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Chlorine Inactivation of PhiX174 Bacteriophage in Treated Sewage and Drinking Water Samples

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CHLORINE is the most frequently used disinfectant in water and wastewater treatment plants all over the world wide. This study investigated the effect of chlorine on phiX174 bacteriophages in treated sewage and drinking water samples which were inoculated separately with phiX174 either with or without autoclaving process. Then the samples were treated with different doses of chlorine solution for 15 and 30min. Our results showed that the phiX174 bacteriophage was more efficiently reduced in the autoclaved samples than in the non-autoclaved samples. In the non-autoclaved treated sewage samples, treatment with 7mg/L a of chlorine for 30 min was sufficient to remove 0.3 ± 0.07 $\log_{10,}$ while treatment with 12 mg/l of chlorine for 15min efficiently remove 0.27 ± 0.05 $\log_{10}$ of the initial phiX174 doses. Furthermore, we observed higher viral reduction in the autoclaved treated sewage samples after treatment with 11mg/L of chlorine for 15min which removed 1 ± 0.1 $\log_{10}$ of the phiX174 initial dose. However, when non-autoclaved and autoclaved drinking water samples were treated with 3 mg/l chlorine for 15 min, it efficiently removed 1.2 ± 0.07 $\log_{10}$ and 1.4 ± 0.17 $\log_{10}$ of phiX174, respectively. This reduction increased to 1.4 ± 0.08 $\log_{10}$ and 2.3 ± 0.21 $\log_{10,}$ respectively, from the initial phiX174 dose when the contact time was increased to 30min. However, phiX174 bacteriophage virus showed high resistance to different doses of chlorine in drinking water and treated sewage water samples.

Keywords: Drinking water, Enteric viruses, PhiX174 bacteriophage, Treated sewage, Water treatment, Wastewater treatment.

Introduction

Waterborne disease outbreaks are caused by many bacterial or non-bacterial agents especially enteric viruses (Wilhelmi et al., 2003; Nimgaonkar et al., 2018). Studies have shown that one hundred and forty serotypes of enteric viruses affect humans, mainly causing gastrointestinal illnesses (Health Canada, 2017). These viruses can also cause acute illnesses such as conjunctivitis, poliomyelitis, meningitis, and respiratory infections (Kocwa-Haluch, 2001; Health Canada, 2017; Nimgaonkar et al., 2018). Rotaviruses mainly cause severe viral gastroenteritis in children (Bányai et al., 2018). Adenoviruses are responsible for various clinical illnesses, such as gastrointestinal, respiratory infections, genitourinary, neurologic, and ophthalmologic diseases (La Rosa et al., 2018). Norovirus commonly causes gastroenteritis in all age groups (Sandkovsky et al., 2014; Riera-Montes et al., 2018). Astrovirus is an important causative agent of viral gastroenteritis (Gofti-Laroche et al., 2003), which causes diarrheal disease in young children and adults (Matsui et al., 2001). Coxsackievirus, echovirus, and AiV cause gastroenteritis in humans through contaminated food or water (Kitajima & Gerba,

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2015; Haramoto et al., 2018). Fecal bacterial indicators such as total coliform, enterococci, and fecal coliform (*E. coli*) bacteria is used to evaluate water quality and protect public health by lowering the incidence of waterborne diseases (Griffin et al., 2001; Kay et al., 2004; Tallon et al., 2005). Enteric viruses are more tolerant to disinfectants such as chlorine in water and wastewater treatment plants than bacterial indicators. Moreover, due to environmental degradation, the absence of fecal coliforms is not indicative of the absence of enteric viruses (Ley et al., 2002; Wu et al., 2011; Fout et al., 2017). According to the World Health Organization (WHO) standards, the bacterial indicators and enteric viruses in water are not correlated (Cho et al., 2000; Hot et al., 2003). Therefore, a viral index has been introduced to indicate the presence/absence of viruses in water samples. Studies have suggested some enteric viruses as possible viral index organisms for viral pathogens, such as adenoviruses (Gerba et al., 2002; Katayama et al., 2008; El-Senousy et al., 2013; Verani et al., 2019), enteroviruses (Grabow, 2007), polyomaviruses (Bofill-Mas et al., 2008), a plant virus Pepper Mild Mottle Virus (Hamza et al., 2011), and bacteriophages (Jofre et al., 2016; Jebri et al., 2017). Therefore, it is crucial to study the effect of chlorine, frequently used to water disinfection in water and wastewater treatment on viral index candidates such as bacteriophages (Shah & McCamish, 1972; Duran et al., 2003; Tree et al., 2003, 2005; Strasser, 2017).

