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The effect of low-electrical voltage as a method to eradicate *Acinetobacter baumannii* bacteria

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ABSTRACT

Introduction: *Acinetobacter baumannii* is one of the bacteria in critical priority according to WHO in 2017 and one of the causes of nosocomial infections in the world. These bacteria usually become resistant to antibiotics (Multi Drug Resistant Organism, MDRO), infect the bloodstream, and cause pneumonia. This study aimed to examine the effect of low-voltage electricity as a method to kill *Acinetobacter baumannii* by using a different electric current.

Methods: This study is experimental research in the Microbiology Laboratory of RSAB Harapan Kita. Experiments were carried out using a solution of *Acinetobacter baumannii* non-MDRO and MDRO bacteria with a parallel design device opposite to the GPS 3030D power supply with a power of 90 W. Each tube was fitted with a 12 cm stainless steel rod conductor with a diameter of 3 mm and delivered amperage 1 mA, 2 mA, 5 mA, and 10 mA and 0.5V with each amperage performed 5 times and measured using DensiCHEK.

Result: From 20 *Acinetobacter baumannii* non-MDRO experiments and 20 MDRO experiments, it was found that from 1 mA, 2 mA, 5 mA, and 10 mA with monitoring time of 30 minutes, 2 hours, and 4 hours, the results of bacterial eradication by DC stimulation with 5 mA and the most optimal time is 30 minutes according to what has been applied.

Conclusion: Using an electric current of 5 mA and a duration of 30 minutes can reduce *Acinetobacter baumannii* MDRO and non-MDRO bacteria with more optimal results in the non-MDRO group.

Keywords: *Acinetobacter baumannii*; low-electrical voltage; biofilms; nosocomial infection.

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INTRODUCTION

Acinetobacter baumannii is the main cause of nosocomial infections in goods and equipment in hospitals. Currently, there is multidrug resistance to antibiotics and there is an urgency to seek other treatments for *Acinetobacter baumannii*. *Acinetobacter baumannii* was determined as a Multidrug Resistant Organism (MDRO) by two methods, namely when it is resistant to one key antibiotic in the presence of cross-resistance or co-resistance to other antibiotics and a strain that is resistant to more than three types. antibiotics from the penicillin, cephalosporin group and with a mixture of their inhibitors, fluoroquinolones, and aminoglycosides.^{1,2}

Acinetobacter baumannii has hydrophobic ability and the ability to form biofilms as a basis for transmission.^{3,4} When a biofilm is formed, it will be easier for bacteria to adhere to materials such as

polystyrene, polypropylene and Teflon, and glass which are the main ingredients of medical devices and make bacteria resistant to antibiotics.⁵⁻⁷

Study has explained that the bioelectric effect has a synergistic effect between an electric voltage and a low current in the liquid surrounding the biofilm and the antibiotic. Electric potential current and electric constant current play a role in controlling biofilm electrochemistry by controlling the biofilm surface and preventing adhesion.⁸ Extracellular polymeric substances in the biofilm matrix can be protonated by forming hydrogen and oxygen bonds in polymer sugars which can cause a reduction in biofilm thickening.⁹

The amount of electric current used to eradicate *Acinetobacter baumannii* will be one part of the research to see the effect of low voltage electricity on non-MDRO and MDRO *Acinetobacter baumannii* bacteria

and the duration of exposure to electricity. This study aimed to examine the effect of low-voltage electricity as a method to kill *Acinetobacter baumannii* using a different electric current.

METHODS

This study uses an experimental study. The research location is in the Microbiology Laboratory of RSAB Harapan Kita. The study population was *Acinetobacter baumannii* non-MDRO and MDRO isolates obtained from the stock of clinical bacterial samples obtained from the Microbiology Lab, University of Indonesia. Bacterial isolates from the growing media will be inoculated into 0.9% PZ NaCl liquid media or 3 mL of distilled water per tube. The bacterial suspension solution will be calculated for the turbidity of the solution and measured with DensiCHEKTM until it reaches 1 McFarland (3 x 10⁸ CFU/mL).

With Federer's formula, 40 bacterial

suspension solutions were obtained for the experiment with 5 samples for each *Acinetobacter* non-MDRO and MDRO bacteria and 4 different electric currents.

