Evaluation of solar light inactivation on multidrug-resistant *Escherichia coli* CGMCC 1.1595

Xiu-Feng Yin, Na Shi, Ting Meng and Ying-Xue Sun

ABSTRACT

This study investigated the simulated solar light disinfection of *Escherichia coli* CGMCC 1.1595, a multidrug-resistant (MDR) strain resistant to tetracycline and ampicillin. With the increase of light intensity, the maximum inactivation efficiency reached 0.74 log in 60 min following visible light irradiation with an intensity of 115.8 mW/cm^2 and following UVA–visible light irradiation, using a 98% UVA-ray contribution at 6.5 mW/cm^2 and 95% contribution at 20.0 mW/cm^2, the inactivation efficiency was up to 6.09 log. The inactivated MDR *E. coli* did not regrow after light irradiation or in the dark after 24 or 48 h after visible light disinfection, demonstrating that visible light disinfection can prevent MDR *E. coli* self-repair. The MDR *E. coli* plasmid electrophoresis band gradually went dark with increase of the light irradiation time and could be completely eliminated by high UVA light intensity treatment, however, simulated sunlight irradiation had minimal influence on both tetracycline and ampicillin resistance of the MDR *E. coli* strain.

Key words | inactivation, multidrug resistant *E. coli* strain, plasmid elimination, solar light radiation, tetracycline resistance shift

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HIGHLIGHTS

- Simulated solar light can inactivate multidrug resistant (MDR) *Escherichia coli* CGMCC 1.1595.
- The inactivation efficiency of MDR *E. coli* reached 0.74 log in 60 min under visible light irradiation.
- The inactivation efficiency of MDR *E. coli* was up to 6.09 log under UVA–visible light irradiation.
- Visible light disinfection can prevent MDR *E. coli* self-repair.
- Simulated sunlight irradiation had minimal impact on the tetracycline and ampicillin resistance of MDR *E. coli*.

INTRODUCTION

The global increase in antibiotic consumption has resulted in large quantities of pharmaceutical origin being found in sewage and wastewater treatment plants (Rizzo *et al.* 2013; Kraemer *et al.* 2019). One of the most severe consequences of antibiotic pollution is the rise in antibiotic resistance (Kümmerer 2009). Antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) are extensively detected in wastewater effluents and aquatic environments worldwide and pose a serious threat to aquatic ecosystems and human health (Ouyang *et al.* 2015; Xu *et al.* 2016; Gao *et al.* 2018). The aquatic environment is considered to be a particularly vast reservoir of ARGs and is also where ARB can emerge following the transfer of ARGs between autochthonous and allochthonous bacteria (Shao *et al.* 2018; Almakki *et al.* 2019). A few chromosomal ARGs transmit to their offspring through vertical gene transfer and most plasmid ARGs may transfer between bacterial strains via horizontal gene transfer (Forbes & Schaberg 1983; Rasmussen & Sørensen 1998; Gao *et al.* 2018).

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The spread of ARBs in aquatic environments is affected by many factors including sunlight radiation, temperature, and dissolved oxygen, and among these sunlight radiation is of significance (García-Fernández *et al.* 2015; Giannakis *et al.* 2018a). Solar light can inactivate microorganisms in surface waters (Boehm *et al.* 2009). Giannakis *et al.* (2018b) found that a 6 log inactivation of a streptomycin-resistant strain could be achieved after 2 h irradiation with a simulated solar light intensity of 1,200 W/m^2. The mechanism of solar light inactivation involves direct photolysis of photo-oxidation and indirect photolysis of photosensitivity reactions through which the absorption of solar radiation by photosensitizers in the water results in the formation of active intermediate action cells (Silverman *et al.* 2013).