Bacteriophages are taxonomically classified into three groups: F-specific coliphages, somatic coliphages, and bacteriophages capable of infecting Bacteroides *spp*. (Jofre et al., 2016; Jebri et al., 2017). They are advantageous over bacterial indicators as they cannot replicate in a natural environment without their host present, are more persistent, more abundant in the environment, and the detection is easy and cost effective (Jofre, 2009; Toribio-Avedillo et al., 2021). Bacteriophages are used as indicators of fecal pollution and the presence of other viral pathogens. They can be used as a surrogate of enteric virus due to similarities in their biological and morphological properties (McMinn et al., 2017; Toribio-Avedillo et al., 2021). F-RNA phages and somatic bacteriophages are used as fecal viral pollution indicators in water treatment processes (Jofre et al., 2016; Jebri et al., 2017). Recently, water quality monitoring rules included bacteriophages as viral indicators of enteric viruses (European, Commission, 2018; Health Canada, 2019). The *Microviridae* family includes bacteriophage phiX174 which consider one of the somatic coli phages (Reyes et al., 2012; Jebri et al., 2017). This phage is a non-tailed virus with an icosahedral capsid and circular single-stranded DNA (Bosch, 1998; Fane et al., 2006). It infects the *E.coli* C strain by attaching to the lipopolysaccharide via its capsid protein followed by the dissolution of the spike protein (Sun et al., 2017). Subsequently, the circular single-stranded DNA genome is transferred inside the cell and replicates using enzymatic machinery of the cell (Sun et al., 2014).

Disinfection of drinking water using free chlorine is performed using two steps: a primary disinfectant step starting with the coagulation, flocculation, sedimentation, and filtration, followed by a secondary step in the final outlet and through the distribution system (AWWA, 2000; Cromeans et al., 2010). The removal of viruses during disinfection depends on several variables, including temperature, turbidity, ionic strength, chlorine concentration, contact time, pH, and viral aggregation (Sharp et al., 1980). Chlorine significantly affects enteric viruses, such as norovirus, echovirus, adenovirus, poliovirus, enterovirus, coxsackievirus, rotavirus, hepatitis A virus, and hepatitis E virus (Engelbrecht et al., 1980; Gerba et al., 2003; Black et al., 2009; Kahler et al., 2010; Kitajima et al., 2010; El-Senousy et al., 2014). However, chlorine causes irritation of the eyes, mucous membranes, and skin, creating considerable discomfort for swimmers (Abad et al., 1994). High doses of chlorine can also produce tri-halomethanes, halo-acetic acid, and N-chloramines which are highly carcinogenic to humans (Pereira et al., 1982; Gerba et al., 2003).

In this study, we used the bacteriophage phiX174 acts as a viral model due to its structural similarity with several human enteric viruses (Grabow, 2001), and it has been used as a surrogate in several previous studies (Moriñigo et al., 1992; Schijven & Hassanizadeh, 2000; Jin & Flury, 2002; Rashed et al., 2022).

Therefore, we investigated the effect of chlorine on the phiX174 bacteriophages in treated sewage and drinking water samples.

Materials and Methods

Sewage and drinking water samples

The treated sewage water samples were collected from the Zenin wastewater treatment plant (WWTP) in the El-Giza Governorate, Egypt. The flow rate in Zenin WWTP was 330,000m^3/ day, and the final chlorine concentration ranged between (0.5–0.8) mg/L. The drinking water samples were collected from the El-Giza drinking water treatment plant in El-Giza Governorate, Egypt, with a final chlorine concentration ranging between (1.9–2.5) mg/L. PhiX174 was separately inoculated into 400mL of treated sewage and drinking water samples. From each sample, 200mL was autoclaved before phiX174 inoculation to remove the organic matter, and animal debris that might influence the effect of chlorine on the virus, and 200 mL was directly inoculated without autoclaving. Eight chlorine doses (5, 6, 7, 8, 9, 10, 11 and 12) mg/L were selected for treating the sewage water samples, while two doses (2 and 3) mg/L were used for treating the drinking water samples. The reduction in virus removal was measured twice at 15 and 30min for all samples.

