Effects of ultraviolet light disinfection on tetracycline-resistant bacteria in wastewater effluents

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ABSTRACT

The ubiquitous use of antibiotics has led to an increasing number of antibiotic-resistant bacterial strains, including strains that are multidrug-resistant, pathogenic, or both. There is also evidence to suggest that antibiotic resistance genes (ARGs) spread to the environment, humans, and animals through wastewater effluents. The overall objective of this study was to investigate the effect of ultraviolet (UV) light disinfection on antibiotic-resistant bacteria. Wastewater effluent samples from a wastewater treatment plant (WWTP) in Texas were evaluated for differences in tetracycline-resistant bacteria before and after UV treatment. The effects of photoreactivation or dark repair on the reactivation of bacteria present in WWTP effluent after UV disinfection were also examined. Culture-based methods were used to characterize viable heterotrophic, tetracycline-resistant heterotrophic, *Escherichia coli*, and tetracycline-resistant *E. coli* bacteria present before and after UV treatment. UV disinfection was found to be as effective at reducing concentrations of resistant heterotrophs and *E. coli*, as it was at reducing total bacterial concentrations. The lowest survival ratio following UV disinfection was observed in tetracycline-resistant *E. coli* showing particular susceptibility to UV treatment. Photoreactivation and dark repair rates were found to be comparable to each other for all bacterial populations.

Key words | antibiotic-resistant bacteria, disinfection, reactivation, wastewater

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INTRODUCTION

The ubiquitous use of antibiotics has led to an increasing number of antibiotic-resistant bacterial strains, including strains that are multidrug resistant, pathogenic, or both. Drug-resistant strains were first identified in hospitals. Resistance for each antibiotic appeared not long after the antibiotic was introduced (Levy & Marshall 2004). Significant amounts of antibiotic resistance genes (ARGs) are not only found in hospital settings, but in the environment as well. Antibiotic-resistant infections, and particularly those which are multidrug resistant, are more difficult to treat and cost more lives than a normal bacterial infection. Some strains of *Escherichia coli* are resistant to six families of antibiotics, similarly, infections of *P. aeruginosa* and *A. baumannii* can be resistant to all or all but one antibiotics (Levy & Marshall 2004). Clearly, ARGs pose a major health threat.

Resistance mechanisms have been identified for all of the major classes of antibiotics (Levy & Marshall 2004). This study focuses on tetracycline resistance because of the widespread use of tetracycline and high incidence of tetracycline resistance. Tetracycline resistance is the most common kind of resistance found in bacteria isolated from the environment as well as from animals (Billington *et al.* 2002). In total, 47–89% of bacteria in various soil and water environments were found to be tetracycline resistant (Esiobu *et al.* 2002). The antibiotics in the tetracycline family are broad-spectrum agents that work against a variety of Gram-positive and Gram-negative bacteria by inhibiting protein synthesis. In addition, they are used for the prevention of malaria and growth promoters in livestock at sub-therapeutic levels (Chee-Sanford *et al.* 2009).

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Significant levels of ARGs have been detected in urban and agricultural environments, in the influent and effluent of wastewater and drinking water treatment plants (Chee-Sanford *et al.* 2001; Pei *et al.* 2006; Pruden *et al.* 2006). There is potential for antibiotic-resistant bacteria to spread to humans because of their environmental presence. Numerous studies have been conducted showing the presence of ARGs and antibiotic-resistant and multidrug-resistant bacteria in wastewater and drinking water treatment plants (Armstrong *et al.* 1981; Schwartz *et al.* 2002; Volkmann *et al.* 2004; Martins da Costa *et al.* 2006; Auerbach *et al.* 2007). There is also evidence to suggest that ARGs spread to the environment, humans and animals, through wastewater effluents. Wastewater treatment plants (WWTPs) have been found to have higher concentrations and more diversity of tetracycline resistance genes than natural waters (Auerbach *et al.* 2007). A study by Pruden *et al.* (2006) showed the presence of *tet*(W) and *tet*(O) genes in treated wastewater, indicating that tetracycline resistance genes can be introduced into the environment through wastewater treatment plant effluents.