Solar light is mainly composed of ultraviolet A (UVA) (320–400 nm), ultraviolet B (UVB) (290–320 nm), ultraviolet C (UVC) (200–290 nm), visible (400–700 nm) and infrared light (Silverman *et al.* 2013). UVB/C can be directly absorbed by DNA, leading to the formation of lesions in pyrimidine and purine bases (Lian *et al.* 2018), however, UVC cannot reach the Earth's surface because of the ozone layer in the stratosphere and most UVB quickly decays in natural water (Kadir & Nelson 2014; Lian *et al.* 2018). UVA's effects on DNA include direct and UVA oxidative damage. Direct damage is the destruction of DNA by the formation of the cyclobutane pyrimidine dimer (Giannakis *et al.* 2016). In a visible light disinfection system, microbacteria can use endogenous photosensitizers such as NADH and cytochromes to absorb photons (Lavi *et al.* 2004) at 400–500 nm which can produce reactive oxygen species (ROS) that attack cell membranes and cause cell damage (Garza *et al.* 2018). UVA rays make up approximately 95% of total UV radiation but only 10% of the solar energy that reaches the Earth's surface, while visible light accounts for 40% of the solar energy. It is therefore important to understand microbial inactivation in surface waters by UVA rays and visible light.

Jiménez-Tototzintle *et al.* (2018) found that the efficiency of *P. aeruginosa* inactivation increased along with the UVA light dose, with a maximum of 6 log achieved after 3 h irradiation with 7.74 kJ/L of accumulated UVA intensity. Lui *et al.* (2016) investigated the ability of semi-commercial LED arrays (270–740 nm) to inactivate *Escherichia coli* K12 ATCC W3110 and *Enterococcus faecalis* ATCC 19433, and found that inactivations of 5 log and greater were consistently achieved after 6 h irradiation with the 270, 365, 385 and 405 nm arrays. Visible light is not as effective at microbacterial disinfection as UVA rays. Li *et al.* (2017) found that the efficiency of *Escherichia coli* DH5α inactivation was less than 1 log after 6 h irradiation using a xenon lamp (300 W) with $\lambda > 420$ nm.

High visible light intensity can promote the absorption of more photons by endogenous cellular photosensitizers, leading to the generation of high levels of ROS, which will damage bacteria (Lubart *et al.* 2011), however, it is difficult for solar light disinfection to eliminate antibiotic resistances in *E. coli*. Giannakis *et al.* (2018b) found that the $bla_{\text{ctx-m-9}}$ gene in *E. coli* ESBL 8543 was not eliminated after 4 h solar light disinfection and Rizzo *et al.* (2012) reported that while solar light radiation can reduce ciprofloxacin resistance (MIC decreased by 33% after 3 h irradiation) in an MDR *E. coli* isolate from a wastewater treatment plant, it did not impact its resistance to amoxicillin (MIC > 256 μg/mL) and sulfamethoxazole (MIC > 1,024 μg/mL).

In this paper, the effect of light irradiation on tetracycline and ampicillin resistance in *Escherichia coli* CGMCC 1.1595, an MDR, was studied using simulated solar light. The inactivation efficiency of different light intensity conditions was analyzed and the photoreactivation and dark repair effects were also investigated. Furthermore, the inactivation mechanism was examined using a plasmid elimination test and observing changes in antibiotic susceptibility.

MATERIALS AND METHODS

Microorganism

The MDR *E. coli* strain (*E. coli* CGMCC 1.1595) was provided by the Institute of Microbiology of the Chinese Academy of Sciences. This strain harbors the plasmid pBR322 which confers resistance to tetracycline (TET) and ampicillin (AMP) (Huang *et al.* 2013; Pang *et al.* 2016).

Sample preparation

The MDR *E. coli* was cultured by removing a single colony from plates, subculturing in nutrient broth (g/L) (peptone:

10.0, beef extract: 3.0, NaCl: 5.0, pH: 7.2) with 16 mg/L TET and 32 mg/L AMP at 37 °C overnight in a rotary shaker (200 rpm). The cells were collected by centrifugation (5 min at 12,000 rpm at 4 °C), washed twice with sterile physiological solution, and resuspended in sterile physiological solution at a concentration of approximately 10^6 CFU/mL.

Light irradiation experiment

Light irradiation experiments were performed using an XPA-7 photochemical reactor (Xujiang Electromechanical Plant, China) equipped with a constant-temperature water tank. The bacterial suspension was continuously stirred in the reactor while light irradiation was provided by 100, 300, 500 W mercury and 1,000 W xenon lamps equipped with 300 nm or 400 nm optical filters to simulate UVA–visible light ($\lambda > 300$ nm) and visible light ($\lambda > 400$ nm). During light irradiation disinfection, light intensities with wavelengths of 300–400 nm and 400–760 nm were measured with UV-A and FZ-A irradiance meters, respectively. The light dose (D) was the product of the light irradiation time (T) and light intensity (I).

