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THE EFFECT OF LED MAGNETIC ON *STAPHYLOCOCCUS AUREUS* BACTERIA

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Abstract

Nosocomial infections, caused by viruses, bacteria, and fungal pathogens, often occur during treatment in health facilities due to cross-contamination from healthcare workers or medical equipment. The study explores the efficacy of static magnetic fields and photodynamic inactivation in inactivating Staphylococcus aureus bacteria, a crucial step in sterilization. The study used high-power blue LEDs and static magnetic fields generated by neodymium magnets. The highest reduction percentage observed was 81.92 ± 7.92%, found in the combination treatment of static magnetic fields (SMF) with LED illumination at the F8 microplate location, with a treatment time of 40 minutes, an LED dose of 11.72 J/cm², and a magnetic field dose of 25.61 mT. The lowest reduction percentage, 52.41 ± 7.64%, was observed at the F8 microplate location with a treatment time of 10 minutes, an LED dose of 2.93 J/cm², and a magnetic field dose of 25.61 mT.

Keywords: Photodynamic Inactivation (PDI), Static Magnetic Field (SMF), High Power LED, Staphylococcus aureus

Abstrak

Infeksi nosokomial adalah infeksi oleh virus, bakteri dan jamur patogen yang menyerang pasien ketika menjalani proses perawatan di fasilitas kesehatan. Hal ini disebabkan oleh infeksi silang dari tangan petugas kesehatan atau peralatan medis yang digunakan. Bakteri *Staphylococcus Aureus* adalah salah satu patogen yang paling terkenal menyebabkan infeksi mulai dari infeksi kulit ringan hingga sepsis fatal atau kegagalan multiorgan. Sterilisasi adalah suatu proses atau metode untuk membasmi atau menghancurkan segala bentuk mikroorganisme seperti bakteri, fungi, jamur dan virus yang ada pada suatu benda atau lingkungan sehingga tidak dapat lagi berkembang biak atau menyebabkan infeksi. Penelitian ini bertujuan menguji pengaruh kombinasi medan magnet statis dengan *photodynamic inactivation* untuk inaktivasi bakteri *Staphylococcus aureus*. Penelitian ini menggunakan *High Power* LED Biru dan Medan magnet Statis yang berasal dari Magnet Neodimium, Nilai persentase reduksi tertinggi adalah 81,92 $\pm$ 7,92 % ditemukan pada perlakuan kombinasi medan magnet statis dengan penyinaran LED di lokasi *microplate* F8 dengan waktu 40 menit, Dosis LED sebesar 11,72 J/cm^2 dan medan magnet statis sebesar 25,61 mT. Dan persentase reduksi terendah yaitu 52,41 $\pm$ 7,64 % ditemukan pada lokasi *microplate* F8 dengan waktu 10 menit, Dosis LED sebesar 2,93 J/cm^2 dan nilai medan magnet statis (SMF) sebesar 25,61 mT. Perlu dilakukan penelitian lebih lanjut menggunakan bakteri gram negatif dan jamur.

Kata kunci: *Photodynamic Inactivation (PDI), Static magnetic field (SMF), High Power LED, Staphylococcus aureus*

1. INTRODUCTION

Health facilities are establishments where individuals can receive health services, such as hospitals, community health centers, and clinics (Supardi, 2016). A hospital is a healthcare institution that offers comprehensive health services, including inpatient, outpatient, and emergency care (Peraturan Menteri Kesehatan Republik Indonesia, 2020). Health services are initiatives or activities aimed at achieving the highest level of health for individuals and society (Astuti et al., 2023). Nosocomial infections are caused by viruses, bacteria, and fungal pathogens that infect patients while undergoing treatment at a health facility (Marfu'ah & Sofiana, 2018). Nosocomial infections can be caused by the patient's endogenous flora or by cross-infection from the hands of healthcare workers or contaminated medical equipment (Lubis et al., 2015). Infections often occur due to a need for the equipment used in the healthcare process to be cleaned more thoroughly. Based on research by Lubis (2015), bacterial contaminants found on nurses' hands include Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Bacillus sp. Contaminated hands of nurses can act as carriers, transferring these bacteria to medical tools used in healthcare services. This aligns with research by Marselina (2023), which found that gram-positive bacteria, such as *Streptococcus sp*., *Staphylococcus epidermidis*, and *Staphylococcus aureus*, frequently contaminate surgical instruments. *Staphylococcus aureus (S. aureus)* bacteria can originate from the contamination of wounds by normal skin flora. In contrast, *Escherichia coli* (*E. coli*) bacteria are found on nurses' hands when they touch contaminated urinary catheters.

