Research Paper

Efficacy of integrated ultraviolet ultrasonic technologies in the removal of erythromycin- and quinolone-resistant *Escherichia coli* from domestic wastewater through a laboratory-based experiment

Owassa Dza Rebecca Annisha, Zifu Li, Xiaoqin Zhou,
Ngomah Madgil Don Stenay Junior and Oscar Omondi Donde

ABSTRACT

Bacterial resistance to antibiotics has become a common occurrence especially in areas where antibiotic drugs are widely used. Moreover, the potential effect of antibiotic pollution and the presence of antibiotic-resistant genes (ARGs) on the composition of bacterial communities in the ecosystems continue to degrade the quality of most ecosystems. Despite the use of ultraviolet (UV) radiation and ultrasound (US) technologies in wastewater purification, little is known on their application in the elimination of fecal pathogenic microorganisms such as *Escherichia coli*. Moreover, their individual and combined potential in the elimination of erythromycin- and quinolone-resistant *E. coli* is a topic that still requires proper understanding. Therefore, this study was aimed at evaluating the individual and combined/integrative potential of UV radiation and ultrasonic technologies in the removal of erythromycin- and quinolone-resistant *E. coli* from domestic effluents using a laboratory experimental-based set-up. The results showed that UV radiation experiment was able to significantly eliminate erythromycin- and quinolone-resistant *E. coli* from the water to a value of 2 log units. Additionally, US technology was equally able to significantly reduce both the erythromycin- and quinolone-resistant *E. coli* to 2 log units. However, on combining the two technologies, there was further reduction to 1 log unit, hence, pointing to the need for adopting the integrative approach in water purification for increased wastewater purification efficiencies and improved ecosystem and human health.

Key words | erythromycin, quinolones, ultrasonic, ultraviolet radiation, wastewater

Owassa Dza Rebecca Annisha (corresponding author)
Zifu Li
Xiaoqin Zhou
School of Energy and Environmental Engineering,
   Beijing Key Laboratory of Resource-oriented
   Treatment of Industrial Pollutants,
National Environment and Energy International
   Science and Technology Cooperation Base,
University of Science and Technology Beijing,
Beijing, 100083,
China
E-mail: *owannie-owas@outlook.com*

Ngomah Madgil Don Stenay Junior
Department of Sciences and Communication
   Skills,
Marien Ngouabi University,
Brazzaville,
Congo

Oscar Omondi Donde
Department of Environmental Science,
Egerton University,
P.O. 536-20115, Egerton,
Kenya

INTRODUCTION

Studies have continued to prove that water resources within the globe will continue to deteriorate and the amount of wastewater produced will continue to rise. Something of

greater concern is the fact that the infrastructure and management systems are not adequately prepared for this increasing wastewater generation (Madbouly 1998). Globally, an estimated two million tons of sewage, industrial and agricultural wastes are discharged into the world's water bodies, and that does not consider the unregulated or illegal discharge of contaminated water. This wastewater

contaminates freshwater and coastal ecosystems, threatening food security, access to safe drinking and bathing water, and is a major health and environmental management challenge (Allen *et al.* 2010; Berendonk *et al.* 2015). The production and cycling of pathogenic- and drug-resistant bacteria will continue to be a big global challenge. This is based on the nature in which food is produced (uses 70–90% of the available fresh water), and much of this water returns back to the system with additional nutrients, pollutants, contaminants and pathogens. Further downstream, agricultural pollution is increasingly joined by human and industrial wastes. Up to 90% of wastewater flows untreated into the densely populated areas such as informal settlements. This has contributed to the growth of freshwater and marine dead zones which lead to further losses in biodiversity and ecosystem resilience, which in turn will undermine prosperity and efforts towards a more water quality sustainability (Romina *et al.* 2018).

The emergence of bacterial resistance to antibiotics has become a common occurrence especially in areas where antibiotic drugs are widely used. Indeed, such incidences have greatly contributed to the rise in antibiotic-resistant bacteria in aquatic environments (Aarestrup *et al.* 1996; Schwartz *et al.* 2003). The rise in various forms of infections in human and animal husbandry have contributed to the increased use of antibiotics. This widespread application of antibiotics in human health as well as intensive animal husbandry is evidence of the increased resistance of most pathogenic bacteria to the existing antibacterial drugs (Schwartz *et al.* 2003; Young *et al.* 2016). The manufacture and use of many forms of human and animal antibiotics have contributed to the emergence of numerous resistant bacteria; furthermore, most of these resistant bacteria have entered and accumulated in most aquatic ecosystems (Su *et al.* 2018). Studies have linked the presence of environmental antibiotic-resistant genes (ARGs) to the composition of bacterial communities within the environment, suggesting that antibiotic pollution and the spreading of ARGs play a big role in the conformation of drug-resistant bacterial communities in wastewater as well as in drinking water sources. Moreover, the potential effect of antibiotic pollution and the presence of ARGs on the composition of bacterial communities in the wastewater prompt the fundamental question about potential effects on bacterial-related

ecosystem services supplied by aquatic ecosystems such as lakes and reservoirs (Huerta *et al.* 2013).

