The effect of chlorine and combined chlorine/UV treatment on coliphages in drinking water disinfection

Alyaa M. Zyara, Eila Torvinen, Anna-Maria Veijalainen and Helvi Heinonen-Tanski

ABSTRACT

Chlorine disinfection is a globally used method to ensure the safety of drinking water. However, it has not always been successful against viruses and, therefore, it is important to find new methods to disinfect water. Seventeen different coliphages were isolated from the treated municipal wastewater. These coliphages and MS2 were treated with different dosages of chlorine in drinking water, and a combined chlorine/ultraviolet irradiation treatment for the chlorine-resistant coliphages. Chlorine disinfection with 0.3–0.5 mg/L total chlorine (free Cl-dosage 0.12–0.21 mg/L) for 10 min achieved 2.5–5.7 Log_{10}-reductions for 11 sensitive coliphages. The six most resistant coliphages showed no reduction with these chlorine concentrations. MS2 was intermediate in chlorine resistance, and thus it is not a good indicator for viruses in chlorine disinfection. In the combined treatment total chlorine of 0.05–0.25 mg/L (free Cl-dosage 0.02–0.08 mg/L) and ultraviolet irradiation (14–22 mWs/cm^2) were more effective than chlorine alone, and 3–5 Log_{10}-reductions were achieved for the chlorine-resistant strains. The chlorination efficiency could be increased by higher dosages and longer contact times, but this could increase the formation of disinfection by-products. Therefore, the combination treatment is a recommended disinfection method.

Key words | chlorine, coliphages, combination, disinfection, ultraviolet irradiation, water

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INTRODUCTION

Drinking water supplies containing human enteric viral pathogens can pose a risk to public health. Enteric viruses can cause a range of disease with severity from self-limiting diarrhea to meningitis and even death. Conventional drinking water treatment process consisting of coagulation and sedimentation followed by filtration can remove from 64.3% to 98.6% (the median being 86.2%) of the viruses present in raw water. When post-filtration disinfection is added to the process, the efficiency increases to 85.8% to 99.9% (the median being 95.1%) (Hurst 1991). Removal of the viruses depends on virus type and performance of the treatment process (Hurst 1991; LeChevallier & Au 2004), and even though the conventional drinking water treatment process is generally quite efficient in removing viruses from water, some may still survive and enter the distribution system and cause epidemics (Kukkula *et al.*1999; Maunula *et al.* 2005).

Chlorine (Cl) is one of the most widely used disinfectants in drinking water and it is usually added at concentrations of 0.2–0.5 mg/L to get around 0.2 mg/L residual free chlorine in the distribution system (WHO 2011). Free residual chlorine is the sum of chlorine gas (Cl_2), hypochlorous acid (HOCl), and hypochlorite ions (OCl^-), to which, chlorine dissociates form in water depending on the pH (Cole 1987; APHA *et al.* 2005). The disinfection mechanism of chlorine is not fully understood but it may act by causing losses in both genome- and protein-mediated functions, the specific targets of which

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depend on chlorine compounds and microorganisms (Wigginton *et al.* 2012).

The effect of chlorination has been found to vary highly depending on virus type. According to Thurston-Enriquez *et al.* (2003), free chlorine concentration of 0.50–0.54 mg/L within 15 s caused above 4 Log_{10}-reduction for calicivirus, and 2 Log_{10}-reduction for adenovirus type 40. Engelbrecht *et al.* (1980) achieved 2 Log reductions for echoviruses, coxsackie virus, and poliovirus types 1 and 2 at the same free chlorine concentration (0.5 mg/L) with exposure time of 1.2–4.5 min. Li *et al.* (2002) inactivated hepatitis A virus completely using 10–20 mg/L free chlorine and 30 min contact time. The main disadvantage of chlorination is its potential to form carcinogenic disinfection by-products when reacting with organic material (Ates *et al.* 2007; Yang *et al.* 2013) and, therefore, alternative disinfection methods are needed.

Ultraviolet (UV) irradiation is an increasingly used method in drinking water disinfection. It has been reported to be efficient against many bacteria and some protozoa, which were inactivated more efficiently than bacterial spores and viruses (Hijnen *et al.* 2006). Adenoviruses have been shown to be among the most resistant microbes against UV (Meng & Gerba 1996; Thurston-Enriquez *et al.* 2003; Rattanakul *et al.* 2014, 2015) and it may even need a UV dose of several hundreds mWs/cm^2 to be inactivated by 6 Log_{10} units (Hijnen *et al.* 2006). Disadvantages of UV are its short-lived bactericidal effect, the possibility for viruses to survive under certain conditions, and dependency on water quality (Sobotka 1993). General advantages, on the other hand, are that UV is safe to use, does not form mutagenic by-products and needs only a short contact time compared to chemical disinfection (EPA 1999).

