ORIGINAL ARTICLE

405 nm UV-LED Application for Pathogen Inactivation on Plasma Donor Contaminated by *Staphylococcus aureus*

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ABSTRACT

Introduction: Bacterial contamination in blood products is a persistent issue in transfusion, with *Staphylococcus aureus* being a common contaminant. Current methods to prevent contamination have limitations, leading the needs of exploration of alternative solutions like UV-LED light for bacterial inactivation. **Materials and methods:** This study used a true experimental design. Plasma samples were collected, inoculated with *S. aureus*, and exposed to 405 nm UV-LED light for varying durations (15, 30, 45, and 60 minutes). Colony counts were measured to determine the effect of UV-LED on bacterial growth and compared to control. **Results:** The results demonstrated a significant reduction in bacterial counts, with a >90% decrease in *S. aureus* contamination at 60 minutes of exposure ($p < 0.05$). Moderate reductions were observed at shorter durations (15, 30, and 45 minutes), with the most pronounced decrease at 60 minutes. **Conclusion:** The findings suggest that 405 nm UV-LED is an effective method for inactivating *S. aureus* in donor plasma, offering a promising alternative for reducing bacterial contamination in blood products. Further studies are recommended to optimize exposure times for maximum efficacy.
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Keywords: Bacterial contamination, Blood donor, Plasma donor, *Staphylococcus aureus*, 405 nm UV-LED

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INTRODUCTION

Blood transfusion is a critical aspect of healthcare, with ensuring the quality of blood products being a primary concern. The preparation of blood bags intended for transfusion requires sterility, free from bacterial, fungal, and other microorganism contamination. To meet this need, blood collection and processing must adhere to aseptic conditions and employ advanced materials for procedures such as phlebotomy, donation, collection, component separation, refrigeration, and freezing (1).

Despite significant advancements and increased scientific research in the past decade, bacterial contamination remains a challenging issue in blood products (2). Unlike viral infections, bacterial contamination poses a higher risk of infection transmission through blood transfusion, making it a prevalent cause of mortality and infections related to transfusion (3). Studies have identified gram-positive and gram-positive bacteria in a notable percentage of blood products (4), with developing countries reporting higher contamination rates compared to developed nations (5). Cases of bacterial contamination in blood products are also frequently reported in African countries (6). Contamination could cause by blood processing, phlebotomy, and might be caused by bacteremia donor (7). Contaminated blood product could cause sepsis to receiver patient by transfusion. Sepsis is a systemic infection which responsible to multiorgan failure and even death (8).

One of the bacteria commonly found contaminating blood products is *Staphylococcus aureus* (9). Previous studies have reported *S. aureus* contamination in blood products in various countries worldwide, such as Congo (10), Morocco (11), Ethiopia (12), and other locations (13). *S. aureus* is a prominent gram-positive pathogenic bacterium frequently found on human skin surfaces (14,15). *S. aureus* have a rounded shape, grape-like irregularly arranged (16). This bacterium poses a significant risk of contaminating blood products and causing serious infections in humans through blood transfusion (17).

As a gram-positive bacterium, *S. aureus* has been widely reported in various blood products; contamination by this bacterium can adversely affect blood recipients as *S. aureus*-contaminated blood products have the potential to cause infections due to the exotoxins they produce (18). Most of bacteria identified in plasma blood products are gram-positive bacteria, capable of triggering transfusion reactions, whereas gram-negative bacterial contamination, though less frequent, carries an increased risk of transfusion-related complications and potential mortality (19).

Various approaches have been undertaken to reduce or eliminate cases of *S. aureus* contamination in blood products, ranging from preventive measures to curative actions such as the use of Pathogen Reduction Technology (PRT) (20). One such effort involves the utilization of SiO2, a technique that enhances blood bags and related medical devices by incorporating organic and inorganic nanomaterials, especially silicon dioxide (SiO2) nanoparticles, known for their antibacterial properties (21). Another strategy is Photodynamic Antimicrobial Chemotherapy (PACT), which entails the production of Reactive Oxygen Species (ROS) through the interaction of oxygen, photosensitizing molecule, and light (22). However, bacterial contamination in blood bags remains a significant challenge that requires resolution to enhance the quality of blood products and patient health. Therefore, further targeted interventions are necessary, particularly concerning bacterial contamination in blood bags, where this study take place.

Several studies have explored the potential of 405 nm Ultra Violet Light Emitting Diode as a promising method for decontamination without the need for additional substance like photosensitizers in PACT (23) and amotosalen in UV-A PRT (24) or additional bag in UV-C PRT (25). 405 nm UV-LED introduced as efficient and safety for human application (26). Additional substance usage such as amotosalen in UV-A PRT is known could reduce platelet function, affecting blood quality. While usage of photosensitizer and additional bag certainly affect efficiency due to additional steps and cost required to achieve ready-to-transfusion blood. 405 nm UV-LED recognized for their ability to decontaminate medical instruments. Previous research has shown that UV LEDs effectively deactivate bacteria such as MRSA and vancomycin-resistant enterococci, as well as certain viruses, with reductions in bacterial counts observed following exposure for specific durations (27). Exposure to 405 nm UV-LEDs can significantly reduce bacterial contamination levels in plasma products by 99%-100% after one hour of exposure (4). These findings underscore the potential of UV light to eliminate microorganisms within a defined timeframe, so this study will take place to contribute the thrive to overcome bacterial contamination in blood bags.

