

Comparison of standard light-emitting diode (LED) and 385 nm ultraviolet A LED (UVA-LED) for disinfection of *Escherichia coli*

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Abstract

UV light has become an integral part of human life especially in performing wide range of disinfection. Most of the research on UVLEDs is limited to UVC region because of comparison with mercury based UV lamps which work typically at 254 nm. Limited research is found on the use of UVA-LEDs for inactivation of microorganisms in healthcare. In this study a standard 3 mm LED has been compared with 385 nm UVA-LED for inactivation of *Escherichia coli*. *E. coli* strains were swabbed on control, LED and UVA-LED petri dishes using cotton bud. The LED and UVA-LED samples were exposed to standard LED light and UVA light respectively for 1 h. The analysis of bacteria by determining Colony forming units (CFU) and log inactivation were carried out to calculate the number of colonies present in each sample. Result showed negligible to none disinfection properties in standard LED light. LED samples had 19×10^6 CFU/ml colonies compared to control which is 27×10^6 CFU/ml. UVA-LED samples achieved maximum inactivation and only had 0.0043×10^6 CFU/ml. Log inactivation results showed that LED samples observed 0.1-log inactivation whereas the UVA-LED had significant inactivation of 3.8-log inactivation corresponding to approximately 99.99% *E. coli* reduction. The results demonstrate that UVA-LED at 385 nm is capable of efficiently providing inactivation of bacteria *E. coli*.

Keywords: Ultraviolet light, UVA, disinfection, light emitting diodes (LEDs), *Escherichia coli* (*E. coli*)

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INTRODUCTION

Disinfection and sterilization have been part of human life for years, providing important means to ensure human well-being is kept at utmost high standards. Disinfection is required for numerous tasks on day to day basis ranging from disinfecting hands before eating to disinfecting medical devices before use. Life without disinfection would cease to exist. Disinfection becomes even more important when it involves healthcare, where every possible effort is made to ensure the highest level of disinfection. A lot of advancements have been made since the beginning of disinfection era however, poor management of disinfection still widely exists worldwide causing millions of deaths each year and costing billions of dollars to treat diseases as a result of poor disinfection practices.

According to World Health Organization (WHO) healthcare-associated infection (HCAI) "... is an infection occurring in a patient during the process of care in a hospital or other health care facility which was not present or incubating at the time of admission" (Organization, 2016). HCAs are considered to be the most common cause of threatening patients safety globally (Allegranzi et al., 2011). Undoubtedly, the exact global impact of HCAs is yet to be determined due to the extreme difficulties experienced in gathering

the reliable data. Poor surveillance of HCAI in most countries has added further complexities in order to obtain accurate statistics. At any given time, in civilized countries 1 in every 20 admitted patient would be affected with HCAI (Koch et al., 2015; Koch et al., 2015; Magill et al., 2014). A study carried by Klevens confirmed that 1.7 million HCAs cases were recorded in US in a single year resulting in a whopping 99,000 deaths and cost an additional \$ 4.5 to 5.7 billion dollars annually (Burke, 2003; Klevens et al., 2007). According to WHO, in Europe these infections account for 37,000 deaths yearly (Organization, 2013). Patients who developed HCAI remained in hospitals 2.5 times longer than average patient and incurred healthcare 3 times the average cost (Narendranath et al., 2017). With such high number of people being affected by HCAI and resultantly its immense costs make the proper disinfection in healthcare absolutely important.

Traditional methods make use of wide range of different procedures to perform disinfection in healthcare. These methods are, but not limited to, heat steam, gas and chemicals. These procedures have been in place for years but sadly not much improvements have been made for their enhancement. Limitations of these existing method as, illustrated in Fig. 1, include the extreme cost required to buy necessary chemical products to keep the disinfection process going. Their tedious and time-consuming processes only make them

worse especially in the hospitals where every single minute is crucial (Matsuyama *et al.*, 1997). Extensive use of these methods not only reduces medical device's performance but also alters the surface structure (Mahoney & Lim, 2012). Furthermore, chemicals sometime cause serious skin related allergies as well as respiratory disorder such as asthma (Arif *et al.*, 2003; Dumas *et al.*, 2012; Kogevinas *et al.*, 2007). A study conducted by Harvard University (Slawson, 2017) which took account of more than 55,185 nurses in US, confirmed that nurses who regularly use disinfectants have as much as 32% risk of developing chronic obstructive pulmonary disease (COPD). Considering the aforesaid limitations, the use of alternative methods such as Ultraviolet (UV) radiation becomes very important to perform disinfection. UV light has been implemented in some hospitals to provide better, reliable and eco-friendly disinfection. UV radiation in disinfection of water has been ongoing for years (Hijnen *et al.*, 2006) but its applications in healthcare is still relatively new and much work needs to be done to make UV a primary source of radiation in the hospitals.

UV light achieves inactivation of microorganisms by directly targeting and damaging the deoxyribonucleic acid (DNA). When DNA absorbs the light, it forms pyrimidine dimers which in result effects the transcription and replication process resultantly disrupting the microorganism ability to reproduce (Rastogi *et al.*, 2010). Mercury based UV lamps are the most common type of UV sources being used in healthcare for disinfection of surfaces to terminal decontamination of pathogen from patients' rooms. Monochromatic and polychromatic are two main types of UV lamps. Monochromatic mercury lamps, also referred as low pressure (LP), emit most of their light at 254 nm wavelength whereas polychromatic can provide light in various wavelengths from 200 - 300 nm (Bohrerova & Linden, 2006). LP mercury lamps are the most common type used in disinfection of water and healthcare.

precautions must be met at all times to ensure safety and to prevent spillage of mercury contents. Moreover, high maintenance cost associated with the lamps adds another layer of distress (Chatzisyneon *et al.*, 2013). Generally, a warm-up time between 2 – 15 minutes is required before operation (Chatterley & Linden, 2010) and frequent replacement of UV lamps is needed due to extremely short lifecycle of 8000 – 10,000 hours (Rasoulifard *et al.*, 2015). Moreover, the cost required for proper disposal of mercury substance after use creates further complications on the continuous use of this technology (Rasoulifard *et al.*, 2015). Unlike mercury lamps, Xeon lamps do not require mercury vapour to produce UV light. Instead, they produce light with the help of Xeon gas and hence considered less hazardous than mercury lamps. Xenex is a Xeon lamp based commercially available product capable of producing entire disinfection spectrum from 200 nm to 320 nm. A study carried out by Michelle (Nerandzic *et al.*, 2015) compared the disinfection effectiveness of Xenon and mercury based devices. The research used Tru-d and Xenex devices for comparison and the results showed both devices were equally strong in inactivating microorganisms. Xenon based UV sources are not as common as mercury lamps due to limitations of their own such as extremely low lifecycle requires frequent lamp replacement, moreover, they are extremely expensive etc. as shown in Fig. 1.