The disinfection techniques combining UV and chemical oxidizing agent are used to get better disinfection efficiency than that using only a single treatment and they have been shown to increase the reduction of viruses in water. Chlorine (Shang *et al.* 2007; Rand *et al.* 2008; Wang *et al.* 2011), peracetic acid (PAA) (Rajala-Mustonen *et al.* 1997; Koivunen & Heinonen-Tanski 2005), or ozone (O_3) (Jung *et al.* 2008) has been used as chemical oxidizing agents in disinfection of drinking or wastewater. The combined process may be sequential, where the primary disinfection step is stopped before applying the secondary disinfectant (UV + oxidant or vice versa) (Rand *et al.* 2008; Cho *et al.* 2011), or simultaneous, where the two disinfectants (UV + oxidant) are present at least partly at the same time. High synergy has been observed in the simultaneous processes causing effective disinfection (Koivunen & Heinonen-Tanski 2005; Shang *et al.* 2007; Vankerckhoven *et al.* 2011; Rattanakul *et al.* 2014, 2015). A possible mechanism for the high synergy observed is the generation of free radicals due to photodegradation of a chemical oxidant by UV (Watts & Linden 2007). So far, there are few studies on the effect of the simultaneous chlorine and UV treatment on the inactivation of viruses. Shang *et al.* (2007) and Rattanakul *et al.* (2014, 2015) used in combination tests chlorine concentrations between 0.15 and 1.5 mg/L and UV doses between 17 and 69 mWs/cm^2.

F-specific RNA-coliphage MS2 is often used as a virus surrogate to estimate the disinfection efficacy to enteric pathogens since its structure, morphology, and composition are closely related to those of enteric viruses (Grabow 1986) and it is fairly easy, safe, and rapid to analyze. However, previous studies have shown that MS2 is not as resistant as some human enteric viruses (Shin & Sobsey 2008; Rattanakul *et al.* 2014). In an effort to identify new human virus surrogates for water treatment, we isolated coliphages from wastewater and analyzed their susceptibility to chlorine and successive exposure to low doses of chlorine and UV irradiation in drinking water.

METHODS

Isolation and purification of coliphages

The coliphages were isolated from wastewater effluent of the Lehtoniemi municipal wastewater treatment plant (WWTP) located in Kuopio, Finland on 1st November, when the maximum outdoor temperature was 4.6 °C. The average influent flow to the WWTP was ca.18,700 m^3/d in 2014. The wastewater samples (1,000 mL) were collected as two parallel subsamples and transported to the laboratory within 2 hours. Two host strains were used in order to isolate different coliphages. One of them was *Escherichia coli* ATCC 13706, which is widely used for detection of somatic coliphages and the other was *E. coli* ATCC 15597, used for

detecting F-specific RNA coliphages (Hurst *et al.* 2007). The hosts were used for the isolation of coliphages from both subsamples according to the protocol described by ISO (1998), using the dilutions of 10^0, 10^{-1}, and 10^{-2} of wastewater and host strains in the Log-phase. The host bacteria were taken from stock cultures with a sterile loop and inoculated in phage THG broth, consisting of tryptose 10 g, yeast extract 5 g, glucose 2 g, NaCl 5 g, and $MgSO_4 \cdot 7H_2O$ 0.25 g added to 1,000 mL of deionized water, and incubated at 37 °C for 24 ± 3 h. After incubation, 3.0 mL of suspension was re-inoculated into 100 mL phage THG broth and incubated at 37 °C for 24 ± 3 h. This overnight culture was rejuvenated in a shaker incubator (Edison, NJ, USA) at 37 °C for 2 h. The coliphages were cultivated by a double-layer technique as described by Adams (1959) using phage THG media with 12 g agar in hard agar and with 6 g agar in semi-solid agar in 1 L of deionized water. The method was modified so that 0.1 mL of 2,3,5-triphenyltetrazolium chloride (TTC) solution was added to 1.0 mL of semi-solid agar. This solution was prepared freshly by adding 0.04 g TTC to 5.0 mL of ethanol (Rajala-Mustonen & Heinonen-Tanski 1994). Different plaques were picked up from the plate depending on their size and morphology of lysogenic and lytic zones (Tan *et al.* 2008) and inoculated into fresh host solutions. The solution was incubated for 4–5 h at 37 °C and then left to stand for 24 h at 4 °C. The suspension was centrifuged at 3,250 × *g* for 15 min to separate bacterial cells and cell debris (in pellet) and coliphages (in supernatant) (Rajala-Mustonen & Heinonen-Tanski 1994). Purity of the coliphage suspension was verified by recultivating the solution two to three times by a double layer technique. The density of coliphages was titered by a double layer technique according to ISO (1998). In total, 17 different coliphages were isolated and purified (Table 1). The concentration of coliphages in these stock solutions was approximately 10^9 PFU/mL. MS2 (strain ATCC 15597-B1), an F-specific RNA coliphage, was similarly rejuvenated and used in the disinfection experiments.

