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ORIGINAL ARTICLE

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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

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Abstract

Background Antibiotic-resistant bacteria, including *Escherichia coli* (*E. coli*), are high-risk waterborne pathogens that pose a vital threat to the general public's health. Therefore, this study aims to develop alternative and affordable treatment approaches. Coliphage treatment is an economically and environmentally sustainable method for eliminating pathogenic bacteria. A significant step toward improving germicidal effectiveness might be to combine coliphage with electron beam treatment.

Results Twelve isolated *E. coli* were used as host bacteria. In addition, eleven coliphages were isolated and characterized to determine their suitable host range and lytic activities. Antibiotic resistance was tested to detect the most antimicrobial-resistant *E. coli* isolates. Results indicated that *E. coli*-2 and *E. coli*-10 were the most resistant bacterial isolates. Both somatic coliphage-3 (S3) and F-specific coliphage-3 (F3) were the most active lytic coliphages. Based on transmission electron microscope analysis, S3 was classified as a member of the *Myoviridae* family, while F3 belonged to the *Leviviridae* family. Genome types were detected; the S3 genome was a linear double-stranded DNA virus, while the F3 genome was a single-strand RNA virus. The adjustment of pH to 7 and temperature to 38 °C increased coliphage activity by 32.2% for S3 and 14% for F3. The optimum multiplicity of infection (MOI) for S3 was 1:1 and 2:1 for F3. From the one-step growth curve, both the latent periods of S3 and F3 were estimated to be 30 and 20 min, and the burst sizes showed 5.8 and 4.6 (PFU)/infected cells, respectively. The D_{10} values of the most two antimicrobial-resistant strains (*E. coli*-2 and *E. coli*-10) were calculated, showing nearly identical values (0.37 and 0.38 kGy), respectively. Both coliphages were used, either alone or in combination with electron beam irradiation (EBI), to eradicate the most multidrug-resistant *E. coli* in domestic wastewater. EBI reduced the counts of *E. coli*-2 and -10 by 59% and 65%, respectively. While the combination of coliphages and EBI completely eradicated these microbes.

Conclusions Combination of each individual coliphage and EBI decreased the growth of *E. coli* in domestic wastewater to an undetectable level.

Keywords Somatic coliphages, F-specific coliphages, *E. coli*, Domestic wastewater treatment, Electron beam irradiation, Combined treatment

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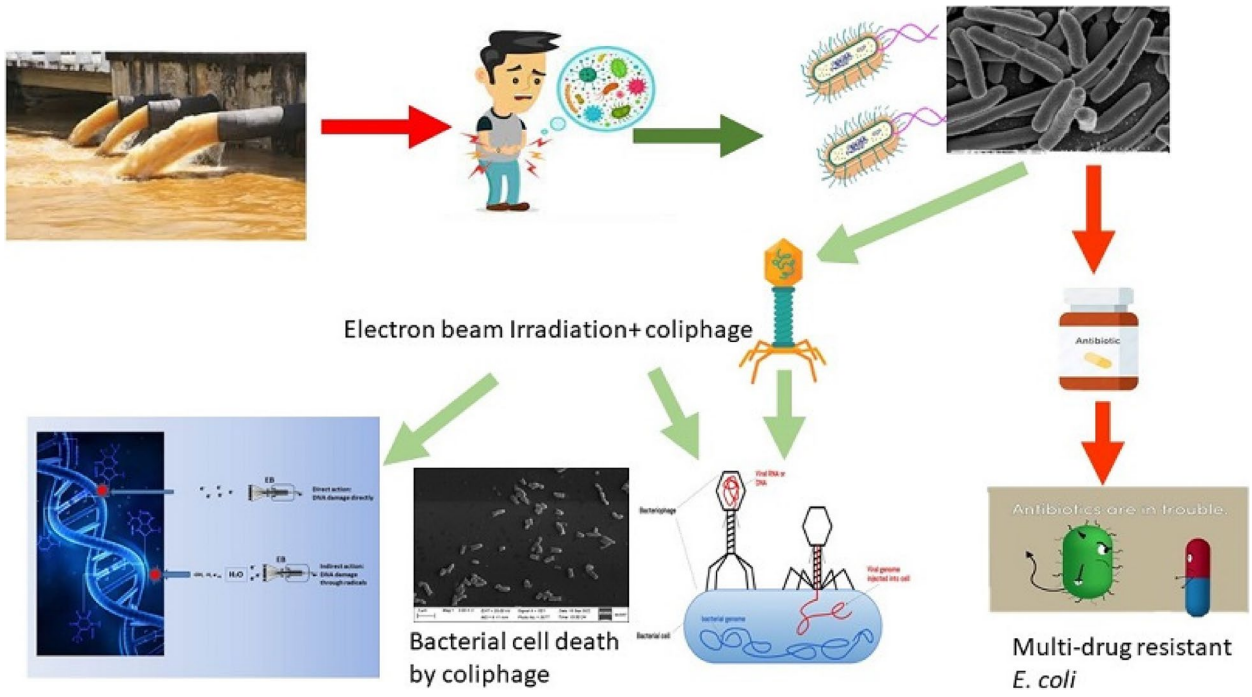
Graphical Abstract



Background

The world's water resources are limited, despite the increase in human demands for safe drinking water due to population growth. Effective wastewater treatment and water reuse are becoming more crucial to reducing this imbalance. The presence of both pathogenic bacteria and viruses in wastewater threatens human health and has become a significant public health concern (Mathieu et al. 2019).

Escherichia coli is the main bacterial pathogen that causes intestinal and extra-intestinal infections such as diarrhea, bacteremia, meningitis, and urinary tract infections. Multidrug-resistant *E. coli* has emerged as a significant source of serious infection in both humans and animals.

There are several physical and chemical techniques that can eliminate the risk of multidrug-resistant bacteria, but since outbreaks still happen, they have not been very effective and are quite expensive (Ahmed et al. 2021). Therefore, more exact, and effective techniques need to be created and applied to control water pollution. One of the many alternative treatments that can be applied to control bacterial cells, particularly multidrug-resistant bacteria, is the use of bacteriophages as biocontrol agents (Mathieu et al. 2019; Gildea et al. 2022). Bacteriophages are a group of viruses that can only infect bacteria with strong host specifications; the level of host preferences varies and is dependent on the bacteriophage (Kwiatek et al. 2020). Bacteriophage treatment is the use of phages or their by-products as bioagents to reduce bacterial infectious illnesses, particularly bacteria with multiple treatment resistances. They are considered a good therapeutic solution because they can affect the target pathogenic bacteria without affecting the beneficial normal flora, they slowly go away after the death of the host, and they can affect bacterial biofilms, which are thought to be the main cause of antibiotic resistance. Additionally, phage treatment does not require multiple dosages of phages as it does with chemical antimicrobials because they can proliferate until the host dies and they are nontoxic, which makes them environmentally sustainable (Ji et al. 2021).

Most of the time, the host-specificity of phages is determined by receptor molecules on the surface of bacteria. Different bacterial parts have been described as having phage receptors (capsule, cell wall, flagella, and pili). The most prevalent phages are somatic phages, which adhere to receptors found in the bacterial cell wall (Jofre et al. 2016). Contrarily, phages that invade bacterial cells via the sex pili and are encoded by the F-plasmid are referred

to as F-specific bacteriophages, male-specific bacteriophages, or sexual coliphages (Fauquet et al. 2005).

It is important to take into consideration a variety of physical and chemical parameters, such as pH, temperature, UV light, and exposure to chemical detergents or disinfectants, which can affect a phage's viability, stability, and inactivation through damage to its structural elements (tail, head, and envelop) and/or DNA structural alternations (Ly-Chatain 2014).

Electron beam irradiation (EBI), as an alternative traditional method, has been used for the treatment of drinking water, wastewater, sludge, food, and healthcare products to inactivate human pathogenic bacteria that have contaminated these materials (Emami-Meibodi et al. 2016; Ebrahim et al. 2022). In recent years, electron beam accelerators have been established in many countries for wastewater treatment due to their effectiveness in removing microbial contamination and other pollutants from the wastewater (Hossain et al. 2018). Combining phage with electron beam treatment could be a crucial step toward enhancing germicidal efficacy. A combination of bacteriophage and an electron beam significantly decreased the development of antibiotic-resistant cells in *E. coli.* According to several studies, combination treatments are more effective at reducing bacterial levels than each medication alone (Hieke and Pillai 2018; Osman et al. 2023; Abou El-Nour et al. 2023). Thus, the primary goals of this study were to isolate and characterize coliphages from different water sources, to improve bacteriophage lytic activity by optimizing various environmental conditions, and to be used either individually or in combination with EBI to eliminate multidrug-resistant *E. coli*, which is the most common contaminant in wastewater treatment plants.

Table 1 Isolation of *E. coli* from different water sources

Water sample	Positive *E. coli*
Ground water 1	1 + ve
Ground water 2	1 + ve
Ground water 3	2 + ve
Ground water 4	1 + ve
Ground water 5	1 + ve
Ground water 6	1 + ve
Ground water 7	1 + ve
Ground water 8	1 + ve
Inlet working pumping station (IWPS)	1 + ve
Treated water	1 + ve
Activated sludge	1 + ve

Table 2 Isolation of different types of coliphages from different water sources

Water sample sources	Somatic coliphage	F-specific coliphage
Ground water 1	+ ve	-ve
Ground water 2	+ ve	-ve
Ground water 3	+ ve	-ve
Ground water 4	+ ve	-ve
Ground water 5	+ ve	-ve
Ground water 6	+ ve	-ve
Ground water 7	+ ve	-ve
Ground water 8	+ ve	-ve
Inlet working pumping station (IWPS)	-ve	+ ve
Treated water	-ve	+ ve
Aerobic activated sludge	-ve	+ ve

(+ ve) contain coliphage, (-ve) not contain coliphage

Results

Isolation of *E. coli*

Twelve *E. coli* isolates were isolated from various water samples, identified, and used as bacterial hosts for coliphages to determine the presence of phages in water samples, as shown in (Table 1).