Preparation of solutions

Preparation of sodium thiosulfate solutions

The following sodium thiosulfate solutions were prepared: 10% solution of $Na_2S_2O_3$ (0.1mg/L of this solution removes up to 15mg/L of residual chlorine from the treated sewage samples) (Willson, 1935). 3% solution of $Na_2S_2O_3$ (0.1mg/L can remove up to 5 mg/L of residual chlorine from drinking water samples) (Willson, 1935). 0.025N (normality) of $Na_2S_2O_3$ solution (6.2g of $Na_2S_2O_3$ dissolved in 1L of distilled water) was used for iodometric titration. To prevent contamination, 5 mL of chloroform or 0.4g of NaOH were added to all solutions (APHA, 2017a).

Chlorine solution

One gram of hypochlorite calcium chloride was dissolved in 1 L of distilled water with stirring to dissolve the powder completely. The solution was incubated overnight and filtrated using filter paper, and the filtrate was used to prepare different doses of chlorine after determining the strength of the chlorine solution (APHA, 2017a).

1% Starch solution and 5% potassium iodide solution

One gram of starch was dissolved in 100mL of distilled water under constant heating and stirring until it completely dissolved. The solution was incubated overnight and then filtrated using filters paper. Approximately 1.25g of salicylic acid / L or a few drops of toluene were added to the clear solution to prevent contamination. The potassium iodide (KI) solution was prepared by dissolving f g of KI in in 100mL of distilled water (APHA, 2017a).

Determination of the strength of chlorine solution

Chlorine solution (10mL) was added to a conical flask containing 2mL of glacial acetic acid and 5mL of 5% KI. Then the solution was titrated against 0.025N of $Na_2S_2O_3$ solution. When the color changed from brown to pale yellow, 1%starch solution droplets were added until the color turned blue. The solution was continuously titrated against 0.025N of $Na_2S_2O_3$ solution until the blue color disappeared. The strength of chlorine was determined using the following equations (APHA, 2017a).

1- $NV_{of\ CL} = NV_{of\ Na2S2O3}$

where, N is normality, V is volume

2- Strength of Chlorine = N_{CL} *equivalent weight of chlorine (35.5) g/L

Determination of chlorine

The chlorine doses were determined using the following equation:

Each 1 ml of chlorine solution= the strength of chlorine (mg/L)

Therefore, based on the required dose, the equivalent volume in mL was taken from the stock solution to 1L of the sample (the sample volume used was 200mL).

Determination of the residual chlorine in the sample

The residual chlorine in the samples was determined after incubation for 15 and 30min. Two mL of glacial acetic acid and 5mL of 5% KI, were added to a conical flask containing 200mL of the sample. Then, this solution was titrated against 0.025N of $Na_2S_2O_3$ solution.

When the color changed from brown to pale yellow, droplets of 1% starch solution were added till the solution turned blue. It was titrated against 0.025N of $Na_2S_2O_3$ solution until the blue color

disappeared (APHA, 2017a).

$$\text{The residual chlorine mg/l} = \frac{\text{NVof Na2S2O3} * 35.5 * 1000}{\text{volume of sample}}$$

Quantification of infectious bacteriophage phiX174 virus

The phiX174 virus was quantified by examining the water and wastewater according to standard methods 23rd edition (APHA, 2017b). All inoculated samples were treated with 3% or 10% $Na_2S_2O_3$ solution (depending on the type of water sample) to remove the residual chlorine before quantifying the infectious bacteriophage. The phiX174 strain (ATCC 13706B1) and *Escherichia coli* strain C (ATCC 13706) were kindly provided by Dr. Maha Al-Khazindar, associate professor of virology, faculty of science, Cairo University.

Statistics

All the experiments were repeated thrice. Statistical analysis was done by One-way ANOVA to all samples except the comparison between the doses of chlorine of treated sewage samples was done by One-way ANOVA followed by Tukey's test (IBM© SPSS© Statistics Version 22).