Bacterial count

Bacterial counts were performed using the spread plate method. Briefly, small amounts of bacterial suspension were diluted according to the expected number of colonies; 100 μL of diluted bacterial suspension was spread onto a nutrient agar plate (g/L) (peptone: 10, beef extract: 3, NaCl: 5, agar: 15, pH: 7.2) and incubated at 37 °C for 24 h. Measurements were made in triplicate and the average values and standard deviation were plotted as CFU/mL. The inactivation efficiency of the sample was expressed as logarithmic inactivation efficiency ($\lg N_0/N_t$, where N_0 and N_t represent the concentration of the bacterial suspension before and after light irradiation disinfection).

Photoreactivation and dark repair

After light irradiation, bacterial suspension samples were transferred to Petri dishes for photoreactivation and dark repair tests. For the photoreactivation capacity, dishes were placed on magnetic stirrers, exposed to light radiation for 48 h and bacterial concentration counted at 24 h intervals. To assess dark repair capacity, dishes were placed on magnetic stirrers in the dark for 48 h and bacterial concentration counted at 24 h intervals. The photoreactivation/dark repair efficiency was calculated using Equation (1):

$$\text{Reactivation efficiency} = \frac{N_r - N_t}{N_0 - N_t} \times 100\% \quad (1)$$

where N_0 and N_t represent bacterial concentration before and after light irradiation disinfection; N_r represents hatchability of the inactivated microbe exposed to light (photoreactivated) or dark (dark repair).

Plasmid elimination test

The plasmid elimination test was conducted with 100, 300, and 500 W mercury and 1,000 W xenon lamps. Strains were inoculated in nutrient broth supplemented with 16 mg/L TET and 32 mg/L AMP at 37 °C and 200 rpm for 14 h, then centrifuged at 12,000 *g* for 5 min, and washed twice with sterile water to remove medium and antibiotics. Bacteria were resuspended in sterile water and added into a tube to eliminate the plasmid. The plasmid was extracted using the Takara Minibest Purification Plasmid Kit ver.4.0 (Takara Plasmid, China).

Antibiotic resistance assay

Bacterial antibiotic resistance before, and after, plasmid elimination was assessed with the Kirby–Bauer method according to Clinical and Laboratory Standards Institute (2013). After plasmid elimination, colonies were transferred into 10 mL of physiological solution to achieve a concentration of 10^8 CFU/mL (0.5 McFarland). Bacterial suspensions were then spread onto Mueller–Hinton agar (g/L) (casein hydrolysate: 17.5, starch: 1.5, beef extract: 5, agar: 12.5, pH: 7.2) using a sterile cotton swab. Antibiotic discs of TET (30 mg) and AMP (30 mg) (Hang Zhou Microbial Reagent Co., Ltd, China) were placed on the surface of each inoculated plate. After incubation for 16–18 h at

35 ± 2 °C, the diameters of the growth inhibition zones were measured.

Stable inheritance of antibiotic resistance after plasmid elimination

After irradiation with either 100, 300, and 500 W mercury or 1,000 W xenon lamps, the MDR *E. coli* was subcultured in nutrient broth containing either no antibiotic, 16 mg/L TET, or 32 mg/L AMP (37 °C, 200 rpm, 14 h). Antibiotic resistance was assessed with the Kirby–Bauer method every two generations to track the variation tendency.

RESULTS AND DISCUSSION

MDR *E. coli* inactivation

Inactivation of MDR *E. coli* using simulated solar light was performed using various light intensities. The light intensities of visible light ($\lambda > 400$ nm) generated by 100, 300, and 500 W mercury and 1,000 W xenon lamps were 18.9, 27.3, 40.2, and 115.8 mW/cm^2, respectively. During the visible light disinfection, the inactivation efficiency increased with irradiation time and reached 0.09, 0.14, 0.28 and 0.74 log at 1 h with light intensities of 18.9, 27.3, 40.2, and 115.8 mW/cm^2 (Figure 1(a)). Furthermore, the inactivation efficiency increased with light intensity and dose at certain irradiation times (Table S1, Supporting Information). Such results might be caused by bacteria using endogenous photosensitizers to produce ROS, which can attack bacteria, disrupt bacterial defense and also prevent bacterial photoreactivation under high visible light intensity (Rincón & Pulgarin 2003).

