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Isolation, visualization and characterization of four somatic coliphages from Egyptian sewage

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Somatic coliphages are a group of bacteriophages that infect *Escherichia coli* and can be used in aquatic environment for risk assessment of viral and fecal pollution. The objective of this work is to isolate bacteriophages specific to *E. coli* strain ATCC-13706, as well as characterization and evaluation of their stability. In this study, an enrichment method was used to isolate phages against *E. coli* strain ATCC 13706 from two wastewater treatment plants, and one opened drain. The isolated phages purified by successive single plaque isolation techniques and results indicated isolation of four different phages. The plaque sizes for isolated phages were between 1.0 and 4.0 mm in diameter with high titer ranging from 19 to 55 × 10^9 PFU/mL. Based on morphology, by Transmission Electron Microscopy, all phages were found to belong to tailed-phages order, *Caudovirales*, and were identified as members of the *Myoviridae* and *Siphoviridae* families. The characterization of these phages revealed high tolerance to temperature degrees ranging from 5 to 75°C, and viability over wide pH values ranging from 3 to 11. Some phages exhibit a wide range of hosts, which could be polyvalent phage, while others were host specific. 1.0 is the optimum ratio of Multiplicity of Infection (MOI) for these phages. In the phage efficiency test, MOI of 1.0 resulted in 63.93, 61.20, 69.39 and 79.14% reduction in bacterial cell count for EcoP1, EcoP2, EcoP3 and EcoP4, respectively, after 5hrs incubation at 37 °C. In one step growth curve, the latent period and burst size of these phages were between 10 – 20 min and 44 – 62 PFU/cell, respectively. Our results indicated that the isolated phages are abundant in Egyptian sewage, with high diversity, and a wide host range, which can be used for biocontrol of some pathogenic bacteria. The isolated phages exhibit high tolerance to a wide range of pH values and temperature degrees, which supports the notion of using phages as indicators of fecal pollution in the Egyptian aquatic environment.

Keywords: Water quality; sewage; *E. coli*; Coliphages, fecal pollution

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INTRODUCTION

Bacteriophages are viruses that infect bacteria, and they are the most prevalent and plentiful living forms on Earth, with an estimated number about 10^{31} particles in the biosphere, and they have been found in various water metrics including fresh, marine, and wastewater (Hendrix et al., 1999; Short and Suttle, 2005; Comeau et al., 2008; Yamaki, et al., 2014; Mushegian, 2020). Phages are a vital element of a sophisticated microbial ecosystem. They exist either as free-floating infectious agents in the environment, including water matrices, or within a bacterium, or absorbed to solid particles (Clokie et al., 2011; Zimmerman et al., 2020). Viruses in general, and bacteriophages in particular, harbor an enormously diverse pool of genes that can be exchanged with other viruses and bacteria (Fuhrman and Schwalbach, 2003; Kenzaka et al., 2010; Jurczak-Kurek et al., 2016). Bacteriophages are recognized for playing a major role in molecular biology science development and are still being heavily utilized in many biotechnological applications, including the food industry, environmental, and medical sectors, due to their unique properties (El-Daly et al., 2018; Wei et al., 2019; El-Telbany et al., 2021; Da Silva et al., 2022). Phages are obligatory parasites of prokaryotes that reproduce by the bacterial and archaeal domains of cellular life, there is some indication that they may interact with eukaryotic by altering their microbiota or bacterial pathogens (Sime-Ngando, 2014; Putra and Lyrawati, 2020).

Water-borne infections caused by bacteria, and viruses have always been considered a major global public health concern (Gall et al. 2015a; Shailemo et al. 2016; WHO, 2020; Ferreira et al. 2021; WHO, 2022), Particularly in developing nations where the socio-economic burden of waterborne illnesses, and viral outbreaks is significant (Célia da Silva Lanna et al., 2019; Adelodun et al., 2021; Shayo et al., 2023). Therefore, the need for an indicator of fecal pollution and viral pollution seems to be vital to assist water quality, avoid water-borne illnesses, and reduce its impact (Anderson et al., 2005; Yates, 2007; Lin and Ganesh, 2013; wen et al., 2020). Several epidemiological studies reported that there is no reliable relationship between conventional fecal indicator bacteria such as *E. coli*, fecal coliform, total coliform, and human enteric viruses, where they

cannot accurately reflect the amount of viral pollution in the environment (Haramoto et al., 2007; Espinosa et al., 2009; Jurzik et al., 2010; Wu et al., 2011; Flannery et al., 2012; McMinn et al., 2017). Because of the weak correlation between fecal indicator bacteria and viruses' fate in the environment, especially in wastewater, bacteriophages have been proposed as an alternative alarming system of fecal and viral contamination of water metrics. Several epidemiological studies have suggested the use of bacteriophages as indicators for human enteric viruses, like human polyomaviruses, adenoviruses, and rotaviruses in addition to enteric pathogenic bacteria (Leclerc et al., 2000; Flannery et al., 2012). Generally, bacteriophages meet both viral, and fecal pollution indicator criteria; they are found at high concentrations in the aquatic environment, economical, fast, and easy to detect (Yahya et al., 2015; Jofre et al., 2016). This is explained by how bacteriophages react to pollutants in the environment; they tend to adsorb to solid particles that are found in wastewater at high concentrations, and they are highly resistant to conventional treatment processes, and most importantly, they are more easily cultivated and detected than human viruses (Grabow, 2001). Two classes of bacteriophages known as somatic and F-specific coliphages are responsible for infecting *E. coli*, and other bacteria found in the gut microbiomes of humans and animals. Both types were proposed as fecal and viral indicators (Armon et al., 1996; Grabow et al., 2001; Jofre, 2007). The most suggested phages for water quality monitoring and management are somatic coliphages, F-specific phages, and crAssphages because of their prevalence, high stability in the environment, and high resistance to treatment processes (Flannery et al., 2012; Jofre et al., 2016; Samhan et al., 2016; Grabow, 2001; Sinton et al*.,* 2002; Arredondo-Hernandez et al., 2017). Compared to conventional markers, somatic coliphages are more enduring to sludge treatments because they are adsorbed to solid particles (Martín-Díaz et al., 2020). Furthermore, due to their lytic capacity, and because bacteriophage infections are specific, they can be used in wastewater treatment facilities as a biological control agent or as a helpful tool for removing pathogenic bacteria (Chen et al., 2013; Ribeiro et al., 2018; Stefanakis et al., 2019).

The American regulatory authorities proposed the use of coliphages in quality control of surface water utilized for recreational and bathing (USEPA, 2015). The Australian guidelines for water quality suggested MS2 bacteriophages as feasible indicators for operational monitoring because of their high stabilities over a wide range of temperature and pH (Amarasiri et al., 2017).

Most bacteriophage's studies in Egypt are focusing on utilizing bacteriophages to control Multi-Drug-Resistant bacteria (Nasr-Eldin and Soliman, 2020; El-Telbany et al., 2021; Soliman *et al., 2*023; Ramadan et al., 2024), while neglecting the environmental applications of bacteriophages. Therefore, the aim of this study is the isolation of somatic coliphages from Egyptian sewage for a better understanding of their diversity, and host specificity in the environment, as well as testing the stability, and viability of isolated phages under some environmental stress, to evaluate the feasibility of using somatic colliphages as fecal and viral pollution indicators in the Egyptian aquatic environment.

MATERIAL & METHODS

Host strain preparation

E. coli strain (ATCC 13706) was kindly provided by Professor Dr. Ibrahim Ahmed Hamza, National Research Centre, Egypt, utilized as the bacterial host, in accordance with the standard method of the International Organization for Standardization, ISO 10705-2 (ISO, 2000). for somatic coliphages isolation. Briefly, 5 mL of Nutrient broth (NB) was inoculated with *E. coli* from a previously prepared slant and incubated overnight for 16 to 20 h. at 36.5 °C.

Sewage samples collection

Raw sewage samples were collected during the winter season of 2022 as grab samples from the influent of two wastewater treatment plants (WWTP) in Egypt, El-Gabal El-Asfar WWTP in EL-Qalyubiyya Governorate, and Zenin WWTP in Bulaq Dakror, in Giza. In addition, wastewater samples were also collected from EL-Mariotia open drain located in EL-Mariotia, Giza. Samples were collected from the influence of the two WWTP, and the selected open drain in sterile bottles, and then transported within 2 hrs. to the laboratory for the isolation of bacteriophage.