Results

The statistical analysis represented that there was a highly significant difference ($P<0.05$) between the non-autoclaved and autoclaved treated sewage samples after the samples were inoculated separately with phiX174 and treated with eight chlorine doses for contact times 15 and 30min. The non-autoclaved and autoclaved treated sewage samples treated for 15min with 7mg/L of chlorine after inoculated with phiX174 showed efficient removal of 0.19 ± 0.02, and 0.88 ± 0.09 $\log_{10}$ respectively. However, at the contact of 30min and treated with 7mg/L of chlorine, the non-autoclaved and autoclaved treated sewage samples have efficient removal of 0.3 ± 0.07, and 0.89 ± 0.16 $\log_{10}$ respectively (Fig. 1). When the time was increased from 15 to 30 min for all doses of chlorine, a significant increasing ($P<0.05$) of bacteriophage phiX174 removal in non-autoclaved treated sewage samples was observed. Chlorine dose of 5mg/L for treatment of the non-autoclaved treated sewage samples was sufficient to remove 0.14 ± 0.02 $\log_{10}$ at contact 15 min and increased to remove 0.24 ± 0.02 $\log_{10}$ from the initial phiX174 dose at contact 30min (Fig. 1). Conversely, there was not significant difference ($P>0.05$) between autoclaved treated sewage samples after the same treatment for contact times 15 and 30min. When the inoculated autoclaved treated sewage samples were treated with 11mg/L of chlorine for 15 and 30min, 1 ± 0.1 $\log_{10}$, and 1.05 ± 0.14 $\log_{10}$ respectively, were removed from the initial phiX174 dose (Fig. 1).

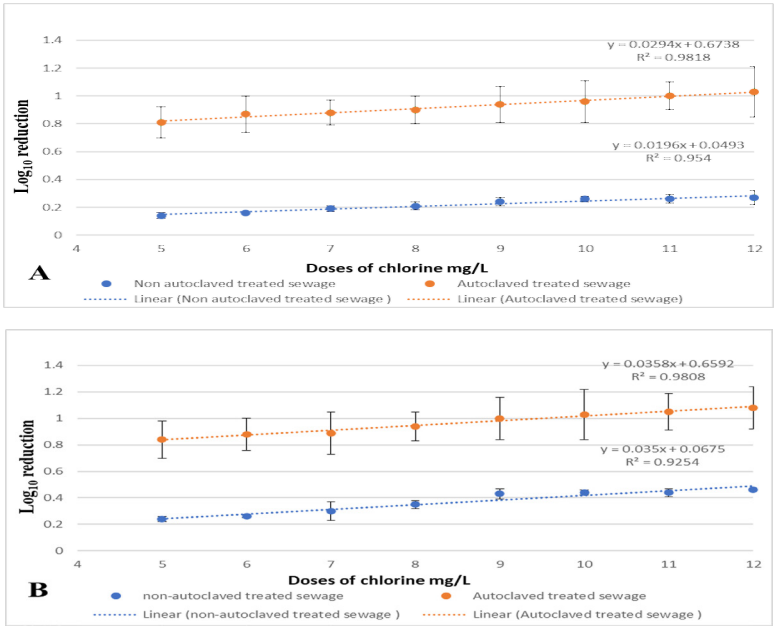

Fig. 1. $\log_{10}$ reduction of phiX174 bacteriophage virus after treatment with eight different chlorine doses (5, 6, 7, 8, 9, 10, 11, and 12) mg/L in non-autoclaved treated sewage and autoclaved treated sewage samples at contact time A: 15min, and B: 30min

The results presented in Fig. 2 showed that the residual chlorine increased from 2.66 ± 0.18mg/L to 8.72 ± 0.43mg/L which significant difference was detected between doses ($P<0.05$) when the chlorine concentration was increased from 5mg/L to 12mg/L in the phiX174 inoculated autoclaved treated sewage water samples treated for 15min. Furthermore, a significant difference was detected also between the doses ($P<0.05$) when the contact time was increased to 30 min, and the residual chlorine ranged between 2.21 ± 0.12mg/L to 7.69 ± 0.26mg/L in the same samples. In contrast, the residual chlorine in the phiX174 inoculated non-autoclaved treated sewage water treated with chlorine for 15min ranged from 2.01 ± 0.15mg/L to 8.02 ± 0.36mg/L, a significant difference was detected ($P<0.05$) between doses. When the contact time was increased to 30min, the residual chlorine ranged from 2 ± 0.09mg/L to 7.38 ± 0.42mg/L also a significant difference was detected ($P<0.05$) between doses.