Above mentioned limitations have caused the development of a new type of UV light called Ultraviolet light emitting diodes (UV-LEDs). They are considered to be one of the most influential alternatives to UV lamps due to numerous advantages. UV-LED basically, is a p-n junction based semiconductor device capable of producing electroluminescence in a narrow spectrum of light in all UV sub-bands (Yoshihiko *et al.*, 2014). When compared with UV lamps, UV-LEDs undoubtedly stand out because they are able to provide highly efficient energy (Zhou *et al.*, 2017), no warm-up time is required (Yoshihiko *et al.*, 2014), they have extremely long lifecycle and able to produce UV light without the use of mercury contents (Wurtele *et al.*, 2011). These LEDs are very cost effective and do not require regular maintenance as is the case with UV lamps. They are completely environmentally friendly and can be easily disposed of without any complications (Yoshinobu *et al.*, 2011).

UV light having wavelength mainly between 200 to 300 nm is considered to be most effective in targeting DNA of the microorganisms. Generally, it is accepted that the maximum absorption wavelength through DNA is around 260 nm (Olson & Morrow, 2012). However, the optimum wavelength is dependent on the type of microorganism hence can vary greatly from one microorganism to another (Song *et al.*, 2016). The main limitation with LP mercury lamps is that it only emits light at wavelength of 254 nm therefore it cannot efficiently target all different sort of microorganism. In contrast, the UV-LEDs can be manufactured at different peak emission wavelengths which has the potential to produce better results in inactivating microbes.

Wavelengths between 254 and 280 nm which fall in UVC region are considered to be most effective in eliminating microorganisms because they fall closely to the DNA maximum absorption rate. Pyrimidine dimers produced as a result of UVC exposure will eventually cause microorganism inability to reproduce (Chatterley & Linden, 2010; Chevremont *et al.*, 2012; Hamamoto *et al.*, 2007). UVLEDs clearly have the ability to efficiently inactivate microorganisms (Chatterley & Linden, 2010; Hamamoto *et al.*, 2007; Mary H Crawford, 2005; Oguma *et al.*, 2013; Oguma *et al.*, 2016). Moreover, it is reported that UV-LEDs at 260 nm are as efficient as traditional LP UV mercury lamps (Sholtes *et al.*, 2016). Majority of the research on UVLEDs has been limited to UVC region because this UV region is often used by mercury lamps. Unfortunately, only a handful of research can be found on the effectiveness of other UV sub-bands namely UVB and UVA respectively.

DNA damage caused by exposure of UVC light is likely to be repaired by the DNA repair mechanisms namely the photo-reactivation and dark repair hence making treatment with UVC less long-lasting (Nebot Sanz *et al.*, 2007; Rodriguez *et al.*, 2014). Since DNA repair mechanism is completely unwanted to achieve maximum


Chemicals, Gas, dry heat	Mercury Lamp	Xeon Lamp
 Skin Irritation	 High Voltage	 Heat Production
 Time Consuming	 Mercury Contents	 Very Short Lifecycle
 Costly	 Long warm-up time	 High Voltage
 Effects Surface	 Short Lifecycle	 Very Expensive
 Enhances Rust	 Heat Production	 Warm-up Time

Fig. 1 Limitations of the existing disinfection methods.

Tru-d is a mercury lamp based commercially available device in the market which is currently being used for disinfection in healthcare. Disinfection using mercury based UV lamps poses some serious limitations (shown in Fig. 1) in the hospitals and their existence in the healthcare is being questioned. Some of the limitations include high operation voltage and current ranging from 110 – 240V AC (Lui *et al.*, 2014) and their extreme operational temperature of 100 °C (Yoshinobu *et al.*, 2011). Mercury contents used in these lamps are extremely hazardous to human and environment (Soloshenko *et al.*, 2006) therefore extreme safety

and long-lasting disinfection therefore this process must be weakened if not eliminated entirely. DNA repair enzymes help in the repair of damaged DNA, however, exposure to UVA light can weaken these enzymes resultantly achieving longer disinfection. Repair enzymes are sensitive to higher UV intensities (Sommer *et al.*, 1998) therefore using UVA instead of UVC could produce better results. Moreover, UVA damages cellular membrane and increases growth delay by indirectly increasing the level of reactive oxygens species in the microorganisms (Berney *et al.*, 2006; Oppezco & Pizarro, 2001; Schuch *et al.*, 2017).

Disinfection through UVA radiation is less efficient as compared to UVC but it still has the ability to carry out disinfection as reported by various studies (Chevrement *et al.*, 2012; Hwang, 2013; Nakahashi *et al.*, 2014; Hamamoto *et al.*, 2007). However, when it comes to prevent DNA repair this is where UVA really stands out. UVA irradiation has not been studied as widely as UVC therefore their exact potential is not fully understood. UVA achieves inactivation when reactive intermediates indirectly cause oxidative damage to DNA as well as other cellular components. Moreover, in comparison with UVC available in the market, UVA-LEDs are much more energy efficient and have higher output power (Harris, *et al.*, 2013; Yoshihiko *et al.*, 2014). Moreover, recent studies carried out confirmed that damage caused by UVA radiation is considered to be irreparable (Oguma *et al.*, 2013; Xiong & Hu, 2013).