Ultraviolet (UV) light disinfection is being commonly used as the final disinfection step during wastewater treatment. UV light reacts with bacterial DNA to cause the formation of pyrimidine dimers, thus inactivating the bacteria. However, many bacterial species can utilize the mechanisms of photoreactivation, and/or, dark repair to undo the DNA damage. Photoreactivation occurs in UV-A or visible light where pyrimidine dimers form a complex with a photoreactivating enzyme which can then undergo photolysis that restores the original monomer (Masschelein 2002). Dark repair requires multiple enzymes to excise the dimers from the DNA, and considerably fewer bacteria can reactivate under dark conditions (Sanz *et al.* 2007).

Little research has been done to date on the effect of UV irradiation on antibiotic-resistant bacteria. One study indicated that UV disinfection does not decrease the number of tetracycline resistance genes present and suggested that treatment did not likely reduce the concentration of *tet*(Q) and *tet*(W) genes in effluent (Auerbach *et al.* 2007). However, this study focused on molecular methods and did not take into account viable counts of resistant bacteria or the possible effects of photoreactivation.

The overall objective of this study was to investigate the bactericidal effect of UV disinfection on antibiotic-resistant bacteria. Wastewater effluent samples from a WWTP in Texas were evaluated for differences in abundance of tetracycline-resistant bacteria before and after UV treatment. A culture-based method was used to characterize viable heterotrophic, tetracycline-resistant heterotrophic, *E. coli*, and tetracycline-resistant *E. coli* bacteria present before and after UV treatment. The effects of photoreactivation and dark repair on the reactivation of bacteria present in WWTP effluent after UV disinfection were also examined.

MATERIALS AND METHODS

Experimental design

Total heterotrophic bacteria, resistant heterotrophic bacteria, total *E. coli*, and resistant *E. coli* were enumerated using a culture-based method from wastewater samples taken before and after UV treatment. One WWTP effluent sample before and two samples after UV treatment were taken at the inlet and outlet of the UV disinfection chamber at the WWTP, respectively. Samples collected at the outlet of the UV disinfection chamber were exposed to reactivating conditions. One sample was kept under visible light conditions and the second sample was kept in darkness to promote photoreactivation and dark repair, respectively. Regular time interval sampling was performed for bacteria enumeration under repair using a culture-based method. Plating was performed in triplicate.

Wastewater samples

Samples were collected from a wastewater treatment plant in southeast Texas on four different dates, summarized in Table 1. Samples were collected in autoclaved 500 mL Pyrex® bottles, leaving approximately 100 mL headspace. On each collection date, one sample was collected immediately before UV treatment and two samples were collected immediately after UV treatment: one in a clear bottle and one in an aluminum-foil-covered bottle. All samples were stirred continuously on a magnetic stir plate under natural light conditions for 48 h at room temperature.

Table 1 | Flow and water quality data for WWTP effluent

Sample collection Date	Flow rate (MGD)	TSS[a] (mg/L)	TDS[b] (mg/L)	*E. coli* (CFU/100 mL)
10/28/2009	6.28	2	520	17
11/02/2009	5.27	2	620	26
11/11/2009	5.96	2	640	42
11/18/2009	5.75	2	580	17

[a]Total suspended solids.
[b]Total dissolved solids.

Bacterial enumeration

Heterotrophic bacteria, tetracycline-resistant heterotrophic bacteria, *E. coli*, and tetracycline-resistant *E. coli* were enumerated from each sample over a 48 h period for heterotrophs and 24 h period for *E. coli*. Bacteria enumeration was performed at time $t = 0$, 0.5, 1, 2, 4, 6, 12, and 24 h for *E. coli* detection and 48 h for heterotrophic bacteria estimation. Ten-fold serial dilutions were performed as required to obtain appropriate colony forming units, and samples were spread plated on Difco® nutrient agar for heterotrophs and MacConkey agar for *E. coli*. For the enumeration of resistant bacteria, 0.03 mM tetracycline was added to the agar after autoclaving. For each dilution level, triplicate plates were spread plated and were kept in the incubator at 37 ± 1 °C for 24 and 48 h for *E. coli* and heterotrophic bacteria, respectively.