Figure 3 showed that no significant difference ($P>0.05$) between the removal efficiency of phiX174 from inoculated non-autoclaved and autoclaved drinking water samples treated with the chlorine doses for contact 15min. The chlorine dose 2mg/L for 15min was sufficient to remove 1 ± 0.13 $\log_{10}$ and 1.2 ± 0.15 $\log_{10}$ from the initial phiX174 dose of inoculated non-autoclaved and autoclaved drinking water samples respectively.

Furthermore, no significant difference ($P>0.05$) between the removal efficiency of phiX174 from inoculated non-autoclaved and autoclaved drinking water samples treated with the chlorine doses for contact 30min. At contact time 30min, chlorine dose of 3mg/L was sufficient to remove 1.4 ± 0.08 $\log_{10}$ and 2.3 ± 0.21 $\log_{10}$ from the initial phiX174 dose of the inoculated non-autoclaved and autoclaved drinking water samples respectively. Also, the statistical analysis indicates that no significant difference ($P>0.05$) between the viral removal from non-autoclaved drinking water samples when the time increased from 15 to 30min. When the contact time was 15min with a chlorine dose of 3mg/L for treated non-autoclaved drinking water samples, the reduction reached 1.2 ± 0.07 $\log_{10,}$ and increased to 1.4 ± 0.08 $\log_{10,}$ when the time increased to 30 min. On the other hand, there was no significant difference ($P>0.05$) of the viral removal of phiX174 from inoculated autoclaved drinking water samples when the time increased from 15 to 30min. The reduction of phiX174 removal from inoculated autoclaved drinking water sample increased from 1.4 ± 0.17 $\log_{10}$ to 2.3 ± 0.21 $\log_{10}$ when the time increased from 15 to 30min with 3mg/L chlorine dose (Fig. 3).

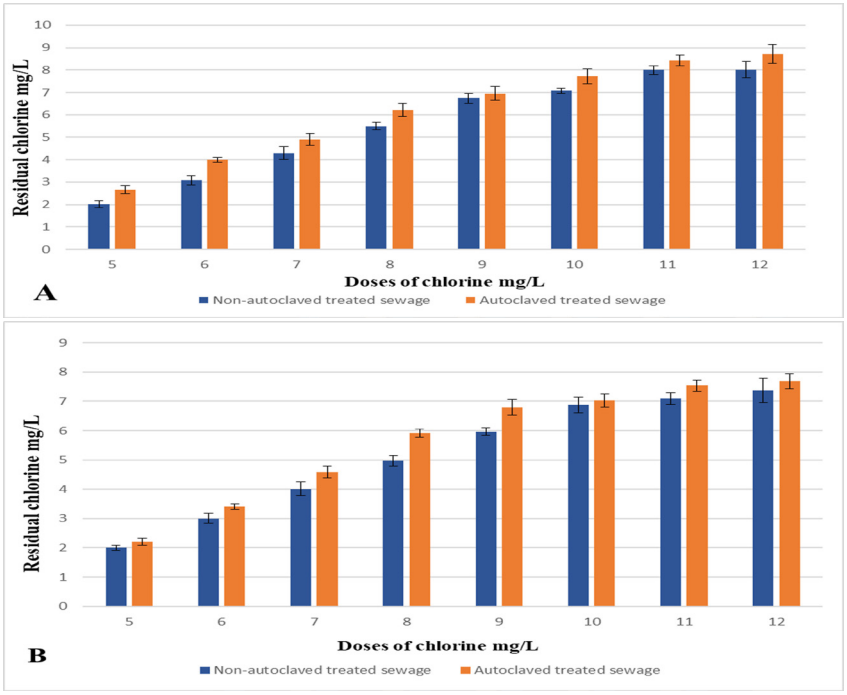

Fig. 2. The residual chlorine (mg/L) after removal of phiX174 bacteriophage after treatment with eight chlorine doses (5, 6, 7, 8, 9, 10, 11, and 12) mg/L in non-autoclaved treated sewage and autoclaved treated sewage samples, for A: 15min, B: 30min