The use of mercury based UV lamps in healthcare disinfection is relatively a new area and research is on-going. A study carried out Dr Deverick (Anderson *et al.*, 2017) on drug resistant bacteria namely methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *enterococci*, *C difficile* and multidrug-resistant *Acinetobacter* using UV lamp confirmed that 30 percent transmission of microorganisms can be controlled by UV. The use of UV-LEDs for disinfection in healthcare has not been explored greatly, undoubtedly missing out all the great benefits that can be achieved through the use of low cost, energy efficient and environmentally friendly LEDs. In this paper, a comparison of standard LED and UVA-LED has been studied in order to understand their behaviour in disinfection of pathogens and to determine their efficiency in inactivation of microorganisms. UVA-LED with peak wavelength of 385 nm has been compared with standard 3 mm LED for the purpose of inactivating *Escherichia coli* (*E. coli*).

MATERIALS AND METHOD

Preparation of microorganism

Escherichia coli (ATCC 11229) was selected to be used in this research study due to its common availability and importance towards human health. *E. coli* strains were cultured on nutrient agar petri dish using an inoculation loop in order to get isolated colonies. The petri dishes were then incubated at 37°C for approximately 24 h. Next, isolated colonies from petri dishes were removed using inoculation loop and about 5 – 7 colonies were added into 1 ml saline solution and mixed gently to even the concentration. The mixture was compared with 0.5 McFarland for turbidity and ensured that the desired concentration of approximately 1.5×10^8 was obtained. After achieving desired concentration, the mixture was swabbed on an agar petri dish using sterilized cotton bud. Petri dishes were left to dry before placing upside down and sealing them with parafilm. This process was repeated for control, LED and UV-LED samples. The *E. coli* swabbed petri dishes were then exposed to their respective light for treatment.

Design of experimental device

A standard super bright 3 mm LED (F33CC4SB-3) with 460 nm wavelength was used to provide light for LED samples. Its compact size, high brightness, low power consumption as well as higher output stability and reliability were some of the key features which made it stand out. A DC constant power supply was used to power on the LED. A voltage and current limiter circuit was designed to driver the LED, to ensure it stays working efficiently and to maintain a constant current flow (30 mA) in the circuit. The total power consumption of

the circuit was around 0.2 W. Similarly, a high power 385 nm wavelength UVA-LED (NVSU233A(T)-D1) from Nichia, Japan was selected for UVA-LED samples in this experimental setting. A constant current of 700 mA was applied to the UVA-LED. The total power consumption of the circuit was 2.45 W. Irradiation dose of 57.6 J/cm² was received by the sample during 1 h exposure to UVA-LED light. Every possible effort was made to make sure the current and voltage did not exceed the maximum limit. The UVA-LED was able to provide maximum output power at 1400 mW. Both LED and UVA-LED ran in continuous mode and the distance between the sample and light source was kept at 70 mm so that the light could effectively perform disinfection. The dimenisions of the LED and UVA-LED can be seen in Fig. 2 (a) and (b) respectively.

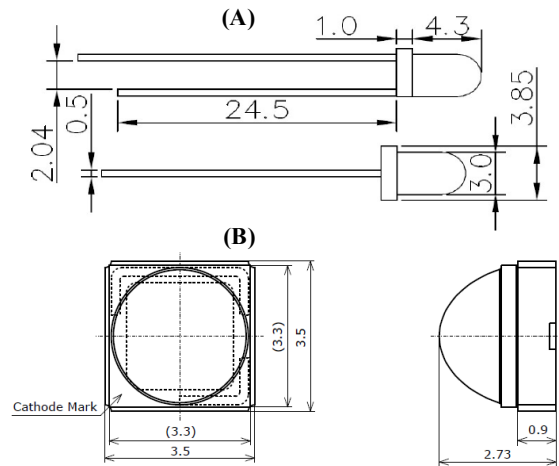


Fig. 2 Dimensions of (A) 3 mm LED and (B) 385 nm UVA-LED.

Determination of bacteria number

Maintenance and growth of microorganisms have been an integral part of microbiology. Colony forming unit (CFU) is a common method used to to determine the number of cells that remained viable in spite of the treatment and were able to form small colonies. To calculate the exact population of viable microorganisms after UV treatment, a serial dilution method was used. In this study, CFU was used to identify which of the two light sources, LED or UVA-LED, is the most efficient one in inactivating microorganisms. The post treatment petri dishes were swabbed with cotton bud and mixed with 1 ml of saline solution. Then, this solution was further added into 9 ml of bacteria-free saline solution based on serial dilution. Approximately 30 µl of each of these dilutions were cultured on petri dish at 37°C for 24 h. Following day, the petri dishes were observed for bacteria growth and the number of colonies were counted. The CFU was calculated using Eq. (1):

$$c = \frac{n \times d}{v} \quad (1)$$

where

c=CFU/ml

n=number of colonies on petri dish

d=dilution factor

v=volume transferred on the plate

Exposure to LED and UV-LED light

Three separate cardboard boxes were used in this experiment. One box was dedicated for control while second and third were used for LED and UVA-LED, respectively. All experiments were conducted in well ventilated and sterilized environment with room temperature approximately at 25°C. During the experiment, petri dishes for LED and UVA-LED were exposed to their respective light for 1 h while the control condition was left without LED and UVA-LED. In the first

experimental setting, no bacteria (*E. coli*) was swabbed on either of the three sample petri dishes. Control, LED and UVA-LED dishes were left in an open environment for 1 h without being covered with their lids. LED dish was exposed to LED light whereas UVA-LED dish was exposed to UV light while control dish was left without LED during the same period of time. After the experiment, the dishes were covered with lids, sealed using parafilm and were kept in an incubator at 37°C for 24 h for microorganism growth.

In the second experimental setting, the control, LED and UVA-LED petri dishes were swabbed with *E. coli* and had their lids sealed off with parafilm. Control sample was untreated whereas LED and UVA-LED dishes were exposed to their respective lights for 1 h. The schematic representation of the experimental setting is shown in Fig. 3. Every possible effort was made to ensure no contamination of the samples dishes, hence safety cabinet was often used. The cardboard boxes were covered to provide dark environment as well as to prevent outside environment influence on the samples. Fig. 4 shows the real-time experiment in progress. After experiment, the dishes were incubated at 37°C for 24 h to observe bacteria growth and disinfection efficiency. CFUs and log-inactivation are two main methods used after petri dishes have been exposed to UV light to calculate the amount of disinfection observed.

