Effectiveness of visible and ultraviolet light emitting diodes for inactivation of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*: A comparative study

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INTRODUCTION

Infections that patients acquire while having treatment in the healthcare are known as healthcare associated infection (HAIs). HAIs are considered to be one the most leading causes of illnesses and deaths worldwide. HAIs can occur in hospitals, care homes, and even in patient’s own house (Van Kleef, Robotham, Jit, Deeny, & Edmunds, 2013). It is reported that in United States alone around 1.7 million people contacted HAIs each year and causing deaths among 99,000 patients (Branigan & Holmes, 2012). Cruickshank and Ferguson estimated that as many as 200,000 HAIs cases are recorded in Australia annually which makes it the most common complication effecting patients (Cruickshank, Murphy, & eds., 2009). Some of the most frequent microorganisms, responsible from HAIs, are the *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Donlan, 2001). They are known to cause infections such as pneumonia, respiratory tract infections, urinary tract infections, and surgical site infections (Al-Tawfiq & Tambyah, 2014).

Medical device associated infections also pose significant risk to the patients and medical personnel in healthcare that resulted in increases morbidity and mortality (Donlan, 2008). Routine disinfection of non-critical medical devices such as stethoscopes, blood glucose meters, and blood pressure cuffs are important to protect vulnerable patients from nosocomial infections. It has been reported that non-critical medical devices rarely get disinfected between uses with different patients which increases the likelihood of infecting any patient getting in contact with these devices (Bukhare, Al-Zahrani, Rubaish, & Abdulmohsen, 2004; Dancer, 2012; Uneke et al., 2014; Uneke, Ogbonna A Fau - Oyibo, Oyibo Pg Fau - Ekuma, & Ekuma, 2008; Zachary et al., 2001). In fact, in some studies the percentage of contamination on these devices could range between 25% to 100% (Bukhari et al., 2004; Chigozie Jesse, 2014; D, S, R, G, & S, 2016; Grewal, Varshney, Thomas, Kok, & Shetty, 2013).

Existing disinfection and sterilization practices in healthcare such as the use of chemicals, dry heat, and steam have significant limitations which make medical personnel to neglect the disinfection protocols. Some of the limitations are as follows: i) long and tedious procedures; ii) cause skin irritation and respiratory diseases; iii) alter surface structure of the medical device. Alternative methods such as ultraviolet (UV) light can help to overcome existing limitations thereby increasing the quality of human life. The importance of UV irradiation in everyday life is ever increasing with its ability in various applications ranging from disinfection to tanning and food preserving and many more. UV light is already replacing traditional disinfection and sterilization practices in healthcare.

The use of mercury based UV lamps is very common when it comes to modern disinfection technologies. UV mercury based lamps are widely used in disinfection of water and now the use of these lamps in healthcare is also increasing. These lamps make use of mercury vapor to produce UV light and can be categorized into two main types:
monochromatic and polychromatic. Monochromatic are referred to as low-pressure (LP) mercury lamps and are a type of monochromatic which emit most of UV light at 253.7 nm wavelength. Whereas polychromatic which is also known as medium pressure (MP) can produce light of various wavelengths (Kowalski, 2009; Linden, Thurston, Schaefer, & Malley, 2007). Tru-D is one of many commercial products making use of mercury vapor principle to produce light. Numerous studies have been carried out to study the effectiveness of these devices in healthcare. The results indicated that Tru-D can efficiently inactivate significant amount to pathogens from healthcare surfaces and devices (Anderson et al., 2013; Mahida, Vaughan, & Boswell, 2013; Nerandzic, Cadnum, Pultz, & Donskey, 2010).