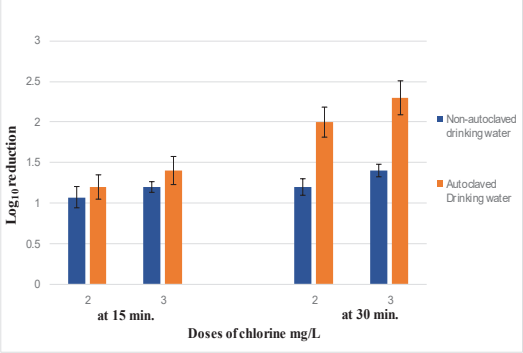

Fig. 3. log_{10} reduction of phiX174 bacteriophage treatment with two different chlorine doses (2, and 3) mg/L for 15 and 30min in non-autoclaved and autoclaved drinking water samples

Figure 4 indicated that the residual chlorine in the autoclaved drinking water samples inoculated with phiX174 was 1.77 ± 0.14mg/L and 2.3 ± 0.16mg/L when treated with 2mg/L and 3mg/L chlorine for 15min, respectively, and a significant difference was detected (P<0.05). However, the residual chlorine was 1.5 ± 0.2mg/L and 2.17 ± 0.27mg/L when samples were treated with 2 mg/L and 3mg/L of chlorine, respectively, for 30min contact time, and no significant difference was detected (P>0.05). Contrastingly, the residual chlorine in the inoculated non-autoclaved drinking water samples was 1.1 ± 0.12mg/L and 1.5 ± 0.1mg/L when treated with 2 and 3mg/L of chlorine, respectively, for 15min, a significant difference was detected (P<0.05). While the residual chlorine was 0.88 ± 0.07mg/L and 1.28 ± 0.13mg/L in the samples treated for 30 mins with 2 and 3mg/L chlorine, respectively, a significant difference was detected (P<0.05).

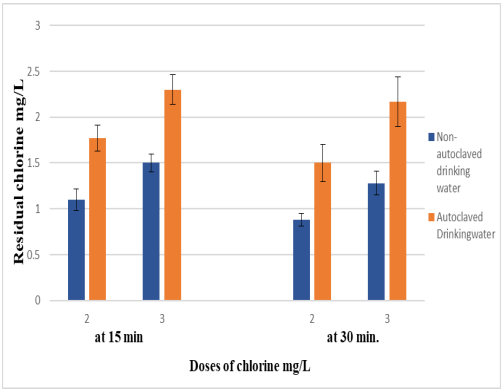

Fig. 4. The residual chlorine mg/L after removal of phiX174 bacteriophage from non-autoclaved and autoclaved drinking water samples after treatment with two chlorine doses (2-3) mg/L for 15 and 30min

Discussion

Monitoring the quality of drinking water and wastewater treatment processes in developed and developing countries is crucial for preventing waterborne diseases. Approximately 6.3% of deaths occur due to insufficient sanitation, unsafe drinking water, and poor hygiene. Moreover, approximately 4% of diseases occurring worldwide can be prevented by improving sanitation, water quality, and hygiene (WHO, 2015; Szálkai, 2019). Disinfection is critical to adequately remove or inactivate of pathogens in the drinking water or wastewater treatment processes (Ngwenya et al., 2013). Chlorine-based disinfectants such as free chlorine (sodium or calcium hypochlorite), chlorine dioxide, and chloramines (monochloramine, dichloramine), are widely used to inactivate waterborne pathogens and provide safe drinking water (Thurston-Enriquez et al., 2005; Kitajima et al., 2010; Ngwenya et al., 2013; Li et al., 2017; Zhang et al., 2020). It is necessary to detect residual disinfectants in the drinking water distribution system and the sewage treatment plant effluents to get partially prevent the growth and contamination of microbes. (WHO) recommends that the residual chlorine of drinking water must be higher than 0.5mg/L for 30min at pH < 8, while the residual chlorine at the distribution system endpoint should be 0.2mg/L (WHO, 2011). The recommended chlorine dose used in traditional wastewater treatment plants is less than 25gm/L, which results in residual chlorine ranging between 0.5 to 1gm/L in the plant's effluent (Kingsley et al., 2017).