Table 1 | Diameter of lytic and lysogenic zones (mm, mean ± SD, n = 10) of plaques for 17 tested coliphages and MS2 coliphage using hosts *E. coli* ATCC 13706 and ATCC 15597

Coliphage ATCC number of isolation host		Host ATCC 13706		Host ATCC 15597	
		Lytic zone	Lysogenic zone	Lytic zone	Lysogenic zone
1	15597	2.09 ± 0.18	No zone	2.40 ± 0.48	1.63 ± 0.49
2	15597	No plaques	No plaques	1.42 ± 0.16	No zone
3	15597	No plaques	No plaques	2.87 ± 0.43	1.31 ± 0.51
4	15597	0.74 ± 0.14	1.17 ± 0.26	1.05 ± 0.37	1.17 ± 0.26
5	15597	2.78 ± 0.04	1.33 ± 0.06	2.72 ± 0.53	1.32 ± 0.32
6	15597	2.23 ± 0.10	2.55 ± 0.16	2.12 ± 0.47	2.64 ± 0.67
7	13706	1.85 ± 0.55	1.05 ± 0.30	2.27 ± 0.16	2.03 ± 0.20
8	13706	1.76 ± 0.28	No zone	3.64 ± 0.26	1.97 ± 0.56
9	13706	4.14 ± 0.30	1.99 ± 0.57	4.43 ± 0.15	2.17 ± 0.19
10	13706	1.34 ± 0.41	No zone	0.80 ± 0.07	0.88 ± 0.11
11	13706	1.80 ± 0.38	No zone	3.65 ± 0.42	3.70 ± 0.60
12	15597	1.73 ± 0.13	No zone	3.56 ± 0.28	3.00 ± 0.36
13	13706	4.69 ± 0.35	1.40 ± 0.16	4.71 ± 0.11	1.66 ± 0.20
14	15597	Not tested	Not tested	0.86 ± 0.40	No zone
15	15597	No plaques	No plaques	2.04 ± 0.32	No zone
16	15597	3.60 ± 0.12	No zone	3.67 ± 1.31	No zone
17	13706	3.56 ± 0.49	1.63 ± 0.31	3.34 ± 0.31	3.33 ± 0.65
MS2	15597	No plaques	No plaques	2.49 ± 0.29	1.21 ± 0.34

Chlorination experiments

Kuopio municipal tap water was used in disinfection experiments. The originally sand bank filtered lake water was treated using coagulation and filtration followed by chlorination with, on average, 0.35 mg free chlorine/L. After treatment water had a color of <5 mg Pt/L, turbidity 0.10 NTU, COD_{Mn} 1.5 mg/L, and numbers of *E. coli* and enterococci 0 CFU/100 mL (Kuopio Water Company 2015).

The tap water was flushed for at least 3 min before six liters of water was collected in a beaker and left at room temperature for 24 h to remove the residual chlorine. Temperature and pH were measured with an HQ40d meter (Hach Company, Loveland, USA), and total chlorine and free chlorine concentrations were measured with a Hach DR2800 spectrophotometer (Hach Company) using colorimetric DPD method according to APHA *et al.* (2005). Sodium hypochlorite solution (0.1%) was prepared daily from 10.5% NaOCl solution by diluting it with sterile deionized water. After that, 0.1% NaOCl was added to 100 mL of water to get the final concentrations of approximately 0.1 mg/L, 0.3 mg/L, and 0.5 mg/L of total chlorine (0.04 mg/L, 0.1 mg/L, and 0.2 mg/L of free chlorine, respectively) for the inactivation tests. The tests were carried out at room temperature of 20 to 21 °C and pH of 7.2 to 7.4.

The water was spiked with 17 coliphage strains and MS2 one at a time by adding 2.0 mL of coliphage solution in 2 L of dechlorinated drinking water to reach the coliphage concentration of approximately 10^6 PFU/mL. The spiked water was divided into four replicate bottles, each containing 500 mL of water. One bottle served as a control and the volumes of the other three bottles were divided into three parallel subsamples (V = 100 mL). Each of these were exposed for 10 min to total chlorine concentrations of 0, 0.1 mg/L, 0.3 mg/L, and 0.5 mg/L, respectively, after which the residual chlorine was quenched by adding 0.05 mL of sodium thiosulfate (18 mg/mL) to 100 mL of water sample (SFS-EN ISO 19480). The concentrations of total and free chlorine were determined immediately after addition of chlorine and again just when the treatment was completed. After the experiment, the number of coliphages was again tested by a double layer technique as described earlier in order to see the effect of chlorine on the coliphages.