Electric Current Intervention

The instrument used is a single output power supply Direct Current type 3030D 90W, which is paralleled into six lines of electricity lines, each of which has a rheostat potentiometer with a magnitude of 30 ohms and 30 watts as a regulator of electric current at the large output voltage and electric current that will come out in each line of the circuit line. The bacterial suspension solution was put into a tube of 3 mL, and 2 stainless steel rods 12 cm long, and 3 mm in diameter were sterilized, and an electric current of 1 mA, 2 mA, 5 mA, and 10 mA was applied with an electric voltage of 0.5 V. Monitoring and recording changes in bacterial colonies every 30 minutes, 2 hours, and 4 hours.

Statistical analysis

This study used SPSS 12.0 for data compilation and tabulation and was statistically tested with variance test, Bonferroni, and t-test.

RESULT

The results were obtained from the administration of low electric voltage with a power of 1 mA, 2 mA, 5 mA, and 10 mA by monitoring the change in bacterial colonies at three different times on *Acinetobacter baumannii* non-MDRO bacteria along with the CFU/ml

table. Table 1 and 2 shows the comparison of the average number of colonies of *Acinetobacter baumannii* non-MDRO and MDRO in CFU/mL with units of time based on 4 different amperes. Table 3 shows the comparison of the mean of bacterial colonies in CFU/mL between MDRO and Non-MDRO for each unit of monitoring time in per ampere.

DISCUSSION

Biofilm on *Acinetobacter baumannii* plays a role in survival and transfer of bacteria in hospital areas on biotic and abiotic surfaces. Biofilm-associated protein (bap) plays an important role in intercellular adhesion. Expression of the blaPER-1 gene makes *Acinetobacter baumannii* easier to attach to respiratory epithelial cells. OmpA (outer membrane protein A) plays a role in the attachment of bacteria to plastic surfaces, where OmpA is the most abundant outer membrane porin.^{10,11} CsuA plays a role in forming biofilms that can adhere to abiotic surfaces. There are many cases where genetic biofilm-forming in *Acinetobacter baumannii*, including csuE, OmpA, and bap, also affects the resistance of bacteria to antibiotics.¹² The electric voltage causes electroporation or the formation of holes in the cell membrane. The amount of electricity to electroporate bacteria is smaller than that of human cell membranes because of the different conductivity. Exposure to electricity that is delivered to lyse bacteria does not affect humans.¹³

According to Wellman et al., 1996,

it was found that 1 mA amperage was added with 5 mg tobramycin/liter, there was bactericidal activity, but at 1 mg/ml added with 1 mA electricity, there was no evidence of bacterial eradication.⁷ It was found in the experimental analysis described in the previous chapter that 1 mA amperage did not have a significant effect on the killing of the bacteria *Acinetobacter baumannii* with the three monitoring times at 30 minutes, 2 hours and 4 hours on both bacteria not having significant results on the p-value. The mean results for both also showed an increase in the p-value without any visible results being significant, with the best results at 30

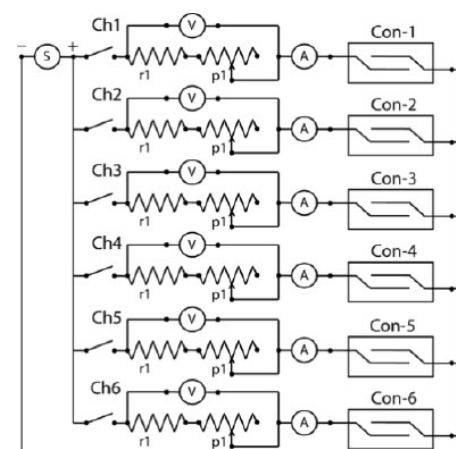


Figure 1. Electrical circuit for the division of 6 channels. S = DC power source, V = voltmeter, A = currentmeter. r1= 2 ohms, p1= rheostat potentiometer 30 ohms 30 watts, Con-1 to 6 (conductors 1 to 6).