Isolation and purification of coliphages

Eleven coliphages were isolated from the different sources of water. Table 2 shows that eight somatic coliphages were isolated from ground water, while three F-specific coliphages were isolated from IWPs, treated water, and activated sludge. The results were based on the visual observation of light fluorescence when sample bottles were exposed to a UV lamp (366 nm), as shown in Fig. 1a. Positively tested samples were further examined by transferring 10 µl of the sample to agar-spot plates. A double-layer agar assay was performed on the diluted phages. Figure 1b demonstrates that the isolated S3 coliphage produced visible plaques on the bacterial lawn with diameters ranging from 1 to 3 mm. While the plaque morphology of F3 coliphages appeared as small circular plaques on the double-layer agar plate, their size was less than 1 mm in diameter, as shown in Fig. 1c. Each individual plaque (representing a single lytic phage) was chosen for phage propagation and purification.

Host range

The host range of all isolated coliphages was checked against the 12 *E. coli* isolates to evaluate the host specificity of each coliphage by using a spot test assay, as represented in (Table 3). The results of the experiments appeared in the lytic spectra of the tested bacteriophages. Overall, *E. coli* isolates were susceptible to one or more phages, demonstrating that bacteriophage is

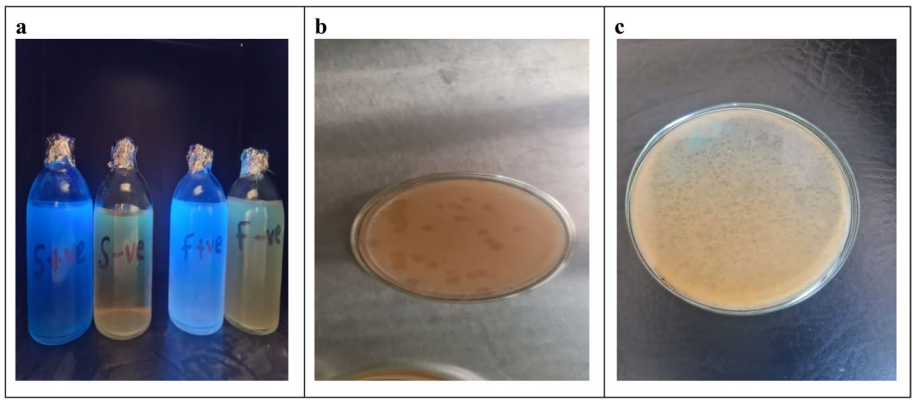


Fig. 1 **a** Formation of fluorescence light when sample bottles exposed to UV-lamp (366 nm) for (+ve) sample while no fluorescence light for (–ve) sample. **b** Plaque morphology of S3 coliphage on DLA plate. **c** Plaque morphology of F3 coliphage on DLA plate

Table 3 Determination of lytic activity of eleven coliphage isolates against different *E. coli* bacterial isolates

E. coli	Infectivity of coliphages										
	S1	S2	S3	S4	S5	S6	S7	S8	F1	F2	F3
1	+	−	−	+	+	−	−	−	+	−	++
2	+	−	++	−	+	+	+	−	−	−	−
3	−	−	+	−	−	−	−	−	+	+	++
4	+	+	−	−	−	−	−	+	−	−	+
5	−	−	−	−	−	−	−	−	−	+	+
6	−	−	+	−	−	+	+	−	−	−	−
7	−	-	+	−	+	−	−	−	−	−	−
8	−	+	++	−	−	+	−	+	−	−	−
9	−	−	++	−	−	−	−	+	−	−	−
10	−	−	−	−	−	−	−	−	+	+	++
11	−	−	+	+	−	−	−	−	−	−	−
12	−	−	+	+	−	−	−	−	−	−	−

(++) Very clear lysis zone, (+) Clear lysis zone, (−) No lysis zone

highly effective against a wide variety of *E. coli* strains. S3 coliphage showed lytic activity against 8 out of 12 tested *E. coli* (66.6%), whereas F3 coliphage lysed 5 out of 12 *E. coli* (41.6%). Both S3 and F3 coliphages have the widest host range and will be used for biocontrol experiments.

Based on the data represented in Table 3, it was possible to observe that all isolated somatic coliphages exhibited lytic activity specifically against *E. coli*-2, while all F-specific coliphages demonstrated lytic activity limited to *E. coli*-10. The potential impact of the various isolated somatic coliphages (S1, S2, S3, S4, S5, S6, S7, S8) and F-specific coliphages (F1, F2, F3) against the multidrug-resistant *E. coli*-2 strain against *E. coli*-10, respectively, was determined and illustrated in Fig. 2. The acquired data showed that, in comparison to the other examined coliphages, S3 and F3 coliphages exhibited the highest phage titers (1.80e + 11 and 1.85e + 11, respectively). Moreover, the specific host of S3 coliphage was *E. coli*-2, while that of F3 coliphage was *E. coli*-10. Thus, in the subsequent tests, S3 and F3 coliphages with their respective *E. coli* hosts were used.

Antibiotic susceptibility

The antibiotic resistance of each tested *E. coli* strain was evaluated. All tested *E. coli* strains responded differently to the antibiotics, with *E. coli*-2 and *E. coli*-10 exhibiting the greatest resistance against most of the tested antibiotics. Among the twelve examined antibiotics, *E. coli*-2 and *E. coli*-10 were resistant to 7 out of 12 and 5 out of 12, respectively, with minimum inhibition zone diameters against the tested antibiotics as shown in (Fig. 3). Moreover, *E. coli*-2 appeared to be highly sensitive to the S3

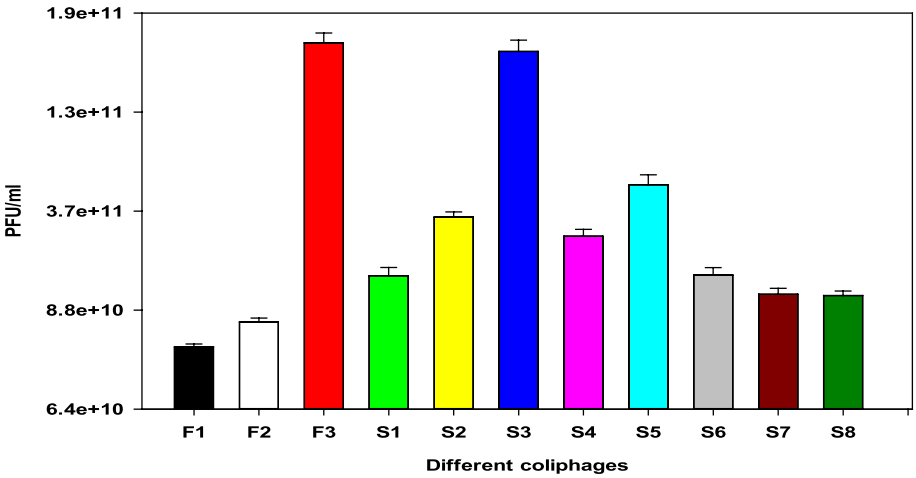


Fig. 2 Effect of different somatic coliphages and F-specific coliphages against the most resistant *E. coli*-2 and *E. coli*-10, respectively. Values represent the mean of three experiments; error bars represent the standard deviation

coliphage, whereas *E. coli*-10 showed high sensitivity to the F3 coliphage, as indicated by plaque assay on an agar plate as previously presented in (Table 3).

Physiological and biochemical identification of the most resistant *E. coli* strains

Two bacterial isolates were selected and characterized based on gram staining and biochemical characteristics, as listed in Table 4. Gram staining indicated that the two isolates were gram-negative bacteria. According to their physiological-biochemical properties, it could be suggested that the two bacterial isolated belonged to Enterobacteriaceae family.

Phage morphology

Transmission electron microscopy analysis of both coliphages (S3 and F3) revealed that the S3 coliphage had a regular elongated icosahedral head with a width of approximately 62.2 ± 3 nm and a contractile tail with a length of approximately 110 ± 3 nm. The evaluation of its morphological characteristics in accordance with the official guidelines of the International Virus Taxonomy Committee revealed that the phage displayed typical features of a phage belonging to the *Myoviridae* family, which comprises a quarter of tailed bacteriophages and includes the *E. coli* phage T4, as represented in Fig. 4a (Ackermann 2009). While the TEM analysis of F3 coliphage suggested that it belonged to the *Leviviridae* family, it had a regular icosahedral head with a length and width of approximately 55.1 ± 2 nm and 54.0 ± 2 nm without tail, respectively, as represented in Fig. 4b.

Acridine orange staining

Acridine orange dye is a quick technique used to differentiate between ssDNA, dsDNA, and ssRNA based on color differences. Figure 5 shows the acridine orange staining under a fluorescence microscope. It can be observed that S3 (Fig. 5a) is double-stranded dsDNA, showing a bright green fluorescence color after phosphate treatment, while F3 (Fig. 5b) is single-stranded ssRNA, displaying a bright red fluorescence color.

pH stability

The resistances of S3 and F3 coliphages to different pH values (4, 5, 6, 7, 8, and 9) were performed to evaluate their stability. The results indicate that both coliphages showed identical behavior; both phages were relatively stable at pH values ranging between 6 and 8, with maximum stability at pH 7. As shown in Fig. 6a, coliphage activity was reduced by 83.2, 84.3, and 88.3% at pH 5, 4, and 10, respectively. Also, F3 at pH 7 exhibited the highest lytic activity. At pH 4, the percentage of reduction was 93.7, followed by 77.5% at pH 5 and 78.9% at pH 10 (Fig. 6b).