The reduction of phiX174 bacteriophages in the autoclaved treated sewage was higher than in non-autoclaved treated sewage at the same dose and contact time which a significant difference detected (P<0.05). This might be due to the breakdown of the suspended solids during the autoclaving process, which led to direct exposure of the virus to chlorine, or due to the breakdown of the organic particles and coagulation of proteins that protect the viruses from chlorine. Furthermore, increasing the time of treatment showed a significant difference (P<0.05) in non-autoclaved and autoclaved treated sewage samples due to increasing the period of exposure of the virus to chlorine (Salonius et al., 1967; Lotrario et al., 1995; El-Senousy et al., 2014). However, the reduction of phiX174 bacteriophages in the autoclaved and non-autoclaved drinking water was

higher than that in the treated sewage (autoclaved and non-autoclaved) samples besides, there was no significant difference (P>0.05) between the non-autoclaved and autoclaved drinking water samples with increasing time from 15min to 30min due to absence of suspended solids and organic matter. The high protein content of bacteria and parasites in the treated sewage might protect the viruses against chlorine exposure as these microorganisms are much larger than the viruses (El-Senousy et al., 2014).

Chlorine is an oxidant that kills pathogens by reacting with the cell components via transport or diffusion across the cell causing cell death (Cho et al., 2010; Lee et al., 2011). Chlorine mainly damages or alters the nucleotide sequences coding for proteins like the murine norovirus affects the nucleotide sequences encoding for capsid proteins (Rachmadi et al., 2018). It can occasionally inactivate nonstructural proteins (Guay et al., 2005). Moreover, higher chlorine concentrations can damage the cell's genome (Nieuwenhuijsen et al., 2000). Two important factors affect the chlorine disinfection: the concentration of residual chlorine C, expressed in mg/L and the contact period T expressed in minutes. These combined values, also called CT values, help to evaluate the disinfection process and facilitate practical applications. The CT value (mg × min/L) indicates the effectiveness of the disinfection process (Collivignarelli et al., 2017). The drinking water guidelines WHO suggest that a CT value is ranging from 2 to 30mg × min/L can achieve a 2-$\log_{10}$ reduction in the viral load (WHO, 2011). Our results showed that the reduction of phiX174 load increased with an increase in the chlorine dose.

Our results showed that significant difference in phiX174 bacteriophage removal (P<0.05) from inoculated treated sewage samples by increasing the dose or time which the suitable dose of chlorine in treatment plants depends on the dose which achieves higher viral reduction at a low cost and ensuring safety by preventing the formation of the carcinogenic trihalomethane compounds, especially in drinking water (Richardson et al., 2007).

Kanna (2016) observed a higher reduction rate with an initial $\log_{10}$ reduction of MS2, 28B, phi6, and phiX174 by 0.34, 2.14, 4.89, and 4.97 $\log_{10,}$ respectively. Moreover, Strasser (2017) reported that the phi6, and phiX174 were surrogates of the enteric viruses have a 5 $\log_{10}$ (99.999%) reduction in hospital sewage treated with initial free chlorine at 2,800 and 3,500mg/L, respectively, for 10min. El-Senousy et al. (2014) showed 4 $\log_{10}$ reduction from the initial dose of human enterovirus, norovirus GGI, GGII, and 3 $\log_{10}$ reductions from the initial dose of human and animal rotavirus in drinking water (autoclaved and non-autoclaved) treated with of 3mg/L chlorine for 15min. However, we observed a reduction of 1.4 $\log_{10}$ and 1.2 $\log_{10}$ of the phiX174 bacteriophage at the same dose and the contact time in autoclaved and non-autoclaved samples, respectively, suggesting that the phiX174 is might be more resistant than human enterovirus, norovirus GGI, GGII, and human and animal rotavirus. Tree et al. (2005) reported that MS2 bacteriophage, showing 1 $\log_{10}$ reduction, was more resistant than poliovirus which reduced by 2.85 $\log_{10}$ after being treated with 30mg/L of chlorine for 30min. Duran et al. (2003) reported that MS2 is more resistant than phiX174 to chlorinated secondary effluent when treated with 20 mg/L of chlorine for 20 – 30min showing a reduction of (1.3 $\log_{10}$, 2.1 $\log_{10}$) and (2.8 $\log_{10}$ – 3.3 $\log_{10}$) respectively. The lower reduction rate observed in our study, even in autoclaved samples might be due to the difference in nature of water samples and the difference in the viral isolates.

Bacteriophages have been suggested as potential enteric virus surrogates (Dias et al., 2018). Bacteriophages infect a specific bacterial host. Somatic and F+ coliphages infect *E. coli* and could be used as adequate surrogates for the presence or absence of enteric viruses (Nappier et al., 2019). Previous studies suggested that the PhiX174 bacteriophage adequately represents enteric viruses in several water treatment processes, (Abbaszadegan et al., 2007; Mayer et al., 2008; Kreißel et al., 2014; Rashed et al., 2022). The phiX174 bacteriophage is an adequate surrogate for the poliovirus and adenovirus (Shirasaki et al., 2016). We observed higher resistance of phiX174 to chlorine than other enteric viruses. Therefore, our findings suggest that bacteriophage phiX174 is a suitable viral index candidate for evaluating treated sewage and drinking water samples.

