents for water pollution, which includes the exploration of phage cocktail design and the utilization in conjunction with adjuvants to mitigate the development of phage resistance.

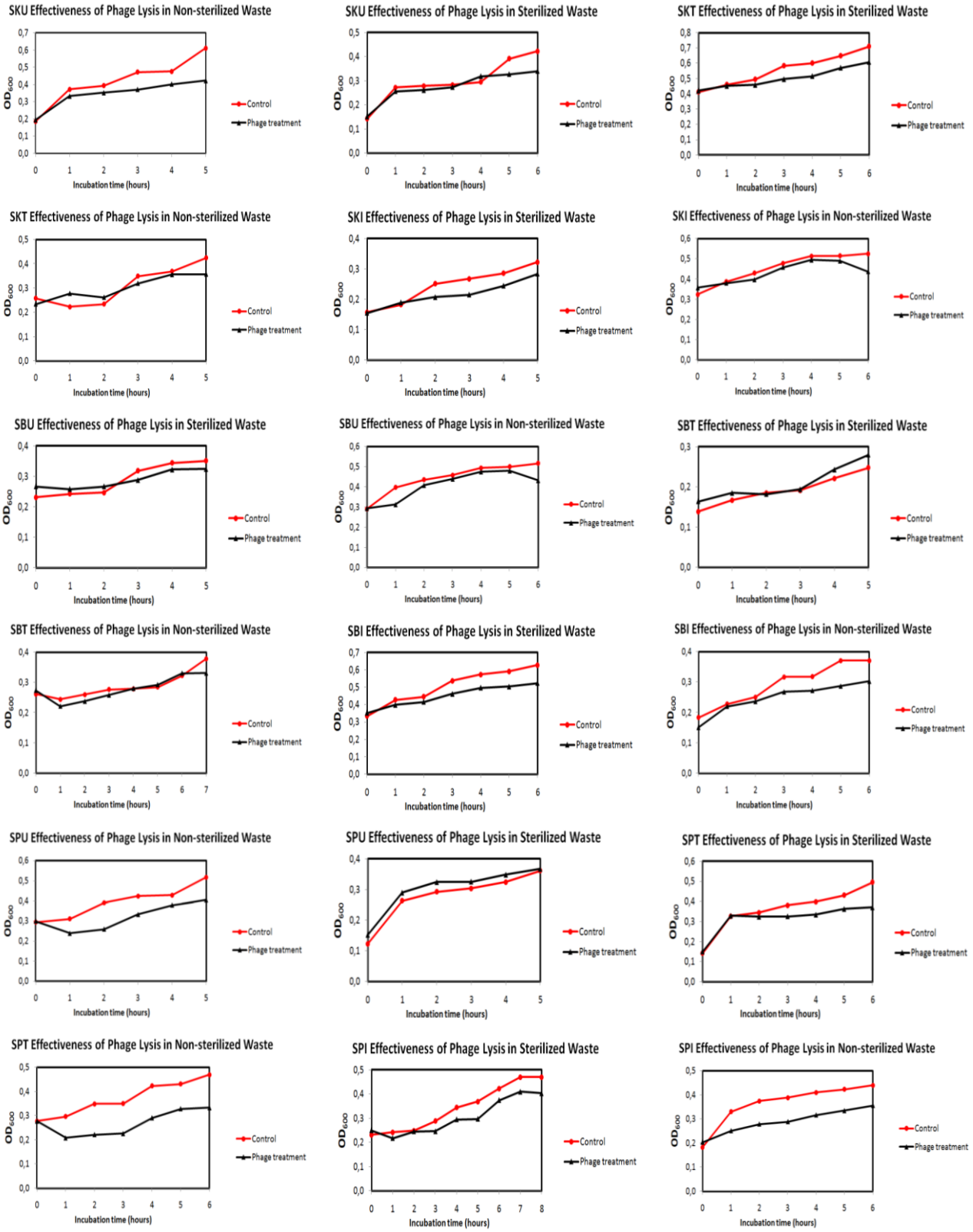


**Figure 4.** The assessment of the effectiveness of bacteriophages as biocontrol

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## REFERENCES

Abedon ST, Kuhl SJ, Blasdel BG, Kutter EM. 2011. Phage treatment of human infections. Bacteriophage 1 (2): 66-85. DOI: 10.4161/bact.1.2.15845.