The availability of fecal-related ARGs has continued to rise in aquatic ecosystems. There has been the co-occurrence of both the fecal coliform and ARGs within the wastewater ecosystems as well as those considered essential for drinking water sources. Furthermore, both the fecal coliform and ARGs have been isolated from the biofilms formed on the wastewater plants and also on the drinking water treatment, delivery and storage facilities such as the delivery pipes, taps and tanks (Bergeron *et al.* 2017). Therefore, there is need to strengthen the available ARG detection and removal technologies for improved water quality and human health. An interesting fact is that studies have also linked certain chemical treatment techniques to increased ARGs in the wastewater and related environments. Liu *et al.* (2018) for the first time showed that chlorination increased the levels of ARG pollution in wastewater treatment plants (WWTPs), and that it enhanced both the extracellular and intracellular ARG pollution. Additionally, *Escherichia coli* showed a positive correlation with the total extracellular ARG concentration after chlorination. This not only confirms the use of *E. coli* as a suitable pollution indicator, but may also depict the possible availability of ARGs in water (Donde *et al.* 2013; Liu *et al.* 2018).

The use of UV radiation in wastewater purification involves the instantaneous neutralization of the microorganisms as they pass by ultraviolet (UV) lamps submerged in the effluent (Brahmi & Hassen 2011). The application of UV light for wastewater disinfection is increasingly getting preferred to the known inefficient and environmental damaging chemical-based disinfection techniques (Pang *et al.* 2016). This preference is on the basis that the UV radiation has proved to be one of the few cost-effective disinfection alternatives that do not create or release carcinogenic by-products into the environment. Ultrasound (US) is a technology that is based on cyclic sound pressure that has a frequency of greater than the upper limit of human hearing. It works on the principle of transmission of sound mechanical energy by pressure waves in a material medium (Farooq *et al.* 2009). The use of ultrasonic technology has also been adopted in wastewater purification for the elimination of pathogenic microorganisms and related resistant genes (Kumar *et al.* 2014). The US technology is applied in water

treatment through the use of US systems such as the Sonic systems, which are manufactured to eradicate the intended specific microbial growth and biofilm formation and their related ARGs (Farooq *et al.* 2009; Kumar *et al.* 2014).

Despite these two technologies (UV radiation and US) being considered highly effective and safer in wastewater purification, little is known on their application in eliminating fecal pathogenic microorganisms such as *E. coli*. Moreover, their individual and combined potential in the elimination of erythromycin- and quinolone-resistant *E. coli* is a topic that still requires proper understanding. Therefore, this study was aimed at evaluating the individual and combined/integrated potential of UV radiation and ultrasonic technologies in the removal of erythromycin- and quinolone-resistant *E. coli* from domestic effluents using a laboratory experimental-based set-up. The study chose on erythromycin- and quinolone-resistant *E. coli* due to the existing wide spread of the bacterial genes that has been reported to pose higher resistant to the erythromycin and quinolone forms of drug, making immunosuppressed individuals like the HIV-infected patients to be at a greater risk (Flanigan 1994; Liu *et al.* 2018).

MATERIALS AND METHODS

Sampling and membrane filtration technique

Wastewater samples were obtained from the effluent of an anoxic/anaerobic municipal WWTP in Xian, China that had an average volume of 50 m³ and an average daily flow rate of 5,000 l (5 m³), giving a hydraulic retention time of 10 days. Wastewater sampling was done monthly for a 3-month period using 500 ml sterile bottles, and the samples were stored at 4 °C and carried to the laboratory under ice condition for further experiment and analyses.

The membrane filtration technique (MFT) was done to quantify the bacterial colony units as stipulated in American Public Health Association (APHA) (2005) and Donde & Bangding (2017). Water samples (100 ml) were filtered through a mixed cellulose ester membrane with a pore size of 0.45 μm and put onto Petri dishes with Chromocult agar (Merck) plates and incubated at 37 °C for 18–24 h. Typical colonies appearing blue were counted as *E. coli* colonies. The numbers of cells were expressed as CFUs (colony-forming units)/100 ml (APHA 2005).