Combined chlorine and UV experiments

The UV irradiation was produced by a low-pressure mercury lamp (Osram HNS 30W, λ = 253.7 nm). It was used as a UV source enclosed in a self-made collimator, where a black plastic tube directed the UV radiation to an exposed area 30.0 cm under the lamp. The intensity of UV irradiation on the sample surface, measured with OL 756 Portable High-Accuracy UV-Visible Spectroradiometer (Optronic Laboratories Inc., USA), was approximately 0.2 mWs/cm^2. The average UV dosage (mWs/cm^2) was calculated as the product of radiation intensity and time in seconds as described by Bolton & Linden (2003). Transmittance of the water was 87% calculated from the absorbance of the water measured with a spectrophotometer (UV-2401PC, Shimadzu, Kyoto, Japan) at the wavelength of 254 nm.

The combined effect of chlorine and UV irradiation disinfection was tested on MS2 and the six coliphages, which had proved most resistant in the chlorination experiment. The studied coliphages were spiked into drinking water to get an initial concentration of 10^6 PFU/mL. The coliphages were exposed to total chlorine concentrations of 0.05–0.25 mg/L corresponding to free chlorine concentrations from 0.02 to 0.08 mg/L with contact times of 1–10 min, which resulted in Ct values between approximately 0.02 and 0.4 mg free chlorine × min/L. Immediately after chlorination without quenching the residual chlorine, 10.0 mL of the spiked water was pipetted into glass Petri dishes, mixed with a magnetic stirrer and exposed to 71 s to 7 min of UV yielding the dosages of 14–82 mWs/cm^2. Coliphage density was determined before and after the treatment as described before.

Calculations and statistical analyses

Inactivation values were calculated as the Log_{10} of N/N_0, where N is the plaque density after the treatment and N_0 the density at the beginning of the experiment. The detection limit for the density of coliphages was 1 PFU/mL. Ct values were calculated by multiplying the concentrations of free chlorine (mg/L) measured at the beginning of the 10 min contact time with the exposure time (min). Non-parametric tests (Related sample Friedman's two-way analysis) were performed in order to determine the statistically significant

differences between the plaque densities before and after treatments. Linear regression equations were calculated to describe the relationship between Log_{10}-reductions of coliphages and free chlorine concentration. To find the statistically significant differences between coliphage strains at $p < 0.05$, the slopes of the linear regression equations were analyzed by non-parametric Kruskall–Wallis test.

RESULTS

Inactivation of coliphages by chlorine

The concentrations of total chlorine and free chlorine were similar before and after the 10 min exposure to applied chlorine. Thus there was no decay of chlorine during the exposure (Table 2).

There was a large variation in the sensitivity of the coliphage isolates to different concentrations of chlorine after 10 min contact time. Eleven of the 17 coliphages isolated were intermediate or sensitive to chlorination (Tables 2 and 3). A free Cl-dosage of 0.21 mg/L (total Cl 0.50 mg/L) with 10 min contact time caused 3.8 to more than 5 Log_{10}-reduction for these coliphages ($p < 0.05$). MS2 coliphage was intermediate and free chlorine concentration as low as 0.04 mg/L achieved 1.7 Log_{10}-reduction ($p < 0.05$). Free Cl-dosage of 0.12 mg/L with 10 min contact time was enough to achieve at least 5.7 Log_{10}-reduction (less than detection limit) of MS2 (Table 2). Six of seven sensitive coliphages were inactivated with free Cl-dosages of 0.04 mg/L (total Cl 0.13 mg/L) more than 1.7 Log_{10}–units resulting in the steep inactivation lines as shown by the slopes of the linear regression equations (Table 2).

Seven of the 17 isolated coliphage strains were resistant against chlorine concentrations tested and in six of seven

Table 2 | Log_{10}-densities (mean ± SD, n = 3) and (Log_{10}-reduction in parenthesis) of MS2 and the 17 isolated coliphages at different concentrations of chlorine and with 10 min contact time