Phages Isolation and Propagation

Different influent raw sewage samples were centrifuged at 6,000 rpm for 10 min to remove the solid impurities then filtered by membrane filter with a 0.45-µm pore-size to remove any bacterial cells. For each sample, 1000 µL of supernatant was added to 10 ml exponential growth *E. coli* strain (ATCC 13706) and

incubated for 12 hrs. to enrich the phages on a shaker incubator. The culture solution was centrifuged at 6000 rpm, for 20 min, 4ºC. The supernatant was filtered through a syringe filter, with a pore size of 0.45 µm, then tested by the spot test assay, by placing 10 µl on nutrient agar seeded with bacterial host. The positive sample was further propagated, purified, and tested by double-layer plaque assay according to Adams, (1959).

Phage Purification:

Successive single plaque isolation method was used to obtain homogenous plaques as described by Sambrook and Russell (2001). For virus concentration, Dextran sulfate-polyethylene glycol (two-phase liquid system) was used for the final purification of isolated phages according to Bachrach and Friedman, (1971). Following plaque purification and concentration, all phage titers for all assays were determined by the double-layer plaque assay as described previously by Adams (1959). Purified phages were stored at 4°C for further analysis.

Morphological Characterization of Isolated Phages

Morphological features of isolated phages were determined using a Transmission Electron Microscope (TEM). For each eluted phage isolate, one drop of high titer phage supernatant was spotted on the surface of carbon coated grid negatively stained with 2% with potassium phosphotungstate (pH 7.2) and examined at various magnitudes under TEM (JEOL-JEM) at the National Research Centre, Giza, Egypt. The diameters (nm) of the capsid and the tail of the isolated phages were determined by electron micrographs at magnification= 100000X. Classification of the isolated phages performed according to Virus Taxonomy: Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses as mentioned by Van Regenmortel, (2000).

Host Range and Cross Infectivity of phages

The spot test was utilized to investigate the host specificity of the isolated phages based on the degree of lysogenicity on the bacterial lawn, according to Li et al*.,* (2016) against 8 species of bacteria from *Enterobacteriaceae* family including *E. coli* strain ATCC13706, *Enterobacter. Cloacae subsp. dissolvens strain LMG 2683(R2017) (NR044789.1)*, *E. coli* strain NRC B- 3703, *E. coli* strain (ATCC25922), *Salmonella typhimurium* ATCC 14028, *Bacillus subtilis* NRC*, Pseudomonas aeruginosa NRC B-32*, and *Staphylococcus aureus* NRRL B-313.

Phage Stability Assay

For evaluation of thermal stability of isolated phages, equal volumes of phages (approximately 10^{10} PFU/ml) were incubated at different temperatures of 5°C, 25 °C, 35 °C, 45 °C, 55°C, 65°C, 75°C and 85°C 95° for 10 mins. according to Kerketta et al., (2014). Phages stability over a wide pH range was examined according to Huang et al., (2018) by treating the phages separately at a pH of 3.0, 5.0, 7.0, 9.0, and 11.0 for 2 hrs. The phage suspensions were used for phage titer determination immediately after the treatment.

Optimal Multiplicity of Infection (MOI)

To detect the optimum infection ratio between the phages and the host bacterial cells. Two different ratios of MOI (0.1, 1.0 PFU/ CFU) were added to *E. coli* strain (ATCC 13706) host cells (approximately 10^7 CFU/ml) and incubated for 10 mins. at room temperature. After incubation at 37 °C, the samples of each MOI were assayed to determine the phage titer by double-layer plaque assay. The MOI with the highest phage titer was considered the optimal MOI (Youqiang et al*.,* 2016).

Evaluation of Bacterial Growth Reduction

Growth reduction assay of *E. coli* strain (ATCC 13706) was performed separately for each phage isolate in NB broth. Overnight bacterial culture only was incubated at 37°C with shaking, and then phages were added separately to bacterial culture with a MOI at 1. Phage-free *E. coli* culture was used as a control sample in experiments. The optical density of test and control samples was recorded at 600 nm and was set to zero at the time of infection every hour for 5 h at 37 ºC (Ghasemian et al*.,* 2017). Bacterial growth was examined by monitoring the OD600 using a spectrophotometer (Genway 6300, UK).

One-Step Growth Curve

A one-step growth curve was performed to determine the burst size and latent period of each phage as described by (Mudgal et al*.,* 2006; Lee and Park, (2015), with some modifications. Briefly, 5 ml of the exponential phase of *E. coli* (ATCC 13706) was centrifuged at 6,000 rpm for 5min at 4°C. The bacterial pellet was resuspended in 10 ml of NB supplemented with 2 mM of CaCl2. Each phage (10^7 PFU/ml) was added separately to the bacterial suspension (1×10^7 CFU/ml) to achieve multiplicity of infection (MOI) 0.1, then this bacteria-phage suspension was incubated at 37°C for 10 min. After incubation, the suspension was centrifuged at 6,000 rpm for 20 min. and the pellets

containing infected bacterial cells were resuspended in 1 ml of NB supplemented with 10 mM of magnesium sulfate and transferred into 9.0 ml of pre-warmed NB. The samples were incubated in a shaking incubator (150 rpm) at 37 ◦C for 1 h and, during the subsequent incubation; samples were taken at 5 min. intervals, serially diluted and plated on EMB agar using the double-layer agar method. Plaque numbers were counted and expressed by PFU per infected cell to obtain the one-step growth curve.

Statistical Analysis

Statistical analysis was performed to determine the effect of temperature and pH on phages survival and to determine whether there were statistical differences between phage-treated samples and controls. All experiments were conducted in triplicates, and the data were analyzed using Python. For statistical analysis, Jupyter Notebooks (version 7.1.2) was utilized. The standard deviation of the mean was represented by error bars.

RESULTS

Isolation bacteriophages

The supernatant obtained from the sewage samples was examined by a spot test and the results indicate the presence of specific phages that had highly strong lytic activity against *E. coli* strain (ATCC 13706) as shown in Figure 1.

Phage Purification

Four specific phages were selected and further purified using a single plaque assay and the phages were named EcoP1, EcoP2, EcoP3, and EcoP4. Phages formed different sizes and appearance of rounded plaques (Figure 2 A, Table 1) on the lawn of *E. coli* strain (ATCC 13706).

Morphological characterization isolated phages.

Based on the Transmission Electron Microscopy (TEM) examination, according to Virus Taxonomy: Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses by Van Regenmortel (2000), the four isolated phages exhibit common morphological characteristics as all of them have tails, thus they belong to the order *Caudovirales*. The obtained TEM data showed that EcoP1 had an icosahedral head with a diameter of (53.67×49.28nm) and a short, contractile tail of (86.53nm) length and (14.18nm) width. While EcoP2 showed an icosahedral head of (51.85×50.11nm) diameters and a long, non-contractile tail of (109.18 nm) length and (6.80) width.

Figure 1. Spot test demonstrating lysis of bacterial growth caused by virulent bacteriophages specific for *E. coli* strain C (ATCC 13,706) ATCC strain arrows indicate lysis spot. (M) indicates the phage isolated from EL-Mariotia open drain sample in Giza, Egypt. (Z) indicates the phage isolated from Zenin WWTP sample in Giza, Egypt. (g) indicates the phage isolated from El-Gabal El-Asfar WWTP sample in EL-Qalyubiyya Governorate, Egypt.

EcoP3 is characterized by an icosahedral small head of (48.42 × 47.72 nm) diameter, a long non-contractile tail of (110.31nm) length, and (12.19 nm) width. Finally, EcoP4 had an icosahedral head of (54.14 ×44.62 nm) diameter, contractile tail of (100.37 nm)

Table 1. Plaque morphology and measurement of the four phages.

Isolated Phages	Plaque Morphology	Diameter (mm)
EcoP1	Small-sized, clear plaque with an irregular margin	1 mm
EcoP2	Small-sized, diffused/opaque plaque with a sharp margin	1.5 mm
EcoP3	Large-sized, diffused/opaque plaque with a sharp margin	4 mm
EcoP4	Large-sized, clear plaque	3 mm