Conclusions

Based on our findings, we conclude that bacteriophage phiX174 requires high doses of chlorine (9mg/L) in the absence of suspended solids and organic matter to remove 1 $\log_{10}$ in treated

sewage samples even after 30min. The viral load can be reduced to 0.5 $\log_{10}$ using 12mg/L chlorine in the presence of suspended solids and organic matters. However, 2 and 3mg/L of chlorine were sufficient to remove 1.2 $\log_{10}$ and 1.4 $\log_{10}$ from the initial phiX174 dose, respectively, after 30 min in the non-autoclaved drinking water samples. After treating samples with 2 and 3mg/L of chlorine for 30min without suspended solids and organic matter, the initial phiX174 doses could be reduced to 2 $\log_{10}$ and 2.3 $\log_{10,}$ respectively.

Conflict of interests: The authors declare no conflict of interest.

Authors' contributions: All authors have contributed equally in conceptualization, methodology design, laboratory work, interpretation of the results, and manuscript preparation.

Ethical approval: Not applicable

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تثبيط الكلور للعاثيات البكتيرية PhiX174 في عينات مياه الصرف الصحي المعالجة ومياه الشرب

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تعتبر الفيروسات والبكتريا والطفليات من أهم مسببات الأمراض المنقولة عن طريق المياه. حيث تعتبر الفيروسات المعوية من أهم العوامل المسببة لتفشي الأمراض. ويمكن استخدامها كمؤشرات فيروسية معوية مثل فيروسات الادينو والروتا والنورو وبعض العاثيات مثل العاثيات البكتيرية PhiX174. يتم اختيار المؤشر الفيروسي وفقًا لبقاء الفيروسات في البيئة، ومقاومة الفيروسات لعمليات المعالجة سواء في محطات الصرف الصحي أو محطات مياه الشرب. حيث يعد الكلور من أكثر أنواع المطهرات المستخدمة في محطات المعالجة للقضاء على مسببات الامراض. والهدف من دراستنا هو إظهار أثر تثبيط الكلور على بعض العاثيات مثل PhiX174 في عينات معقمة وغير معقمة من مياه الصرف الصحي المعالجة ومياه الشرب باستخدام جرعات مختلفة من الكلور لإظهار مدي مقاومة هذه العاثيات للكلور ومدي جدیتها لاستخدامها كمؤشر فيروسي للفيروسات المعوية. وقد أظهرت نتائج الدراسة إلى مقاومة قوية لهذه العاثيات PhiX174 لجرعات مختلفة من الكلور في عينات المياه ومياه الصرف الصحي المعالجة وخاصتا في عينات المياه المعقمة عن عينات المياه الغير معقمه (عينات الصرف الصحي المعالجة) وذلك لتأثير عملية التعقيم على تقليل الحمل العضوي والميكروبيولوجي في هذه العينات. ولوحظ أيضا عدم وجود اختلاف كبير في معدلات التثبيط بين عينات المياه المعقمة وغير المعقمة لمياه الشرب نتيجة عمليات المعالجة في المحطات. وقد اعتمدت هذه المقاومة على: جرعة الكلور المستخدمة وفترة التلامس بين الكلور وهذه العاثيات؛ فكلما زادت جرعة الكلور المضافة زاد معدل التثبيط؛ وكلما زادت فترة التلامس زادت معدلات التثبيط ايضا ولكن هناك عدة عوامل تتحكم في استخدام هذه الجرعات داخل المحطات مثل عدم تكوين مركبات التراي هالوميثان المسرطنة والمسببة للعديد من الامراض للإنسان بجانب النظرة الاقتصادية لاستخدام هذه الجرعات من الكلور. وقد خلصت هذه الورقة البحثية إلى تقارب كبير بين مقاومة هذه العاثيات PhiX174 وفيروسات الادينو التي رشحت من قبل كثير من العلماء لاستخدامها كمؤشرات فيروسية للدلالة على التلوث الفيروسي البرازي.