The simulated solar light of $\lambda > 300$ nm was produced using the 100, 300, and 500 W mercury and 1,000 W xenon lamps combined with 300 nm optical filters. The visible light intensities were 18.9, 27.3, 40.2, and 115.8 mW/cm^2, while the UVA light intensities were 6.5, 10.0, 20.0 and 2.83 mW/cm^2, respectively. Figure 1(b) shows the inactivation efficiency of light radiation of $\lambda > 300$ nm. During this light radiation disinfection, the inactivation efficiency increased with the irradiation time, reaching 6.09, 1.17, 6.09 and 1.16 log at 1 h (Figure 1(b)). While the inactivation

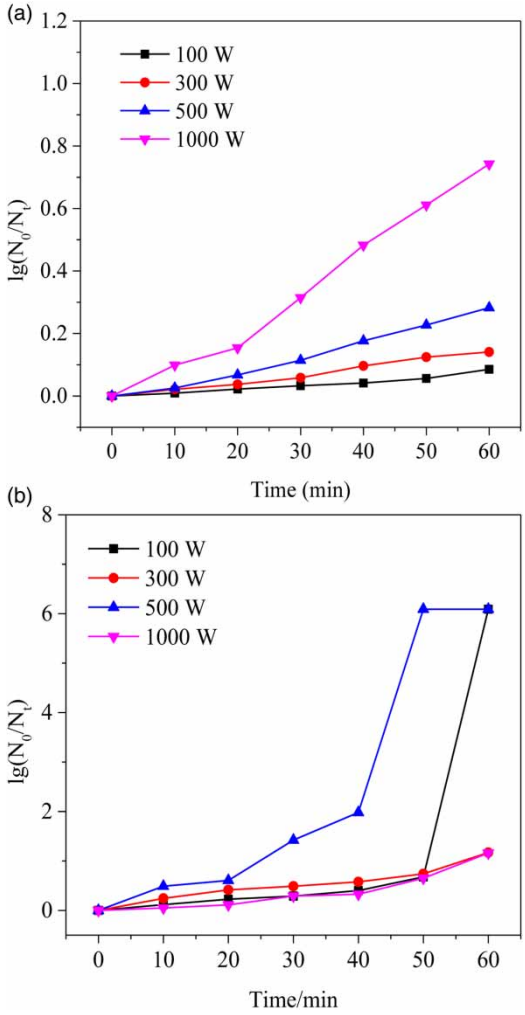

Figure 1 | Inactivation of MDR *E. coli* under (a) $\lambda > 400$ nm and (b) $\lambda > 300$ nm light irradiation.

efficiency was up to 6.09 log under $\lambda > 300$ nm light radiation, UVA–visible light disinfection efficiency was higher.

With $\lambda > 300$ nm light radiation for 1 h, the contributions of UVA rays to the inactivation rate were approximately 98%, 88%, 95% and 36%, where the light intensities were 6.5, 10.0, 20.0 and 2.83 mW/cm^2, respectively. In the UVA–visible light disinfection system the inactivation efficiency was controlled by the UVA light intensity and dose (Table S2). UVA can lead the photosensitizer to absorb UVA photons and generate ROS, which attacks DNA and causes oxidative damage, resulting in bacterial damage (Baier *et al.* 2006; Ito *et al.* 2007). Moreover, high light intensity may obtain a high flow of photons, and

increased photons can directly attack bacteria to increase the inactivation efficiency (Xiong & Hu 2013). The inactivation increased rapidly with the light radiation of $\lambda >$ 300 nm within 50 min irradiation time, and then exhibited a lag phase, which may be due to the accumulation of UVA oxidation-related injury (Giannakis *et al.* 2016).