Sterilization is a process or method used to eradicate or destroy all forms of microorganisms, including bacteria, fungi, and viruses, on an object or environment to prevent them from reproducing or causing infections. Sterilization aims to ensure an object's cleanliness and safety from harmful germs or microorganisms. Among all living organisms, bacterial spores are the most resistant to sterilization. The effectiveness of sterilization depends on the amount and type of contamination by other substances and the presence or absence of protective places for microorganisms on the equipment (Komariyah, 2020).

In the healthcare industry, sterilization is a crucial process for maintaining cleanliness and preventing the spread of infection. However, current sterilizers have certain limitations in their effectiveness and practicality. The autoclave is considered the gold standard for sterilization. It operates using high-pressure saturated steam and requires approximately 45 minutes for sterilization (holding time).

Autoclaves' disadvantages include high electrical power consumption, long waiting time to reach the target temperature (penetration time), and heavy weight of the equipment. Additionally, the success of sterilization with an autoclave heavily depends on the steam quality (Ma'at, 2009). *Autoclaves* are classified as a type of wet sterilization.

There is a hot air sterilizer for dry sterilization, which operates using hot air. This tool sterilizes materials or instruments that cannot be sterilized with water vapor. The sterilization method involves an oxidation process that can damage the sterilized material. The advantage of this method is that the material or instrument remains dry. However, the diffusion or penetration of dry air is slow, requiring a relatively long sterilization time. The working temperature ranges from 140°C to 200°C, with sterilization times varying from 30 to 180 minutes.

Thus, a more practical alternative method for inactivating contaminant bacteria is needed, such as using Photodynamic Inactivation (PDI) combined with static magnetic fields (SMF). Photodynamic Inactivation is a method that inhibits cell metabolic activity by utilizing the interaction between light and photosensitizer molecules, resulting bacterial cell death (Astuti et al., 2020). This technique breaks down target cells through oxidation, which causes cell lysis and inactivation of membrane proteins (Pereira et al., 2018). Combining light and a photosensitizer with the appropriate spectrum can induce cell photoinactivation. Many microbial cells are susceptible to killing by blue light (400–470 nm) due to the natural accumulation of photosensitizers such as porphyrins and flavins (Yin et al., 2013). Naturally, some bacteria produce endogenous porphyrin compounds, endogenous photosensitizer molecules sensitive to light (Astuti et al., 2020). Combining light with a static magnetic field (SMF) in dynamic phototherapy can enhance the photoinactivation of pathogenic bacteria. Magnetic fields can contribute additional energy by inducing a strong magnetic field in photosensitivity (Arifianto et al., 2023). Variations in the dose of static magnetic field (SMF) energy and LED energy density are used to determine the optimal dose during in vitro testing on *Staphylococcus aureus* bacteria (Gram-positive). Based on an in vitro study, light with a wavelength of 405 nm and a radiation exposure of 15 J/cm² achieved an inactivation efficacy of 90% against *Staphylococcus aureus* bacteria (Yin et al., 2013).

2. RESEARCH METHOD

2.1 Time and Place of Research

The research was conducted from April 2024 to June 2024 at the Biophysics and Medical Physics Laboratory, Department of Physics, Faculty of Science and Technology, Airlangga University.