Abiodun O-AO, Oluwaseun O, Oladayo OK, Abayomi O, George AA, Opatola E, Orah RF, Isukuru EJ, Ede IC, Oluwayomi OT, Okolie JA, Omotayo IA. 2023. Remediation of heavy metals using biomass-based adsorbents: Adsorption kinetics and isotherm models. Clean Technol 5 (3): 934-960. DOI: 10.3390/cleantechnol5030047.

Agún S, Fernández L, González-Menéndez E, Martínez B, Rodríguez A, García P. 2018. Study of the interactions between bacteriophage phiIPLA-RODI and four chemical disinfectants for the elimination of *Staphylococcus aureus* contamination. Viruses 10 (3): 1-12. DOI: 10.3390/v10030103.

Asakura Y, Kobayashi I. 2009. From damaged genome to cell surface: Transcriptome changes during bacterial cell death triggered by loss of a restriction-modification gene complex. Nucleic Acids Res 37 (9): 3021-3031. DOI: 10.1093/nar/gkp148.

Atterbury RJ, Van Bergenet MAP, Ortiz F, Lovell MA, Harris JA, Boer AD, Wagenaar JA, Allen VM, Barrow PA. 2007. Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. Appl Environ Microbiol 73 (14): 4543-4549. DOI: 10.1128/AEM.00049-07.

Bao H, Zhang P, Zhang H, Zhou Y, Zhang L, Wang R. 2015. Bio-control of *Salmonella* enteritidis in foods using bacteriophages. Viruses 7 (8): 4836-4853. DOI: 10.3390/v7082847.

Boyd CE. 2015. Water quality: An introduction. Springer, Berlin. DOI: 10.1007/978-3-319-17446-4_15.

Brüssow H. 2016. Targeting the gut to protect the bladder: Oral phage therapy approaches against urinary *Escherichia coli* infections?. Environ Microbiol 18 (7): 2084-2088. DOI: 10.1111/1462-2920.13310.

Chan BK, Abedon ST, Loc-Carrillo C. 2013. Phage cocktails and the future of phage therapy. Future Microbiol 8 (6): 769-783. DOI: 10.2217/fmb.13.47.

Chan BK, Sistrom M, Wertz JE. Kortright KE, Narayan D, Turner PE. 2016. Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*. Sci Rep 6: 1-8. DOI: 10.1038/srep26717.

Chen P, Sun H, Ren H, Liu W, Li G, Zhang C. 2020. LamB, OmpC, and the Core Lipopolysaccharide of *Escherichia coli* K-12 function as receptors of bacteriophage Bp7. J Virol 94 (12): 10-1128. DOI: 10.1128/JVI.00325-20.

Chibani-Chennoufi S, Sidoti J, Bruttin A, Kutter E, Sarker S, Brüssow H. 2004. In vitro and in vivo bacteriolytic activities of *Escherichia coli* phages: Implications for phage therapy. Antimicrob Agents Chemother 69 (9): 5364-5371. DOI: 10.1128/aac.48.7.2558-2569.2004.

Christine G, Budiarti S, Astuti I. 2018. Diversity of urinary tract infection bacteria in children in Indonesia based on metagenomics approach. Biodiversitas 19 (4): 1375-1381. DOI: 10.13057/biodiv/d190425.

Dalmasso M, Strain R, Neve H, Franz CMAP, Cousin FJ, Ross RP, Hill C. 2016. Three new *Escherichia coli* phages from the human gut show promising potential for phage therapy. PLoS ONE 11 (6): e0156773. DOI: 10.1371/journal.pone.0156773.

Domingo-Calap P, Delgado-Martínez J. 2018. Bacteriophages: Protagonists of a post-antibiotic era. Antibiotics 7 (66): 1-16. DOI: 10.3390/antibiotics7030066.