Photoreactivation and dark repair

The photoreactivation and dark repair percentages of the MDR *E. coli* varied between the visible light and UVA–visible inactivation groups (Figure 2). The photoreactivation percentages of MDR *E. coli* inactivated by 1 h exposure to the visible light intensities of 18.9, 27.3, 40.2 and 115.8 mW/cm^2 were −355%, −231%, −64% and −22% after 24 h continuous light irradiation and −346%, −229%, −77% and −22%, respectively after 48 h (Figure 2(a)). Although the inactivation efficiency of visible light disinfection with 115.8 mW/cm^2 was lower compared with UVA–visible, the inactivated MDR *E. coli* did not regrow after light irradiation for 24 h and 48 h. This might be due to the high flow of photons that a high light intensity obtains, which can prevent bacterial reactivation (under visible light) (Xiong & Hu 2013).

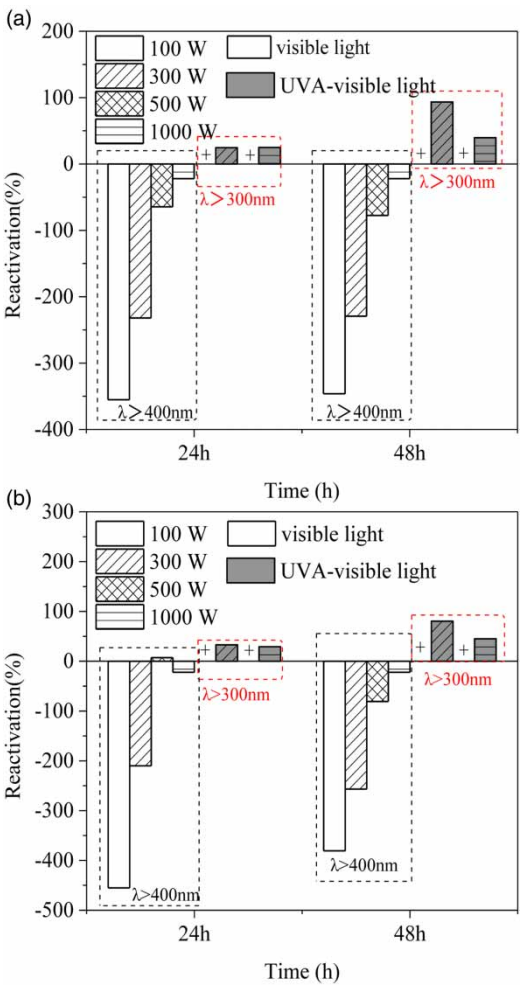

Figure 2 | (a) Photoreactivation and (b) dark repair of the MDR *E. coli*.

There was no photoreactivation of MDR *E. coli* inactivated by 1 h of UVA–visible light simulated by the 100 W (with UVA and visible light intensity of 6.5 and 25.4 mW/cm^2, respectively) and 500 W mercury lamps (with UVA and visible light intensity of 20.0 and 61.2 mW/cm^2, respectively). The photoreactivation percentages of the MDR *E. coli* inactivated by 1 h of UVA–visible light simulated by the 200 W mercury (with UVA and visible light intensity of 10.0 and 27.3 mW/cm^2, respectively) and 1,000 W xenon lamps (with UVA and visible light intensity of 2.83 and 115.8 mW/cm^2, respectively) were 24% and 24.8% after 24 h continuous light irradiation, and 93% and 39.5% after 48 h. The photoreactivation ability of an organism depends on a number of factors including light dose, wavelength, light intensity, and exposure time (Benabbou *et al.* 2007). Xiao *et al.* (2018) found that *E. coli* ATCC 15597 and 700891 resurrected after 4 h irradiation under an LED lamp (10 klx) after UVA–visible pre-radiation and that the effect of a low dose of UVA–visible light (1.08×10^5 J/cm^2) on ROS production was not enough to cause damage to the bacteria. Photoreactivation may rely on pyrimidine dimers that were not completely damaged by light irradiation and the photolyase was still active after binding to the dimer (Wen *et al.* 2019).