2.2 Equipment and Materials

The equipment used in this study included a magnetic LED-based sterilizer, Gram-positive bacteria Staphylococcus aureus, distilled water, aluminum foil, plastic wrap, spirits, tissues, 70% alcohol, TSA (Tryptic et al.) and TSB (Tryptic et al.) media, physiological saline (a mixture of distilled water and NaCl), microplates, autoclave, vortex mixer, incubator, magnetic stirrer, digital scale, micropipette, spatula, inoculating loop, Erlenmeyer flask, measuring cup, weighing paper, cuvette, Bunsen burner, and stirring rod.

2.3 Research Design

This research is an experimental study with a completely randomized design using a factorial pattern. It consists of High Power LED energy density and static magnetic field (SMF) energy dose. The energy factor combines power and time and is categorized into four energy doses. The study is conducted with six variations of locations on the microplate. Figure 1 shows the research design diagram.

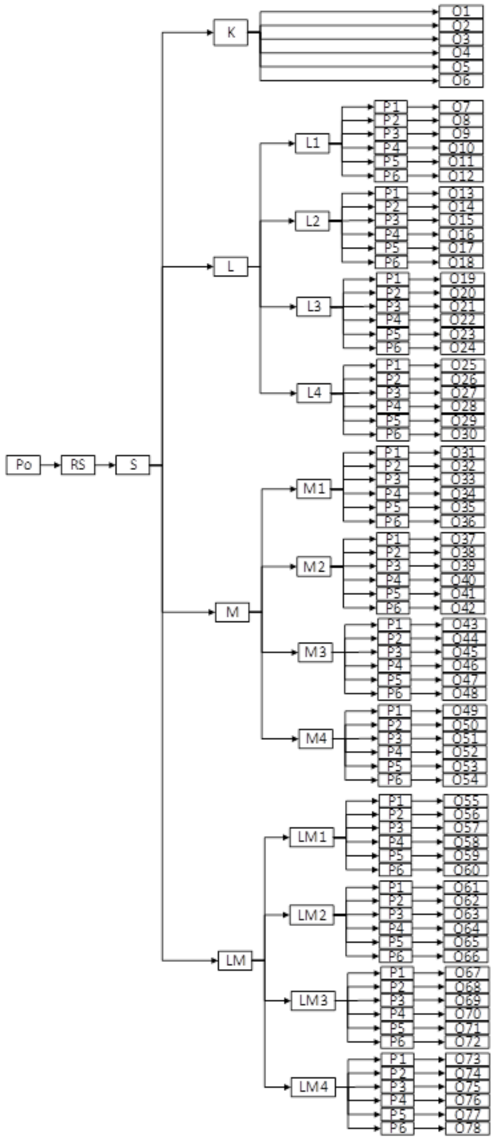

Figure 1. Research Design Diagram

2.3 DATA ANALYSIS.

Data analysis was conducted quantitatively after the bacteria underwent various treatments and time variations. This was followed by calculating the number of colonies. The method used to determine the number of bacteria is the Total Plate Count (TPC) method. The percentage decrease in bacterial colonies was calculated using Equation 1.

$$\%\,Reduction\ bactery = \left|\frac{\sum treatment - \sum control}{\sum control}\right| \times 100\% \quad (1)$$

Further analysis was conducted using the IBM SPSS (Statistical Package for the Social Sciences) program. The Two-Way ANOVA Factorial Test was performed, which determines the effect of each factor and the interaction between factors. The Two-Way ANOVA Factorial Test requirements include typically distributed data and homogeneous variances, with the data being at least at the interval scale. The null hypothesis (H0) is rejected if the significance value (p) is less than α, where α = 0.05.

Subsequently, a Post Hoc Test using the Tukey method was performed to examine differences between sample factors, with the condition that the p-value must be less than 0.05 ($p < 0.05$). The results of this test will help identify the most effective treatment for inactivating *Staphylococcus aureus* bacteria.