Elshayeb AA, Yagoub SO, Yousif AS, Abedalkareem EA, El-Hag SM, Elagib AA. 2011. Identification of protein profiles of *Escherichia coli*, *Staphylococcus aureus* and their corresponding phages. Am J Biotechnol Mol Sci 1: 39-44. DOI: 10.5251/ajbms.2011.1.2.39.44.

Foschino R, Perrone F, Galli A. 1995. Characterization of two virulent *Lactobacillus fermentum* bacteriophages isolated from sour dough. J Appl Bacteriol 79 (6): 677-683. DOI: 10.1111/j.1365-2672.1995.tb00954.x.

Galtier M, De Sordi L, Sivignon A, De Vallée A, Maura D, Neut C, Rahmouni O, Wannerberger K, Darfeuille-Michaud A, Desreumaux P, Barnich N, Debarbieux L. 2017. Bacteriophages targeting adherent invasive *Escherichia Coli* strains as a promising new treatment for Crohn's disease. J Crohns Colitis 11 (7): 840-847. DOI: 10.1093/ecco-jcc/jjw224.

Garvey M. 2022. Bacteriophages and food production: Biocontrol and bio-preservation options for food safety. Antibiotics 11 (10): 1-10. DOI: 10.3390/antibiotics11101324.

Gill JJ, Hyman P. 2010. Phage choice, isolation, and preparation for phage therapy. Curr Pharm Biotechnol 11 (1): 2-14. DOI: 10.2174/138920110790725311.

Hoyland-Kroghsbo NM, Maerkedahl RB, Lo Svenningsen S. 2013. A quorum-sensing-induced bacteriophage defense mechanism. mBio 4 (1): e00362-12. DOI: 10.1128/mBio.00362-12.

Jatmiko YD, Purwanto AP, Ardyati T. 2018. Uji aktivitas bakteriofage litik dari limbah rumah tangga terhadap *Salmonella Typhi*. Jurnal Biodjati 3 (2): 134-147. DOI: 10.15575/biodjati.v3i2.3471. [Indonesian]

Javed MA, Poshtiban S, Arutyunov D, Evoy S, Szymanski CM. 2013. Bacteriophage receptor binding protein-based assays for the simultaneous detection of *Campylobacter jejuni* and *Campylobacter coli*. PLoS ONE 8 (7): e0069770. DOI: 10.1371/journal.pone.0069770.

Kauppinen A, Siponen S, Pitkänen T, Holmfeldt K, Pursiainen A, Torvinen E, Miettinen IT. 2021. Phage biocontrol of *Pseudomonas aeruginosa* in water.Viruses 13 (5): 1-16. DOI: 10.3390/v13050928.

Kim KS. 2016. Human meningitis-Associated *Escherichia coli*. EcoSal Plus 7 (1): 1-15. DOI: 10.1128/ecosalplus.ESP-0015-2015.

Koda E, Miszkowska A, Sieczka A. 2017. Levels of organic pollution indicators in groundwater at the old landfill and waste management site. Appl Sci 7 (6): 1-22. DOI: 10.3390/app7060638.

Kropinski AM, Waddell T, Meng J, Franklin K, Ackermann H-W, Ahmed R, Mazzocco A, Yates J, Lingohr EJ, Johnson RP. 2013. The host range, genomics and proteomics of *Escherichia coli* O157:H7 bacteriophage rV5. Virol J 10 (76): 1-12. DOI: 10.1186/1743-422X-10-76.

Kudva IT, Jelacic S, Tarr PI, Youderian P, Hovde CJ. 1999. Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. Appl Environ Microbiol 65: 3767-3773. DOI: 10.1128/AEM.65.9.3767-3773.1999.

Lee DS, Lee S-J, Choe H-S. 2018. Community-acquired urinary tract infection by *Escherichia coli* in the era of antibiotic resistance. BioMed Res Intl 2018: 7656752. DOI: 10.1155/2018/7656752.

Leptihn S. 2019. Welcome back to the pre-penicillin era. why we desperately need new strategies in the battle against bacterial pathogens. Infect Microbes Dis 1 (2): 33. DOI: 10.1097/IM9.0000000000000009.