Temperature stability

The two coliphages thermal stability under varying temperature degrees (18, 28, 38, 48, and 58 °C) was examined. The PFU of S3 coliphage was highest after incubation at 38 °C, while the plaque titer significantly declined at the other tested temperatures. The least S3 coliphage lytic activity was recorded at 58 °C (86.9% reduction as compared with 38 °C). At 18, 28, and 48 °C, the plaque titer lytic activity decreased by 76.5, 58.8, and 82.1%, respectively, when compared to 38 °C, as represented in Fig. 7a. Meanwhile, the highest lytic

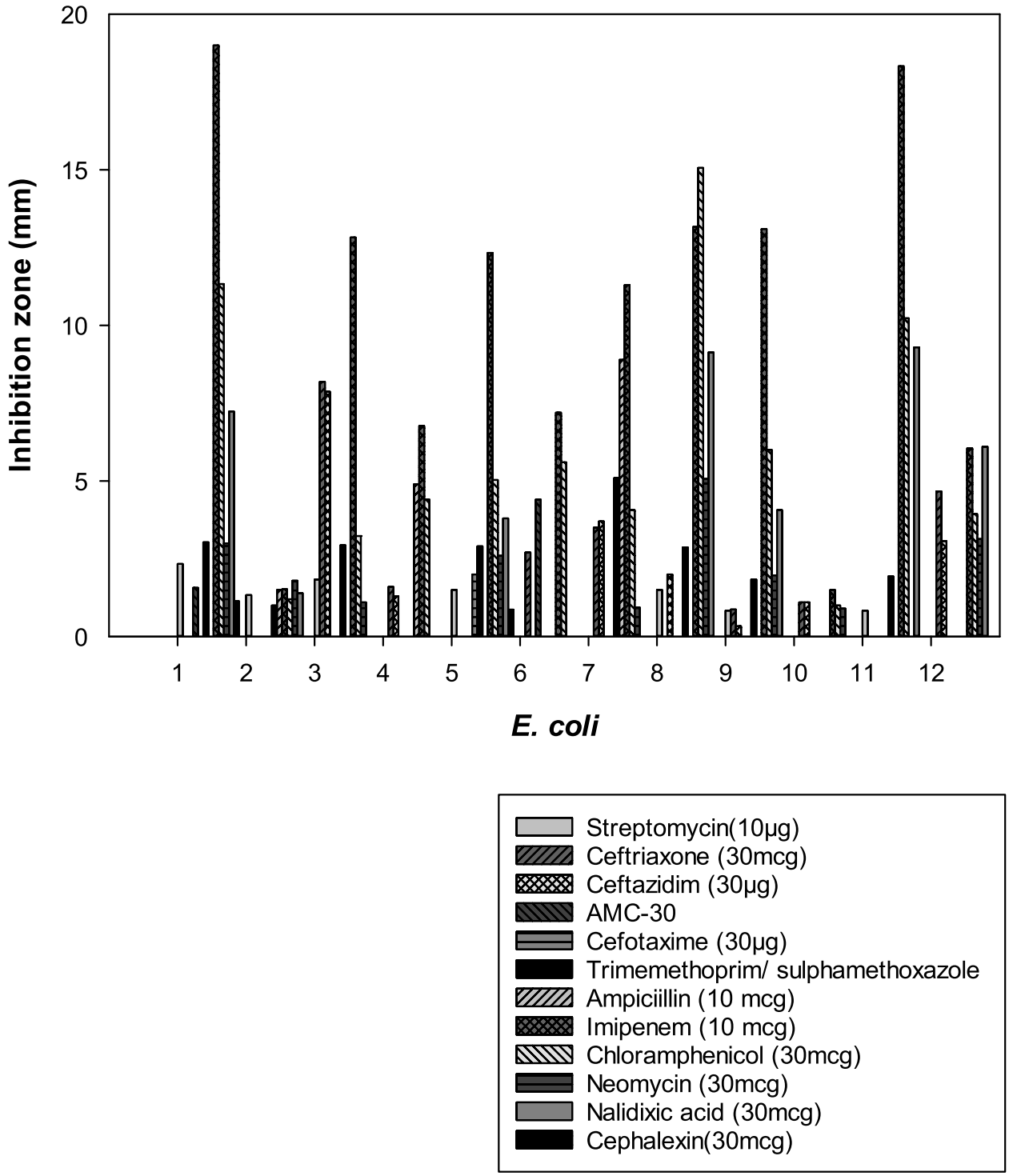


Fig. 3 Effect of different antibiotics against twelve *E. coli* isolates

activity of F3 was also recorded at 38 °C, and at 58 °C, the minimum F3 coliphage titer (85.5% reduction compared with 38 °C) was observed. The lytic activity of F3 was significantly reduced by 44.1, 18.6, and 82.6% at 18, 28, and 48 °C, respectively (Fig. 7b). The results of the temperature stability assay revealed that both coliphages exhibited the same behavior: as the temperature increased above 38 °C, the phage stability

Table 4 Biochemical tests of the most resistant *E. coli* strains (*E. coli*-2 and *E. coli*-10)

Biochemical test	*E. coli*-2	*E. coli*-10	Biochemical test	*E. coli*-2	*E. coli*-10
Ala-phe ProArylamidase	–	–	**L-Arabitol**	–	–
H_2S production	–	–	**D-glucose**	+	+
Beta-glucosidase	–	–	**D-mannose**	+	+
L-proline Arylamidase	+	–	**Tyrosine arylamidase**	+	+
Saccharose/cucrose	–	–	**Citrate (Sodium)**	–	–
L-Lactate alkalinisation	+	–	**Beta-*N*-Acetyl Galactoseaminidase**	–	–
Glycine Arylamidase	–	–	**L-Histidine assimilation**	–	–
O/129 resistance (comp. vibrio)	+	+	**Ellman**	+	+
Adonitol	+	–	**D-cellobiose**	–	–
Beta-*N*-Acetyl-Glucosaminidase	–	–	**Gamma-Glutamyl transferase**	–	–
D-maltose	+	+	**Beta-Xylosidase**	–	–
Lipase	–	–	**Urease**	–	–
D-Tagatose	–	–	**Manitol**	–	–
Alpha-Glucose	–	–	**Alpha-Galactosidase**	+	+
Ornithine Decarboxylase	–	–	**Coumarate**	+	+
Glu-Glu-Arg-Arylamidase	–	–	**L-Lactate assimilation**	–	–
L-Pyrrolydonyl-Arylamidase	–	–	**Beta-Galactosidase**	+	+
Glutamyl Arylamidase PNA	–	–	**Fermentation/glucose**	+	+
D-Mannitol	+	+	**Beta-Alanine arylamidase pNA**	–	–
Palatinose	–	–	**D-sorbitol**	+	+
D-Trehalose	+	+	**5-Keto-D-Gluconate**	–	+
Succinate alkalinisation	+	+	**Phosphatase**	+	–
Lysine Decaboxylase	+	+	**Beta-Glucuronidase**	+	+
L-Malate assimilation	–	–			

Where (+): positive result and (–): negative result

decreased. The optimal temperature for the lytic activity of the two bacteriophages was determined to be 38 °C.

Multiplicity of infection

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). MOI was investigated to determine the optimal phage-host concentrations for optimum phage activity. Host bacteria were infected with S3 and F3 coliphages at different MOIs. Based on the finding in Fig. 8a, the S3 phage titer reached its maximum when the MOI was 1, indicating that an equal concentration of S3 coliphage against the same concentration of host strain resulted in the highest titer. In contrast, the F3 phage titer reached its maximum when the MOI was 2, indicating that the concentration of F3 coliphage must be double the host strain concentration to obtain the highest titer, as represented in Fig. 8b.

One step growth

Plotting phage infection time against PFU/ml of phage allowed for the construction of the one-step growth curve, which calculated the latent period, burst period, burst size, and rise period. A triphasic growth curve comprising the latent, rise, and plateau phases of the two bacteriophages was obtained. Figure 9a, b shows the one-stop growth curves of S3 and F3 coliphages, respectively. It is obvious from the curves that the latent periods of S3 and F3 were approximately 30 and 20 min, respectively. The rise periods were 40 and 30 min for S3 and F3 coliphages, respectively. For S3 and F3 coliphages, the burst period was approximately 70 and 50 min, respectively, and the burst sizes were 5.8 and 4.6 PFU/infected bacterial cell (burst size = number of phage particles released at the plateau level / the initial number of infected bacterial cells).

Mode of action

Growth curve

The potential of the isolated coliphages to reduce the growth of the host bacteria was assessed by construction the bacterial growth reduction curve. The growth of bacterial cells alone, *E. coli*-2 (B.C-2), *E. coli*-10 (B.C-10), and bacterial cells with its coliphages (*E. coli*-2 + S3 phage) and (*E. coli*-10 + F3 phage) with MOI 1 and 2,