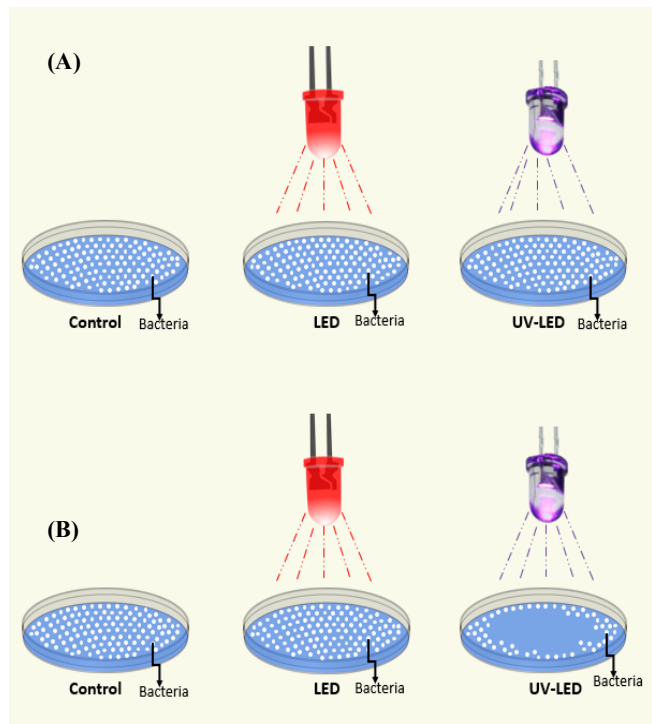


Fig. 3 Schematic representation of experimental setting (A) before and (B) after treatment.

Log inactivation level

To keep huge numbers in manageable position, microbiologists often use scientific notation to express numbers easily. Similarly, when calculation of microorganism is required, a logarithmic scale

(log scale) is used frequently. Log inactivation is a suitable tool used to express the number or percentage of microbes inactivated as a result of disinfection test. Generally, a 1-log reduction (inactivation) means that the disinfection process was able to inactivate 90% of the microorganisms whereas a 3-log reduction shows 99.9% microorganisms reduction. In this paper log scale was used to calculate the efficiency of LED and UVA-LED in inactivating microbes. Log inactivation was calculated using Eq. (2):

$$\text{Log inactivation ratio} = \log \left(\frac{N_t}{N_0} \right) \quad (2)$$

where

N_t = Number of colonies post UV treatment
 N_0 = Number of colonies before UV treatment

RESULTS AND DISCUSSION

Bacteria Inactivation

In the first experimental setting where the experiments were conducted without bacteria on the agar surface, the control samples showed relatively high number of CFU (Table 1). The LED, compared to control had less bacterial colonies. However, UV light was able to stop bacteria growth and hence, the least number of colonies were found on the UVA-LED petri dish.

Table 1 Number of viable colonies present on petri dishes.

	Control	LED	UVA-LED
Number of colonies	8	3	1

Fig. 5 shows the control, LED and UVA-LED treated petri dish. Control dishes had 10 colonies with dotted circle. LED and UVA-LED dishes had 3 and 1 bacterial colony, respectively. UVA was proven to be effective even when the petri dishes were exposed to all kind of different bacteria present in the laboratory environment.

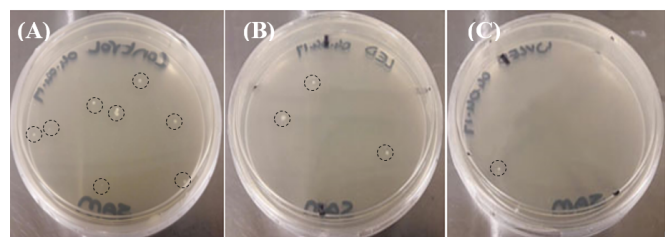


Fig. 5 Petri dishes without *E. coli* after treatment with their respective lights (A) control (B) LED (C) UVA-LED.

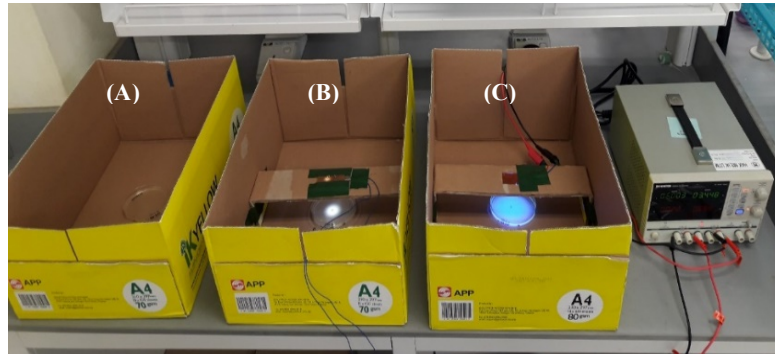


Fig. 4 Experimental setting setup in real time (A) control (B) LED (C) UVA-LED.

The second experimental setting where *E. coli* was swabbed on the petri dishes was carried out to calculate the efficiency of LED and UVA-LED in inactivating *E. coli*. In order to assess the disinfection capabilities of LED and UVA-LED, the samples (control, LED and UVA-LED) were first observed qualitatively after treatment. Post-treatment results are illustrated in Fig. 6 and Fig. 7. Entire control sample petri dish was covered with overgrown colonies and no single isolated colony was observed as illustrated in Fig. 6 and 7 (a). With respect to the LED sample, similar behaviour was observed indicated no significant differences between control and LED sample as shown in Fig. 6 and 7 (b). From mere observance it was easily concluded that no disinfection have been observed by the LED petri dish from exposure to standard LED light.

The situation with the UVA-LED sample (Fig 6 and 7 (c)) is completely different from its counterparts. The UVA-LED sample clearly showed almost no bacteria colonies at the center of the petri dish marked with "X" where the light intensity was at maximum indicating high level of disinfection properties. A much wider disinfection (circled with dotted line) is visible which is not present in the LED sample. The colonies concentration increased as moved towards the edge of the petri dish highlighting that the intensity of light reduced as move further away from the center. This is due to the fact that only one UVA-LED was used in this study and the spot area was not significantly enough to disinfect the entire petri dish. This limitation can easily be overcome with the introduction of more UV-

LEDs. UVA-LED treated sample outweighed disinfection of microorganism when compared with standard LED, which did not produce any disinfection.