3. RESULTS AND DISCUSSION

In this study, a bacterial inactivation test was conducted using the Total Plate Count (TPC) method to determine the percentage of bacterial reduction. *Staphylococcus aureus* was tested with three treatments: LED irradiation, exposure to static magnetic fields (SMF), and a combination of LED and static magnetic fields (SMF). Variations in time and location on the microplate were also considered. Figure 2 shows the percentage reduction of *Staphylococcus Aureus* bacteria (a) location A12 (b) location H1 (c) location B6 (d) location G6 (e) location C8 (f) location F8.

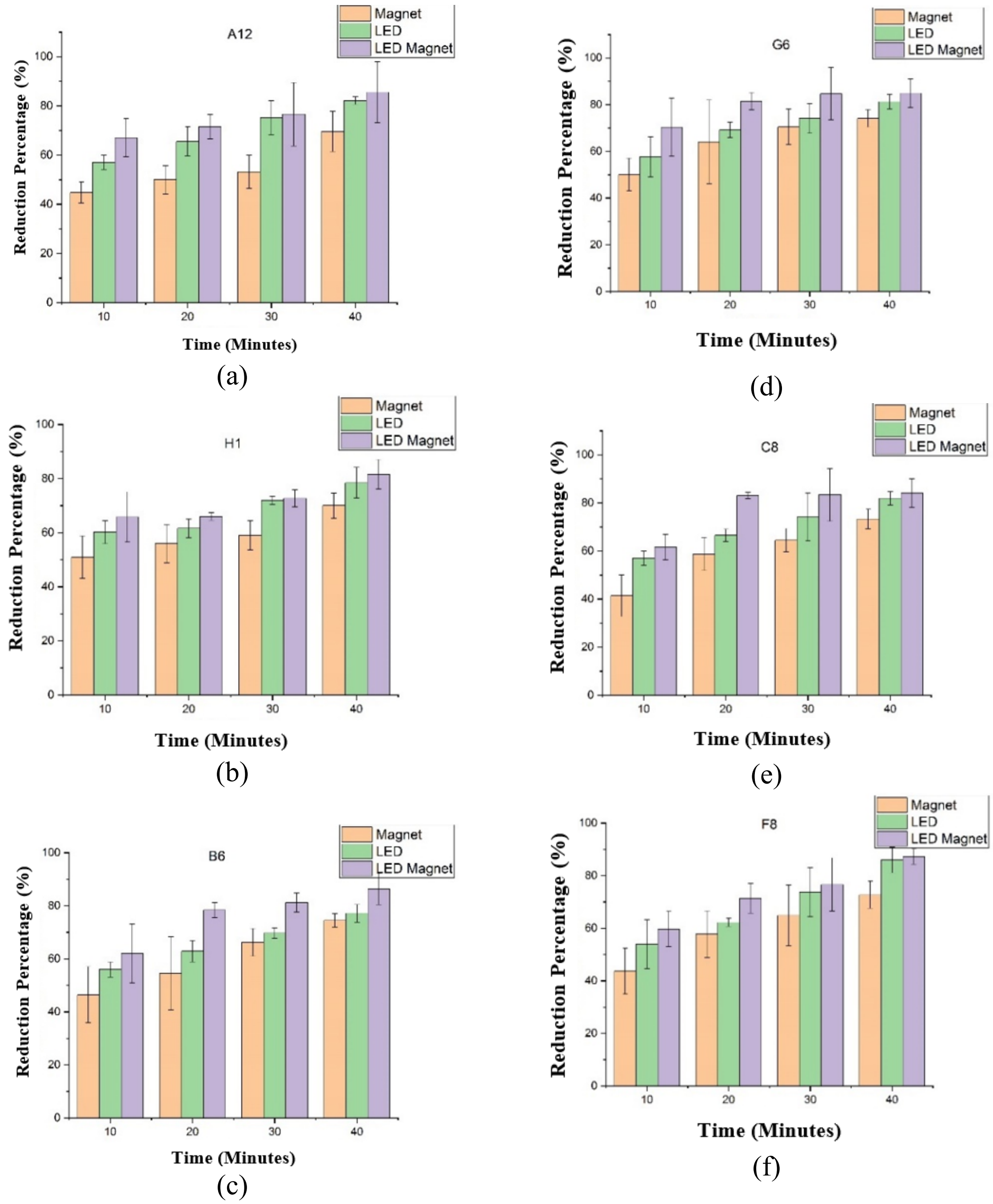

Figure 2. Percentage reduction of Staphylococcus Aureus bacteria (a) location A12 (b) location H1 (c) location B6 (d) location G6 (e) location C8 (f) location F8.