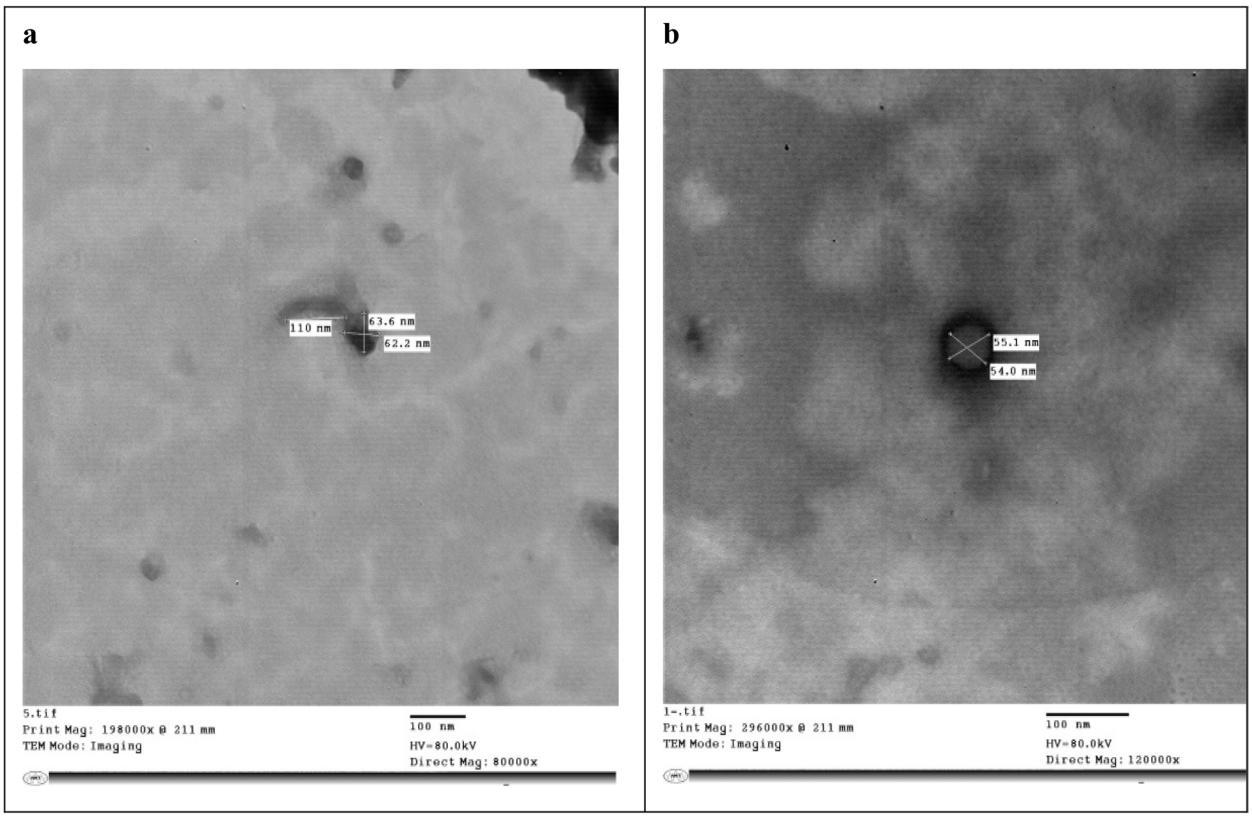


Fig. 4 Transmission electron microscopy (TEM) of the phage (**a**) S3, (**b**) F3 coliphages with scale bar 100 nm

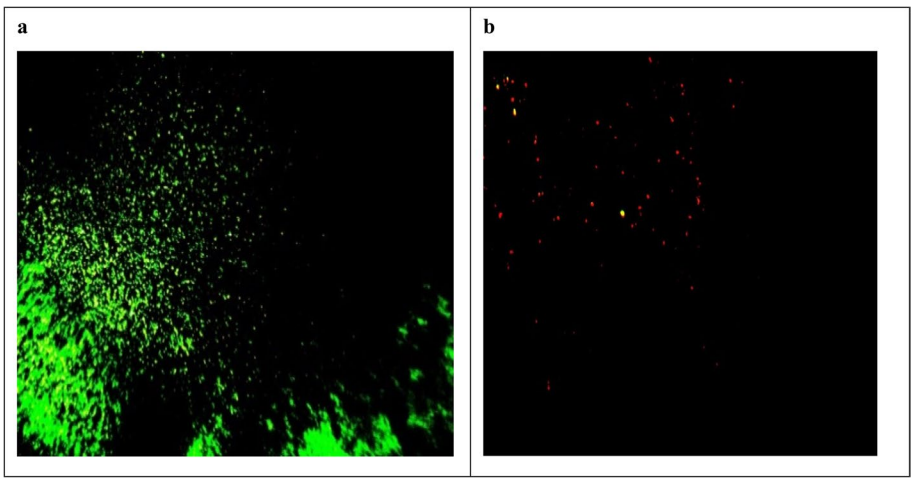


Fig. 5 Staining of bacteriophage nucleic acid using acridine orange stain under florescence microscope. **a** Bright green fluorescence color dsDNA S3 coliphage. **b** Bright red fluorescence color ssRNA F3 coliphage

respectively, was estimated every 10 min for 90 min. The growth curves of both groups were constructed by plotting the results of their 37 °C incubation and the optical density over time. The increase in optical density (OD_{600}) of non-infected bacterial cells (B.C-2 and B.C-10) over the course of 90 min of incubation indicated their normal

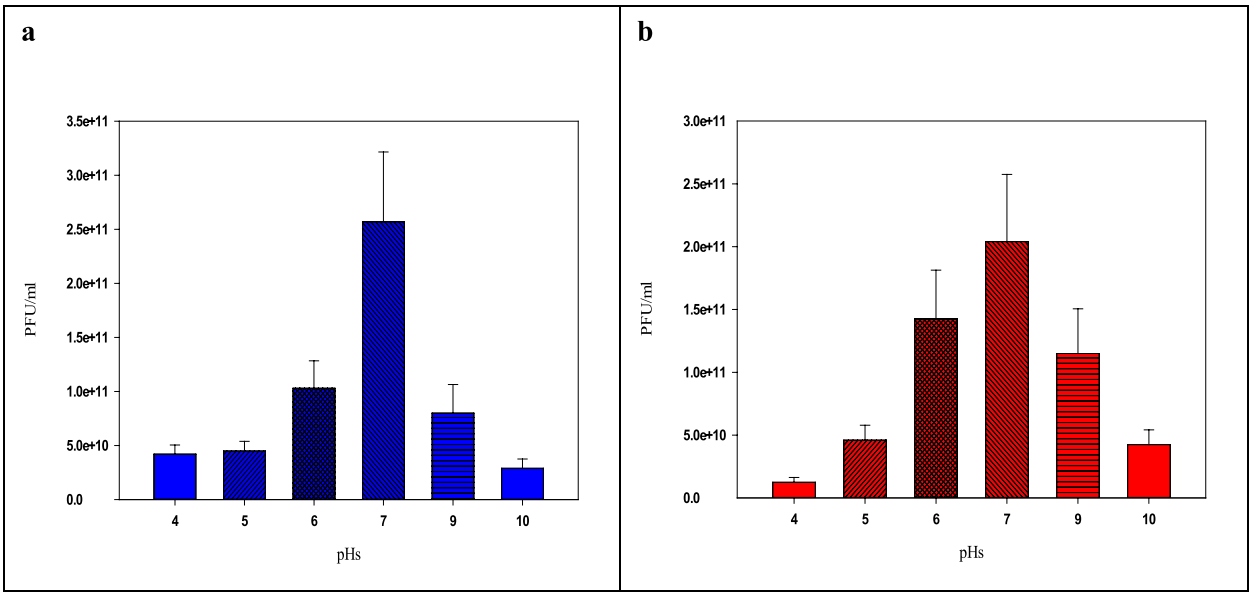


Fig. 6 The effect of pH on bacteriophage stability of **a** S3, **b** F3 coliphages. The phage lysate treated at different pH values for one hour followed by calculating phage titer; values represent the mean of three experiments; error bars represent the standard deviation

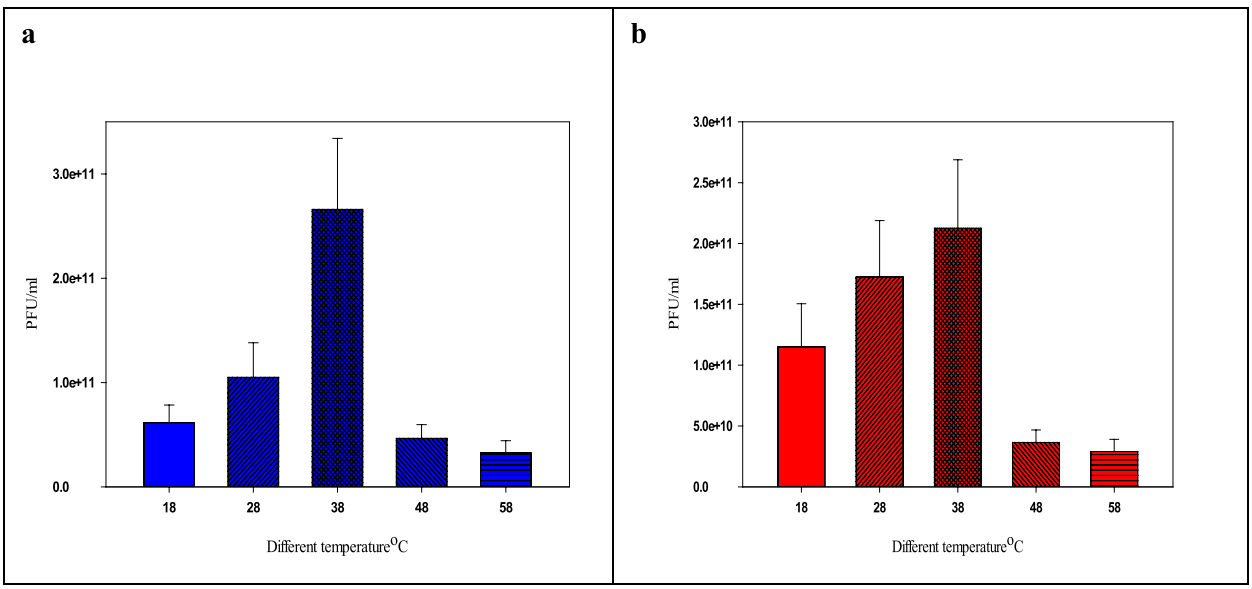


Fig. 7 Effect of different temperature on bacteriophage efficiency **a** S3, **b** F3. The phage lysate treated at different temperatures for 1 h followed by calculating phage titer; values represent the mean of three experiments; error bars represent the standard deviation

growth pattern; however, after 90 min of incubation of *E. coli*-2 + S3 phage and *E. coli*-10 + F3 phage, both bacteriophages suppressed bacterial growth by 71.5% and 55.1%, respectively, as shown in Fig. 10.