Determination of CFU

In order to identify the inactivation efficiency, quantitatively, the number of bacteria took place in LED and UVA-LED samples post-treatment. Control and LED samples did not show any inhibition zones. Both samples was observed to be identical highlighting no disinfection properties. However, on the contrary, the situation for UVA-LED sample was completely different. A clear microorganism-free inhibition zone was observed instantly indicating that the exposure to UVA light was successful in inactivating microorganisms. No colonies were found at the center area of the petri dish, where the intensity of the light source was at maximum, however colonies were visible and became more concentrated as travelled further away from the center of the petri dish. This is due to the fact that only one UVA-LED was used in this experiment. However, if multiple LEDs were used, this limitation would have been easily overcome.

The CFU was calculated using serial dilution method. Different dilution factors had different number of colonies present in them. Some colonies were so highly dense that it was impossible to count each and every one of them while other dilution factors had so little colonies that it would provide statistically unreliable results as shown in Fig. 8.

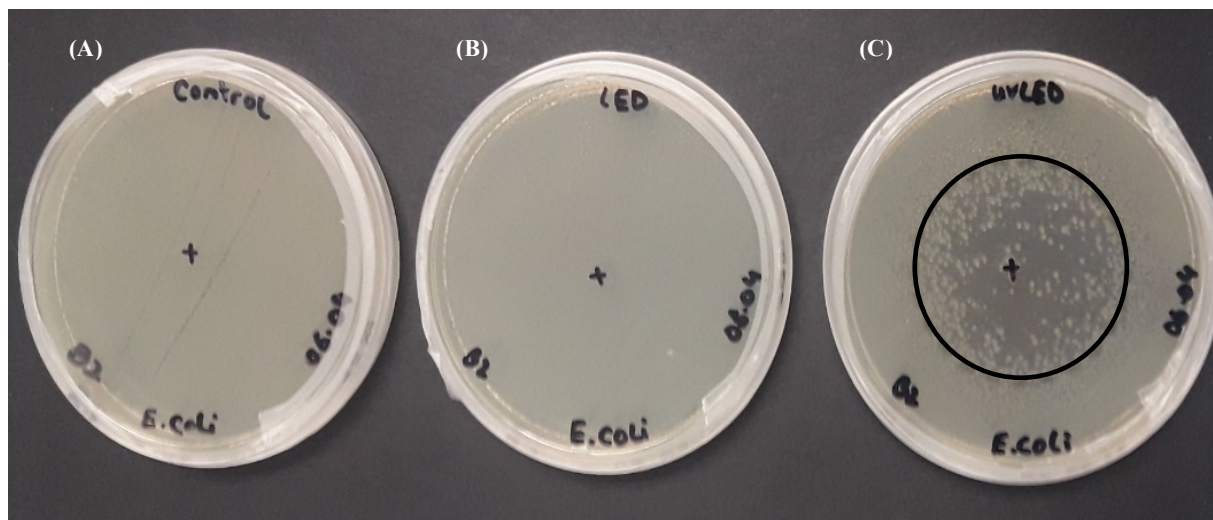


Fig. 6 Petri dishes after treatment with their respective lights. (A) control (B) LED (C) UVA-LED.

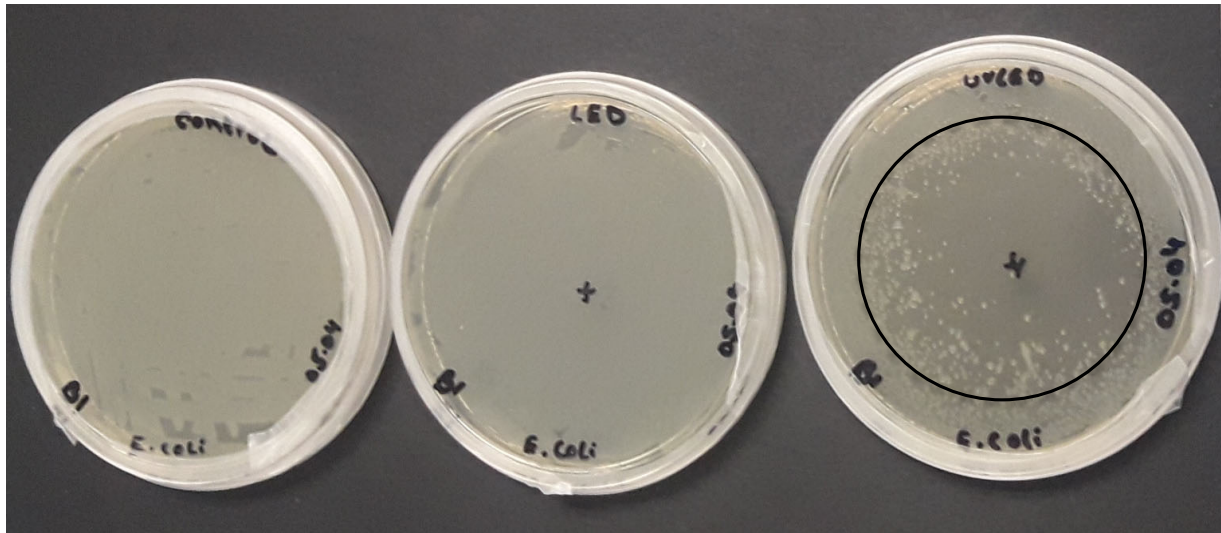


Fig. 7 Petri dishes post-light treatment (A) control (B) LED (C) UVA-LED.

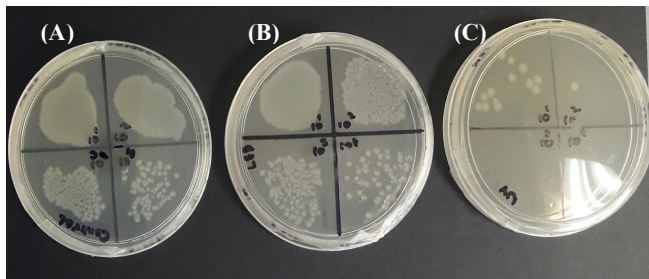


Fig. 8 Serial dilution for calculation of colony-forming units (CFU). (A) Control sample (B) LED (C) UVA-LED.