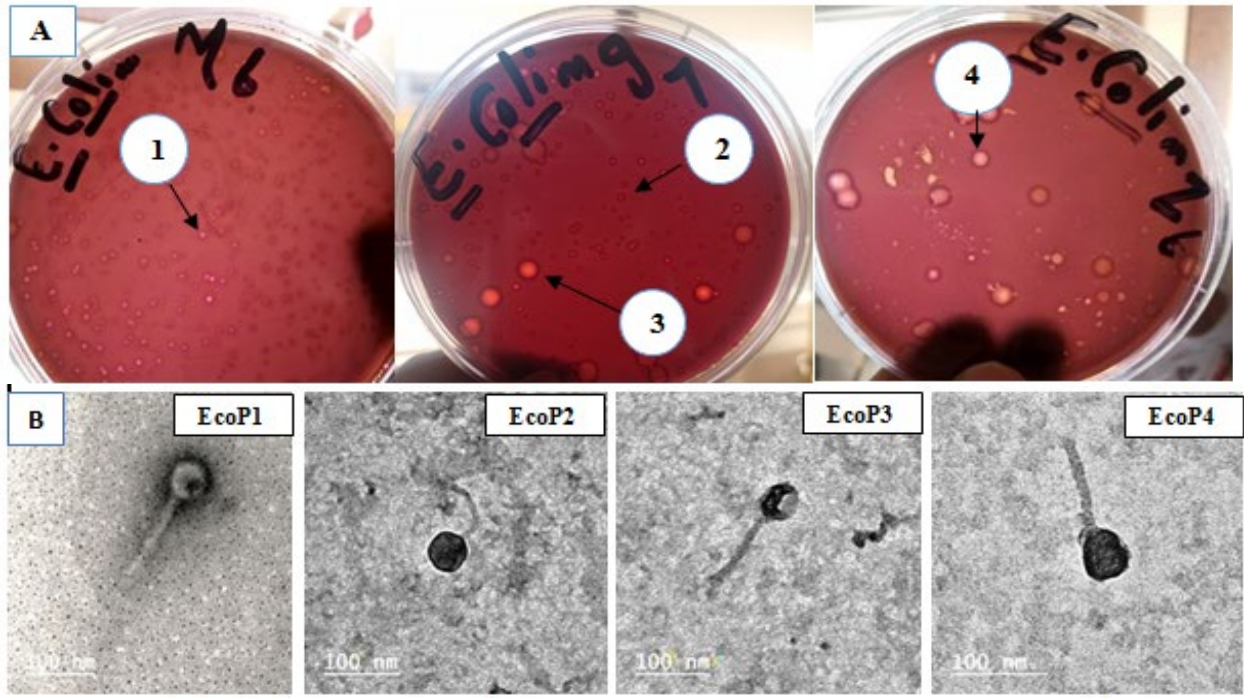

Figure 2. Morphological observation of phage EcoP1, EcoP2, EcoP3 and EcoP4. (A) Plaques produced on doubled-layer agar plates. EcoP1 was isolated from EL-Mariotia (M) open drain, EcoP2 and EcoP3 were isolated from El-Gabal El-Asfar (g) WWTP, and Phage EcoP4 was isolated from Zenin (Z) WWTP. (B) Virion morphology observed using transmission electron microscopy, magnification=100000X.

Table 2. Host range for lysogenized and non-lysogenized bacterial strain by isolated phages.

Tested bacterial strains	Phage Sensitivity			
	EcoP1	EcoP2	EcoP3	EcoP4
E. coli strain (ATCC13706)	L +	L +	L +	L +
E. coli strain NRC B-3703	L +	L +	-	L +
E. coli strain (ATCC25922)	-	-	-	-
E. cloacae subsp. dissolvens strain LMG 2683 (R2017) (NR044789.1).	-	-	L+	L +
Pseudomonas aeruginosa NRC B-32	-	-	-	-
Salmonella typhimurium ATCC 14028	L +	L +	L +	L +
Staphylococcus aureus NRRL B-313	L+	L+	L+	-
Bacillus subtilus NRC	-	L+	L +	-

L (+): means lysis. (−): means no lysis.

length and (11.07 nm) width. Based on morphological studies, Phage EcoP1 and Phage EcoP4 are allotted to the family *Myoviridae.* However, Phages EcoP2 and EcoP3 showed appearances typical of *Siphoviridae* family members as demonstrated in Figure 2B.

Host Range and Cross Infectivity of Phages

The host range testing revealed that phages were highly specific to their host *E. coli strain (ATCC 13706)* and showed a high degree of lysogenability to the main host, *E. coli strain (ATCC 13706)*, and variable degrees of activity against the other tested bacteria as showed in Table 2.

Phages Characterization
Thermal and pH Stability

As shown in Figure 3, phages suspensions at concentrations (1×10^7 PFU) were found to be active at temperatures ranging between 5°C to 75 °C for 10 mins. with different degrees of viability for the isolated four phages. For Ecop1 and Ecop2, the highest activity was recorded between 35 to 65°C, while for Ecop3, the highest activity was recorded between 25 to 45°C, and Phage Ecop4 was highly active between 25 to 55°C. At high temperature 75 °C, Ecop1, Ecop2, and Ecop3 were active but there was a reduction in their titer, while Ecop4 lost its activity. All four phages lost their viability at temperatures higher than 75°C, and the highest activity recorded between 35 to 55°C. Phage Ecop2 was more tolerant to higher temperatures than the other phages.

The obtained data on PH stability indicates that isolated phages did not differ greatly in their degree of viability and stability at a wide range of pH degrees, from pH 1 to 13. All four isolated phages Ecop1, Ecop2, Ecop3, and Ecop4 were active and had high phage titer at pH values from 5 to 9. There was a reduction in phages titer at extreme alkaline (pH 11), and at extreme acidic conditions (pH 3). Ecop2 was the only phage that remained active at pH 3, while the other three phages could not. At highly alkaline conditions (pH 13), all four phages lost their activity as seen in Figure 4.

Detection of Optimal Multiplicity of Infection (MOI)

The obtained data indicated that the MOI ratio of 1.0 produced the highest phage titter, and the maximum phage yield compared to the 0.1 ratio. Thus, MOI of 1.0 is considered the optimal and has been selected for the following experiments, as shown in Figure 5.

Evaluation of Bacterial Growth Reduction

The phages activities in vitro against their host *E. coli* strain (ATCC 13706) were evaluated by measuring the optical density (OD600) in liquid medium during the growth of host bacteria at 37°C with MOI of (1.0). Reduction in OD600 was observed 63.93, 61.20, 69.39 and 79.14% reduction in bacterial cell count for EcoP1, EcoP2, EcoP3, and EcoP4, respectively, comparing to phage-free bacterial growth curve (control), which reached 0.513 at OD 600 after 5hrs. (Figure 6).

One-Step Growth Curve

The one step growth curve indicated that the latent periods for the four isolated *E. coli* phages were 15, 20, 15, and 10 min for Ecop1, Ecop2, Ecop3, and Ecop4, respectively, while the burst sizes were 44, 36, 33, and 62 phages per infected cell respectively as shown in Figure 7.

DISCUSSION

Phages are present in raw sewage at high multitude and considerable diversity (Ballesté et al., 2022). Several studies in Egypt have reported the isolation and characterization of different bacteriophages from sewage samples, especially bacteriophages which are specific for multidrug resistant bacterial strains (Mahmoud et al., 2018; Askoura et al., 2020; Al-Mohammadi et al., 2020). As populations served by El-Gabal El-Asfar WWTP are approximately 12,000,000 inhabitants, and the treated sewage water from it is used for the irrigation of 150,000 acres of landscaping, while Zenin WWTP serves one Million inhabitants, and the treated effluent from it is also used for tree irrigation. This treated sewage water could be a potential source of environmental contamination by viral and bacterial pathogens if the treatment process is deficient. Therefore, the main purpose of the present study is to isolate phages specific to *E. coli* strain ATCC-13706 which can be used for environmental risk and water quality assessment, as well as biological control of some pathogenic bacteria.

In the present study, we isolated four different bacteriophages that showed lytic activity against E. coli strain ATCC- 13706, from two WWTP (Zenin, and EL-Gabl EL-Asfer WWTP), and EL-Mariotia open drain in Giza by using the spot test and double layer plaque assay technique. The isolated phages were classified into types based on the shape, size, and appearance of their plaques (Abedon and Yin, 2009). Morphological variation of the plaques indicated a difference in phage strain (Ghasemian et al., 2017). Morphological examination by TEM showed four different bacteriophages, assigned to *Siphoviridae* and *Myoviridae* families, according to the International Committee on Taxonomy of Viruses (ITV). Our results agree with previous studies indicating that the majority of known somatic coliphages found in municipal wastewater and identified by the host strains advised in standard operating procedures are members of the *Myoviridae, Siphoviridae, Podoviridae, and Microviridae* families (Rajala-Mustonen and Heinonen-Tanski, 1994; Muniesa et al. 1999). Our results are also in agreement with Samhan et al., 2016, who reported the isolation of somatic coliphage

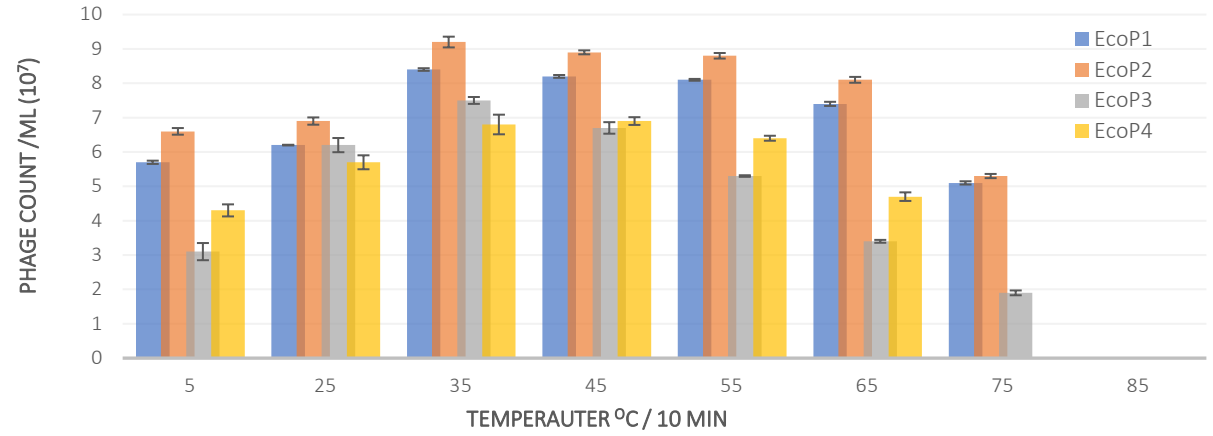

Figure 3. The thermal stability of the four *E coli* strain (ATCC 13706) isolated phages at various temperature degrees. (The standard deviation of the mean was represented by error bars.).