Impact of coliphages on their *E. coli* host

Utilizing a scanning electron microscope (SEM), control sample (*E. coli*-2 and *E. coli*-10 without exposure to S3 and F3 coliphages, respectively) and the impact of S3 and F3 coliphages on their *E. coli* hosts was examined. When compared to the control sample, each phage had a significant impact on both bacterial cells after incubation with

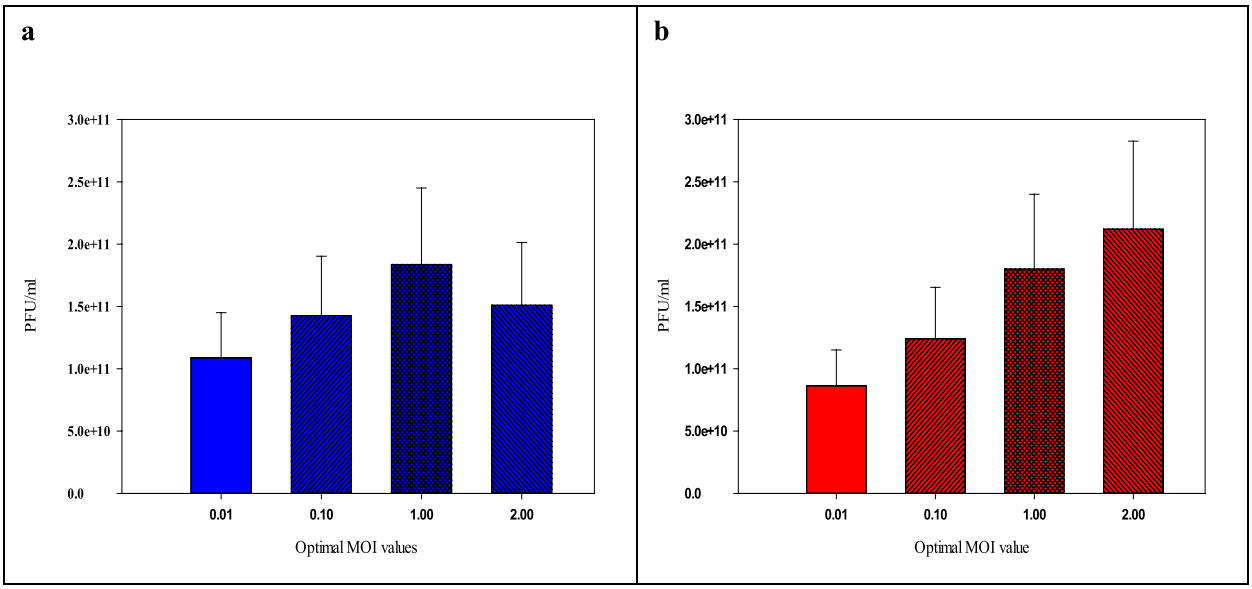


Fig. 8 Effect of different MOI values on **a** S3 and **b** F3. Values represent the mean of three experiments; error bars represent the standard deviation

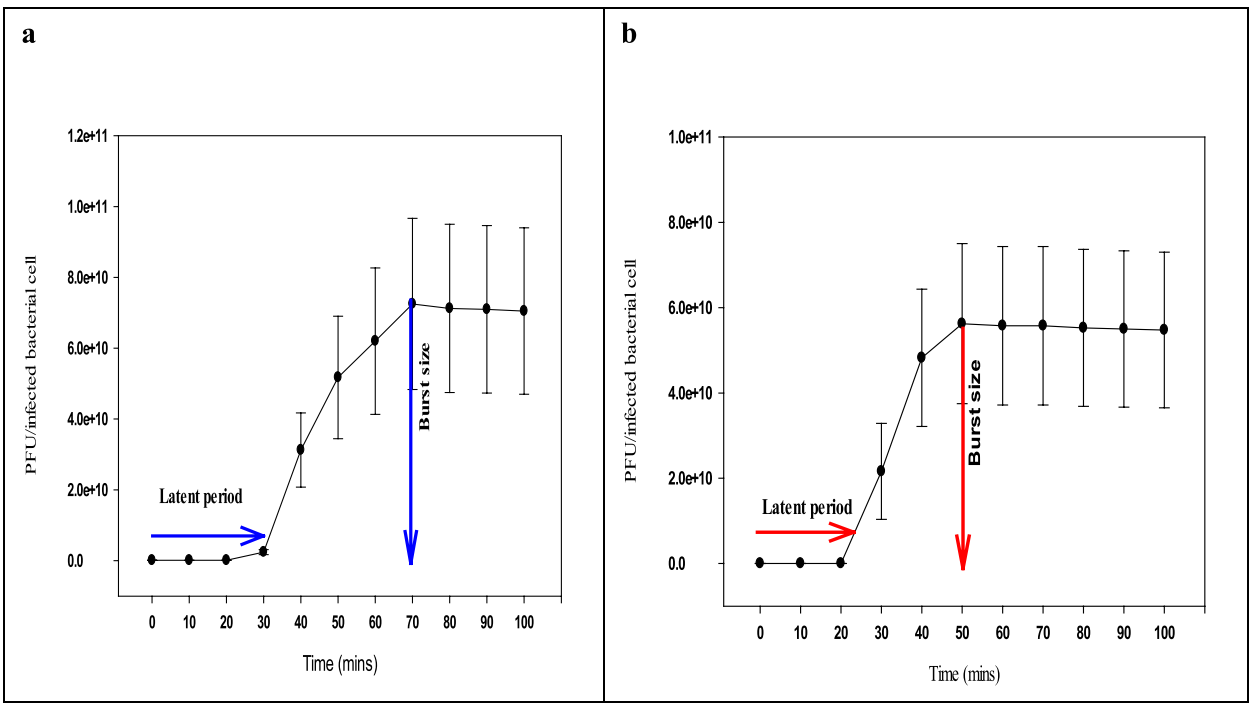


Fig. 9 One step growth curve for estimation of the latent period and burst size of **a** S3 and **b** F3. Each data point is the mean of three experiments

its respective bacterial host and SEM analysis. Figure 11 showed that both coliphages caused lysis to its host cells, which were visible by SEM as shortening of bacterial cells (due to flaws in the ratio of the length to width axis) and a collapse of bacterial bodies. The findings suggest that both bacteriophages can induce structural changes at the bacterial level.

Response of *E. coli* to irradiation

The EB radiation sensitivity of the two *E. coli* isolates was investigated by calculating their D_{10} values from

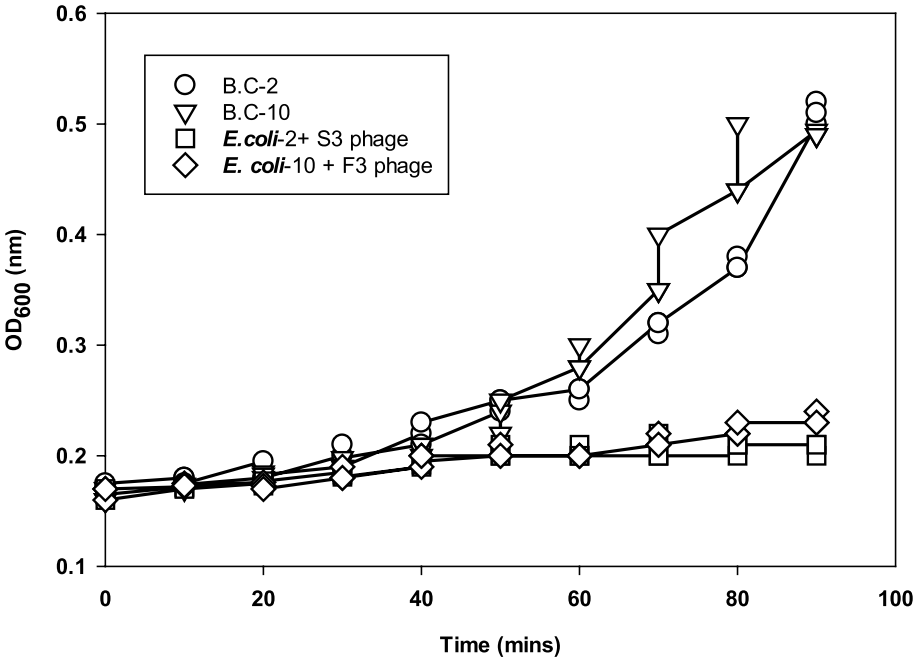


Fig. 10 Bacterial growth reduction assay for coliphage S3 and F3. The OD of infected and non-infected bacterial culture with bacteriophage is compared. Where *E. coli*-2 (B.C-2), *E. coli*-10 (B.C-10), *E. coli*-2 + S3 phage, and *E. coli*-10 + F3 phage

their radiation-dose response curves (Fig. 12). It is obvious that their radiation-dose response curves could be described as exponential curves. The D_{10} values of the two *E. coli* isolates (*E. coli*-2 and *E. coli*-10) were nearly identical (0.37 and 0.38 kGy, respectively).

Application of coliphages and irradiation

In this experiment, domestic wastewater was artificially inoculated with *E. coli* isolates and exposed to 2.0 kGy according to their D_{10} value. Table 5 shows that the initial counts of *E. coli*-2 inoculated into domestic wastewater samples were 8.37 logs. After 24 h of storage at 37 °C, 2.0 kGy EBI reduced the counts by 3.4 logs (a 59.3% reduction). On the other hand, the initial log counts of *E. coli*-10 inoculated into samples of wastewater were 8.4 logs. When contaminated wastewater was exposed to 2.0 kGy EBI, the colony count decreased by 3.28 logs (a 60.95% reduction) after 24 h of storage at 37 °C.

Table 5 also shows that the log counts of *E. coli*-2 inoculated into wastewater samples with the S3 coliphage at 37 °C were reduced to 1.9 logs (a 77.2% reduction). On the other hand, the log counts of *E. coli*-10 inoculated in wastewater samples with F3 decreased to 3.2 logs (a 61.9% reduction). Individually, S3 with its *E. coli*-2 host, and F3 with its *E. coli*-10 host were inoculated into domestic wastewater samples. In combination treatment, each group was exposed to 1.5 kGy of EBI. From the obtained results, it could be observed that a combination of coliphages and 1.5 kGy EBI led to the complete elimination of both *E. coli* under investigation that had been artificially inoculated into wastewater samples as counts of *E. coli* were below detectable level.

Discussion

Bacteriophages as a biocontrol agent can be used as an alternative to antibiotics to inactivate and control several waterborne pathogenic bacteria (Li et al. 2021; Dewonggana 2022). This biotechnology has many advantages over other traditional techniques, such as high specificity and efficacy, no harmful impact on humans, normal microbiota, animals, and plants or the environment, being fast and cost-effective, and persisting as long as target bacteria are present (Kwiatek et al. 2020; Ji et al. 2021).

In this study, twelve *E. coli* isolates were isolated from different water sources. They were identified and used as host bacteria to detect and isolate somatic and F-specific coliphages simultaneously in water samples by a standardized method (ISO 2000; US-FDA 2022).