Unfortunately, these lamps pose many drawbacks which makes their use in the modern era highly unfavorable. The lamps are generally made of fragile quartz material hence the risk of mercury leakage is always present throughout the lifecycle of the lamps (Shin, Kim, Kim, & Kang, 2016). The lamps require warm-up time before operation therefore instantaneous disinfection cannot be carried out. Moreover, they require high voltage and produce a lot of heat during operation (Yoshihiko, Masahiro, & Suguru, 2014). The frequent replacement of these lamps is very common due to extremely short lifecycle (Hölz, Lietard, & Somoza, 2017). Additionally, it can only be used in continuous mode and thus, have to be remained switched on throughout entire disinfection process. Aforesaid limitations do not allow these lamps to be used in point-of-care (POC) disinfection applications (Chen, Loeb, & Kim, 2017).

One of the most feasible alternatives to UV lamps are the UV light emitting diodes (UVLEDs). The use of UVLEDs is on the rise due to latest technical advancements in this technology. Furthermore, UVLEDs offer benefits that seem impractical using conventional lamps. These environmentally-friendly lamps do not make use of mercury contents and produce ozone-free UV light (Eskandarian, Choi, Fuzli, & Rasoulifard, 2016). Highly compact size allows them to be used in portable and POC applications. Almost all the energy is converted into UV light and only small amount of energy is wasted as heat (McDermott, Walsh, & Howard, 2008; Vilhunen, Särkkä, & Sillanpää, 2009). These LEDs are available in UVC, UVB, and UVA regions and the wavelengths can be selected based on the type of microorganisms to be disinfected. Extremely long lifetime, low operation voltage, and no warm-up time required are some of many advantages of UVLEDs (Chatterley & Linden, 2010; Matafonova & Batoev, 2018; Messina et al., 2016).

The efficiency of UVLEDs in disinfection of water (Chatterley & Linden, 2010; Matafonova & Batoev, 2018; Oguama, Kita, & Takizawa, 2016), food (Kim, Kim, & Kang, 2016; Shin et al., 2016; Shirai, Watanabe, & Matsuki, 2017), and healthcare (Donlan, 2008; G. Messina, Burgess, Messina, Montagnani, & Cevenini, 2015; Gabriele Messina et al., 2016; Omotani et al., 2018) has been reported by numerous studies which suggest that UVLEDs can be effectively used for said applications. The UVLEDs are already in process of replacing traditional UV lamps (Yoshihiko et al., 2014). Comparative studies between UVLEDs and UV lamps have also been carried out to determine the most effective technology in disinfection. The results concluded that in some cases UVLEDs are as effective as UV lamps (Beck et al., 2017; Sholtes et al., 2016) while in other they are even more effective (Li, Wang, Hua, Lu, & Hu, 2017).

Majority of research has been directed towards UVC-LEDs for disinfection applications and comparatively limited research can be found on UVA-LEDs. It is well established that UVC-LEDs have higher inactivation efficiency than UVA, however, both have disinfection properties. Unfortunately, damaged caused by UVC can be easily repaired using photoreactivation process whereas UVA can withstand photoreactivation hence making UVA long-lasting disinfection as compared to UVC. Furthermore, UVA-LEDs are much more energy efficient, have higher optical output power, and are far cheaper than UVC (Ooyagi et al., 2011; Harris, Pagan, & Batomi, 2013; Yoshihiko et al., 2014). In this study, inactivation efficiency of a standard visible light LED, and UV LED has been compared for disinfection of Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli.
Exposure to light source

For each experiment, a set of three petri dishes were used: for control, LED, and UVA LED samples. Each petri dish was kept in a separate box with light source on top, facing downwards. Both light sources had similar source/sample distance and were operated in continuous wave (CW) throughout the experimental period to investigate their effects on the bacteria. All experiments were conducted in well ventilated and sterilized environment as to reduce contamination. All boxes including control were covered with lids to avoid outer light influence on the samples. Exposure time was varied while constantly exposing the petri dishes to their respective lights. Different exposure times (1, 5, 15, 30, and 60 min) were examined for all three microorganisms. The UV dose induced to the sample after 1 h of continuous exposure was 57.6 mJ/cm². After treatment, the petri dishes were sealed with parafilm and incubated at 37°C for approximately 24 h to observe the bacteria growth.