Li X, Gonzalez F, Esteves N, Scharf BE, Chen J. 2020. Formation of phage lysis patterns and implications on co-propagation of phages and motile host bacteria. PloS Comput Biol 16 (3): 1-22. DOI: 10.1371/journal.pcbi.1007236.

Liao Y-T, Sallvador A, Harden LA, Liu F, Lavenburg VM, Li RW, Wu VCH. 2019. Characterization of a lytic bacteriophage as an antimicrobial agent for biocontrol of Shiga toxin-producing *Escherichia coli* O145 strains. Antibiotics 8 (2): 1-19. DOI: 10.3390/antibiotics8020074.

Lindberg HM, McKean KA, Wang I-N. 2014. Phage fitness may help predict phage therapy efficacy. Bacteriophage 4 (4): 1-7. DOI: 10.4161/21597073.2014.964081.

Lingga R, Budiarti SRI, Rusmana I, Wahyudi AT. 2020. Isolation, characterization and efficacy of lytic bacteriophages against pathogenic *Escherichia coli* from hospital liquid waste. Biodiversitas 21 (7): 3234-3241. DOI: 10.13057/biodiv/d210745.

Litt PK, Jaroni D. 2017. Isolation and physiomorphological characterization of *Escherichia coli* O157: H7-infecting bacteriophages recovered from beef cattle operations. Intl J Microbiol 2017: 1-13. DOI: 10.1155/2017/7013236.

Liu Y, Zhu M, Fu X, Cai J, Chen S, Lin Y, Jiang N, Chen S, Lin Z. 2021. *Escherichia coli* causing neonatal meningitis during 2001-2020: A study in eastern China. Intl J Gen Med 14: 3007-3016. DOI: 10.2147/IJGM.S317299.

Mohsen IH, Mohsen AH, Zaidan HK. 2019. Health effects of chlorinated water: The meaning of lucidity. Pak J Biotechnol 16 (3): 263-267. DOI: 10.34016/pjbt.2019.16.3.24.

Mruk I, Kobayashi I. 2014. To be or not to be: Regulation of restriction-modification systems and other toxin-antitoxin systems. Nucleic Acids Res 42 (1): 70-86. DOI: 10.1093/nar/gkt711.

Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. Front Microbiol 10 (539): 1-24. DOI: 10.3389/fmicb.2019.00539.

Naureen Z, Dautaj A, Anpilogov K, Camilleri G, Dhuli K, Tanzi B, Maltese PE, Cristofoli F, De Antoni L, Beccari T, Dundar M, Bertelli M. 2020. Bacteriophages presence in nature and their role in the natural selection of bacterial populations. Acta Biomed 91 (1): 1-13. DOI: 10.23750/abm.v91i13-S.10819.

Necel A, Bloch S, Nejman-Faleńczyk B, Grabski M, Topka G, Dydecka A, Kosznik-Kwaśnicka K, Grabowski Ł, Jurczak-Kurek A, Wołkowicz T, Węgrzyn G. 2020. Characterization of a bacteriophage, vB_Eco4M-7, that effectively infects many *Escherichia coli* O157 strains. Sci Rep 10 (1): 3743. DOI: 10.1038/s41598-020-60568-4.

Oechslin F. 2018. Resistance development to bacteriophages occurring during bacteriophage therapy. Viruses 10 (7): 351. DOI: 10.3390/v10070351.

Park D-W, Lim G-y, Lee Y-d, Park J-H. 2020. Characteristics of lytic phage vB _ EcoM- ECP26 and reduction of shiga-toxin producing *Escherichia coli* on produce romaine. Appl Biol Chem 63 (19): 1-9. DOI: 10.1186/s13765-020-00502-4.

Park M, Lee JH, Shin H, Kim M, Choi J, Kang DH, Heu S, Ryu S. 2012. Characterization and comparative genomic analysis of a novel bacteriophage, SFP10, simultaneously inhibiting both *Salmonella enteric* and *Escherichia coli* O157: H7. Appl Environ Microbiol 78: 58-69. DOI: 10.1128/AEM.06231-11.