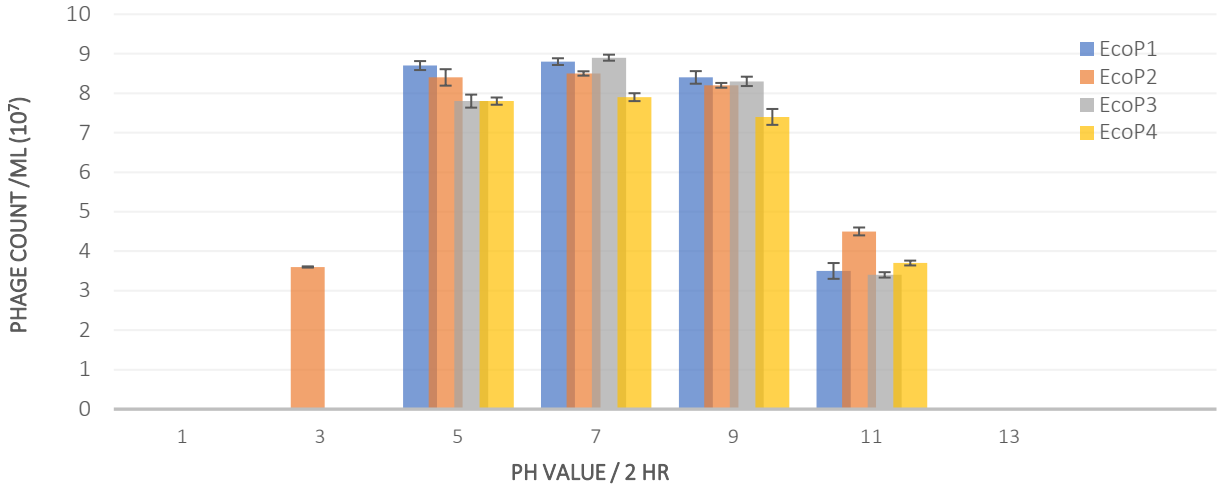

Figure 4. The pH stability of isolated *E coli* strain (ATCC 13706) phages at different pH values. (The standard deviation of the mean was represented by error bars).

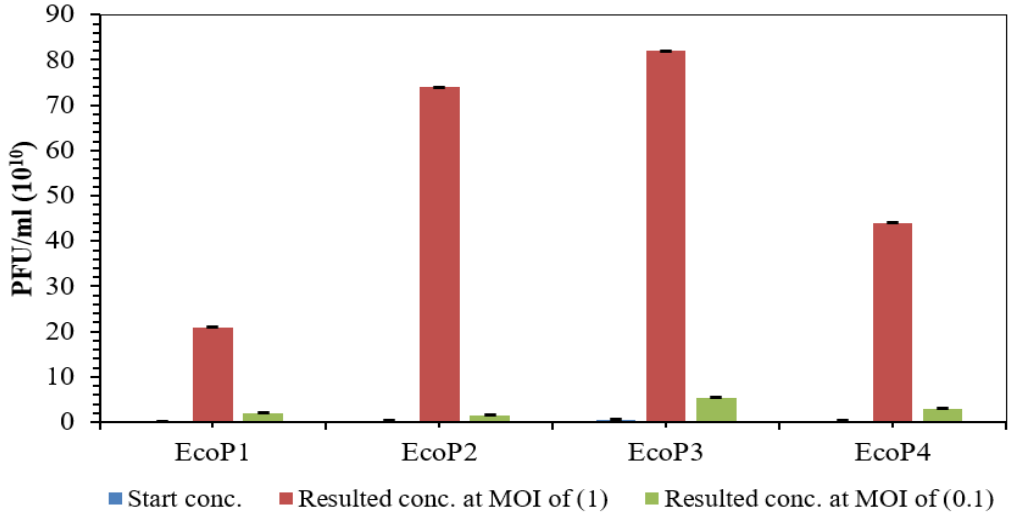

Figure 5. The effect of MOI ratios (1.0 and 0.1) on the isolated phages titer (The standard deviation of the mean was represented by error bars.)

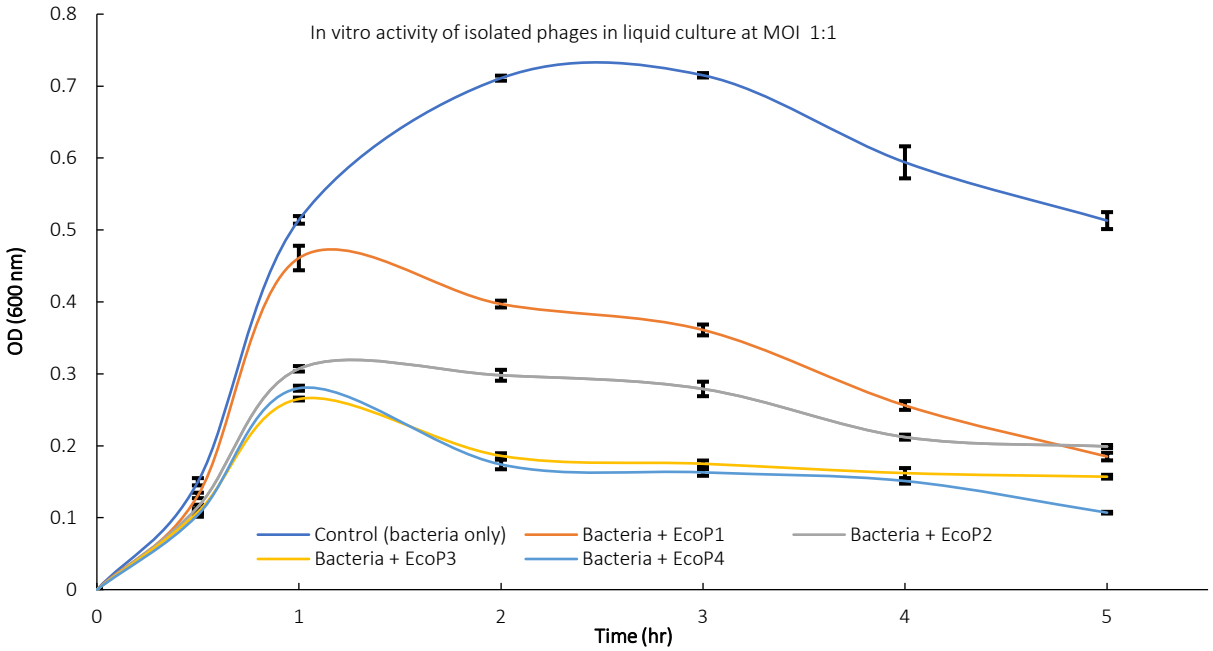

Figure 6. Growth reduction and lytic ability of isolated phages using the growth curves of the *E. coli* strain (ATCC 13706) host (The standard deviation of the mean was represented by error bars).

belonging to the *Siphoviridae* family from the Ismailia Canal, and with Nasr-Eldin and Soliman, (2020), who described similar morphological features for a *Myoviridae* phage isolated from Qalyubia Governorate, Egypt, sewage samples. Lytic activity of the four phages was identical concerning their main host *E. coli* strain ATCC 13706 strain, while differed with other tested bacterial strains of Gram-positive, and Gram-negative bacteria. EcoP1, EcoP2, EcoP3, and EcoP4 exhibit a wide host range in line with the findings of Bielke et al. (2007), who noted that the range of phage host is not always limited by genera. All isolated phages showed strong lytic activity against their main host *E. coli* strain C (ATCC 13706), while all of them failed to show any lytic ability against *Pseudomonas aeruginosa* NRC B-32, and *E. coli* strain (ATCC25922). In addition, EcoP1, and EcoP4, phages could not show lytic activity against *Bacillus subtilus* NRC, while EcoP3, and EcoP4 exhibit strong lytic activity against *E. cloacae subsp. dissolvens* strain LMG 2683(R2017) (NR044789.1) than EcoP1 and, EcoP2 which seem to be unable to infect it. The three phages EcoP1, EcoP2, and EcoP3 showed strong lytic activity against *Staphylococcus aureus* NRRL, B-313, while EcoP4 phage did not show any lytic potential against it. The results also showed that the isolated phages EcoP2 and EcoP3 have a wider host range than EcoP1 and EcoP4 phages. Interestingly, all four isolated phages exhibit lytic activity against *Salmonella sp*.