Strain	Cl 0 mg/L	Cl_{tot}: 0.13 ± 0.01 mg/L[a]; Cl_{free}: 0.04 ± 0.03 mg/L[a]	Cl_{tot}: 0.33 ± 0.02 mg/L[b]; Cl_{free}: 0.12 ± 0.04 mg/L[b]	Cl_{tot}: 0.50 ± 0.03 mg/L[c]; Cl_{free}: 0.21 ± 0.08 mg/L[c]	Linear regression equation: = Log_{10}-reduction; x = Cl_{free} (mg/L)
1	4.54 ± 0.30	4.19 ± 0.04 (0.35)	4.81 ± 0.07 (−0.27)	4.57 ± 0.15 (0.03)	$y = 0.49x$ ($R^2 = 0.003$)
5	5.14 ± 0.04	5.02 ± 0.10 (0.12)	5.06 ± 0.11 (0.07)	5.11 ± 0.13 (0.02)	$y = -0.42x$ ($R^2 = -0.21$)
7	4.23 ± 0.05	4.02 ± 0.01 (0.21)	4.14 ± 0.11 (0.10)	4.16 ± 0.11 (0.07)	$y = -0.62x$ ($R^2 = -0.64$)
6	5.41 ± 0.29	5.41 ± 0.07 (0)	5.03 ± 0.07 (0.38)	5.17 ± 0.25 (0.24)	$y = -1.20x$ ($R^2 = 0.13$)
17	4.84 ± 0.42	4.58 ± 0.19 (0.26)	4.44 ± 0.12 (0.40)	4.34 ± 0.06 (0.50)	$y = -2.99x$ ($R^2 = 0.51$)
14	5.62 ± 0.35	5.47 ± 0.13 (0.15)	4.84 ± 0.50 (0.77)	5.03 ± 0.26 (0.59)	$y = -5.21x$ ($R^2 = 0.36$)
13	2.26 ± 0.10	1.99 ± 0.02 (0.27)	0.31 ± 0.39 (1.95)	LDL (2.26)*	$y = -9.84x$ ($R^2 = 0.79$)
MS2	**5.71 ± 0.36**	**4.01 ± 0.04 (1.70)**	**LDL (5.71)***	**LDL (5.71)***	$y = -27.55x$ ($R^2 = 0.91$)
11	5.58 ± 0.21	5.37 ± 0.07 (0.21)	1.65 ± 0.31 (3.92)	0.41 ± 0.15 (5.16)*	$y = -30.66x$ ($R^2 = 0.93$)
4	5.36 ± 0.22	3.34 ± 0.21(2.02)	LDL (5.36)*	LDL (5.36)*	$y = -31.67x$ ($R^2 = 0.97$)
3	5.52 ± 0.18	3.81 ± 0.30 (1.71)	LDL (5.52)*	LDL (5.52)*	$y = -32.59x$ ($R^2 = 0.99$)
10	4.69 ± 0.16	2.05 ± 0.36 (2.64)	LDL (4.69)*	LDL (4.69)*	$y = -33.03x$ ($R^2 = 0.88$)
9	4.62 ± 0.14	2.59 ± 0.27 (2.03)	LDL (4.62)*	LDL (4.62)*	$y = -37.03x$ ($R^2 = 0.90$)
2	5.74 ± 0.09	5.52 ± 0.22 (0.22)	3.28 ± 0.07 (2.46)	LDL (5.74)*	$y = -40.98x$ ($R^2 = 0.88$)
12	3.80 ± 0.58	LDL (3.80)*	LDL (3.80)*	LDL (3.80)*	$y = -43.43x$ ($R^2 = 0.83$)
16	4.41 ± 0.31	2.38 ± 0.38 (2.02)	LDL (4.41)*	LDL (4.41)*	$y = -45.99x$ ($R^2 = 0.85$)
15	5.28 ± 0.06	3.49 ± 0.33 (1.78)	0.27 ± 0.56 (5.00)*	LDL (5.28)*	$y = -46.08x$ ($R^2 = 0.91$)
8	4.95 ± 0.25	0.13 ± 0.38 (4.82)*	LDL (4.95)*	LDL (4.95)*	$y = -157.3x$ ($R^2 = 0.99$)

Statistically significant differences from the control (Cl 0 mg/L), assessed by Related sample Friedman's two-way analysis of variance, are indicated with asterisks *$p < 0.05$. LDL = less than the detection limit <1 PFU/mL.

[a]Concentrations of total and free chlorine after 10 min contact time were 0.13 ± 0.01 mg/L and 0.04 ± 0.02 mg/L, respectively.

[b]Concentrations of total and free chlorine after 10 min contact time were 0.31 ± 0.01 mg/L and 0.10 ± 0.03 mg/L, respectively.

[c]Concentrations of total and free chlorine after 10 min contact time were 0.47 ± 0.04 mg/L and 0.19 ± 0.07 mg/L, respectively.

Table 3 | Grouping of coliphage strains to chlorine-resistant[a], intermediate[b], and sensitive[c] based on statistically significant differences between the slopes of linear regression equations (Log_{10}-reduction = k*Cl_{free})[d] of the strains (*$p < 0.05$, Kruskal–Wallis test)

	Resistant[a]							Intermediate[b]			
Groups	**Strains**	**1**	**5**	**7**	**6**	**17**	**14**	**13**	**MS2**	**11**	**4**	**3**
Sensitive[c]	**10**	0.015*	0.038*	0.043*								
	9	0.004*	0.011*	0.013*	0.022*	0.049*						
	2	0.002*	0.006*	0.007*	0.013*	0.029*						
	12	0.001*	0.003*	0.003*	0.006*	0.015*	0.027*	0.049*				
	16	0.001*	0.002*	0.002*	0.005*	0.012*	0.022*	0.040*				
	15	0.000*	0.002*	0.002*	0.004*	0.100*	0.020*	0.036*				
	8	0.000*	0.000*	0.000*	0.001*	0.002*	0.005*	0.010*	0.026*	0.040*		

The table shows only the statistically significant *p*-values.
[a]Resistant coliphage strains are statistically significantly different from 4 to 7 sensitive strains.
[b]Intermediate coliphage strains are statistically significantly different from 0 to 1 sensitive strains.
[c]Sensitive coliphage strains are statistically significantly different from 3 to 9 resistant strains.
[d]Data for linear regression equation are presented in Table 2.

cases the slopes and R^2 values of linear regression equations were low indicating no or almost no reduction of coliphages (Table 2).