Photoreactivation and dark repair kinetics

Equation (1) was used to determine the specific growth rate for the exponential growth phase of each bacterial culture. The exponential growth phase was estimated graphically. These rates were used to model growth curves for the cultures:

$$\mu = \frac{\ln(X/X_0)}{t} \quad (1)$$

where μ = specific growth rate for exponential bacterial growth (min^{-1}); X = number of organisms at time t (CFU/mL); X_0 = number of organisms at time, $t = 0$ (CFU/mL); t = time (min).

Statistical analysis

One-way analysis of variance (ANOVA) with $\alpha = 0.05$ was performed to test the statistical significance in treatment means within and among treatments. Rate of UV repair of various bacteria in municipal wastewater effluent was also analyzed using one-way ANOVA with $\alpha = 0.05$.

RESULTS AND DISCUSSION

Disinfection efficiency immediately after ultraviolet exposure was determined for total heterotrophs (96%), resistant heterotrophs (89%), total *E. coli* (77%), and resistant *E. coli* (100%). Similar efficiencies were reported in previous studies for total heterotrophic bacteria and *E. coli* (Lindenauer & Darby 1994). UV treatment effectively disinfected tetracycline-resistant heterotrophs and *E. coli*. However, the disinfection efficiency for tetracycline-resistant heterotrophs was only 89%, lower than the 96% disinfection of total heterotrophs.

Bacterial growth

Figures 1(a) through 1(d) compare the cell concentration in each of the three treatments (before UV, after UV kept in light, and after UV kept in dark) at various times for heterotrophic bacteria, resistant heterotrophic bacteria, *E. coli*, and resistant *E. coli*. Bacterial cell concentrations were transformed to the log concentration and plotted at various enumeration times. For all the figures, the mean values from the triplicate plates were plotted. Error bars show plus and minus one standard deviation of the triplicates.

Figures 1(a) and 1(b) show that for both total heterotrophs and resistant heterotrophs UV treatment is effective at reducing the initial concentration of bacteria. However, over time the concentration of UV treated heterotrophs and resistant heterotrophs increases and becomes greater than the initial concentration. This illustrates that UV treatment is effective and reducing the concentration of both heterotrophs and resistant heterotrophs but regrowth and reactivation is occurring after exposure to UV. This occurred in both the treatments (kept in light or kept in dark) which indicates that both light and dark repair mechanisms could