Isolation of antibiotic-resistant *E. coli*

The random selection of 10 *E. coli* colonies was done from the mixed cellulose ester membrane and subjected to the polymerase chain reaction to detect the presence of erythromycin- and quinolone-resistant genes (*ere(A)* and *qnrA*, respectively) as stipulated in Momtaz *et al.* (2012). The primer details are provided in Table 1. To further screen and confirm the antibiotic-resistant colonies, the filtered membranes were placed on separate m-FC agar plates with 16 mg/l of erythromycin and 16 mg/l of quinolones. All the *E. coli* colonies positive for the resistant genes were then cultivated to mid-log phase at 37 °C in 20 ml of nutrient broth. Each culture was centrifuged at 5,000 rpm/min for 15 min and the pellet was then washed twice with sterile distilled water. This procedure was repeated to ensure that only pure resistant *E. coli* were obtained and used as the test organism. About 500 ml of wastewater sampled from the domestic WWTP was sterilized at 121 °C for 30 min using air tight pressure heater and aseptically cooled to room temperature. Two 100 ml sets of the sterilized wastewater were separately mixed with 16 mg/l of erythromycin-resistant *E. coli* and 16 mg/l of quinolone-resistant *E. coli*. In total, 10 ml of each of the pelleted resistant *E. coli* with the bacterial concentration of 52,000 CFUs

Table 1 | Primer details for the detection of erythromycin- and quinolones-resistant genes in *E. coli*

Antimicrobial agent	Resistant gene	Sequence	Size (bp)	Annealing temperature (°C)	References
Erythromycin	ere(A)	(F) GCCGGTGCTCATGAACTTGAG (R) CGACTCTATTCGATCAGAGGC	419	52	Momtaz *et al.* (2012)
Quinolones	qnrA	(F) GGGTATGGATATTATTGATAAAG (R) CTAATCCGGCAGCACTATTTA	670	50	Momtaz *et al.* (2012)

for erythromycin-resistant *E. coli* and 48,000 CFUs for quinolone-resistant *E. coli* were put into separate 250 ml sterile flasks and then separately seeded with 90 ml of sterile primary wastewater, giving an initial approximate concentration of 52,000 and 48,000 viable cell counts/100 ml, respectively, for erythromycin-resistant *E. coli* and quinolone-resistant *E. coli*. The seeded antibiotic-resistant *E. coli* were then subjected to UV and US purification experiment.

UV radiation and ultrasonic purification experiments

A total of 20 ml of wastewater seeded with erythromycin- and quinolone-resistant *E. coli* were placed into a properly labeled 50 ml transparent (95% transparent for 360 nm light) test tube with screw caps. Each seeded drug-resistant *E. coli* viable counts (erythromycin- and quinolone-resistant *E. coli*) were exposed to three doses (0, 300 and 600 mW·s·cm^{-2}) of UV radiation that were fixed in a chamber. The sample was mixed gently and continuously using a sterile magnetic stir bar. The seeded *E. coli* were then aseptically sampled at different time intervals (0, 30, 60, 120 and 240 min) and CFU quantified using the MFT. For each of the tested seeded drug-resistant *E. coli*, a UV radiation experiment was performed in triplicate. The collimated UV light was provided by a 15-W mercury vapor 254-nm lamp which was directed onto the transparent tubes containing the seeded erythromycin- and quinolone-resistant *E. coli* within a closed chamber. The intensity of UV light was measured using a radiometer equipped with a UV 254 detector. All the tests were carried out at *E. coli* optimum growth temperature condition of 37.5 °C. For the ultrasonic experiment, the seeded erythromycin- and quinolone-resistant *E. coli* were put in a properly labeled 50 ml transparent test tube without screw caps and then subjected to different frequencies of ultrasonic sounds (35 and 130 kHz) under 250 W power. The seeded *E. coli* were then aseptically sampled at different time intervals (0, 30, 60, 120 and 240 min) and CFU quantified using the MFT. For each tested seeded erythromycin- and quinolone-resistant *E. coli*, ultrasonic experiment was performed in triplicate. To evaluate the potential of combined/integrated UV radiation and ultrasonic wastewater treatment technology in the eradication of erythromycin- and quinolone-resistant *E. coli*, an additional experimental run was set which involved combined UV radiation and ultrasonic purification technology set-ups. The samples were then subjected to the combined set-up conditions. The seeded erythromycin- and quinolone-resistant *E. coli* viable counts that were subjected to the combined condition were then aseptically sampled at different time intervals (0, 30, 60, 120 and 240 min) and CFU quantified using the MFT. For each of the tested seeded erythromycin- and quinolone-resistant *E. coli*, the combined UV radiation–ultrasonic experiment was performed in triplicate. A summary of UV and US dosage with respective sample identities for the different time durations are provided in Table 2.