The dark repair percentages of MDR *E. coli* inactivated by 1 h of visible light intensities of 18.9, 27.3, 40.2 and 115.8 mW/cm^2, were −455%, −209.9%, 7.18% and −22% after 24 h in the dark, and −380%, −256.7%, −80.1% and −22% after 48 h (Figure 2(b)). Similar to the photoreactivation results, MDR *E. coli* inactivated by visible light with 115.8 mW/cm^2 intensity did not regrow after 24 and 48 h in the dark. It is possible that in the visible light disinfection system, ROS could persist and prevent the dark repair capacity of MDR *E. coli* (Xiong & Hu 2013). Furthermore, there was no dark repair observed in MDR *E. coli*

inactivated with 1 h of UVA–visible light simulated by the 100 and 500 W mercury lamps. The dark repair percentages of MDR *E. coli* inactivated by 1 h of UVA–visible light simulated by the 200 W mercury and 1,000 W xenon lamps were 32.8% and 29% after 24 h in the dark, and 80.4% and 45% after 48 h. This might be because the UVA light irradiation dose was lower than 1.8×10^5 J/cm^2 and could not therefore inhibit bacterial repair (Xiao *et al.* 2018). The completely inactivated MDR *E. coli* did not self-repair after 48 h in the dark, which is consistent with a previous study (Benabbou *et al.* 2007). Visible light has a residual disinfection effect, which prevents MDR *E. coli* self-repair. UVA disinfection requires a high light dose to prevent MDR *E. coli* performing the dark repair process.

Effect of light irradiation on plasmid elimination

TET and AMP resistance of MDR *E. coli* did not change during 1 h light irradiation under the study conditions. Figure S1 (Supporting Information) illustrates the change of TET inhibition zone diameter under $\lambda > 400$ nm and $\lambda > 300$ nm light irradiation and shows that its diameter was less than 11 mm. MDR *E. coli* did not form an AMP inhibition zone in this light irradiation system. This suggests that the MDR *E. coli*'s TET and AMP resistance was not affected by $\lambda > 400$ nm and >300 nm light radiation, which agrees with a previous study which showed that the $bla_{\text{ctx-m-9}}$ gene in *E. coli* ESBL 8543 was not eliminated after solar light disinfection for 4 h (Giannakis *et al.* 2018b).

To evaluate the effects of full wavelength light irradiation on the antibiotic resistance of MDR *E. coli*, the influence of light irradiation on plasmid elimination was explored. The plasmid was digested with *Hin*dIII and the reaction product separated on a 0.7% agarose gel by electrophoresis at 110 V (Figure 3). Quantitative analysis using ImageJ software to measure the gray value of the electrophoresis bands with the marker used as an internal reference is shown in Figure 4. With increasing light irradiation time the relative gray value of the plasmid electrophoresis band gradually decreased. Light irradiation dose was also an important factor that influenced plasmid elimination. The relative gray value of the plasmid band decreased from 0.38 to 0 during 10 min light irradiation time under the 500 W mercury lamp, which indicates that the plasmid can be completely eliminated by high UVA light intensity.

Xenon lamp irradiation (mainly visible light) had a lower impact on plasmid elimination than mercury lamp irradiation (mostly UVA). During visible light disinfection, endogenous cellular photosensitizer bacteria can absorb the visible light photons, generating ROS which cause bacterial damage (Lubart *et al.* 2011). UVA could penetrate the cell membrane and cytoplasm, and covalently bind two thymine bases adjacent to the bacterial DNA to form dimers, thus damaging the pyrimidines and purine bases (McGuigan *et al.* 2012) and the configuration of DNA and RNA, interfering with its normal replication (Horai *et al.* 2017).

Changes in antibiotic susceptibility of MDR *E. coli*

The Kirby–Bauer test was used to assess MDR *E. coli* resistance to TET (Figure 5). The resistance, mediation, and sensitivity of *E. coli* CGMCC 1.1595 to TET and AMP were judged according to the diameter of the inhibition zone. According to the standard of Clinical and Laboratory Standards Institute (2013), the TET tolerance of *Enterobacteriaceae* is determined by the inhibition zone diameter (D) as follows: D > 15 mm is sensitive, D between 12 and 14 mm is intermediary, and D < 11 mm is resistant. AMP tolerance of *Enterobacteriaceae* is determined by the inhibition zone diameter (D) as follows: D > 17 mm is sensitive, D between 14 and 16 mm is intermediary, D < 13 mm is resistant. In brief, the smaller the inhibition diameter, the higher the bacterial resistance to the antibiotic.