Payus C, Ying TS, Kui WN. 2016. Effect of heavy metal contamination on DNA mutations in Nepenthes plants from abandoned mines. Res J Environ Toxicol 10 (4): 193-204. DOI: 10.3923/rjet.2016.193.204.

Peng Q, Yuan Y. 2018. Characterization of a newly isolated phage infecting pathogenic *Escherichia coli* and analysis of its mosaic structural genes. Sci Rep 8 (1): 1-10. DOI: 10.1038/s41598-018-26004-4.

Pirnay J-P, Verbeken G, Ceyssens P-J, Huys I, De Vos D, Ameloot C, Fauconnier A. 2018. The magistral phage. Viruses 10 (2): 64. DOI: 10.3390/v10020064.

Poolman JT, Wacker M. 2016. Extraintestinal pathogenic *Escherichia coli*, a common human pathogen: Challenges for vaccine development and progress in the field. J Infect Dis 213: 1-8. DOI: 10.1093/infdis/jiv429.

Manohar P, Tamhankar AJ, Lundborg CS, Ramesh N. 2018. Isolation, characterization and in vivo efficacy of *Escherichia* phage myPSH1131. PLoS ONE 13 (10): e0206278. DOI: 10.1371/journal.pone.0206278.

Prestinaci F, Pezzotti P, Pantosti A. 2015. Antimicrobial resistance: A global multifaceted phenomenon. Pathog Glob Health 109 (7): 309-318. DOI: 10.1179/2047773215Y.0000000030.

Razif M. 2022. BOD, COD, and TSS predictions from DO measurement results for the Surabaya River, Indonesia. J Civil Eng Plann Des 1 (1): 1-7. DOI: 10.31284/j.jcepd.2022.v1i1.3047.

Romero-Calle D, Guimarães BR, Góes-Neto A, Billington C. 2019. Bacteriophages as alternatives to antibiotics in clinical care. Antibiotics (Basel) 8 (3): 138. DOI: 10.3390/antibiotics8030138.

Santos SB, Kropinski AM, Ceyssens P-J, Ackermann H-W, Villegas A, Lavigne R, Krylov VN, Carvalho CM, Ferreira EC, Azeredo J. 2011. Genomic and proteomic characterization of the broad-host-range *Salmonella* phage PVP-SE1: Creation of a new phage genus. J Virol 85 (21): 11265-11273. DOI: 10.1128/JVI.01769-10.

SNI. 2009. Indonesian National Standard (SNI 6989.72:2009); Water and wastewater–Section 2:Biochemical Oxygen Demand (BOD) testing methods. National Standardization Agency, Jakarta. http://sispk.bsn.go.id/sni/DetailSNI/8217.

SNI. 2019. Indonesian National Standard (SNI6989.2:2009); Waterandwastewater-Part2: Test method for chemical oxygen demand (COD) with closed reflux spectrophotometrically. National Standardization Agency, Jakarta. http://sispk.bsn.go.id/sni/DetailSNI/7861.

SNI. 2004. Indonesian National Standard (SNI06-6989.3:2004); Water and waste water-Part3: Test method for total suspended solids (TSS) gravimetrically. National Standardization Agency, Jakarta. http://sispk.bsn.go.id/sni/DetailSNI/6725.

SNI. 2009. Indonesian National Standard (SNI6989.4:2009); Water and waste water-Part4: Method of the assay for iron (Fe) by Atomic Absorption Spectrophotometry (AAS)-flame. National Standardization Agency, Jakarta. http://sispk.bsn.go.id/sni/DetailSNI/7864.

SNI. 2005. Indonesian National Standard (SNI06-6989.38:2005); Water and waste water-Part 38: Method of testing the levels of Cadmium (Cd) with an Atomic Absorption Spectrophotometer(AAS)in a carbon furnace.National Standardization Agency, Jakarta. http://sispk.bsn.go.id/sni/DetailSNI/7011.