Inactivation of coliphages with the combined chlorine and UV treatment

First applying chlorine with Ct values of 0.1–0.4 mg free Cl × min/L (0.02 mg/L to 0.08 mg/L free chlorine for 5 min) and then UV with a dosage of 14 mWs/cm^2, without quenching the residual chlorine, achieved 0.6 to above 5.4 Log_{10}-reductions reaching the detection limit for the tested chlorine-resistant coliphage strains (Figure 1). Approximately 3–5.4 Log_{10}-reductions were achieved when using Ct value of 0.3 mg free chlorine × min/L (0.03 mg/L free chlorine for 10 min) followed by 22 mWs/cm^2 UV. The chlorine dosages as low as 0.05 mg/L and only 5 min contact time (Ct 0.1 mg free chlorine × min/L) followed by 22 mWs/cm^2 UV gave 1–2 Log_{10}-reductions. Statistical analysis showed that the combination with Cl and UV treatment was significantly more effective than chlorine alone for six Cl-resistant coliphages tested ($p < 0.05$). In combined disinfection, increasing either dosage of chlorine or UV, while keeping the other constant, typically increased the disinfection efficiency and gave significantly higher reductions of coliphages ($p < 0.05$) (Figure 1).

MS2 was more sensitive to the combined Cl/UV treatment than the chlorine-resistant coliphages as can be seen in Figure 1. MS2 experienced 2.2 Log_{10}-reductions at Ct value of 0.1 mg free chlorine × min/L (0.02 mg/L free chlorine for 5 min) already followed by 14 mWs/cm^2 UV dosage. The Ct value of 0.4 mg free chlorine × min/L (0.08 free chlorine for 5 min) followed by 22 mWs/cm^2 UV achieved more than 6.0 Log_{10}-reductions for MS2.

DISCUSSION

The present study showed that the coliphage strains isolated by us have a high variation in their resistance against chlorine disinfection (Table 2). Coliphages were grouped into three categories: chlorine-resistant, intermediately chlorine-resistant, and sensitive to chlorine (Table 3). The chlorine treatment can easily destroy the intermediate and sensitive coliphages. In our work, as many as 7 of 18 tested coliphages (almost 40%) belonged to the chlorine-resistant group and they were difficult to destroy with chlorine concentrations typical in drinking water disinfection.

Li *et al.* (2002) reported that enteric viruses, such as hepatitis A virus, were completely inactivated at pH 7 only if the free chlorine concentration was 10 or 20 mg/L with a 30 min contact time, a concentration which is not possible in regular use for drinking water (WHO 2011). The study of Engelbrecht *et al.* (1980) with enteric viruses, such as coxsackie, polio type 1 and 2, and echoviruses, showed a wide range of susceptibility to the concentration of approximately 0.5 mg/L free chlorine within 1.2 min to 4.5 min

required to inactivate 2 Log_{10} of these viruses at pH around 7.8 and temperature of 5 ± 0.2 °C. Also, Thurston-Enriquez *et al.* (2003) demonstrated the variable effect of chlorine on different viruses such as polio type 1, calici- and adenovirus 40 in drinking water. In their study, the free chlorine concentration was 0.50–0.53 mg/L within 15 s contact time at 5 °C and pH 6–8. In our study performed at 20–21 °C and pH 7.2–7.4, free chlorine at a concentration of 0.21 mg/L for 10 min caused less than 1 Log_{10}-reduction for the most chlorine-resistant coliphage strains.

In our experiment MS2 and some other coliphages were classified to intermediately resistant to chlorine and MS2 achieved 5.71 Log_{10}-reduction with free chlorine concentration of 0.12 mg/L within 10 min contact time (Ct value 1.2 mg free chlorine × min/L). Shin & Sobsey (2008) showed that at pH 6 and temperature of 5 °C MS2 was inactivated by 5 Log_{10}-units at 1 mg/L concentration of free chlorine and only 20 s contact time (Ct value 0.3 mg free chlorine × min/L). Rattanakul *et al.* (2014), on the other hand, showed that at pH 7 and 20 °C, only 2.5 Log_{10}-reduction of MS2 was achieved with the Ct value of 1 mg free chlorine × min/L. Temperature and pH of our study are close to those of Rattanakul *et al.* (2014) but the different matrix, phosphate buffer in Rattanakul *et al.* (2014) and drinking water in our study, and different hosts used may explain the different results in spite of almost similar Ct value. When compared to the pathogenic enteric viruses, MS2 has been shown to be as sensitive to chlorination as norovirus, but more sensitive than poliovirus (Shin & Sobsey 2008). MS2 thus seems to be a poor surrogate for the chlorine-resistant viruses and better surrogates could be found among the chlorine-resistant coliphages. These, however, should be studied in more detail and to compare their chlorine resistance to those of resistant enteric viruses, such as polioviruses.