The average inhibition zone diameter for TET before light irradiation was 8.5–9.7 mm, and after 10 min light irradiation, the diameter was less than 11 mm (Figure 5). This indicates that MDR *E. coli* resistance to TET was not altered by solar light irradiation. MDR *E. coli* did not form an AMP inhibition zone after either mercury or xenon lamp irradiation. Giannakis *et al.* (2018b) reported that the $bla_{\text{ctx-m-9}}$ gene in *E. coli* ESBL 8543 was not eliminated after 4 h solar light disinfection and Rizzo *et al.* (2012) found that while solar light radiation can influence ciprofloxacin resistance (MIC decreased by 33% after 180 min of irradiation), it had no impact on amoxicillin (MIC > 256 μg/mL) and sulfamethoxazole (MIC > 1,024 μg/mL) resistances. From our results, we see that solar light

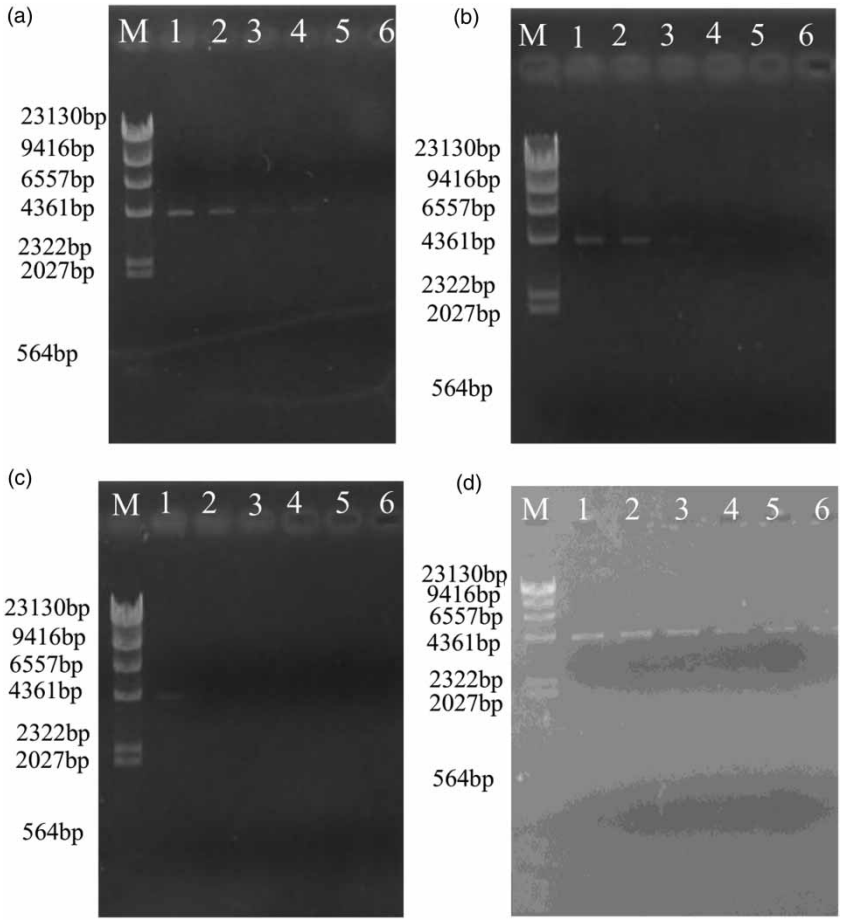

Figure 3 | Agarose gel electrophoretogram of the plasmid DNA after light irradiation stimulated by (a) 100 W, (b) 300 W, (c) 500 W mercury and (d) 1,000 W xenon lamps; 1–6 represent 0, 2, 4, 6, 8, 10 min, respectively.