SNI. 2009. Indonesian National Standard (SNI6989.8:2009); Water and waste water-Part8: Method of the assay for Lead (Pb) by Atomic Absorption Spectrophotometry (AAS)–flame. National Standardization Agency, Jakarta.http://sispk.bsn.go.id/sni/DetailSNI/8199.

SNI. 2009. Indonesian National Standard (SNI6989.19:2009); Water and wastewater–Part19: Method of chloride (Cl-) test by the argentometric method. National Standardization Agency, Jakarta. http://sispk.bsn.go.id/sni/DetailSNI/7862.

Srivastava P, Mishra CP, Nath G. 2022. Bacteriophages can make a difference in water quality: Evidence from a community-based study from North India. Cureus 14 (8): e27551. DOI: 10.7759/cureus.27551.

Steimle A, Autenrieth IB, Frick J-S. 2016. Structure and function: Lipid modifications in commensals and pathogens. Intl J Med Microbiol 306: 290-301. DOI: 10.1016/j.ijmm.2016.03.001.

Storms ZJ, Teel MR, Mercurio K, Sauvageau D. 2020. The virulence index: A metric for quantitative analysis of phage virulence. Phage 1 (1): 27-36. DOI: 10.1089/phage.2019.0001.

Sunarti RN, Budiarti S, Verawaty M, Siregar BA, Hariani PL. 2022. Diversity of antibiotic-resistant *Escherichia coli* from rivers in Palembang City, South Sumatera, Indonesia. AIMS Environ Sci 9 (5): 721-734. DOI: 10.3934/envionsci.2022041.

Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, Ouellette M, Outterson K, Patel J, Cavaleri M, Cox EM, Houchens CR, Grayson ML, Hansen P, Singh N, Theuretzbacher U, Magrini N. 2018. Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 18 (3): 318-327. DOI: 10.1016/S1473-3099(17)30753-3.

Vandersteegen K, Mattheus W, Ceyssens P-J, Bilocq F, De Vos D, Pirnay J-P. 2011. Microbiological and molecular assessment of bacteriophage ISP for the control of *Staphylococcus aureus*. PLoS ONE 6 (9): e0024418. DOI: 10.1371/journal.pone.0024418.

Verawaty M, Apriani N, Tarigan LR, Aprian ET, Laurenta WC, Muharni. 2020. Antibiotics-resistant *Escherichia coli* isolated from aquatic ecosystems in Palembang, South Sumatra, Indonesia. Biodiversitas 21 (1): 86-97. DOI: 10.13057/biodiv/d210113.

Von Wintersdorff CJH, Penders J, Van Niekerk JM, Mills ND, Majumder S, Van Alphen LB, Savelkoul PH, Wolffs PF. 2016. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Front Microbiol 7: 173. DOI: 10.3389/fmicb.2016.00173.

Washizaki A, Yonesaki T, Otsuka Y. 2016. Characterization of the interactions between *Escherichia coli* receptors, LPS and OmpC, and bacteriophage T4 long tail fibres. Microbiol Open 5: 1003-1015. DOI: 10.1002/mbo3.384.

World Health Organization (WHO). 2017. WHO Publishes a List of Bacteria for Which New Antibiotics Are Urgently Needed. WHO, Swiss. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.

World Health Organization (WHO). 2021. Ten Global Health Issues to Track In 2021.WHO, Swiss.https://reliefweb.int/report/world/10-global-health-issues-track-2021.

Yulinery T, Triana E, Suharna N, Nurhidayat N. 2019. Isolation and anti-*Escherichia coli* biofilm activity of lytic bacteriophages isolated from water environment in vitro. IOP Conf Ser: Earth Environ Sci 308: 012010. DOI: 10.1088/1755-1315/308/1/012010.

Zhang J, Li Z, Cao Z, Wang L, Li X, Li S, Xu Y. 2015. Bacteriophages as antimicrobial agents against major pathogens in swine: A review. J Anim Sci Biotechnol 6 (36): 1-7. DOI: 10.1186/s40104-015-0039-7.

Zou X, Xiao X, Mo Z. 2022. Systematic strategies for developing phage-resistant *Escherichia coli* strains. Nat Commun 13: 4491. DOI: 10.1038/s41467-022-31934-9.