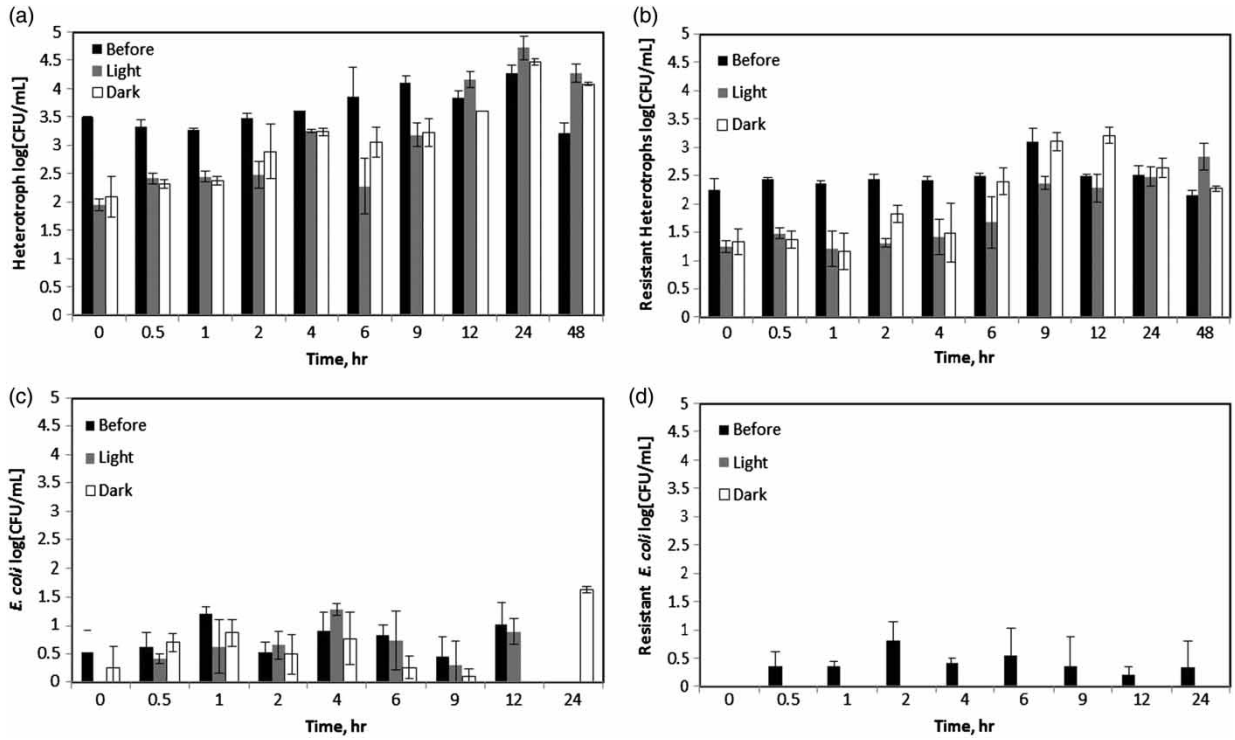

Figure 1 | Viable heterotrophic bacteria in wastewater before UV disinfection and after UV disinfection kept in light and dark for (a) heterotrophic bacteria, (b) resistant heterotrophic bacteria, (c) *E. coli*, and (d) resistant *E. coli*.

be occurring in these samples. The amount of survival under dark repair conditions for both total heterotrophic and resistant heterotrophic bacteria was considerably higher than that reported in previous studies, including those by Lindenauer & Darby (1994) and Sanz *et al.* (2007). A possible explanation is that these studies enumerated either pure cultures or indicator organisms, while these data were acquired from the entire culturable heterotrophic community in the wastewater effluent, and thus could potentially include organisms with higher rates of dark repair than those used in the previous studies.

Figures 1(a)–1(d) show that *E. coli* bacteria make up a small fraction of the total heterotrophic population. Figure 1(c) also illustrates that UV treatment was less effective at reducing the concentration of *E. coli* than heterotrophs. However, it should be noted that concentrations of zero do not indicate that *E. coli* were not present but that the concentration was below the detectable level. Figure 1(d) shows that resistant *E. coli* were not present at a detectable level after UV exposure for both the light and dark treatments. Survival of resistant *E. coli* under dark repair conditions was similar to that reported by Quek & Hu (2008), while photoreactivation survival was lower. The Quek & Hu (2008) study used pure *E. coli* cultures at a concentration of about 1×10^8 CFU/mL, much higher than the concentrations in the WWTP effluent. Results from this study indicate that UV disinfection is an effective method for the reduction of tetracycline-resistant *E. coli*. Figures 1(a)–1(d) clearly show that growth continues to occur in these treatments over time with the exception of resistant *E. coli* exposed to UV. The specific growth rates for these treatments are shown in Table 2.

Table 2 | Specific growth rates (1/min) of heterotrophs and *E. coli* before and after UV treatment

	Heterotrophs		*E. coli*	
Treatment	**Total**	**Resistant**	**Total**	**Resistant**
Before UV	0.006	0.009	0.023	0.027
After UV – Photoreactivation	0.007	0.007	0.022	[a]
After UV – Dark repair	0.011	0.011	0.015	[a]

[a]These cultures did not reach exponential phase growth.