In our work the inactivation of coliphages by 1–10 min chlorination without quenching followed by UV irradiation improved highly the reductions of all tested, chlorine-resistant coliphages (Figure 1). Our work confirmed the results by Shang *et al.* (2007) and Rattanakul *et al.* (2014), who showed that the combination of chlorine and UV, at concentrations and doses used in real drinking water disinfection, was more effective in disinfection of MS2 than either chlorine or UV treatment alone. Shang *et al.* (2007) used free chlorine concentration of 1 mg/L (Ct 0.41 mg free chlorine × min/L) and UV dose of 17 mWs/cm^2 in the simultaneous process and could demonstrate 2.4 Log_{10}-inactivation which was clearly higher than found with the chlorine treatment alone. Our results show that 2.2–6.0 Log_{10}-reductions for MS2 can be achieved with similar or lower Ct values and UV dose of 14 mWs/cm^2. Rattanakul *et al.* (2014, 2015) compared the process, where water was treated first with chlorine without quenching the chlorine and then with UV, to the process where water was exposed to both chlorine and UV simultaneously. Their results showed that when chlorination (1 mg/L free chlorine) was used before UV (23 mWs/cm^2) without quenching the chlorine, the inactivation was between 1 and 3 Log_{10} units supporting our result. Besides the above-mentioned lab-scale studies, Vankerckhoven *et al.* (2011) could show the beneficial effect of the chlorine and UV on free-living mixed bacterial suspensions in a pilot-scale study. The result is promising regarding the full-scale drinking water treatment even though biofilm attached bacteria have not shown similar responses and still require further research (Vankerckhoven *et al.* 2011).

Efficiency of the combined use of chemical oxidant and UV has been demonstrated also with other combination techniques. The combination of PAA/UV disinfection in wastewater studied by Rajala-Mustonen *et al.* (1997) and Koivunen & Heinonen-Tanski (2005) was shown to be more efficient against coliphages than PAA disinfection alone when the UV irradiation was started 10 min or 30 s after the chemical disinfection. The possible explanation for the high inactivation of the resistant coliphages in the simultaneous, or partly simultaneous chlorine/UV treatment such as ours, is that chlorine may damage the viral capsid and thus the virus gets more sensitive to UV which targets the nucleic acids in the next step (Rattanakul *et al.* 2014). In addition, the radicals formed in the simultaneous presence of chlorine and UV irradiation may be responsible for the damage to virus particles (Watts & Linden 2007).

The benefit of our study for water treatment is the high efficiency of the combined treatment for inactivation of the resistant viruses, which allowed using lower disinfection dosages and lower contact times. The combined use of chlorine and UV irradiation would mean that the dosages of chlorine could be decreased, which leads to a saving in