However, it is likely that the isolated phages could be polyvalent phage, capable of infecting both *E. coli sp*. and *Salmonella sp*. as their main host strains. This finding agrees with Mahmoud et al., (2018), who reported the isolation of polyvalent phage belonging to the families *Siphoviridae* and *Myoviridae* from broilers in Egypt and with Saad et al., (2018), who reported the isolation of polyvalent phage from Higashi-Hiroshima, sewage in Japan. Similarly, in Tennessee, USA, Bryan *et al*., (2023) reported isolation of polyvalent phage from sewage that can infect both *E. coli sp*. and *Salmonella sp.*

Considering that the environment's acidity and alkalinity have a significant impact on the stability of the phage, and for the potential practical downstream applications, the stability of the isolated phages was evaluated, and the results indicated high tolerance to temperatures, and stability over various pH values. All isolated phages remained active at high temperatures, and were able to survive temperatures up to 75 ^{o}C, except phage EcoP4 which showed less tolerance to high temperatures (active only until 65 °C), and completely lost its virulence at 75 °C, with optimal temperature for most phages between 35°C to 55°C. The isolated phages in the present study were more tolerant to temperatures than what was reported by Nasr-Eldin and Soliman, (2020), who isolated three different phages from Egyptian sewage

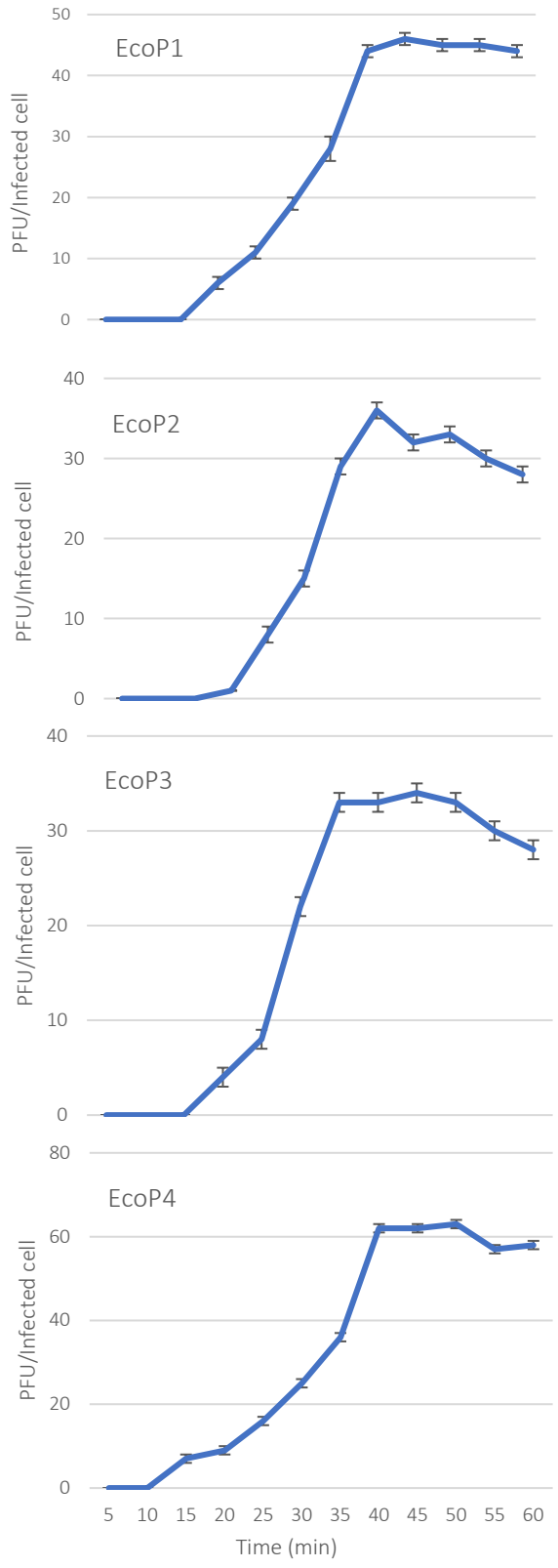

Figure 7. One step growth curve of the four *E.coli* phages (The standard deviation of the mean was represented by error bars).

that remained virulent at temperatures from 25°C to 55°C, but they were completely inactivated at temperatures 65°C. Moreover, our results indicated higher temperature stability than what was reported by Samhan et al., (2016), who reported that three somatic coliphages, isolated from the Ismailia Canal, lost their infectivity at temperatures >60 °C. This difference in thermal stability could be attributed to the difference of heat exposure time, water source of phage isolation, and variation in environmental stresses between sewage and marine water. Our results agree with (Jurczak-Kurek et al., 2016), who reported the survival of *E. coli* lytic phages isolated from urban sewage in Poland, at temperatures up to 62 °C for 40 min, which is longer exposure time than the tested time in our present study which was for 10 mins. Similarly, Jamal et al., (2015) reported stability of isolated phage of the family *Myoviridae* against Multi-Drug Resistant *E. coli* between 37°C and 65°C, and complete inactivation at 70°C. Hasanien et al., (2024) reported thermal stability of three bacteriophages isolated from soil at high temperature up to 80°C, which is higher than what reported in the present study, this extreme thermal stability could be attributed to the different source of phage isolation (soil). Furthermore, it has been reported earlier that thermo-stable phage belonging to the *Siphoviridae* family exhibit stability at 65°C (Lin et al., 2010).

Regarding pH stability, the virulence of the four isolated phages in the present study was tested over a wide range of pH starting from pH 1 to 13 for 2 hrs. The obtained data indicated that isolated phages exhibit similarity in their degree of viability, and all of them showed stability at a wide range of pH values. All phages were stable at pH ranging from 5 to 11, with high titer at optimum pH from 5 to 9, but at pH 11, there was reduction in the phages' titer. EcoP2 phage was more tolerant to acidity and was the only one that was able to survive at pH 3. At highly alkaline conditions, pH 13, all four phages lost their activity. The isolated phages showed high tolerance to different pH values and maintained their viability over a broad range of pH ranging from 3 to 11. However, infectivity declined towards extreme acidic and alkaline conditions. pH values of 5, 7, and 9, were optimum for infectivity.

Our results agree with Nasr-Eldin and Soliman, (2020), who reported that the optimum pH value for their three isolated phages from Egyptian sewage, ranging from 7 to 9, with one phage exhibiting stability at pH ranging from 5 to 9, after 30 min and it retained its lytic ability up to 12 hrs. at pH ranging from 7 to 9. Our

results are also in agreement with Hasanien et al., (2024) who reported pH stability of three bacteriophages belonging to *Myoviridae*, and *Podoviridae* families isolated from soil over pH ranging from 3 to 10. Moreover, Yıldirim et al., (2021), indicated that phages retained their activity among pH 4 to 11, and 4–9. However, a great reduction in the phage's titer was recorded at pH values below 2 or pH above 12. Jurczak-Kurek et al., (2016), reported similar results, showing phage virulence at pH ranging from 4 to 12 for 1 h. and also documented the isolation of extremely thermo stable phage from sewage, belonging to family Myoviridae, which survived at a temperature of 95°C for 5 min. Similar findings were reported later by Topka et al., (2019), showing that phage was sensitive to a low pH value of 4, and to high temperatures above >62 °C. In contrast, Peng and Yuan, (2018), reported less resistance against pH treatment for *E. coli* lytic phage, where the phage was stable at pH between 3 and 9, while they lost their infectivity at pH 11. Thus, both very high temperature and very low pH values were lethal for their isolated phage. According to research by Iriarte et al., (2007), excessive heat and pH extremes can destroy phage viability by dissolving lipids, denaturing DNA and proteins, and damaging phage structure.