Table 2 | Sample identities for UV and ultrasonic dosages on different time duration

E. coli type	Dosage	Time (min)		
		0 min	60 min	240 min
Erythromycin-resistant	0 mW·s·cm^{-2}	ER-0-0	ER-60-0	ESR-240-0
	300 mW·s·cm^{-2}	ER-0-300	ER-60-300	ER-240-300
	600 mW·s·cm^{-2}	ER-0-600	ER-60-600	ER-240-600
Quinolone-resistant	0 mW·s·cm^{-2}	QR-0-0	QR-60-0	QR-240-0
	300 mW·s·cm^{-2}	QR-0-300	QR-60-300	QR-240-300
	600 mW·s·cm^{-2}	QR-0-600	QR-60-600	QR-240-600
Erythromycin-resistant	35 kHz	ER-0-35	ER-60-35	ER-240-35
	130 kHz	ER-0-130	ER-60-130	ER-240-130
Quinolone-resistant	35 kHz	QR-0-35	QR-60-35	QR-240-35
	130 kHz	QR-0-130	QR-60-130	QR-240-130
Erythromycin-resistant	600 mW·s·cm^{-2} and 130 kHz	ER-0-600-130	ER-60-600-130	ER-240-600-130
Quinolone-resistant	600 mW·s·cm^{-2} and 130 kHz	QR-0-130	QR-60-600-130	QR-240-600-130

Limitation and scope of the study

This study was limited to erythromycin- and quinolone-resistant *E. coli* but did not include other forms of bacteria. However, *E. coli* being a traditional pollution indicator, its removal through the technology has a stronger evidence that the technology can also be applied in the removal of other bacteria that harbors resistant genes. The scope of the study covered domestic wastewater. It did not include drinking water or wastewater from industries or agricultural farms. Domestic wastewater was chosen as it is the main reservoir for pathogenic bacteria.

Statistical analysis

Minitab statistical package version 14 was used in all the statistical analysis. Normality tests were run for every data set prior to analysis. Mean values were calculated for bacterial CFUs. Mean comparisons were performed using one-way analysis of variance (ANOVA) at 95% confidence level. Where there was a significant difference between the means, Tukey's test was run as post-hoc test to determine points of mean variation. Student's *t*-test was used in comparing the mean of erythromycin- and quinolone-resistant *E. coli* at the highest dosages and longest time between UV radiation and ultrasonic treatments.

RESULTS

UV radiation purification experiment

The findings for UV radiation experiment on the removal of erythromycin-resistant *E. coli* are presented in Figure 1. From an initial value of 8 log units of the erythromycin-resistant *E. coli*, there was a reduction to a final value of 2 log unit at time 240 min and UV dosage of 600 mW·s·cm^{-2} (ER-240-600). There was a statistical difference in the mean CFU of sulfonamide-resistant *E. coli* between the different UV radiation dosages except between ER-60-600 and ER-240-600 ($P < 0.05$). Results on the UV radiation experiment for the removal of quinolone-resistant *E. coli* are presented in Figure 2. From an initial value of 8 log units of the tetracycline-resistant *E. coli*, there was a reduction to a
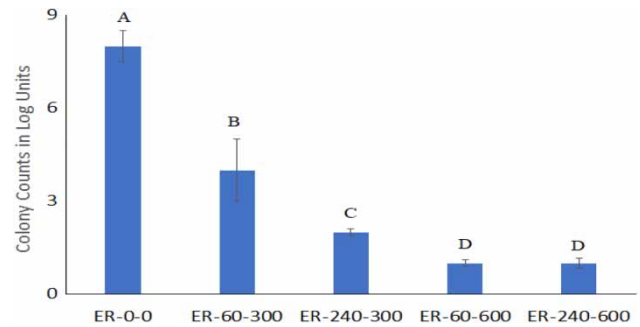
Figure 1 | CFUs for erythromycin-resistant *E. coli* at different UV radiation dosages for different time durations. Time 0 and no UV dosage (ER-0-0), time 60 min at 300 mW·s·cm^{-2} UV dosage (ER-60-300); time 240 min at 300 mW·s·cm^{-2} UV dosage (ER-240-300); time 60 min at 600 mW·s·cm^{-2} UV dosage (ER-60-600); time 240 min at 600 mW·s·cm^{-2} UV dosage (ER-240-600).
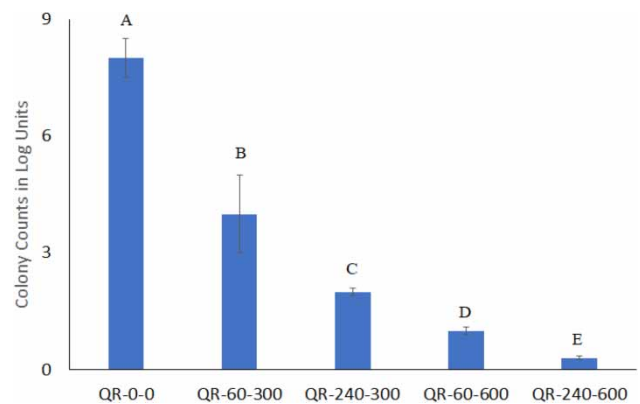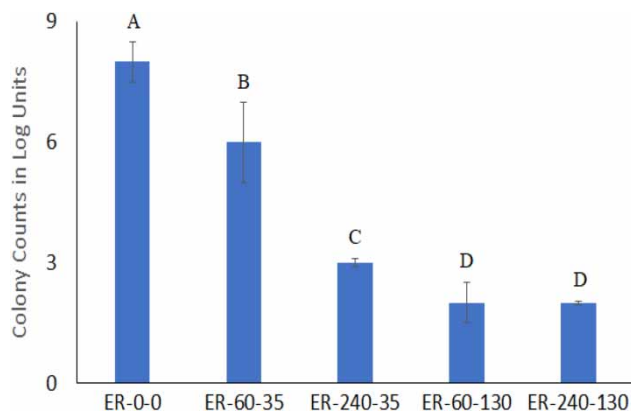