Determination of inhibition zones

To determine antibacterial efficiency of LED and UVA LED sources on the bacteria, zone of inhibition test was conducted. As the diameter of the zone directly corresponds to the sensitivity of light source on the microbes, therefore higher inhibition zone would correlate with high inactivation efficiency and vice versa. The zone of inhibition was measured and recorded in millimeter unit.

RESULTS AND DISCUSSION

Inactivation effects of LED on microorganisms

Effects of LED and UVA LED on the bacteria were compared as a function of varied exposure time (Fig. 3). The center of the petri dish was marked with “X” to highlight that the intensity of light source was at its maximum at the said point. From Fig. 3, it can be concluded that LED light source did not produce any observable inactivation regardless of exposure time. This pattern was observed for all studied bacteria. A summary of the inactivation efficiency of LED at various exposure time with respect to control sample is shown in Table 1. The results clearly demonstrate that LED at 460 nm wavelength does not possess any observable antibacterial activity irrespective of the exposure time.

Table 1 Effects of varied exposure time on bacterial inactivation.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>1 min</th>
<th>5 min</th>
<th>15 min</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>LED</td>
<td>UV</td>
<td>C</td>
<td>LED</td>
</tr>
<tr>
<td>S. aureus</td>
<td>NOI</td>
<td>NOI</td>
<td>NOI</td>
<td>NOI</td>
<td>NOI</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>NOI</td>
<td>NOI</td>
<td>NOI</td>
<td>NOI</td>
<td>NOI</td>
</tr>
<tr>
<td>E. coli</td>
<td>NOI</td>
<td>NOI</td>
<td>NOI</td>
<td>NOI</td>
<td>NOI</td>
</tr>
</tbody>
</table>

Table 2 Post treatment Inhibition zone.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>LED</th>
<th>UVA-LED</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>0</td>
<td>45</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>0</td>
<td>67</td>
</tr>
<tr>
<td>E. coli</td>
<td>0</td>
<td>55</td>
</tr>
</tbody>
</table>

The biggest inhibition zone diameter was observed for P. aeruginosa followed by E. coli and S. aureus. Although E. coli and S. aureus petri dishes were not as clearly inactivated as P. aeruginosa but the concentration of microorganisms at the center of the dish was significantly lower.

Inhibition zone

For inhibition zone of LED and UVA LED, petri dishes were observed at all time period for all microorganisms. No inhibition zone was observed for any of the LED-treated petri dishes indicating no germicidal properties of LED at 460 nm wavelength. In addition, the variation of exposure time for standard LED did not have any effect on the bacterial reduction. On the other hand, UVA-treated petri dishes showed inactivation for all microorganisms. Exposure time influenced the inactivation significantly. The diameter of inhibition zone, after 60 min of exposure time, is recorded in Table 2.
2007). However, investigation of wavelengths closer to the visible spectrum have not been studied in detail. The finding of this research study clearly shows that UVA LEDs, regardless of being known as less effective, still possess germicidal activities and have higher inactivation capabilities compared to standard LED. UVA LEDs may well be used in numerous applications for inactivation of pathogenic microorganisms.

**CONCLUSION**

Effectiveness of standard LED and UVA LED was compared for inactivation of frequently isolated pathogens in hospitals. UVA LED showed tremendous inactivation properties as compared to LED light. Inactivation efficiency was studied as a function of varied exposure time and calculation of inhibition zone was carried out to determine the disinfection effectiveness. Highest inactivation was achieved for *P. aeruginosa*. A linear relationship was witnessed between exposure time and log inactivation. As the exposure time increased so did the inactivation hence proving the importance of exposure time in achieving higher log reduction. The absence of LED’s germicidal properties demonstrated that UVLED at 460 nm cannot to be used for disinfection applications.

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