Based on the results observed in the bar chart of the percentage of bacterial reduction shown in Figure 2, it is evident that the combination of LED irradiation and static magnetic field (SMF) produces the highest reduction compared to treatments with only SMF or LED irradiation alone. The highest percentage of bacterial reduction, 81.92 ± 7.92%, was observed at location F8 with a combination of LED irradiation and SMF for a duration of 40 minutes. At this location, the SMF value was 25.61 mT, and the LED power was 1.38 watts. This indicates that the addition of SMF significantly enhances the bacterial reduction percentage in *Staphylococcus aureus.* Table 1 indficates the results of statistical analysis on *Staphylococcus Aureus* bacteria.

Table 1. Results of statistical analysis on Staphylococcus Aureus bacteria.

Treatment	Group	N	Reduksi		Factorial Test	
			Rerata	SD	Significance	Conclusion
Locations	A12(1)	60	66,51	12,78	P=0,001	Significance
	H1(2)	60	66,18	9,08		
	B6(3)	60	67,94	12,14		
	G6(4)	60	71,86	10,90		
	C8(5)	60	69,13	13,03		
	F8(6)	60	67,47	12,97		
Energy	10 Minutes(A)	90	55,86	8,67	P=0,000	Significance
	20 Minutes(B)	90	65,60	9,30		
	30 Minutes(C)	90	71,78	8,47		
	40 Minutes(D)	90	79,49	6,46		
Interaction	1A	15	56,27	9,66	P=0,057	Not Significance
	1B	15	62,34	9,55		
	1C	15	68,32	11,78		
	1D	15	79,11	7,79		
	2A	15	58,99	6,95		
	2B	15	61,19	4,51		
	2C	15	67,89	6,73		
	2D	15	76,68	5,48		
	3A	15	54,81	7,58		
	3B	15	65,26	10,86		
	3C	15	72,37	6,83		
	3D	15	79,33	6,79		
	4A	15	59,36	9,42		
	4B	15	71,60	8,70		
	4C	15	76,41	7,03		
	4D	15	80,10	4,93		
	5A	15	53,35	9,36		
	5B	15	69,41	10,64		
	5C	15	73,97	8,67		
	5D	15	79,77	5,08		
	6A	15	52,41	7,64		
	6B	15	63,77	6,40		
	6C	15	71,75	6,60		
	6D	15	81,97	7,92		

Based on the calculation of the percentage of bacterial reduction, statistical analysis was performed using the SPSS (Statistical Package for Social Sciences) program. Two statistical tests were conducted: the normality test and the Two-Way ANOVA Factorial test.

The normality test, using the One-Sample Kolmogorov-Smirnov test, resulted in a significance value of 0.291, indicating that the data is normally distributed. This is because the requirement for normality is $p > \alpha = 0.05$, and this criterion was met.

The Two-Way ANOVA Factorial test showed a p-value of 0.057, which is greater than 0.05. This indicates that there were no significant differences in bacterial reduction between different locations and energy variations.

4. CONCLUSIONS

The most effective inactivation results were observed with the combination treatment of LED and static magnetic field (SMF) at the F8 microplate location. The percentage reduction was 81.92 ± 7.92% with a treatment duration of 40 minutes, an LED energy density of 11.72 J/cm², and an SMF of 25.61 mT. Future research could expand on this by applying the Magnetic LED test to other types of bacteria (both Gram-positive and Gram-negative) or fungi.

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