MATERIALS AND METHODS

This study was conducted from August until September 2023 using a true experimental design. The research was carried out at the Laboratory of Blood Bank Technology at Stikes Rajekwesi Bojonegoro, East Java, Indonesia. This study has obtain Ethical approval by the ethics committee under the number 031/PRO/KEPK/VIII/2023 from STIKES Guna Bangsa, Yogyakarta, Indonesia. The sample used in this study consisted of plasma from donors, which was subsequently inoculated with *Staphylococcus aureus*. Liquid plasma obtained from conventional blood donors was processed following Standard Operating Procedures (SOP) for blood separation using double blood bags. The production process was conducted by professional blood service technicians at Laboratory of Blood Component, Bojonegoro Blood Donor Unit, East Java, Indonesia.

Sample Preparation

Aliquots of stock solution were incubated for 24 hours at 37°C in nutrient broth to produce *S. aureus* samples. Gathered liquid plasma from traditional blood donors and processed using a double blood bag as part of the Standard Operational Procedure for Blood Separation. The process of collecting blood at the Bojonegoro Blood Donor Unit is managed by a qualified blood technician. Preparation of bacterial suspension *Staphylococcus aureus* was inoculated on Nutrient Agar (NA) media and incubated at 37°C for 24 hours. After the incubation process, a single colony loop was taken from the solid agar media and transferred to a test tube containing 5 mL of NaCl solution. The turbidity of the colony suspension was adjusted to the 0.5 McFarland standard before being used for subsequent research. The suspension then diluted until 10-5 and ready to added to blood plasma.

The Inoculation of Staphylococus aureus on Blood Plasma

After the bacterial suspension was prepared, the next step was to inoculate the processed donor plasma with the bacteria. 1 mL of the bacterial suspension was added to 9 mL of donor plasma, followed by homogenization process. All procedures were conducted using aseptic techniques to ensure successful inoculation and prevent contamination.

Pathogen Inactivation Procedure

A total of 200 µL of plasma inoculated with *S. aureus* was transferred into a microplate u-96 well and exposed to UV LED light at 405 nm for various durations (15, 30, 45, and 60 minutes). This process was repeated six times for each treatment and control group. Total of 100 µL sample then inoculated into NA media then incubated at 37°C for 24 hours to evaluate the colony count. Colony growth was then observed and counted using a colony counter, and compared between treatment and control groups.

Statistical Analysis

Obtained data then analyzed using SPSS25 software and analyzed using one way ANOVA after knowing the data were distributed normally by Shapiro Wilk test. The differences between groups were using significant value decided less than 0,05 ($p<0,05$).

RESULTS

The result in this study revealed how the UV exposure could affect on decrease of bacterial contaminant in culture sample. Exposure of 405 nm UV LED on Plasma

contaminated by S.aureus exhibiting varied colony growth quantities in NA culture. There is dramatic decrease of colony growth at 60 minutes of UV LED Exposure compared to control (Fig.1). In this study, the observed bacterial growth appeared to have a difference colony count and decreased the number of colonies compared to control group.

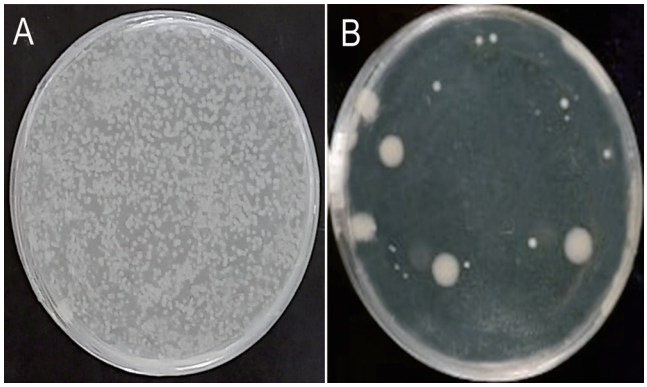

Fig. 1.: *S. aureus* growth on Media Cultures (A. Control group; B. 60 minutes 405 nm UV-LED Exposure)

The effect of 405 nm UV-LED on *S. aureus* inoculated in human plasma exhibit a contamination decrease on every exposure time. The results indicate that the most significant decrease occurred at 60 minutes ($p < 0.05$), with a reduction in bacterial count by >90% compared to the control where were not expose to 405 nm UV-LED. The decrease occurred gradually in accordance with the increase in exposure duration, showing a moderate decrease at 15, 30, and 45 minutes of exposure, followed by a dramatic decrease at 60 minutes of exposure duration (Fig. 2). This research demonstrated that 405 nm UV-LED acted in reducing the quantity of *S. aureus* contamination in donor plasma. However, more testing on various exposure times is required to establish the optimum exposure period that can minimize and prevent the growth of *S. aureus* in donor plasma. UV LED as an instrument is structured by flexible and adjustable mercury lamp. Thus, make it easy to use and can be applied without heating the instrument.