Eight somatic coliphages and three F-specific coliphages were simultaneously isolated from different water sources by infecting *E. coli.* The utility of *E. coli* for simultaneously detecting and isolating somatic and F-specific coliphages from water has been demonstrated and confirmed by Agullo-Barce et al. (2016).

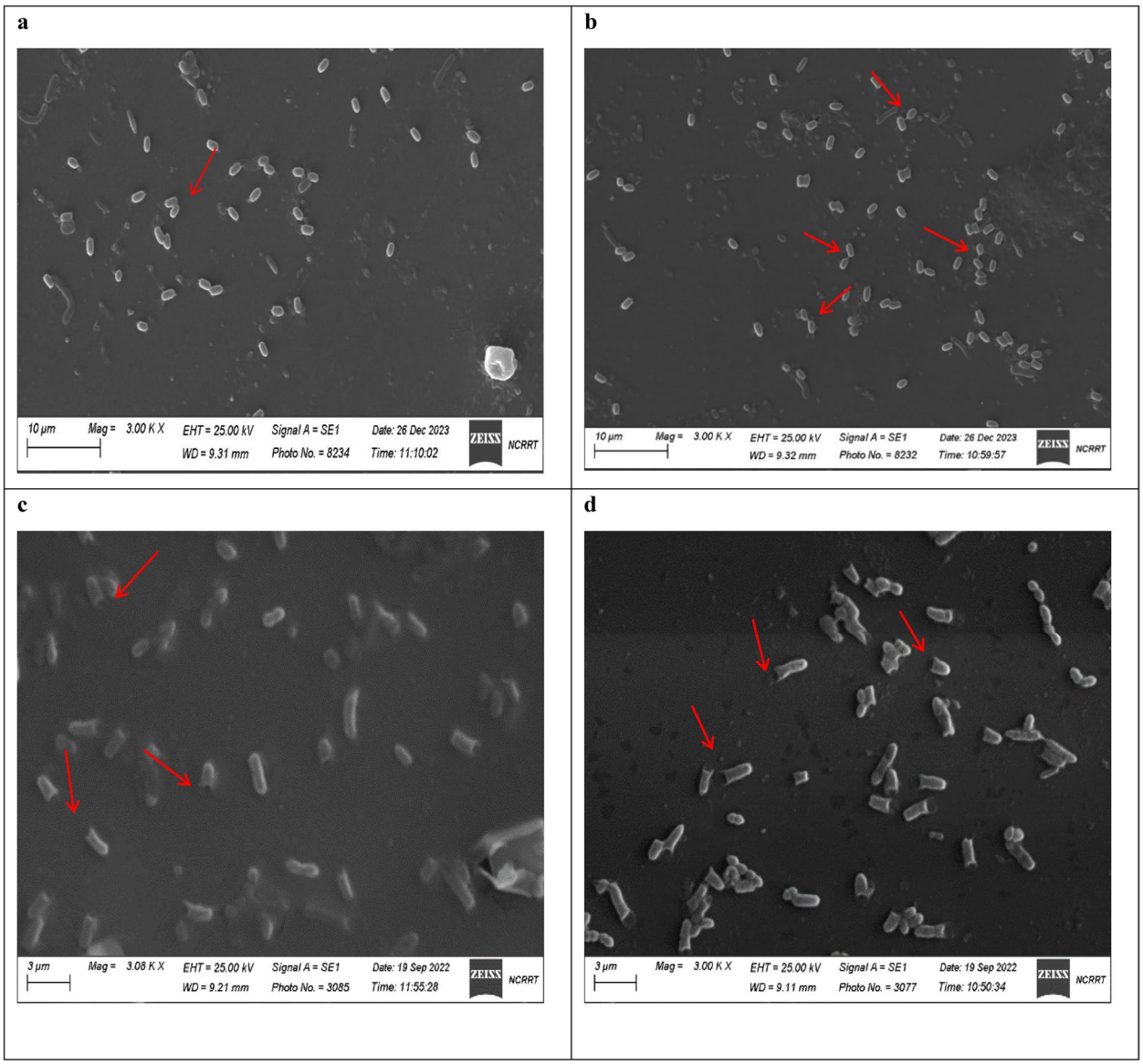


Fig. 11 Scanning electron microscopy (SEM) of **a***E. coli*-2 cells (not exposed to S3 coliphages) (arrow indicates the normal cell). **b** *E. coli*-10 cells (not exposed to F3 coliphages) (arrows indicate the presence of pili). **c***E. coli*-2 infected with S3 coliphages and **d** *E. coli*-10 infected with F3 coliphages. The arrows highlight bacterial cell death (i.e., "ghosts or remnants")

The twelve *E. coli* isolates were used to evaluate the host specificity of eleven somatic and F-specific isolated coliphages using a spot test. The data presented in this manuscript demonstrates that S3 coliphage effectively lysed 66.6% of all tested *E. coli* isolates, with the highest lysis activity against *E. coli*-2, whereas F3 coliphage showed lytic activity against 41.6% of all tested *E. coli* isolates, with the highest lytic activity against *E. coli*-10. The lysis activity of somatic and F-specific coliphages against *E. coli* hosts has also been reported by several researchers (Agullo-Barce et al. 2016; Dewanggana et al. 2022). The most lytic bacteriophages attach to certain receptors found in the host cell wall, infecting and ultimately killing the host bacteria. It has been reported that somatic coliphages infect host bacteria via the cell wall (Jofre et al. 2016), while F-specific coliphages infect host bacteria via sex pili (Fauquet et al. 2005). Based on the available data, it is possible to conclude that somatic and F-specific phages could be used safely as biocides against these bacteria. Differences in proteins in the fibers and tail spikes, which should detect receptor molecules on bacterial cell walls, could explain the difference in host range between

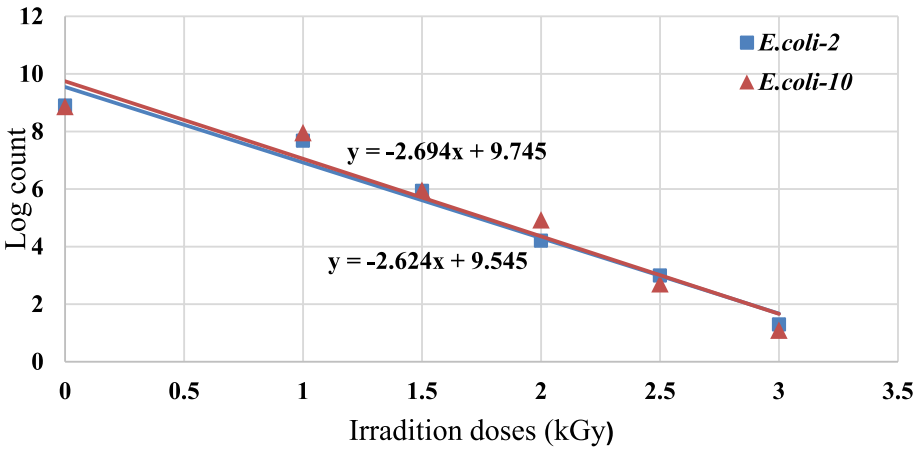


Fig. 12 Radiation dose response curve for *E. coli*-2 and *E. coli*-10

Table 5 Effect of EBI (2.0 kGy), coliphage, and combined of EBI (1.5 kGy) + coliphage on *E. coli* in domestic wastewater

Treatment	*E. coli*-2		*E. coli*-10	
	Log value	% reduction	Log value	% reduction
Control	8.37	–	8.40	–
EBI (2.0 kGy)	3.4	59.3	3.28	60.95
S3 coliphage	1.9	77.2	–	–
F3 coliphage	–	–	3.2	61.9
Combined treatment (1.5 kGy EBI + S3 coliphage)	< 1	> 99.99%	–	–
Combined treatment (1.5 kGy EBI + F3 coliphage)	–	–	< 1	> 99.99%

bacteriophages with high genomic similarity and phages that lyse multiple bacteria (Witte et al. 2021).

The observed morphological differences between bacteriophages classify them into distinct families. S3 coliphage belongs to the *Myoviridae* family, according to TEM analysis, while F3 coliphage belongs to the *Leviviridae* family. Several studies have demonstrated the effectiveness of the *Myoviridae* bacteriophage in reducing pathogenic *E. coli* O157:H7 in foods (Ferguson et al. 2013).

Bacteriophage genome types were estimated by using an acridine orange stain, which indicated that S3 coliphage fluorescence was green (had double-stranded DNA genomes) and F3 coliphage had single-stranded RNA genomes (fluorescence was red), as described by Bradley (1965). Ackermann (2001) reported that somatic coliphages infecting *E. coli* through the cell wall may have double-stranded genomes belonging to *Myoviridae, Siphoviridae*, and *Podoviridae*.

For successful applications of bacteriophage technology to inactivate microorganisms in water, it is essential to conduct prior experiments to determine the optimum conditions for the highest lytic activity against host bacteria. It has been found that pH and the thermal stability of bacteriophages have a great impact on lysis activity because they influence so many aspects of the biological system (Taj et al. 2014).

Our results in this concept showed that the lytic activity of the two investigated coliphages (S3 and F3) against *E. coli*-2 and -10, respectively, was stable over a broad pH range of 6 to 9, with the highest activity at pH 7. This finding is in line with the results of Taj et al. (2014), who found that the lysis activity of T4 bacteriophage against *E. coli* remained constant between pH values of 4 and 10. Ly-Chatain (2014) stated that phages lose their infectivity below pH 5.

According to the temperature results, the optimal temperature for maximum lysis activity for both coliphages was 38 °C. This agrees with the findings of Taj et al. (2014), who reported that the optimal temperature for T4 bacteriophage lysis activity against *E. coli* B121 was 37 °C. The lowest lysis activity was recorded at 58 °C. This reduction is mainly attributed to phage protein denaturation and damage to the phage's physical structure, which compromises its biological control ability (Liu et al. 2012). The present results and previously reported data corroborate the findings of Xu et al. (2016), who reported the survival rate of Phage QL01's at different temperatures. The assessment of heat stability revealed that within 40 min of incubation at 50 °C, over 80% of the phages were still alive. While substantial declines in survival rates were noted during a 40-min incubation period

at 60 and 70 °C. At 70 °C, fewer than 1% of the phage particles were active. Furthermore, Iona and Mark (2014) concluded that an increase in temperature reduces virus survival and activity.