Figure 2 | CFUs for quinolone-resistant *E. coli* at different UV radiation dosages for different time durations. Time 0 and no UV dosage (QR-0-0), time 60 min at 300 mW·s·cm^{-2} UV dosage (QR-60-300); time 240 min at 300 mW·s·cm^{-2} UV dosage (QR-240-300); time 60 min at 600 mW·s·cm^{-2} UV dosage (QR-60-600); time 240 min at 600 mW·s·cm^{-2} UV dosage (QR-240-600).
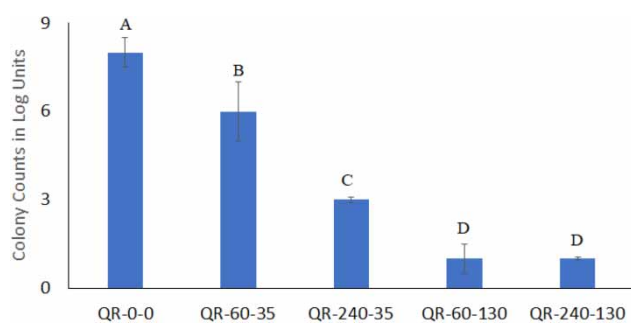
final value of 1 log unit at time 240 min and UV dosage of 600 mW·s·cm^{-2} (QR-240-600). There was a statistically significant difference in the mean CFU of quinolone-resistant *E. coli* between all the different UV radiation dosages ($P < 0.05$).

Ultrasonic purification experiments

Results on the ultrasonic experiment for the removal of erythromycin-resistant *E. coli* are presented in Figure 3. From an initial value of 8 log units of the sulfonamide-resistant *E. coli*, there was a final reduction value of 2 log units at time 240 min and ultrasonic dosage of 130 kHz
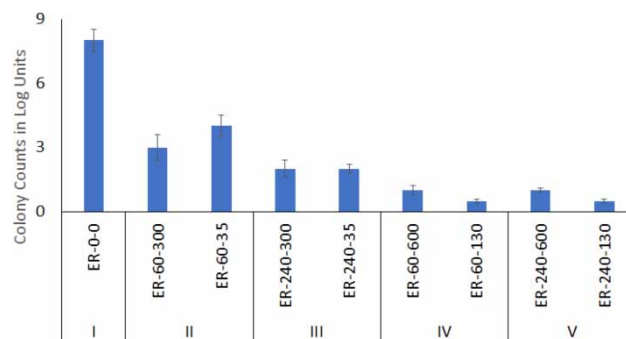
Figure 3 │ CFUs for erythromycin-resistant *E. coli* at different ultrasonic dosages for different time durations. Time 0 and no ultrasonic dosage (ER-0-0), time 60 min at 35 kHz of ultrasonic dosage (ER-60-35); time 240 min at 35 kHz of ultrasonic dosage (ER-240-35); time 60 min at 130 kHz of ultrasonic dosage (ER-60-130); time 240 min at 130 kHz of ultrasonic dosage (ER-240-130).

Figure 4 │ CFUs for quinolone-resistant *E. coli* at different ultrasonic dosages for different time durations. Time 0 and no ultrasonic dosage (QR-0-0), time 60 min at 35 kHz of ultrasonic dosage (QR-60-35); time 240 min at 35 kHz of ultrasonic dosage (QR-240-35); time 60 min at 130 kHz of ultrasonic dosage (QR-60-130); time 240 min at 130 kHz of ultrasonic dosage (QR-240-130).