To deal with this situation, viable count standard was used which identifies the correct dilution factor based on the number of colonies present. Typically, a dilution factor having 30 – 300 colonies is considered accurate. The same process was repeated for control, LED and UVA-LED samples. It can be easily seen in Fig. 8 that in 10^{-2} dilution quadrant, the control and LED samples had huge concentration of bacterial colony so much so that it was impossible to calculate each colony whereas UVA-LED sample only had 1 colony in the same dilution factor. The results overwhelmed suggested that UVA-LED with 385 nm wavelength was capable of producing significant disinfection.

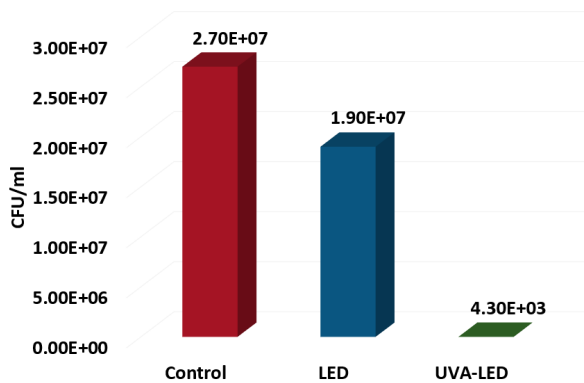


Fig. 9 Number of colony-forming unit (CFU) after exposure to respective light.

Log Inactivation Level

Another method to identify quantitatively the level of disinfection occurred in LED and UVA-LED was to use the log inactivation method. Log inactivation is a convenient way of

representing the numeric or percentage value of the total amount of microorganisms inactivated through the disinfection process. Log inactivation was calculated using equation (Eq. 2), given previously. The results indicated that LED treated sample experienced 0.1-log inactivation as shown in Fig. 10. The UVA treated sample, however, showed incredible amount of disinfection at whopping 3.8-log inactivation which in percentage is approximately 99.99%. The results showed the standard LED in comparison with UVA-LED produced negligible microorganisms' inactivation. This research finding also summarized that UVA-LED at 385 nm wavelength is capable of providing significant disinfection of *E. coli*.

The disinfection system designed in this study was on a smaller scale and there is a possibility of having some challenges when extended to a large system. This experiment used one LED and UVA-LED, however, when multiple LEDs are combined, the overall inactivation efficiency could be altered. The output power produced by both sources were not the same because generally, the standard LEDs are not designed to withhold high current e.g. 1 A whereas UVA-LED used in this study could handle up to 1.4 A. This problem can easily be solved by using LED which has either similar output power or can handle high currents. The experiment was conducted using continuous mode in which light sources remained on during the whole experiment. In future, pulsed mode could also be introduced which can provide higher current for limited period of time for inactivation of pathogen. Pulsed mode has the ability to disinfect as efficiently as continuous mode. Research studies (Li *et al.*, 2010; Wengraitis *et al.*, 2013) reported that pulsed mode performed better compared to continuous mode for microorganism inactivation.

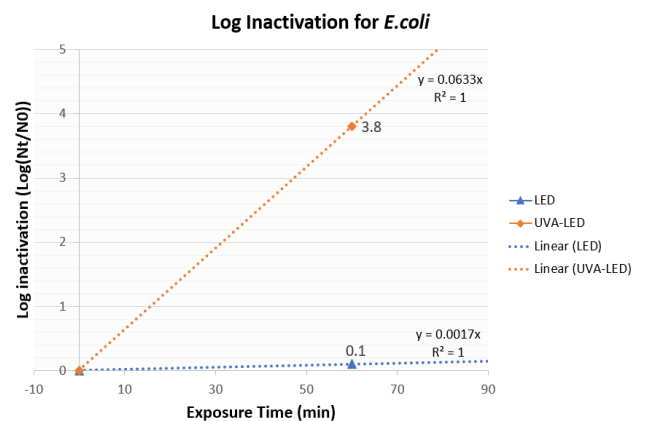


Fig. 10 Log inactivation level post-light treatment.

UV light is an amazing alternative to traditional methods having excellent advantages unbeatable by the existing methods. This proposed device has the potential to be used in hospitals saving millions of dollars each year spent annually on buying disinfection related chemicals etc. Moreover, UVA-LED is completely environmentally friendly hence its importance and contribution in our life is beyond measure (Davididou et al., 2017). Moreover, greater output power of UVA-LEDs as compared to UVC could enhance the disinfection process. In near future, further experiments will be conducted where larger scale applications will be tested to develop a practical device capable of disinfection. Moreover, a combination of multiple sources will be applied in near future to deliver required UV dose in limited time possible to achieve higher bacteria inactivation. The overall size of the device will be reduced to accommodate portability function and hence making the device easier to carry in hard to reach places.

CONCLUSION

A comparison study was carried out to understand the behaviour of standard LED and UVA-LED as well as their efficiency in inactivation of pathogens. The results clearly indicated that the standard LED possess very minimum to none disinfection abilities. The UVA-LED with 385 nm wavelength, on the other hands, is capable of providing disinfection beyond a shadow of a doubt and was able to inactivate approximately 99.99% of *E. coli*. The research study carried out has the potential to be used in various applications for disinfection such as food, water treatment and healthcare settings.