The ratio of a bacteriophage to the concentration of its host (MOI) must be optimized to ensure effective phage-bacteria interaction and to implement phage infection (Chibani-Chennoufi et al. 2004). The lytic activity of the two coliphages (S3 and F3) was assessed at various MOIs ranging from 0.01 to 2.0. The optimum concentration of S3 coliphage to *E. coli*-2 was 1:1 (MOI = 1), while F3 was double (MOI = 2), which allowed for an effective reduction in the bacterial population. These results are consistent with those of Mozaffari et al. (2022), who discovered that a bacteriophage with a multiplicity of infection (MOI) of 1 was capable of substantially decreasing *E. coli* O157:H7. Conversely, Nakai (2010) mentioned that the actual original dosage of bacteriophage may not be necessary because of the self-replication nature of phages, as demonstrated by an increase in phage titers alongside bacteria.

The so-called bacteriophage growth parameters, which describe the bacteriophage multiplication cycle, are crucial to understanding and quantifying in practical applications. Latent period, burst size, rise period, and burst time figure out how well the bacteriophage works against the target host bacteria. Typically, these parameters are derived from a one-step growth curve (Hyman and Abedon 2009). In the present study, the concentrations of two coliphages (S3 and F3) were measured over time to construct a one-step growth curve. The one-step curves of the two tested coliphages for their respective latent periods exhibited dissimilar behavior (30 and 20 min, respectively). During this period, the attachment, entry, replication, transcription, translation, and assembly of progeny occurred. Since no new phage particles are being released, the number of plaques stays the same. During the rise phase, lysis happens, and new batches of virus particles are released, which causes extracellular phages to appear. During this phase, the concentration of phage particles increases rapidly. The rise periods for S3 and F3 coliphages were 40 and 30 min, respectively. Burst period (the plateau); this period represents the end of all infected host cell lysis. Due to high dilution, newly released phage particles fail to reach uninfected host cells. Therefore, during this phase, the plaque count remains constant. S3 and F3 coliphages reached a growth plateau in 70 and 50 min, respectively. Jurač et al. (2019) reported that the appropriate design, regulation, and optimization of bacteriophage production processes require the correct growth parameter factor values. The burst size of a phage determines its level of virulence, which is necessary for productive infection and effective treatment (Khan-Mirzaei and Nilsson 2015). From the one-step growth of the S3 and F3 coliphages, the burst period was approximately 5.8 and 4.6 PFU/infected bacterial cells, respectively.

Bacteriophages have a high potential for lysing (killing) target host bacterial cells during the incubation period. Thus, the effectiveness of S3 and F3 against *E. coli*-2 and *E. coli*-10 with MOI 1 and 2, respectively, was tested by comparing the bacterial growth curve reduction to a control culture. After 90 min of incubation at 38 °C, S3 coliphage inhibited the growth of *E. coli*-2 by 71.5%, while F3 coliphage inhibited the growth of *E. coli*-10 by 55.1%, indicating that S3 was more effective than F3 coliphage against their target host bacteria. The growth of the target bacteria was inhibited by TSE phages eight h after infection and for the following 18 h, according to Khawaja et al. (2016). Haq et al. (2012) stated that the results of the bacterial growth reduction assay can be used to figure out how phages can be used in phage therapy and their viability.

Scanning electron microscopy has demonstrated the bacteriolytic activity of both coliphages (S3, F3) against their multidrug-resistant *E. coli* hosts (*E. coli*-2 and *E. coli*-10, respectively), revealing cell destruction and lysis. Figure 11 shows that both coliphages may connect with receptors on the surface of bacteria to identify specific regions and infect specific hosts. According to Jofre et al. 2016, the somatic phages connect to receptors present in the bacterial cell wall. Instead, phages encoded by the F-plasmid that enter bacterial cells via the sex pili are known as F-specific bacteriophages (Fauquet et al. 2005). Phage was sufficient to break down the bacteria's cell walls and kill them. These findings are in agreement with Wang et al. 2022, who reported using scanning electron microscopy to investigate the alterations in bacterial cells following bacteriophage treatment against Gram-positive (*Staphylococcus aureus* 1606BL1486) and Gram-negative (*E. coli* O157 and *Shigella dysenteriae* KUST9) bacteria that are multi-drug resistant. Leakage of internal contents, bacterial lysis, and extensive damage were induced by bacteriophages. It has been proposed that the lytic enzyme present in bacteriophages might damage the bacterial cell walls, and cause leakage of intracellular contents, thereby leading to the lysis and death of the bacterial cell.

Ionizing radiation in the form of its three applied radiations (gamma radiation, electron beams, and X-rays) has all been shown to be very effective at repressing and controlling pathogenic bacteria, including multidrug-resistant strains in food, wastewater, sludge, and health-care products (Emami-Meibodi et al. 2016; Munir and Federighi 2020).

In the application experiments, *E. coli*-2 and *E. coli*-10 were each artificially inoculated in wastewater for this portion of the investigation and exposed to 2.0 kGy of EBI. The findings show that the log counts of *E. coli*-2 and *E. coli*-10 were reduced from 8.37 and 8.4 logs to 3.4 and 3.28 logs (59.4 and 60.9 logs reductions percent, respectively) after exposure to this irradiation dose. This significant inactivation of *E. coli* cells resulted from the direct and indirect effects of ionizing radiation, mainly on the DNA cells. The absorption of EBI's high photon energy had direct effects, causing single- or double-strand breaks in nucleic acids. Indirect effect resulting from the water radiolysis of highly reactive free radicals such as °OH, °H, and solvated electrons, which have lethal biologically significant functions for a microbe (Farooq et al. 1993; Sommer et al. 2001).

Investigations were done into the individual efficacy of S3 and F3 coliphages in inactivating *E. coli*-2 and *E. coli*-10 inoculated in wastewater, respectively. According to the results, S3 decreased *E. coli*-2 log counts to 1.9 logs (a 77.2% reduction) and F3 decreased *E. coli*-10 log counts to 3.2 logs (a 61.9% reduction) in just 24 h at 37 °C, respectively. This suggests that both coliphages were more effective in reducing tested *E. coli* than EBI and S3 coliphage was more successful than F3 coliphage in suppressing *E. coli* in wastewater. When coliphages infect *E. coli*, they create numerous progeny that are released during the replication process known as the lytic cycle.

The tested coliphage S3 reduced the target *E. coli*-2 level by 6.5 logs, while the F3 coliphage decreased *E. coli*-10 by 5.2 logs, exceeding the maximum recommended reduction of 5 logs for the safety of food and drinking water. In an in vivo experiment, El-Shibiny (2016) found that phage EC3 reduced the amount of *E. coli* to undetectable levels after 120 min of infection. To minimize the irradiation dose required for inactivation, recent advancements in irradiation technology use combination treatments to inactivate pathogenic bacteria in food or water. Thus, combination treatments of EB irradiation (1.5 kGy) combined with coliphages to inactivate *E. coli* in wastewater were examined. The outcomes demonstrate that after just one day of treatment, these combination therapies completely eradicated *E. coli*. To our knowledge, no combination of EB irradiation and coliphage treatment has been used to inactivate *E. coli* in wastewater.

One could draw the conclusion that the use of coliphage and low EBI was very successful in completely eliminating *E. coli* in wastewater. Somatic coliphage alone was more effective than F-specific coliphage in reducing the number of *E. coli* in wastewater. *E. coli* was moderately sensitive to EBI in comparison with other previously reported bacteria. Somatic coliphages and F-specific coliphages can be isolated simultaneously from different water sources.

Conclusion

Antibiotic- and multi-drug resistant strains of *E. coli* frequently spread in water sources, causing a potential health threat. Thus, a new approach to controlling these strains is highly needed. In this study, the isolation and characterization of two effective coliphages (somatic coliphage-3 and F-specific coliphage-3) from different water sources were estimated. The experiments demonstrated that these two coliphages had lytic activity against multi-drug resistant *E. coli* at a wide range of pH values and temperatures, with maximum lytic activity at pH 7 and 38 °C. The application of each individual coliphage individually or combined with an electron beam irradiation as novel techniques resulted in the complete eradication of multi-drug resistant *E. coli* inoculated in domestic wastewater, making this water safe for reuse in different fields.

Methods

Sources of the samples

Eleven water samples were collected from ground water at different places (Assiut, El-Mania, El-Marg, and Ein-Shams, Egypt), an inlet working pumping station (IWPS), treated water, and aerobic activated sludge (El-Gabal El-Asfar Stage-2, Cairo government, Egypt domestic wastewater treatment plant). The samples were immediately placed in sterile plastic bottles and stored at 4 °C for further research.

Isolation, identification, and enumeration of bacterial strains

Twelve *E. coli*, which was used as a coliphage host, were isolated from the previous water samples, and enumerated using the Charm Peel Plate EC Microbial Test (Kit Code: PP-EC-100 k). The Association of Analytical Communities (AOAC) research institute has approved this test as a performance-tested technique under License Number (061501/2021). One milliliter of each water sample was cultured for 18–24 h on Charm Peel Plate EC at 35 ℃ ± 1. *E. coli* appeared as round blue or black colonies, which were counted as colony-forming units (CFU/ml). One separated *E. coli* colony was selected from each sample. These *E. coli* colonies were confirmed by streaking on eosin methylene blue agar (Oxoid, England) as a selective medium. To produce pure colonies, each bacterial isolate was subcultured on LB agar plates for 24 h at 37 ℃ ± 1. The VITEK2 system, Version 08.01 (BioMerieux, Inc., Hazelwood, Mo. 63042), was used to ensure the identification of selected *E. coli* isolates. Prior to each assay, one hundred microliters of each *E. coli* strain suspension were

aseptically transferred to 10 ml of tryptic soy broth (TSB, Oxoid) and incubated at 37 °C ± 1 overnight to achieve an optical density of OD_{600} = 0.5 nm (corresponding to a cell density of 10^8 colony-forming units (CFU)/ml). Bacterial cultures were kept in TSB at 4 ℃ ± 1. All bacterial strains were preserved as glycerol stocks at − 80 ℃ for further usage.