(ER-240-130). There was a statistically significant difference in the mean CFU of erythromycin-resistant *E. coli* between the different ultrasonic dosages except between *ER-60-130* and *ER-240-130* ($P < 0.05$). Results on the ultrasonic experiment for the removal of quinolone-resistant *E. coli* are presented in Figure 4. From an initial value of 8 log units of the quinolone-resistant *E. coli*, there was a reduction to a final value of 1 log units at time 240 min and ultrasonic dosage of 130 kHz (QR-240-130). There was a statistically significant difference in the mean CFU of sulfonamide-resistant *E. coli* between the different ultrasonic dosages except between QR-60-130 and QR-240-130 ($P < 0.05$).
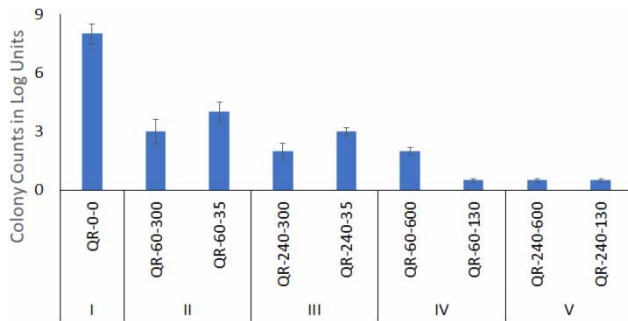

Figure 5 │ CFUs for erythromycin-resistant *E. coli* at different UV radiation and ultrasonic dosages for different time durations. Time 0 and no UV and ultrasonic dosage (ER-0-0), time 60 min at 300 mW·s·cm^{-2} UV dosage (ER-60-300); time 60 min at 35 kHz of ultrasonic dosage (ER-60-35); time 240 min at 300 mW·s·cm^{-2} UV dosage (ER-240-300); time 240 min at 35 kHz of ultrasonic dosage (ER-240-35); time 60 min at 600 mW·s·cm^{-2} UV dosage (ER-60-600); time 60 min at 130 kHz of ultrasonic dosage (ER-60-130); time 240 min at 600 mW·s·cm^{-2} UV dosage (ER-240-600); time 240 min at 130 kHz of ultrasonic dosage (ER-240-130).

Comparison between UV radiation and ultrasonic purification experiments

Results on comparison between the UV radiation and ultrasonic experiment in the removal of erythromycin-resistant *E. coli* are provided in Figure 5. There was a statistically significant difference between 300 mW·s·cm^{-2} for 60 min of UV dosage (ER-60-300) and 35 kHz for 60 min of ultrasonic dosage (ER-60-35) and also between 300 mW·s·cm^{-2} for 60 min of UV dosage (ER-240-300) and 35 kHz for 240 min of ultrasonic dosage (ER-240-35). However, there were statistical differences between 600 mW·s·cm^{-2} for 240 min of UV dosage (ER-240-600) and 130 kHz for 240 min of ultrasonic dosage (ER-240-130) and also between 600 mW·s·cm^{-2} for 60 min of UV dosage (ER-60-600) and 130 kHz for 60 min of ultrasonic dosage (ER-60-130) ($P > 0.05$).

Results on comparison between the UV radiation and ultrasonic experiments in the removal of quinolone-resistant *E. coli* are provided in Figure 6. There was a statistically significant difference between 300 mW·s·cm^{-2} for 60 min of UV dosage (QR-60-300) and 35 kHz for 60 min of ultrasonic dosage (QR-60-35); between 300 mW·s·cm^{-2} for 240 min of UV dosage (QR-240-300) and 35 kHz for 240 min of ultrasonic dosage (QR-240-35) and also between 600 mW·s·cm^{-2} for 60 min of UV dosage (QR-60-600) and 130 kHz for 60 min of ultrasonic dosage (QR-60-130). However, there was no statistical significant difference between 600 mW·s·cm^{-2} for 240 min of UV dosage (QR-240-600)
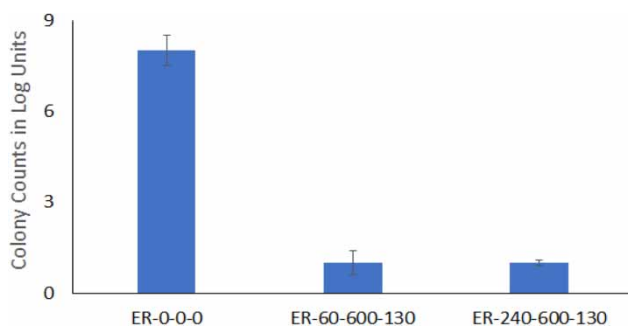
Figure 6 | CFUs for quinolone-resistant *E. coli* at different UV radiation and ultrasonic dosages for different time durations. Time 0 and no UV and ultrasonic dosage (TR-0-0), time 60 min at 300 mW·s·cm^{-2} UV dosage (QR-60-300); time 60 min at 35 kHz of ultrasonic dosage (QR-60-35); time 240 min at 300 mW·s·cm^{-2} UV dosage (QR-240-300); time 240 min at 35 kHz of ultrasonic dosage (QR-240-35); time 60 min at 600 mW·s·cm^{-2} UV dosage (QR-60-600); time 60 min at 130 kHz of ultrasonic dosage (QR-60-130); time 240 min at 600 mW·s·cm^{-2} UV dosage (QR-240-600); time 240 min at 130 kHz of ultrasonic dosage (QR-240-130).

and 130 kHz for 240 min of ultrasonic dosage (QR-240-130) ($P < 0.05$).