The ratio of phage concentration (PFU/ml) to the concentration of host bacterial cells (CFU/ml) is referred to as the multiplicity of infection (MOI). Two MOI were investigated to determine the optimal phage-host concentrations for optimum phage activity. Out of the two MOI that were tested in this study (1.0 and 0.1), 1.0 MOI ratio produced the maximum phage titter and was recorded as the optimal MOI. Lin et al., (2010) reported similar MOI values for activity against specific host. In our study with MOI of a ratio 1.0 at OD600, there was 63.93, 61.20, 69.39 and 79.14% reduction in bacterial cell count for EcoP1, EcoP2, EcoP3, and EcoP4, respectively, compared to the control, indicating a high activity against the bacterial host. Notably, EcoP4 had the strongest activity out of the three phages. This result is like Ghasemian et al., (2017) who determined the efficacy of phage on *E. coli* at MOI 1 at OD 600 after 9hrs. The phages titer in the present study reached its maximum when the MOI was 1.0, this finding was in agreements with that of Fathy et al., (2024), who mentioned that *E. coli* phage titer reached its maximum when the MOI was 1. As well as Sada and Tessema, (2024), who indicated that the optimal MOIs of two *E. coli* lytic phage isolates was 1.0, which gave the highest production of phage progeny. One of their Phage isolates had the highest MOI (1.0) which was also comparable to the host range test result, but the other phage had a broad host range with a slight difference in MOI. In general, phage with a broader host range had the highest MOI and vice versa.

The one step growth curve indicated that the latent periods for the four isolated phages were 15, 20, 15, and 10 min for EcoP1, EcoP2, EcoP3, and EcoP4 respectively, while the burst sizes were 44, 36, 33, and 62 phages per infected cell, respectively. Karami et al., (2024) reported same latent period (10 min) for *E. coli* lytic phage, which is like our result for Ecop4 phage but with different burst size of 152 PFU / host cell. Our result is different from what was previously reported by Yıldirim et al., (2021), and Litt and Jaroni (2017), who reported longer Latent periods and larger burst sizes. On the other hand, Sillankorva et al. (2008) previously reported a small burst size and less latent period, indicating that both latent time and burst size vary among different bacteriophages.

Finally, the idea of using bacteriophages in environmental application as an effective bio control agent are getting more attention in the recent years due to its cost effectiveness, and safety. Azzam et al., (2023), has suggested using a polyvalent phages mixture to reduce *E. coli* strains, coliforms, and other *Enterobacteriaceae sp*. in River Nile and drains outlets based on their strong lytic activity, which enable these phages of reducing the pathogenic bacteria population after two hours of incubation. Furthermore Azzam et al., (2024), isolated and identified three novel Coliphages from the River Nile and the drainage water along the Rosetta branch, these isolated phages showed a remarkable ability to inhibit the growth of seven different pathogenic bacterial strains under neutral conditions, and suggesting the use of a polyvalent phage-based wastewater pollution management strategy for safe reuse of treated drainage water. Fathy et al., (2024), reported using somatic coliphage and F-specific coliphage either alone or in combination with electron beam irradiation to fight multidrug-resistant *E. coli* in sewage as an environmentally sustainable method.

CONCLUSION

Based on the study findings, somatic coliphages are abundant in Egyptian sewage, with high diversity, and a wide host range, which can be used for bio control of some pathogenic bacteria. The isolated phages exhibit high tolerance to a wide range of pH values

and temperature degrees, which supports the notion of using phages as indicators of fecal pollution in the Egyptian aquatic environment. A priority objective of our future research will be to identify the nucleotide and protein sequence of the isolated phages.

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REFERENCES

Abedon, S.T. and Yin, J. (2009). Bacteriophage plaques: Theory and analysis. *Methods and Molecular Biology,* 501: 161–174.

Adams, M.H. (1959). Bacteriophages. New York, NY: Inter Science Publishers Inc.

Adelodun, B.; Ajibade, F.O.; Ighalo, J.O.; Odey, G.; Ibrahim, R.G.; Kareem, K.Y.; Bakare, H.O.; Tiamiyu, A.O.; Ajibade, T.F.; Abdulkadir, T.S.; Adeniran, K.A. and Choi, K.S. (2021). Assessment of socioeconomic inequality based on virus-contaminated water usage in developing countries: *A review Environmental Research.* 192,110309, 0013- 9351.

Al-Mohammadi, A-R., El-idamony, G., Abd El Moneem, M.S., Elshorbagy, I.M., Askora, A. and Enan, G. (2020). Isolation and Characterization of Lytic Bacteriophages Specific for Campylobacter jejuni and *Campylobacter coli. Zoonotic Diseases*, 2, 59–72.

Anderson, K. L.; Whitlock, J. E. and Harwood, V. J. (2005). Persistence and differential survival of fecal indicator bacteria in subtropical waters and sediments. *Applied Environmental Microbiol*ogy, 71(6): 3041–3048.

Amarasiri, M., Kitajima, M., Nguyen, T. H., and Okabe, S. (2017). Bacteriophage removal efficiency as a validation and operational monitoring tool for virus reduction in wastewater reclamation: review. *Water Research.* 121, 258–269.

Askoura, M., Saed, M., Enan, G. and Askora, A. (2020). Characterization of polyvalent bacteriophages targeting multidrug-resistant klebsiella pneumonia with enhanced anti-biofilm activity. *Applied Biochemistry and Microbiology*, 57 (1):117–126.

Arredondo-Hernandez, L.J.R.; Diaz-Avalos, C.; Lopez-Vidal, Y.; Castillo-Rojas, G. and Mazari-Hiriart, M. (2017). F RNA Bacteriophages as Viral Indicators of Faecal Contamination in Mexican Tropical Aquatic Systems. *PLOS ONE.*DOI: 10.1371/journal.pone.0170399.

Armon, R., Kott, Y. (1996). Bacteriophages as indicators of pollution. *Crit. Rev. Environmental Science Technology*, 26, 299–335.

Azzam, M.I., Faiesal, A.A., Mohammed, F.A., and Korayem, A.S. (2023). An innovative approach using lytic phage mix for wastewater management and pathogen control. *Egypt. J. of Appl. Sci.,* 38, 3-4.

Azzam, M.I., ElSayed, E.E., Gado, M.M. and Korayem, A.S., (2024). New phage-based wastewater pollution control solution with safe reuse. Environmental Nanotechnology, Monitoring and Management, 21, 100951.

Bachrach, U. and Friedmann, A. (1971). Practical Procedures for the Purification of Bacterial Viruses. *Applied Microbiol*ogy, 22:706-715.

Ballesté, E., Blanch, A.R., Muniesa, M., García-Aljaro, C., Rodríguez-Rubio, L., Martín-Díaz, J., Pascual-Benito, M. and Jofre, J. (2022). Bacteriophages in sewage: abundance, roles, and applications*. FEMS Microbes,* 3, 1–12.

Bielke, L., Higgins, A., Donoghue, D., Hargis, B.M. (2007) Salmonella host range of bacteriophages that infect multiple genera. *Poultry Science,* 86, 2536-2540.

Bryan, D.W., Hudson, L.K., Wang, J., and Denes, T.G. (2023). Characterization of a Diverse Collection of Salmonella Phages Isolated from Tennessee Wastewater. PHAGE, 4(2), 90-84(2).

Célia da Silva Lanna, M., Viancelli, A., Michelon, W., Castro Carvalho, S.V., de Almeida dos Reis, D., Fernandez de Salles, L.A., Sant'Anna, I.H., Resende, L.T., de Souza Ferreira, C., Aparecido das Chagas, I., Hernández, M., Treichel, H., Rodríguez-Lázaro, D., Fongaro, G. (2019). Household-based biodigesters promote reduction of enteric virus and bacteria in vulnerable and poverty rural area. *Environmental Pollution,* 252, 8–13.

Chen, L-K.; Liu, Y-L.; Hu, A.; Chang, K-C.; Lin, N-T.; Lai, M-J.; and Tseng, C-C. (2013). Potential of bacteriophage ΦAB2 as an environmental biocontrol agent for the control of multidrug-resistant Acinetobacter baumannii. *BMC Microbiology* 13:154.

Clokie, M. R. J., Millard, A. D., Letarov, A. V., and Heaphy, S. (2011). Phages in nature. *Bacteriophage,* 1, 31–45. doi: 10.4161/bact.1.1.14942.

Comeau, A. M., Hatfull, G. F., Krisch, H. M., Lindell, D., Mann, N. H., and Prangishvili, D. (2008). Exploring the prokaryotic virosphere. *Research in Microbiology,* 159, 306–313.

Da Silva, D.T.G., Ebdon, J., Dancer, D., Baker-Austin, C. and Taylor, H. (2022). A Longitudinal Study of Bacteriophages as Indicators of Norovirus Contamination of Mussels (Mytilus edulis) and Their Overlying Waters. *Pollutants*, 2, 66–81.