Coliphage isolation and purification

Somatic and male-specific coliphages (F-specific coliphages) were isolated from the previously mentioned water samples. Using Charm Sciences Fast Phage EPA test kits (FP-SOM-25 K and FP-FPLUS-25 K), which are equivalent to USEPA Method 1601. The double-layer agar (DLA) approach was utilized to determine the presence or absence of bacteriophage in the final filtered liquid through a sterilized 0.22 µm diameter pore-size membrane to remove any remaining bacterial cells (syringe filters, CHROMAFIL® Xtra PES, 20–25 mm, Item number: 729012 Macherey–Nagel GmbH&Co.KG, Germany). Serial dilutions (10^1–10^{10}) of each coliphage in its final filtered liquid were prepared in phosphate buffer solution (PBS) (Yang et al. 2010) to determine optimum phage concentration. Purification of the isolated coliphages was accomplished according to the method described by Kropinski et al. (2009). The purified coliphages were kept at 4 °C for a short time and at − 20 °C for a longer period (6 months). The phages' high-titer lysates (10^{10} PFU/ml) were prepared as directed by Swanstorm and Adams (1951). The reported results are from two independent experiments that were carried out in duplicate.

Coliphages titer measurement

The purified coliphages were enumerated with their host *E. coli* by counting the plaques that represent patches of dead bacteria and one virus apiece, according to Sambrook and Michael (2012). Plaque-forming units (PFU/ml) are used to express the viral titer of each phage lysate.

Coliphages cultivation

Tryptone Soy Broth (TSB) medium was employed for phage cultivation with its host bacteria, *E. coli*. For phage propagation, 1 ml of the host bacterial cells was infected with 1 ml of individual phage and incubated at 37 °C ± 1 for 3 h with shaking at 120–150 rpm until visual clearance. This mixture was then filtered through a sterilized 0.22 µm membrane (Sambrook et al. 1989). Finally, the phage stock solution with 10^8–10^{10} PFU/ml was stored at 4 °C for future use.

Host range analysis and efficiency of plating analysis

A spot assay was used to assess the phage host range (Kutter 2009). Bacterial sensitivity to a phage was established by a lysis cleared zone at the spot. According to the clarity of the spot, bacteria were differentiated into three categories: very clear zone (+++), clear lysis zone (+), and no lysis zone (−). Each experiment was performed in triplicate.

Using the double-layer agar method (Adams 1959), the efficiency of plating (EOP) was determined for bacteria that exhibited positive spot tests (a clear lysis zone). The potential effects of different isolated somatic coliphages (S1, S2, S3, S4, S5, S6, S7, and S8) on the most resistant strain of *E. coli*-2 were studied. Also, the effects of F-specific coliphages (F1, F2, and F3) on *E. coli*-10 were estimated, and phage titers were calculated.

Antibiotic susceptibility test

The eleven *E. coli* isolates were subjected to antimicrobial sensitivity tests using the disc diffusion method as described by Humphries et al. (2021) to detect the most antimicrobial-resistant *E. coli* isolates. Streptomycin (10 µg), Ceftriaxone (30 µg), Ceftazidim (30 µg), AMC-30, Cefotaxime (30 µg), Trimemethoprim/sulfamethoxazole, Ampicillin (10 µg), Imipenem (10 µg), Chloramphenicol (30 µg), Neomycin (30 µg), Nalidixic acid (30 µg), and Cephalexin (30 µg) were the commonly used substances.

Biochemical characteristics of the most resistant *E. coli* (*E. coli*-2 and *E. coli*-10)

Pure colonies of the most resistant *E. coli* (*E. coli*-2 and *E. coli*-10) were characterized using biochemical tests. A standard technique was used to inoculate the isolates onto the following identification cards of the automated The VITEK2 system, Version 08.01 (BioMerieux, Inc., Hazelwood, Mo. 63,042), was used to ensure the identification of selected *E. coli* isolates. Two selected isolates were stained with gram's stains to characterize their morphology.

Characterization of coliphages

Transmission electron microscopy

The morphological features of both purified coliphages (S3 and F3) were described by a TEM (JEM-1400 Electron Microscope, USA, located at Cairo University Research Park (CURP)) operating at 80 kV. According to Ackermann (2009) procedures, coliphage samples were prepared for electron microscopy examination.

Staining of coliphage nucleic acids with acridine orange

According to Mayor and Hill (1961), the fluorescent dye acridine orange has been used to identify viral nucleic acids under fluorescence microscope (HDCE-50B Digital Camera with high quality IR Cutoff Filter, China).

Influence of pH and temperature on coliphage lysis activity

The impact of various pHs and temperatures on the coliphages stability and lysis activity was evaluated by enumerating the virus titer (based on DLA) after incubation at different pHs and temperatures, according to Taj et al. (2014). Each experiment was carried out at least three replicates.

Multiplicity of infection assay

The multiplicity of infection (MOI) for bacteriophage was assayed according to Peng et al. (2020). Lytic activity of both coliphages was evaluated at different MOIs (0.01, 0.1, 1.00, and 2.00). The proportion of phage concentration to host cells (MOI) was calculated according to the formula:

$$\mathrm{MOI} = \frac{\mathrm{Concofphage}}{\mathrm{Concofbacteria}}$$

One-step growth assay

According to Bibi et al. (2016), the experiments of one-step growth curve were carried out three times. The results are the mean ± standard deviation of three independent samples.

Bacterial growth reduction assay

According to Haq et al. (2012), the optical density (OD) was estimated at time intervals of every 10 min for a 90-min period after incubation at 37 °C with shaking at 120 rpm; no phage inoculation was taken as a control.

Scanning electron microscope of the host strain after exposure to phage treatment

To detect the morphological alternations that were exploited by both S3 and F3 coliphages on *E. coli*-2 and *E. coli*-10, respectively, in comparison with *E. coli*-2 and *E. coli*-10 that were not exposed to their specific coliphages, the samples were mounted on stubs using double-sided tape and coated with gold using sputtering. The samples were examined at magnifications of 3.00 Kx using a SEM Zeiss Evo 15, Germany, at NCRRT.

Determining the sensitivity of *E. coli* to irradiation

The sensitivity of *E. coli* to irradiation was detected by the so-called D_{10}-value, which is defined as the irradiation dosage necessary to kill 90% of the population or to reduce the population by one $\log_{10}$ cycle. In this experiment, *E. coli*-2 and *E. coli*-10 cell suspensions (approximately 10^8 CFU/ml) were prepared. Ten milliliters of individual *E. coli* cell suspensions were inserted in plastic bags that had been sterilized with radiation (15.0 kGy). The bags were exposed to various doses of electron beam irradiation (EBI) (0, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy). Three replicates were employed for each dose. The survival counts of each *E. coli* strain were enumerated using the pour-plate technique. The slope of the radiation decimal-reduction curve, which was created by plotting log survival counts against the applied irradiation doses, was used to calculate the D_{10}-value for each host bacterium. Using Excel, Microsoft Office Professional Plus, and a linear regression, the slope of the dose–response curve was calculated using the following equation:

$$\mathrm{D_{10}Value} = -\frac{1}{\mathrm{b}} \quad \mathrm{b} = \frac{\sum \mathrm{xy} - \mathrm{nxy}}{\sum \mathrm{x^2} - \mathrm{nx^2}}$$

where:

x = dose level (kGy), y = logarithmic survival rate following x dose of radiation, and n = number of calculated points.

Irradiation process

Electron beam irradiations were carried out at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt, using an electron beam accelerator (ICI, VIVI RAD CO., France). The EB parameters during the experiments were beam current 1–2 mA, energy 2.6 MeV, and speed 9.5–15.4 m/min.

Artificial contamination of treated domestic wastewater

Two and a half liters of treated domestic wastewater were autoclaved and divided into 100 ml in heat-sealed polyethylene bags previously sterilized by gamma irradiation (15.0 kGy). These bags were divided into eight groups, each consisting of three bags representing triplicates. In the first

group, 1 ml of individual *E. coli*-2 cell suspension (approximately 10^8 CFU/ml) was aseptically added to each bag and served as the control. In the second group, 1 ml of *E. coli*-10 cell suspension was added to each bag and served as the control. The third group was inoculated with *E. coli*-2 and exposed to 2.0 kGy of EBI. The fourth group was inoculated with *E. coli*-10 and subjected to 2.0 kGy of EBI. The fifth group was inoculated with *E. coli*-2 and 1 ml of pure S3 coliphage suspension (Ps). In the six groups, 1 ml of *E. coli*-10 and 2 ml of pure F3 coliphage (Pf). The seventh group was inoculated with *E. coli*-2 plus S3 coliphage and exposed to 1.5 kGy EBI (Ps + EBI). The eighth group was exposed to 1.5 kGy after being inoculated with *E. coli*-10 plus F3 coliphage (Pf + EBI).

Statistical analysis

All the experiments in this study were carried out in three replicates. The results are shown as mean values, with error bars displaying the standard deviations of the obtained data. Minitab statistics software was used to perform statistical analyses (V 19, State College, PA, USA). To ascertain whether there was a significant distinction between the chosen strains, a one-way analysis of variance (ANOVA) was carried out. The significance level for all statistical analyses was set at a P value < 0.05. The analysis software Sigma Plot 12.0 was used for all calculations (Systat Software, Inc., Chicago, USA).

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KATON

Contest to participate
Not applicable.

Authors' contributions
The authors confirm contribution to the paper as follows: conceived and designed the analysis: Salwa A. Abou El-Nour, Reham fathy; collected the data: Reham Fathy, Salwa A. Abou El-Nour; contributed data: Ali A. Hammad, Amal S. Eid; performed the analysis Salwa A. Abou El-Nour, Amal S. Eid; wrote the paper: Reham fathy, Ali A. Hammad. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
This work did not require ethical approval under the research governance guidelines operating at the time of the research.

Consent for publication
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Competing interests
The authors declare no competing interests.

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