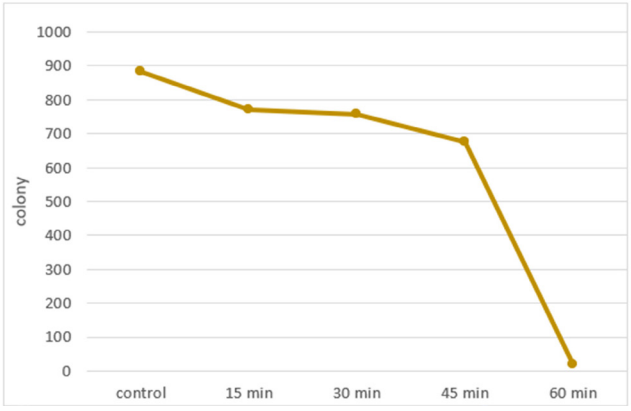

Fig. 2: *S. aureus* colony count exposed by UV LED

DISCUSSION

This study has successfully revealed the effect of 405 nm UV-LED to contaminant bacterial, especially *S. aureus* in plasma donor. We found that 405nm UV LED exposure could reduce *S. aureus* at culture media after certain time of exposure. In addition, 60 minutes of UV LED exposure was resulting a dramatic reduction of *S. aureus* bacterial count. This finding suggest that UV LED exposure could be a potential alternative to applied as effective bacterial decontamination and due to its ease to operate and doesn't need much of resources to operate, this could be an efficient tool of decontamination either. The utilization of UV LED at various wavelength has been shown and reported in many studies that targeted to food (28) and hospital tool's surface (29). *S. aureus* is an opportunistic pathogenic gram-positive bacterium usually presence in various type and place of natural environment that cause a wide range of clinical infections (30). Commonly we could find *S. aureus* on the human skin and recognized as the most common pathogen causing a wide range of infection. Infection from the bacteria could start from superficial skin and soft tissue infections to deadly conditions such as osteoarticular infection, endocarditis, pneumonia, and bacteremia (14,31,32). *S. aureus* was characterized by polysaccharide capsule or thin membrane that role in its virulence (33).

Previous study reported that exposure of UV LED was significantly effective to reduce Pseudomonas aeruginosa contamination and had decontamination effect up to 90% at 405 nm wavelength at 60 minutes of exposure (34). Another study reported that UV LED was significantly effective to reduced MRSA contamination and thus had antibacterial activity by eliminate majority of contaminant on 365 nm wavelength at 24 hours (35). Bacterial contamination of environmental surfaces is widely recognized as a critical factor in the indirect transmission of infections especially within healthcare settings. Decontamination was a crucial process to prevent bacterial contamination that could cause infection and affected human health (36). Therefore, utilization of UV LED as one of Pathogen Reduction Technology technique that address bacterial contaminant is of considerable interest. This study provide evidence that contaminant bacterial are susceptible to inactivation by UV LED at 405 nm within certain exposure time. These demonstrated that 405 nm UV LED exhibit antibacterial activity against examined bacteria.

The UV exposure time and bacterial susceptibility play an important role to achieve the maximum effect on bacterial inactivation. On previous study there are various time required to obtained the maximum antibacterial activity. The exposure time required to obtained maximum antibacterial activity of UV LED exposure addressed MRSA, Candida Auris, bacteriophage MS2 and bacteriophage Phi X174is 48 h (35). In other

hand, another study reported that UV exposure time to achieved maximum bacterial inactivation is way shorter that only need 30-60 minutes (37).

UV light as one method for inactivating bacterial contamination, employing various mechanisms of action that causing specific biological effects. DNA Damage is primary biological effect experienced after UV exposure. Reaction to UV Light caused adjacent thymine to form thymine dimers that result in alteration in cell properties, that further could cause in disruption of bacterial cell membrane (38). Likewise, other biomolecules such as lipids and proteins are become primary target of ROS that produced by UV light exposure and involved indirectly to cell breakdown (39).

Bacterial susceptibility affects in required UV time exposure investigated *S. aureus*, K. pneumoniae, E. coli and E. faecalis reported that *S. aureus* is the most susceptible to inactivation. (40) In this study we use only gram-positive bacteria as a sample. However, there are several reports that shown optimum exposure time needed between gram-positive versus gram-negative contaminant bacteria. Studies reported that there is no significant difference between gram-negative and positive bacteria that required similar optimum exposure time to achieve near-complete reduction (41). Otherwise, another finding shown there is different sensitivity between gram-positive and negative bacteria which gram-negative bacteria was more resilient (42)

CONCLUSION

405 nm UV LED treatment is a potential technology to bacterial inactivation in blood products. A significant effect was observed with 405 nm UV LED exposure on the contaminant bacteria *S. aureus*, where a 60-minute exposure duration resulted in a dramatic reduction in bacterial count. The use of 405 nm UV LED is expected to serve as an alternative solution for reducing bacterial contamination in blood components and to generally improve the quality of blood products. The effectiveness of 405 nm UV LED also offers beneficial effect.

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