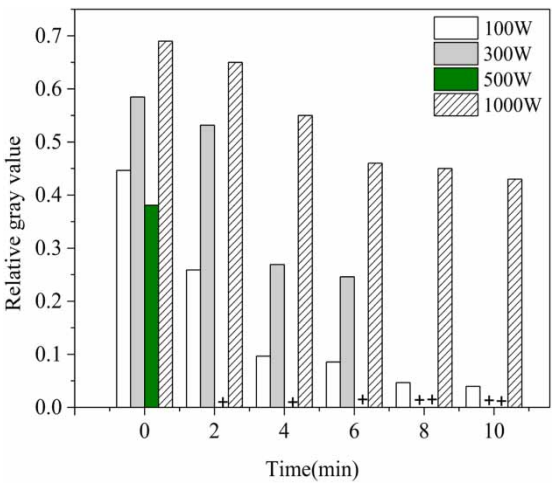

Figure 4 | Relative gray value of the electrophoresis bands of plasmid DNA after light irradiation.

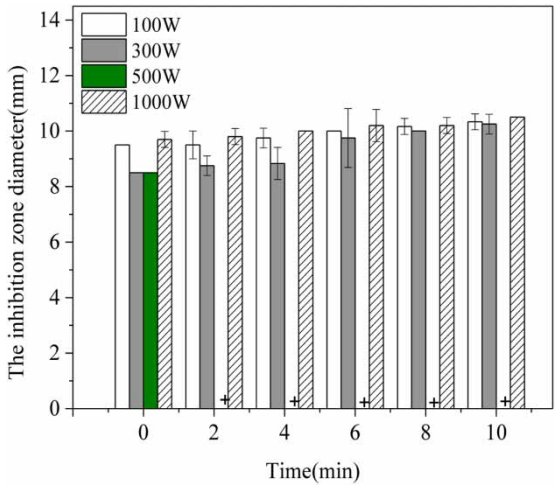

Figure 5 | Tetracycline (TET) inhibition zone diameter before and after plasmid elimination.

Table 1 | Stable inheritance of TET resistance after plasmid elimination

Light source	Medium	Generation					
		0	2	4	6	8	10
100 W	LB	10.3	10.5	9.5	9.2	10.5	8.7
	LB + TET + AMP	10.3	9.3	9.2	9.7	10.0	8.7
300 W	LB	10.3	10.0	9.2	9.3	9.3	8.5
	LB + TET + AMP	10.3	9.2	9.3	9.5	9.7	10.0
500 W	LB	N/A	N/A	N/A	N/A	N/A	N/A
	LB + TET + AMP	N/A	N/A	N/A	N/A	N/A	N/A
1,000 W	LB	10.5	10.5	10.2	9.0	9.5	9.5
	LB + TET + AMP	10.5	10.0	10.0	10.0	9.2	9.3

N/A, not available.

irradiation had no influence on AMP and TET resistance in the MDR *E. coli*.

After plasmid elimination by light irradiation, the MDR *E. coli* were cultured in nutrient broth containing no antibiotics, 16 mg/L TET, or 32 mg/L AMP for ten generations. During this process, MDR *E. coli* did not form an AMP inhibition zone (Table 1) and resistance to TET and AMP did not change as the number of passages increased. In addition, there was no difference in the change of TET resistance between the MDR *E. coli* cultured in nutrient broth containing no antibiotics, TET, or AMP. This suggests that the antibiotic itself might not have an inductive effect on antibiotic resistance.

CONCLUSION

This study examined the inactivation of the MDR *E. coli* strain (*E. coli* CGMCC 1.1595) by simulated solar light. During the visible light disinfection, inactivation efficiency increased along with light intensities, reaching 0.74 log at 1 h irradiation time at a light intensity of 115.8 mW/cm^2. Under UVA–visible light irradiation, the inactivation efficiency reached 6.09 log, with UVA rays contributing 36% to 98% to this efficiency at UVA light intensities of 2.83 to 20.0 mW/cm^2. Although the inactivation efficiency of the visible light disinfection at an intensity of 115.8 mW/cm^2 was lower than that of the UVA–visible, the inactivated MDR *E. coli* did not regrow after either 24 or 48 h in light irradiation or in the dark, demonstrating that visible light disinfection can prevent MDR *E. coli* self-repair. As light irradiation time increased, the resistance plasmid electrophoresis band gradually went dark and the plasmid could be eliminated completely by high UVA light intensity. Overall, light irradiation had no influence on TET and AMP resistance of MDR *E. coli*. This may also show that the antibiotic itself may not have an obvious inductive effect on antibiotic resistance.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at https://dx.doi.org/10.2166/ws.2020.124.

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