The growth after UV treatment in the dark was faster than either growth before UV treatment or growth after UV treatment in the light for both heterotrophs and resistant heterotrophs (Table 2). This indicates that for heterotrophic bacteria the dark repair mechanism is more effective than photoreactivation. This is contradictory to the *E. coli* population where the growth before UV treatment and after UV treatment in light was similar, while the growth in dark repair was slower. The resistant heterotrophic bacteria and total heterotrophic bacteria had the same growth rates after UV treatment both in light and in dark. However, the resistant heterotrophs had faster growth before UV treatment than the total heterotrophs. Thus, it appears that the growth rates for heterotrophs and resistant heterotrophs are similar and the resistant heterotrophs have a longer lag time. Growth rates for tetracycline-resistant *E. coli* could not be compared since these cultures did not achieve exponential growth. This indicates that UV treatment is highly effective at disinfecting resistant bacteria specifically *E. coli* however it is not effective at maintaining disinfection.

Table 3 shows the fractions of the total bacteria that were tetracycline-resistant before UV disinfection and after UV disinfection and exposed to light or dark over time. Overall the resistant fraction varied over time. For heterotrophs, the resistance fraction after 48 h was slightly higher than the initial concentration. The heterotrophs exposed to UV all had a lower resistant fraction at 48 h than at 0 h, which is likely due to the longer lag phase observed in the growth of the resistant heterotrophs. At 0 h, there was a higher fraction of resistant heterotrophic bacteria in the samples taken after UV than before UV. At 48 h the fraction of resistant bacteria after UV was less than the fraction of resistant bacteria before UV, which is also consistent with the difference in kinetic constants between total heterotrophic bacteria and resistant heterotrophic bacteria. This indicates that exposure to UV light can initially select for tetracycline-resistant bacteria but the non-resistant bacteria quickly recover and no long term selection is seen. For *E. coli*, the resistant fraction was 0 after UV in light and dark. The fraction of resistant *E. coli* before UV treatment was slightly higher at 12 h than at 0 h. Once again this indicates that UV is highly effective at reducing resistant bacteria concentrations.

Table 3 | Fractions of resistant heterotrophs and *E. coli* before and after UV treatment kept in light and dark

	Heterotroph			*E. coli*		
Time, h	Before	Light	Dark	Before	Light	Dark
0	0.06	0.20	0.14	0.08	0	0
0.5	0.12	0.12	0.12	0.53	0	0
1	0.12	0.07	0.08	0.18	0	0
2	0.09	0.06	0.06	0.58	0	0
4	0.07	0.02	0.03	0.68	0	0
6	0.03	0.04	0.16	0.86	0	0
9	0.11	0.14	0.70	NA	0	0
12	0.04	0.01	0.43	0.09	0	0
24	0.02	0.01	0.02	NA	0	0
48	0.08	0.04	0.02	NA	NA	NA

CONCLUSIONS

The overall objective of this study was to investigate the effect of UV light disinfection on antibiotic-resistant bacteria. Wastewater effluent samples from a WWTP in Texas were evaluated for differences in abundance and diversity of tetracycline-resistant bacteria before and after UV treatment. Culture-based methods were used to characterize viable heterotrophic, tetracycline-resistant heterotrophic, *E. coli*, and tetracycline-resistant *E. coli* bacteria present before and after UV treatment. The effects of photoreactivation or dark repair on the reactivation of bacteria present in WWTP effluent after UV disinfection were also examined.

Overall, UV disinfection was found to be at least as effective for reducing concentrations of tetracycline-resistant heterotrophs and *E. coli* as it was for reducing concentrations of total heterotrophs and *E. coli*. UV disinfection was found to be most effective at eliminating resistant *E. coli* since no resistant *E. coli* were present at a detectable level after UV treatment. However, results from this study indicate that UV treatment of heterotrophs, resistant heterotrophs, and *E. coli* experience regrowth and reactivation after UV exposure. All three treatments returned to the concentration before UV treatment within 12 h of UV exposure. This could indicate that UV treatment is not effective at maintaining low concentrations of bacteria and that the use of chlorine or ozone might provide better

maintenance of disinfection over time. However, further research is needed to determine their effect on resistant bacteria concentrations.