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REFERENCES

- Allegranzi, B., Nejad, S. B., Combescure, C., Graafmans, W., Attar, H., Donaldson, L., Pittet, D. (2011). Burden of endemic health-care-associated infection in developing countries: Systematic review and meta-analysis. *The Lancet*, 377(9761), 228-241.
- Anderson, D. J., Chen, L. F., Weber, D. J., Moehring, R. W., Lewis, S. S., Triplett, P. F., Sexton, D. J. (2017). Enhanced terminal room disinfection and acquisition and infection caused by multidrug-resistant organisms and *Clostridium difficile* (the benefits of enhanced terminal room disinfection study): A cluster-randomised, multicentre, crossover study. *The Lancet*, 389(10071), 805-814.
- Arif, A. A., Delclos, G. L., Whitehead, L. W., Tortolero, S. R., Lee, E. S. (2003). Occupational exposures associated with work-related asthma and work-related wheezing among U.S. workers. *American Journal of Industrial Medicine*, 44(4), 368-376.
- Berney, M., Weilenmann, H.-U., Egli, T. (2006). Flow-Cytometric study of vital cellular functions in *Escherichia coli* during solar disinfection (SODIS). *Microbiology*, 152(Pt 6), 1719-1729.
- Bohrerova, Z., Linden, K. G. (2006). Assessment of DNA damage and repair in *Mycobacterium terrae* after exposure to UV irradiation. *Journal of Applied Microbiology*, 101(5), 995-1001.
- Burke, J. P. (2003). Infection control - a problem for patient safety. *The New England journal of medicine*, 344(7), 651-656.
- Chatterley, C., Linden, K. (2010). Demonstration and evaluation of germicidal UV-LEDs for point-of-use water disinfection. *Journal of Water and Health*, 8(3), 479-486.
- Chatzisympson, E., Foteinis, S., Mantzavinos, D., Tsoutsos, T. (2013). Life cycle assessment of advanced oxidation processes for olive mill wastewater treatment. *Journal of Cleaner Production*, 54(Supplement C), 229-234.
- Chevremont, A. C., Farnet, A. M., Coulomb, B., Boudenne, J. L. (2012). Effect of coupled UV-A and UV-C LEDs on both microbiological and chemical pollution of urban wastewaters. *Science of The Total Environment*, 426, 304-310.
- Chevremont, A. C., Farnet, A. M., Sergent, M., Coulomb, B., Boudenne, J. L. (2012). Multivariate optimization of fecal bioindicator inactivation by coupling UV-A and UV-C LEDs. *Desalination*, 285, 219-225.
- Davididou, K., McRitchie, C., Antonopoulou, M., Konstantinou, I., Chatzisympson, E. (2017). Photocatalytic degradation of saccharin under UV-LED and blacklight irradiation. *Journal of Chemical Technology & Biotechnology*.
- Dumas, O., Donnay, C., Heederik, D. J. J., Héry, M., Choudat, D., Kauffmann, F., Le Moual, N. (2012). Occupational exposure to cleaning products and asthma in hospital workers. *Occupational and Environmental Medicine*, 69(12), 883-889.
- Hamamoto, A., Mori, M., Takahashi, A., Nakano, M., Wakikawa, N., Akutagawa, M., Kinouchi, Y. (2007). New water disinfection system using UVA light-emitting diodes. *Journal of Applied Microbiology*, 103(6), 2291-2298.
- Harris, T. R., Pagan, J. G., Batoni, P. (2013). Optical and Fluidic Co-Design of a UV-LED Water Disinfection Chamber. *ECS Transactions*, 45(17), 11-18.
- Hijnen, W. A. M., Beerendonk, E. F., Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water: A review. *Water Research*, 40(1), 3-22.
- Hwang, K. Y. T. S. (2013). Combination of light emitting diode at 375 nm and photo-reactive TiO₂ layer prepared by electrostatic spraying for sterilization. *Journal Of Electrical Engineering & Technology*, 8(5), 1169-1175.
- Klevens, R. M., Edwards, J. R., Richards Jr, C. L., Horan, T. C., Gaynes, R. P., Pollock, D. A., Cardo, D. M. (2007). Estimating health care-associated infections and deaths in US hospitals, 2002. *Public Health Reports*, 122(2), 160-166.
- Koch, A. M., Nilsen, R. M., Dalheim, A., Cox, R. J., Harthug, S. (2015). Need for more targeted measures – Only less severe hospital-associated infections declined after introduction of an infection control program. *Journal of Infection and Public Health*, 8(3), 282-290.
- Koch, A. M., Nilsen, R. M., Eriksen, H. M., Cox, R. J., & Harthug, S. (2015). Mortality related to hospital-associated infections in a tertiary hospital; repeated cross-sectional studies between 2004-2011. *Antimicrobial Resistance and Infection Control*, 4 (57), 1-8.
- Kogevinas, M., Zock, J.-P., Jarvis, D., Kromhout, H., Lillienberg, L., Plana, E., Antó, J. M. (2007). Exposure to substances in the workplace and new-onset asthma: an international prospective population-based study (ECRHS-II). *The Lancet*, 370(9584), 336-341.
- Li, J., Hirota, K., Yumoto, H., Matsuo, T., Miyake, Y., Ichikawa, T. (2010). Enhanced germicidal effects of pulsed UV-LED irradiation on biofilms. *Journal of Applied Microbiology*, 109(6), 2183-2190.
- Lui, G. Y., Roser, D., Corkish, R., Ashbolt, N., Jagals, P., Stuetz, R. (2014). Photovoltaic powered ultraviolet and visible light-emitting diodes for sustainable point-of-use disinfection of drinking waters. *Science of The Total Environment*, 493, 185-196.
- Magill, S. S., Edwards, J. R., Bamberg, W., Beldavs, Z. G., Dumyati, G., Kainer, M. A., Fridkin, S. K. (2014). Multistate point-prevalence survey of health care-associated infections. *The New England journal of medicine*, 370(13), 1198-1208.
- Mahoney, J. J., Lim, C. G. (2012). Effect of disinfectants on glucose monitors. *Journal of Diabetes Science and Technology*, 6(1), 81-85.
- Crawford, M. H., Banas, M. A., Ross, M. P., Ruby, D. S., Nelson, J. S., Boucher, R., Allerman, A. A. (2005). Final LDRD Report: Ultraviolet Water Purification Systems for Rural Environments and Mobile Applications. *Sandia Report*.
- Matsuyama, M., Usami, T., Masuda, K., Niimi, N., Ohta, M., Ueda, M. (1997). Prevention of infection in dental procedures. *Journal of Hospital Infection*, 35(1), 17-25.
- Nakahashi, M., Mawatari, K., Hirata, A., Maetani, M., Shimohata, T., Uebanso, T., Takahashi, A. (2014). Simultaneous irradiation with different wavelengths of ultraviolet light has synergistic bactericidal effect on *Vibrio parahaemolyticus*. *Photochemistry and Photobiology*, 90(6), 1397-1403.
- Narendranath, V., S. Nandakumar, B., S. Sarala, K. (2017). Epidemiology of hospital-acquired infections in a tertiary care teaching hospital in India: a cross-sectional study of 79401 inpatients. *International Journal Of Community Medicine And Public Health*, 4(2), 335-339.
- Nebot Sanz, E., Salcedo Dávila, I., Andrade Balao, J. A., Quiroga Alonso, J. M. (2007). Modelling of reactivation after UV disinfection: effect of UV-C dose on subsequent photoreactivation and dark repair. *Water Research*, 41(14), 3141-3151.
- Nerandzic, M. M., Thota, P., Sankar C, T., Jencson, A., Cadnum, J. L., Ray, A. J., Donskey, C. J. (2015). Evaluation of a pulsed xenon ultraviolet disinfection system for reduction of healthcare-associated pathogens in hospital rooms. *Infection Control and Hospital Epidemiology*, 36(2), 192-197.
- Oguma, K., Kita, R., Sakai, H., Murakami, M., Takizawa, S. (2013). Application of UV light emitting diodes to batch and flow-through water disinfection systems. *Desalination*, 328, 24-30.