El-Daly, R.A., Askora, A.; Barakat, A.B. and Hassan, E.E. (2018). Characterization and utilization of lytic bacteriophages for controlling pathogenic Escherichia coli serovars from duckling farms in Egypt. *Middle East Journal of Applied Sciences,* 8(4): 1413-1424.

El-Telbany, M.; El-Didamony, G.; Askora, A.; Ariny, E.; Abdallah, D.; Connerton, I.F. And El-Shibiny, A. (2021). Bacteriophages to Control Multi-Drug-Resistant Enterococcus faecalis Infection of Dental Root Canals. *Microorganisms*, 9, 517.

Espinosa, A. C., Arias, C. F., Sánchez-Colón, S. and Mazari-Hiriart, M. (2009). Comparative study of enteric viruses, coliphages and indicator bacteria for

evaluating water quality in a tropical high-altitude system. *Environmental Health,* 8(1):1–10.

Fathy, R., Eid, A.S., Hammad, A.A. and El-Nour, S.A.A. (2024). Isolation and characterization of coliphages from different water sources and their biocontrol application combined with electron beam irradiation for elimination of *E. coli* in domestic wastewater. *Annals of Microbiology,* 74(1), 10.

Ferreira, D.C.; Graziele, I.; Marques, R.C.; Gonçalves, J. (2021) Investment in drinking water and sanitation infrastructure and its impact on waterborne diseases dissemination: The Brazilian case. *Science Total Environment*, 779:146279.

Flannery, J.; Keaveney, S.; Rajko-Nenow, P; O'Flaherty, V. and Doré, W. (2012). Concentration of norovirus during wastewater treatment and its impact on oyster contamination. *Applied. Environmental Microbiology,* 78 (9): 3400–3406.

Fuhrman, J.A., and Schwalbach, M. (2003). Viral influence on aquatic bacterial Communities. *The Biological Bulletin,* 204, 192–195.

Gall, A.M., Mariñas, B.J., Lu, Y., Shisler, J.L. (2015a). Waterborne viruses: a barrier to safe drinking water. *PLoS Pathogen*, 11(6), e1004867.

Ghasemian, A., Bavand, M., and Moradpour, Z. (2017). A broad-host range coliphage against a clinically isolated *E. coli* O157: isolation and characterization. *Journal of Applied Pharmaceutical Science.* 7(3), 123-128.

Grabow, W.O.K. (2001). Bacteriophages: update on application as models for viruses in water. *Water SA*, 27:251–268.

Hendrix, R. W., Smith, M. C. M., Burns, R. N., Ford, M. E., and Hatfull, G. F. (1999). Evolutionary relationships among diverse bacteriophages and prophages: all the world's phage. *In Horizontal gene transfer* (pp. 133-VI). *Academic Press*

Haramoto, E.; Katayama, H.; Oguma, K. and Ohgaki, S. (2007). Quantitative analysis of human enteric adenoviruses in aquatic environments. *Journal of Applied microbiology.* 103(6): 2153– 2159.

Huang, C., Virk, S.M., Shi, J., Zhou, Y., Willias, S.P., Morsy, M.K., Abdelnabby, H.E., Liu, J., Wang, X. and Li, J. (2018). Isolation, Characterization and Application of Bacteriophage LPSE1 against Salmonella enteric in Ready To Eat (RTE) Foods. *Frontier in Microbiology,* 9, 1046.

Iriarte, F., Balogh, B., Momol, M., Smith, L., Wilson, M., Jones, J. (2007). Factors affecting survival of bacteriophage on tomato leaf surfaces. *Applied and environmental microbiology*, 73(6):1704-1711.

Jamal, M., Hussain, T., Das, C.R., and Andleeb, S. (2015). Isolation and Characterization of a *Myoviridae* MJ1 Bacteriophage against Multi-Drug-Resistant *Escherichia coli*. *Journal of Microbiology*, 8(11): e25917.

Jofre, J. (2007). Indicators of waterborne enteric viruses. *Perspectives in Medical Virology*, 17, 227-249.

Jofre, J., Lucena, F., Blanch, A.R. and Muniesa, M. (2016). Coliphages as Model Organisms in the Characterization and Management of Water Resources. *Water,* 8, 199.

Jurczak-Kurek, A., Gąsior, T., Nejman-Faleńczyk, B., Bloch, S., Dydecka, A., Topka, G., Necel, A., Jakubowska-Deredas, M., Narajczyk, M.; Richert, M., Mieszkowska, A., Wróbel, B., Węgrzyn, G. and Węgrzyn, A. (2016). Biodiversity of bacteriophages: morphological and biological properties of a large group of phages isolated from urban sewage. *Scientific Reports,* 6, 34338.DOI: 10.1038/srep34338.

Jurzik, L.; Hamza, I. A.; Puchert, W.; Überla, K. and Wilhelm, M. (2010). Chemical and microbiological parameters as possible indicators for human enteric viruses in surface water. *Environmental Health*. 213(3): 210–216.

Karami. M, Goudarztalejerdi. A; Mohammad A and Berizi. E. (2024). In vitro evaluation of two novel Escherichia bacteriophages against multiple drug resistant avian pathogenic Escherichia coli. *BMC J. Infectious Diseases* .24:497. https://doi.org/10.1186/s12879-024-09402-0.

Kenzaka, T., Tani, K., and Nasu, M. (2010). High frequency phage mediated gene Transfer in freshwater environments determined at single cell level. The *ISME Journal,* 4, 648–659.

Kerketta, P., Agarwal, R.K., Rawat, M., Jain, L., Kumar, P.P., Dhanze, H., Kumar, M.S. and Kumar, A. (2014). Isolation and Characterization of Lytic Bacteriophage (Φstiz1) Against Salmonella enterica serovars typhimurium. Journal *of Pure and Applied Microbiology*, 8(6), 4719-4726.

Leclerc, H., Edberg, S., and Pierzo, V. (2000). Bacteriophages as indicators of enteric viruses and public health risk in ground waters. *Journal of Applied Microbiology*, 88, 5–21.

Lee, Y.D. and Park, J.H. (2015) Characterization and application of phages isolated from sewage for reduction of Escherichia coliO157:H7 in biofilm. LWT. *Food Science and Technology*, 60, 571-7.

Li, E., Wei, X., Ma, Y., Yin, Z., Li, H., Lin, W., Wang, X., Li, C., Shen, L., Zhao, R., Yang, H., Jiang, A., Yang, W., Yuan, J. and Zhao, X. (2016). Isolation and Characterization of a Bacteriophage Phieap-2 Infecting Multidrug Resistant *Enterobacter aerogenes. Scientific Reports*, 6, 28338. DOI: 10.1038/Srep28338.

Lin, J., and Ganesh, A. (2013). Water quality indicators: Bacteria, coliphages, enteric viruses. *International Journal of Environmental Health Research*, 23(6), 484–506.

Lin, L., Hong, W., Ji, X., Han, J., Huang, L., and Wei, Y. (2010). Isolation and characterization of an extremely long tail Thermus bacteriophage from Tengchong hot springs in China. *Journal of basic microbiology*, 50 (5), 452-456.

Lin, N. T., Chiou, P. Y., Chang, K. C., Chen, L. K., and Lai, M. J. (2010). Isolation and characterization of ϕAB2: a novel bacteriophage of *Acinetobacter baumannii*. *Research in microbiology*, 161(4), 308-314.

Litt, P.K. and Jaroni, D. (2017) Isolation and physiomorphological characterization of *Escherichia coli* O157:H7-infecting bacteriophages recovered from beef cattle operations. *International Journal of Microbiology,* 2017, 7013236.

Mahmoud, M., Askora, A., Barakata, A.B., Rabie, O. E. and Hassan, S.E. (2018). Isolation and characterization of polyvalent bacteriophages infecting multi drug resistant *Salmonella* serovars isolated from broilers in Egypt. *International Journal of Food Microbiology*, 266, 8–13.

Martin-Diaz, J., Lucena, F., Blanch, A.R., Jofre, J. (2020). Indicator bacteriophages in sludge, biosolids, sediments and soils. *Environmental research*, 182:109133.

McMinn, B.R.; Ashbolt, N.J. and Korajkic, A. (2017). Bacteriophages as indicators of faecal pollution and enteric virus removal. *Letters in Applied Microbiology,* 0266-8254.

Mudgal, P., Breidt, F. Jr., Lubkin, S. R. and Sandeep, K. P. (2006). "Quantifying the significance of phage attack on starter cultures: A mechanistic model for population dynamics of phage and their hosts isolated from fermenting sauerkraut," *Applied and Environmental Microbiology*, 72(6), 3908–3915.

Muniesa, M., Lucena, F. and Jofre, J. (1999). Study of the potential relationship between the morphology of infectious somatic coliphages and their persistence in the environment. Journal of Applied Microbiology, 87, 402–409. [CrossRef]

Mushegian, A. R. (2020). Are there 10 31 virus particles on earth, or more, or fewer? *Journal of bacteriology.* 202, e00052–20. doi: 10.1128/JB.00052-20.