Survival and repair of heterotrophic bacteria and *E. coli* under dark repair conditions were found to be much higher than those reported in previous studies (Lindenauer & Darby 1994; Sanz *et al.* 2007; Quek & Hu 2008). Survival and repair under dark repair conditions in the present study were found to be comparable to survival and repair under photoreactivation conditions. This finding suggests that the bacterial strains found in WWTPs may have higher rates of dark repair than the laboratory strains used in the previous studies.

REFERENCES

Armstrong, J. L., Shigeno, D. S., Calomiris, J. J. & Seidler, R. J. 1981 Antibiotic-resistant bacteria in drinking water. *Applied Environmental Microbiology* **42**, 277–283.

Auerbach, E. A., Seyfried, E. E. & McMahon, K. D. 2007 Tetracycline resistance genes in activated sludge wastewater treatment plants. *Water Research* **41**, 1143–1153.

Billington, S. J., Songer, J. G. & Jost, B. H. 2002 Widespread distribution of a *tet* W determinant among tetracycline-resistant isolates of the animal pathogen *Arcanobacterium pyogenes*. *Antimicrobial Agents and Chemotherapy* **46** (5), 1281–1287.

Chee-Sanford, J. C., Aminov, R. I., Krapac, I. J., Garrigues-Jeanjean, N. & Mackie, R. I. 2001 Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. *Applied Environmental Microbiology* **67** (4), 1494–1502.

Chee-Sanford, J. C., Mackie, R. I., Koike, S., Krapac, I. G., Lin, Y. F., Yannarell, A. C., Maxwell, S. & Aminov, R. I. 2009 Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *Journal of Environmental Quality* **38** (3), 1086–1108.

Esiobu, N., Armenta, L. & Ike, J. 2002 Antibiotic resistance in soil and water environments. *International Journal of Environmental Health Research* **12**, 133–144.

Levy, S. B. & Marshall, B. 2004 Antibacterial resistance worldwide: causes, challenges, and responses. *Nature* **10** (12), S122–S129.

Lindenauer, K. G. & Darby, J. L. 1994 Ultraviolet disinfection of wastewater: effect of dose on subsequent photoreactivation. *Water Research* **28** (4), 805–817.

Martins da Costa, P., Vaz-Pires, P. & Bernardo, F. 2006 Antimicrobial resistance in *Enterococcus* spp. isolated in inflow, effluent and sludge from municipal sewage water treatment plants. *Water Research* **40** (8), 1735–1740.

Masschelein, W. J. 2002 *Ultraviolet Light in Water and Wastewater Sanitation*. Lewis Publishers, Boca Raton, FL, USA.

Pei, R., Kim, S. C., Carlson, K. H. & Pruden, A. 2006 Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Research* **40**, 2427–2435.

Pruden, A., Pei, R., Storteboom, H. & Carlson, K. H. 2006 Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. *Environmental Science and Technology* **40**, 7445–7450.

Quek, P. H. & Hu, J. 2008 Indicators for photoreactivation and dark repair studies following ultraviolet disinfection. *Journal of Industrial Microbiology and Biotechnology* **35**, 533–541.

Sanz, E. N., Dávila, I. S., Balao, J. A. A. & Alonso, J. M. Q. 2007 Modeling of reactivation after UV disinfection: Effect of UV-C dose on subsequent photoreactivation and dark repair. *Water Research* **41**, 3141–3151.

Schwartz, T., Kohen, W., Jansen, B. & Obst, U. 2002 Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiology Ecology* **43**, 325–335.

Volkmann, H., Schwartz, T., Bischoff, P., Kirchen, S. & Obst, U. 2004 Detection of clinically relevant antibiotic-resistance genes in municipal wastewater using real-time PCR (TaqMan). *Journal of Microbiological Methods* **56** (2), 277–286.

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