- Oguma, K., Rattanukul, S., Bolton, J. R. (2016). Application of UV light-emitting diodes to adenovirus in water. *Journal of Environmental Engineering*, 142(3). doi:doi:10.1061/(ASCE)EE.1943-7870.0001061
- Olson, N. D., Morrow, J. B. (2012). DNA extract characterization process for microbial detection methods development and validation. *BMC Research Notes*, 5(668), 1-14.
- Oppizzo, O. J., Pizarro, R. A. (2001). Sublethal effects of ultraviolet A radiation on Enterobacter cloacae. *Journal of Photochemistry and Photobiology B*, 62(3), 158-165.
- World Health Organization (2013). *Exploring patient participation in reducing health-care-related safety risks*. Copenhagen: World Health Organization.
- World Health Organization (2016). *Global Guidelines for the Prevention of Surgical Site Infection*: World Health Organization. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK401132/?report=classic>
- Rasoulifard, M. H., Fazli, M., Eskandarian, M. R. (2015). Performance of the light-emitting-diodes in a continuous photoreactor for degradation of Direct Red 23 using UV-LED/S2O8²⁻ process. *Journal of Industrial and Engineering Chemistry*, 24, 121-126.
- Rastogi, R. P., Richa, Kumar, A., Tyagi, M. B., Sinha, R. P. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of Nucleic Acids*, 2010, 1-32.
- Rodriguez, R. A., Bounty, S., Beck, S., Chan, C., McGuire, C., Linden, K. G. (2014). Photoreactivation of bacteriophages after UV disinfection: role of genome structure and impacts of UV source. *Water Research*, 55, 143-149.
- Schuch, A. P., Moreno, N. C., Schuch, N. J., Menck, C. F. M., Garcia, C. C. M. (2017). Sunlight damage to cellular DNA: Focus on oxidatively generated lesions. *Free Radical Biology and Medicine*, 107(Supplement C), 110-124.
- Sholtes, K. A., Lowe, K., Walters, G. W., Sobsey, M. D., Linden, K. G., Casanova, L. M. (2016). Comparison of ultraviolet light-emitting diodes and low-pressure mercury-arc lamps for disinfection of water. *Environmental Technology*, 37(17), 2183-2188.
- Slawson, N. (2017, 11 Septmeber). Regularly using bleach linked to higher risk of fatal lung disease. *The Guardian*. Retrieved from <https://www.theguardian.com/uk-news/2017/sep/11/regularly-using-bleach-linked-to-higher-risk-of-fatal-lung-disease>. (Accessed: 24 September 2017).
- Soloshenko, I. A., Bazhenov, V. Y., Khomich, V. A., Tsiolko, V. V., Potapchenko, N. G. (2006). Comparative research of efficiency of water decontamination by UV radiation of cold hollow cathode discharge plasma versus that of low- and medium-pressure mercury lamps. *IEEE Transactions on Plasma Science*, 34(4), 1365-1369.
- Sommer, R., Haider, T., Cabaj, A., Pribil, W., Lhotsky, M. (1998). Time dose reciprocity in UV disinfection of water. *Water Science and Technology*, 38(12), 145-150.
- Song, K., Mohseni, M., Taghipour, F. (2016). Application of ultraviolet light-emitting diodes (UV-LEDs) for water disinfection: A review. *Water Research*, 94(Supplement C), 341-349.
- Wengraitis, S., McCubbin, P., Wade, M. M., Biggs, T. D., Hall, S., Williams, L. I., Zulich, A. W. (2013). Pulsed UV-C disinfection of Escherichia coli with light-emitting diodes, emitted at various repetition rates and duty cycles. *Photochemistry and Photobiology*, 89(1), 127-131.
- Wurtele, M. A., Kolbe T Fau - Lipsz, M., Lipsz M Fau - Kulberg, A., Kulberg A Fau - Weyers, M., Weyers M Fau - Kneissl, M., Kneissl M Fau - Jekel, M., Jekel, M. (2011). Application of GaN-based ultraviolet-C light emitting diodes--UV LEDs--for water disinfection. *Water Research*, 45(3), 1481-1489.
- Xiong, P., Hu, J. (2013). Inactivation/reactivation of antibiotic-resistant bacteria by a novel UVA/LED/TiO₂ system. *Water Research*, 47(13), 4547-4555.
- Yoshihiko, M., Masahiro, K., Suguru, N. (2014). Development and future of ultraviolet light-emitting diodes: UV-LED will replace the UV lamp. *Semiconductor Science and Technology*, 29(8), 084004.
- Yoshinobu, A., Misaichi, T., Kaoru, Y., Masahito, K., Nobuhito, Y., Naoyuki, K., Yasushi, N. (2011). Inactivation of bacterial viruses in water using deep ultraviolet semiconductor light-emitting diode. *Journal of Environmental Engineering*, 137(12), 1215-1218.
- Zhou, X., Li, Z., Lan, J., Yan, Y., Zhu, N. (2017). Kinetics of inactivation and photoreactivation of Escherichia coli using ultrasound-enhanced UV-C light-emitting diodes disinfection. *Ultrason Sonochem*, 35(Pt A), 471-477.