Combined UV radiation and ultrasonic purification experiments

Results on the combined UV radiation and ultrasonic experiment for the removal of erythromycin-resistant *E. coli* are presented in Figure 7. From an initial value of 8 log units of the erythromycin-resistant *E. coli*, there was a reduction to a final value of 1 log unit at UV radiation dosage of 600 mW·s·cm^{-2} and ultrasonic dosage of 130 kHz for 240 min (ER-240-600-130). Results on the combined UV radiation and ultrasonic experiment for the removal of quinolone-resistant *E. coli* are presented in

Figure 8. From an initial value of 8 log units of the quinolone-resistant *E. coli*, there was a reduction to a final value of 1 log unit at UV radiation dosage of 600 mW·s·cm^{-2} and ultrasonic dosage of 130 kHz for 240 min (SR-240-600-130).

DISCUSSION

Even though untreated wastewater can spread diseases, especially arising from resistant pathogens, most water quality institutions still give little thought to what happens to their wastewater, and the availability of safe, clean drinking water is often taken for granted (Schijven *et al.* 2019). In the past decade, cholera and other wastewater-related diseases were generally viewed as threats only for the less developed countries, but due to the rise in wastewater-related resistant genes, the crisis is currently global, and no region or country is safe (Allen *et al.* 2010; Berendonk *et al.* 2015; Schijven *et al.* 2019). UV radiation is known to inhibit cell growth and induce gene damage and has been used as a method to sterilize water and other medical instruments, because it not only kills the bacteria present but also disrupts bacterial reproduction (Vermeulen *et al.* 2008). Bacterial pathogens on various media respond differently to UV light exposure, and this highly depends on the physical characteristics of the medium (Adhikari *et al.* 2015). Indeed, various forms of UV such as UV–TiO$_2$ photocatalysis technology has been shown to be a promising non-chemical and residue-free method with reduced water usage and more environment friendly for ensuring microbiological safety and maintaining the nutritional quality of fresh blueberries and other fresh
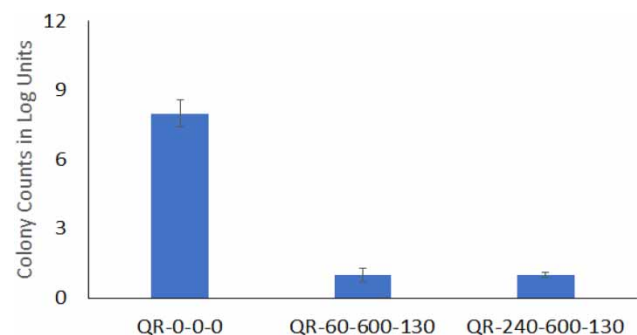

Figure 7 | CFUs for erythromycin-resistant *E. coli* at combined UV radiation and ultrasonic dosages for different time durations. Time 0 and no UV and ultrasonic dosage (ER-0-0); time 60 min at 600 mW·s·cm^{-2} UV and 130 kHz of ultrasonic dosage (ER-60-600-130); time 240 min at 600 mW·s·cm^{-2} UV and 130 kHz of ultrasonic dosage (ER-240-600-130).

Figure 8 | CFUs for quinolone-resistant *E. coli* at combined UV radiation and ultrasonic dosages for different time durations. Time 0 and no UV and ultrasonic dosage (TR-0-0); time 60 min at 600 mW·s·cm^{-2} UV and 130 kHz of ultrasonic dosage (TR-60-600-130); time 240 min at 600 mW·s·cm^{-2} UV and 130 kHz of ultrasonic dosage (TR-240-600-130).

produce during postharvest processing (Mijin *et al.* 2018). For proper wastewater management, there is a need to intrinsically link the process to management of the entire water chain. It is essential that wastewater management is considered as a part of the integrated and ecosystem-based management that operates across sectors and borders such as freshwater and marine (Romina *et al.* 2018).