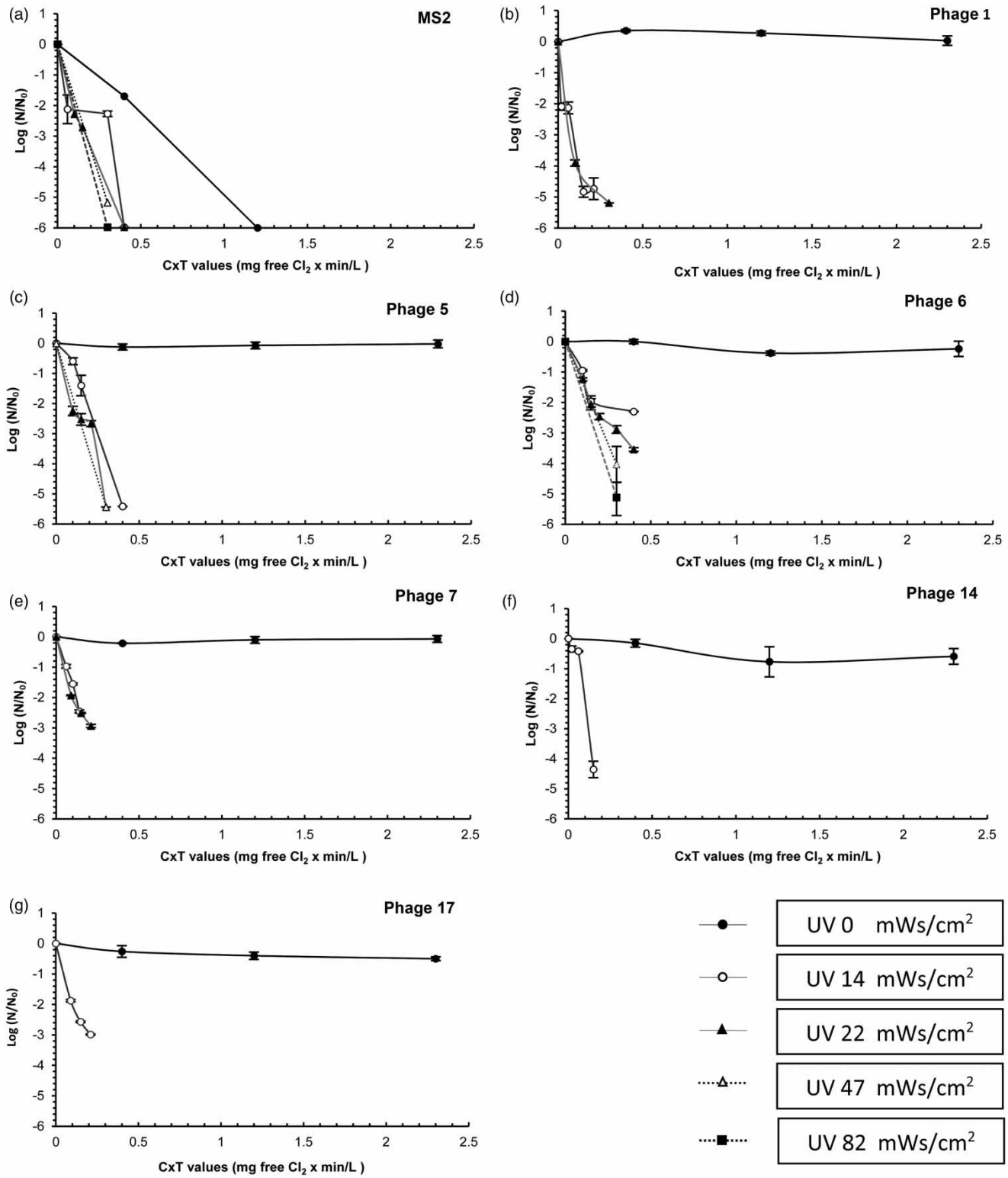

Figure 1 | Inactivation of MS2 and six chlorine-resistant coliphages at different Ct values (mg free Cl × min/L) followed with different UV dosage (mWs/cm^2) in the combined Cl/UV treatments: (a) coliphage MS2, (b) coliphage 1, (c) coliphage 5, (d) coliphage 6, (e) coliphage 7, (f) coliphage 14, and (g) coliphage 17. In the combined treatments the concentrations of free chlorine varied from 0.02 to 0.08 mg/L and time from 1 to 10 min. The pH of the water was 7.2–7.4 and the temperature was 20–21 °C.

chemical costs, as discussed also by Vankerckhoven *et al.* (2011). Also, by using a combination, there could be a decrease in the toxicity of residual chlorine and less negative changes in the taste and smell of water (Wigginton *et al.* 2012). In our tests the water used had a good quality as can be seen in the section Materials and methods. Further, it should be considered that our experiment was done in a collimator device where the UV penetration is good. If the water quality were worse, there could have been a need for higher doses of chlorine and/or UV irradiation.

In this study we found that approximately 40% of the isolated coliphages were chlorine resistant. The chlorine concentrations tested, up to 0.5 mg total chlorine/L or 0.2 mg free chlorine/L, are at the same level as those used to disinfect water in real drinking waterworks (WHO 2011) and our results indicate that a considerable proportion of coliphages may survive during disinfection treatments. If chlorine resistance among human viruses is as usual as it seems to be among coliphages, disinfection of viruses should be studied more. The work should be continued by comparing the resistance of our isolates with those of enteric viruses. More studies are also needed about the effect of the combined chlorine and UV disinfection in water and biofilms against other microorganisms which are known to be resistant to different water disinfection methods.

CONCLUSIONS

In conclusion, we noticed a high variation in the sensitivity of different coliphage strains to chlorine. The sensitive strains were inactivated already at the dosage of 0.04 mg/L free chlorine (0.1 mg/L total chlorine) and 10 min contact time but the most resistant strains were not inactivated even with 0.2 mg/L free chlorine. In contrast, the most chlorine-resistant strains could be efficiently inactivated when a low dosage of chlorine was combined with low dosages of UV showing that the combination is more efficient in disinfection than the chlorination alone and suitable especially against the most chlorine-resistant coliphages. MS2, generally used as a surrogate virus, was intermediately resistant to chlorine, and thus it is not an optimal choice to indicate resistant viruses.

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