Nasr-Eldin, M., and Soliman, A. A. (2020). Evaluation of Novel Virulent Phages Infecting the Aeromonas Hydrophila and Escherichia coli Isolated from Sewage Water Samples. *Egyptian Journal of Botany,* 60 (3), 805-823.

Peng, Q., and Yuan, Y. (2018). Characterization of a newly isolated phage infecting pathogenic Escherichia coli and analysis of its mosaic structural genes. *Scientific Reports,* 8(1), 8086.

Putra, R. D., and Lyrawati, D. (2020). Interactions between Bacteriophages and Eukaryotic Cells. *Scientifica,* 2020:3589316. doi: 10.1155/2020/3589316.

Rajala-Mustonen, R.L. and Heinonen-Tanski, H. (1994). Sensitivity of host strains and host range of coliphages isolated from Finnish and Nicaraguan wastewater. *Water Research,* 28, 1811–1815.

Ramadan, A., Abdel-Monem, M.O., El-Dougdoug, N.K., Mekky, A.E., Elaskary, S.A., Al-Askar, A.A., Metwally, S.A., El-Sayed, A.F., AbdElgayed, G., Saied, E. and Khedr, M., (2024). Fully Characterized Effective Bacteriophages Specific against Antibiotic-Resistant Enterococcus faecalis, the Causative Agent of Dental Abscess. *Medicina*, 60(3), p.501.

Ribeiro, K.V.G.; Ribeiro, C.; Dias, R.S.; Cardoso, S.A.; de Paula, S. O.; Zanuncio, J. C. and de Oliveira, L.L. (2018). Bacteriophage Isolated from Sewage Eliminates and Prevents the Establishment of Escherichia Coli Biofilm. *Advanced Pharmaceutical Bulletin,* 8(1), 85-95.

Saad, A.M., Askora, A., Kawasaki, T., Fujie, M. and Yamada, T. (2018). Full genome sequence of a polyvalent bacteriophage infecting strains of *Shigella, Salmonella, and Escherichia*. *Archives of Virology,* 163, 3207–3210.

Sada. T. S. and Tessema T. S. (2024). Isolation and characterization of lytic bacteriophages from various sources in Addis Ababa against antimicrobial-resistant diarrheagenic Escherichia coli strains and evaluation of their therapeutic potential. BMC *Infectious Diseases*, 24:310. https://doi.org/10.1186/s12879-024-09152-z.

Sambrook, J. and Russell, D.W. (2001). Picking Bacteriophage a. Plaques, In Molecular Cloning: A Laboratory Manual, 3rd ed, Vol.1 2.32. New York, NY: Cold Spring Harbor Laboratory Press.

Samhan, F.A., Askora, A.A., Ezzat, S.M. and Abu El-Nil, E.I. (2016). Differential Effects of Physical and Chemical Factors on Infectivity of Three Coliphages used as Water Quality Indicator. *Egyptian Journal of Microbiology*. 51, 45 – 62.

Shailemo, D.H.P.; Kwaambwa, H.M.; Kandawa-Schulz, M.; Msagati, T.A.M. (2016). Antibacterial activity of Moringa ovalifolia and Moringa oleifera methanol, N-hexane and water seeds and bark extracts against pathogens that are implicated in water borne diseases. *Green Sustain Chemistry*, 06(02), 71–77.

Shayo, G.M., Elimbinzi, E. Shao, G.N. and Fabian, C. (2023). Severity of waterborne diseases in developing countries and the effectiveness of ceramic filters for improving water quality. *Bulletin of the National Research Centre*, 47, 113.

Short, C.M. and Suttle, C.A. (2005). Nearly identical bacteriophage structural Gene sequences are widely distributed in both marine and freshwater environments. *Applied Environmental Microbiology*, 71,480–486.

Sillankorva, S., Neubauer, P. and Azeredo, J. (2008). *Pseudomonas fluorescens* biofilms subjected to phage phiIBB-PF7A. *BMC Biotechnology*. 8:79.

Sime-Ngando, T. (2014). Environmental bacteriophages: viruses of microbes in aquatic ecosystems. *Frontiers in Microbiology*. 5,355.

Sinton, L. W., Hall, C. H., Lynch, P. A., and Davies-Colley, R. J. (2002). Sunlight inactivation of 615 fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and 616 saline waters. *Applied Environmental Microbiology*, 68, 1122–1131.

Soliman, R. M., Othman, B.A., Shoman, S.A., Azzam, M.I., Gado, M.M. (2023). Biocontrol of multi-drug-resistant pathogenic bacteria in drainage water by locally isolated bacteriophage. *BMC Microbiology*, 23, 118.

Stefanakis, A. I., Bardiau, M., Trajano, D., Couceiro, F., Williams, J. B., Taylora, H., et al. (2019). Presence of bacteria and bacteriophages in full-scale trickling filters and an aerated constructed wetland. *Sciences Total Environment*. 659, 1135–1145.

Topka, G., Bloch, S., Nejman-Faleńczyk, B., Gąsior, T., Jurczak-Kurek, A., Necel, A., Dydecka, A., Richert, M., Węgrzyn, G. and Węgrzyn, A. (2019). Characterization of Bacteriophage vB-EcoS-95, Isolated from Urban Sewage and Revealing Extremely Rapid Lytic Development. *Frontiers in Microbiology,* 9, 3326.

USEPA Office of Water. (2015). Review of Coliphages as Possible Indicators of Fecal Contamination for Ambient Water Quality. EPA 820-R-15-098; USEPA Office of Water: Washington, DC, USA, 2015.

Van Regenmortel, M.H., Fauquet, C., Bishop, D., Carstens, E., Estes, M. (2000). Virus Taxonomy: Classification and Nomenclature of Viruses. Seventh Report of the International Committee on Taxonomy of Viruses. San Diego, USA: Academic Press; 2000.

Wei, S.; Chelliah, R.; Rubab, M.; Oh, D-H.; Uddin, M.J. and Ahn, J. (2019). Bacteriophage as potential tools for detection and control of *Salmonella spp*. In food systems. *Microorganisms*, 7, 570.

Wen, X.; Chen, F.; Lin, Y.; Zhu, H.; Yuan, F.; Kuang, D.; Jia, Z. and Yuan, Z. (2020). Microbial indicators and their use for monitoring drinking water quality—A review. *Sustainability,* 12(6), 2249.

World Health Organization (WHO). (2020). State of the world's sanitation: an urgent call to transform sanitation for better health, environments, economies and societies.

World Health Organization (WHO) (2022). Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda (4th ed + 1st add + 2nd add). World Health Organization. https:// apps. who. int/ iris/ handle/ 10665/ 352532.

Wu, J., Long, S. C., Das, D. and Dorner, S. M. (2011). Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. J. Water Health, 9(2), 265–278.

Yahya, M., Hmaied, F., Jebri, S., Jofre, J. and Hamdi, M. (2015). Bacteriophages as indicators of human and animal faecal contamination in raw and treated wastewaters from Tunisia. *Journal of Applied Microbiology*, 118, 1217–1225.

Yamaki, S., Omachi, T., Kawai, Y. and Yamazaki, K. (2014). Characterization of a novel Morganella morganii bacteriophage FSP1 isolated from river water. FEMS microbiology letters, 359(2), 166–172.

Hasanien, Y.A., Abdel-Aal, M.H., Younis, N.A., Askora, A., and El Didamony, G. (2024). Bacteriophages as Promising Agents for Biocontrol of Ralstonia solanacearum Causing Bacterial Wilt Disease. *Egypt. J. Bot*. 64 (1), 1277-291.

Yates, M.V. (2007). Classical indicators in the 21st century far and beyond the coliform. Water Environmental Research, 79(3), 279–286.

Yıldirim, Z., Sakin, T., Akçelik, M., and Akçelik, N. (2021). Identification and characterization of lytic bacteriophages specific to foodborne pathogenic *Escherichia coli* O157: H7. *Food Science and Technology International*, 27(1), 56-72.

Youqiang, X.u., Yuyue, M., Yao, S., Jiang, Z., Pei, J., and Cheng, C. (2016). Characterization, Genome Sequence, and Analysis of Escherichia Phage CICC 80001, a Bacteriophage Infecting an Efficient L-Aspartic Acid Producing *Escherichia coli*. *Food and Environmental Virology*, 8, 18–26.

Zimmerman, A. E., Howard-Varona, C., Needham, D. M., John, S. G., Worden, A. Z., Sullivan, M. B., et al. (2020). Metabolic and biogeochemical consequences of viral infection in aquatic ecosystems. *Nature Reviews Microbiology,* 18 (1), 21–34.