Apart from the nature of the media and the form of UV radiation, there are numerous factors that may also contribute to the efficiency of UV application in wastewater purification. Findings by Oliveira *et al.* (2018) characterized the inactivation of *E. coli* O157:H7 and *Listeria innocua* under different experimental conditions, such as UV concentration, time of light exposure, incubation temperature, pH and chemical oxygen demand content. The findings of the present study were in agreement with the previous findings where UV radiation experiment was able to significantly eliminate erythromycin- and quinolone-resistant *E. coli* from the water. The UV technology was able to reduce the erythromycin-resistant *E. coli* CFUs from 8 log units to below 2 log units within 60 min at 600 mW·s·cm^{-2} UV dosage (ER-60-600). However, for the elimination of quinolone-resistant *E. coli*, the initial 8 log units were reduced to below 2 log units at 240 min at 600 mW·s·cm^{-2} UV dosage (QR-240-600). This indicated higher resistance of the quinolone-resistant than the erythromycin forms of *E. coli*. Therefore, the different resistant ability by different forms of *E. coli* is a factor for consideration in developing wastewater purification technologies, as had been reported by Donde *et al.* (2018).

Efforts have also been invested to provide a deeper understanding on the design and operation parameters necessary for scaling up the US technology as a disinfection stage in municipal wastewater treatment trains (Li *et al.* 2016). It is also important to evaluate the contribution of US on wastewater treatment by considering its role in the removal of both chemical and biological parameters such as nitrogen, phosphorus, dissolved organic matter and other more resistant pathogens, and associated resistant genes (Leonel *et al.* 2018). Investigation on the application of US technology has identified various action mechanisms to different microbial species. For instance, the impairment of the cell membrane, the inactivation of enzymatic activity and the inhibition of metabolic performance are involved in the process of sterilization of *E. coli* and *Staphylococcus aureus* (Jiao *et al.*

2016). Moreover, the study by Jiao *et al.* (2016) reported that the initial concentrations of *E. coli* and *S. aureus* had no significant relationship with the extent of US-induced damage under experimental conditions. This is due to the variation in the destructive mode of action and varying target sites by US. For instance, the target sites may be the outer membrane, the cell wall, the cytoplasmic membrane and the inner structure, and the variation depends on the target organism. The ultrasonic treatment can be used to eliminate both vegetative cells of Gram-positive and Gram-negative bacteria from both the bacterial suspensions and phytoviruses. Moreover, a higher number of pathogenic bacteria are inactivated by using a higher ultrasonic power than the lower one (Antanas *et al.* 2018). The presented study agreed with the previous findings where US technology was able to significantly reduce both the erythromycin- and quinolone-resistant *E. coli* CFUs from 8 log units and 8 log units, respectively, to below 2 log units within 60 min and the ultrasonic dosage of 130 kHz (Adhikari *et al.* 2015; Antanas *et al.* 2018).

Unfortunately, neither the UV radiation nor the lethal effects of US irradiation on microorganisms' spore and some phytoviruses have been sufficient to eliminate various forms of pathogenic *E. coli* to near zero CFU. In this regard, US in combination with other microorganism inactivation techniques such as UV radiation has always been recommended (Antanas *et al.* 2018). This was concurrent with the findings from this study, where the integration of UV and US technologies further reduced both the erythromycin- and quinolone-resistant *E. coli* to near zero values. Indeed, studies have suggested the combination of US treatment with other treatment techniques for increased pathogen reduction and microbial safety on water and food (Hun *et al.* 2011).

CONCLUSION AND RECOMMENDATIONS

The study showed that UV radiation experiment was able to significantly eliminate erythromycin- and quinolone-resistant *E. coli* from the wastewater to or less than 100 CFU. Additionally, US technology was equally able to significantly reduce both the erythromycin- and quinolone-resistant *E. coli* to below 2 log units. However, on combining the two technologies, there was further reduction to 1 log unit. Hence, pointing to the need for integrative approaches in wastewater

purification for increased wastewater treatment efficiencies and improved ecosystem and human health. How wastewater is treated and reused is the key to successfully meeting the vast water requirements of an urban population. This must transcend the entire water supply, wastewater treatment and disposal chain, involving the production and treatment of wastewater. Therefore, the study lays more emphasis on reducing the volume and extent of water pollution through preventive practices, capturing and properly treating water once it has been polluted or contaminated, treating polluted water using appropriate technologies such as integrated UV radiation and US techniques for return to the environment. Indeed, where feasible, safely reuse and recycling wastewater should be embraced, thereby conserving water also provides a platform for the development of new and innovative technologies and management practices within the wastewater quality management sector. Additionally, a paradigm shift is required towards new integrative innovative approaches that not only include wiser management and technological innovation. This is because not one size fits all but ensuring that wastewater treatment technologies are appropriate to all the wastewater from various sources. Such innovation is necessary at both ends of the pipe to reduce the volume and contamination of wastewater produced. This may also enhance the approaches on how to treat or even reuse the wastewater in an affordable and sustainable manner. Hence, the study recommends the incorporation of integrated UV–ultrasonic technologies in the removal of erythromycin- and quinolone-resistant *E. coli* from domestic wastewater for improved water quality and health.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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