Table 1. Comparison of the average number of colonies of *Acinetobacter baumannii* Non-MDRO in CFU/mL with time units-based on four different amperes

Ampere	Average (SD) CFU Time			p-value	Comparison (p-value)		
	30 (1)	120 (2)	240 (3)		1 vs 2	1 vs 3	2 vs 3
1 mA (a)	2.526 (0.16)	2.520 (0.15)	2.436 (0.13)	0.539	1.000	0.984	1.000
2 mA (b)	2.502 (0.08)	2.486 (0.13)	2.480 (0.15)	0.962	1.000	1.000	1.000
5 mA (c)	2.922 (0.04)	2.850 (0.56)	2.802 (0.04)	0.008	0.119	0.007*	0.449
10 mA (d)	2.814 (0.11)	2.706 (0.10)	2.646 (0.11)	0.096	0.464	0.107	1.000
p-value between Amperes	0.000	0.001	0.000				
a vs b	1.000	1.000	1.000				
a vs c	0.000*	0.003*	0.000*				
a vs d	0.005	0.149	0.047*				
b vs c	0.000*	0.001*	0.002*				
b vs d	0.002*	0.059	0.174				
c vs d	0.845	0.439	0.232				

Note: * The mean difference is significant below 0.05 level.

Table 2. Comparison of the average number of colonies of *Acinetobacter baumannii* MDRO in CFU/mL with time units-based on four different amperes

Ampere	Average (SD) CFU Time			p-value	Comparison (p-value)		
	30 (1)	120 (2)	240 (3)		1 vs 2	1 vs 3	2 vs 3
1 mA (a)	3.036 (0.08)	3.018 (0.08)	3.000 (0.08)	0.794	1.000	1.000	1.000
2 mA (b)	3.054 (0.10)	2.988 (0.15)	3.000 (0.10)	0.686	1.000	1.000	1.000
5 mA (c)	2.616 (0.07)	2.562 (0.08)	2.520 (0.12)	0.333	1.000	0.442	1.000
10 mA (d)	2.472 (0.04)	2.460 (0.07)	2.496 (0.09)	0.850	1.000	1.000	1.000
p-value between Amperes	0.000	0.000	0.000				
a vs b	1.000	1.000	1.000				
a vs c	0.000*	0.000*	0.000*				
a vs d	0.000*	0.000*	0.000*				
b vs c	0.000*	0.000*	0.000*				
b vs d	0.000*	0.000*	0.000*				
c vs d	0.077	0.862	1.000				

Note: * The mean difference is significant below 0.05 level.

Table 3. Comparison of the mean number of bacterial colonies in CFu/mL between MDRO and Non-MDRO for each unit of monitoring time per ampere

(A) 1 miliampere				(B) 2 miliampere			
Time	CFU (10 ⁸)		p-value	Time	CFU (10 ⁸)		p-value
	Non-MDRO	MDRO			Non-MDRO	MDRO	
30	2.526 (0.16)	3.036 (0.08)	0.282	30	2.502 (0.08)	3.054 (0.10)	0.388
120	2.520(0.15)	3.018(0.08)	0.328	120	2.486(0.13)	2.988(0.15)	0.543
240	2.436(0.09)	3.000(0.08)	0.466	240	2.480(0.15)	3.000(0.10)	0.404
(C) 5 miliampere				(D) 10 miliampere			
Time	CFU (10 ⁸)		p-value	Time	CFU (10 ⁸)		p-value
	Non-MDRO	MDRO			Non-MDRO	MDRO	
30	2.922 (0.04)	2.616 (0.07)	0.437	30	2.814 (0.11)	2.472(0.04)	0.061
120	2.850(0.05)	2.562(0.08)	0.207	120	2.706(0.10)	2.460(0.07)	0.144
240	2.802(0.04)	2.520(0.12)	0.093	240	2.646(0.11)	2.496(0.15)	0.675

minutes. In both MDRO and Non-MDRO tables, several results show an increase at 4 hours, indicating instability to be used as optimal amperage.

With a low amperage of 2 mA, it can be seen that in the results table there is still an imbalance of experimental results in several tubes where there is an increase at 4 hours in both MDRO and Non-MDRO. The p-value did not show statistically

significant results for MDRO and Non-MDRO with $p > 0.05$. The p value on the comparison of the mean time for both MDRO and Non-MDRO did not show any significant results by showing the lowest time at 30 minutes.

Ampere 5 mA showed significant results in *Acinetobacter baumannii* non-MDRO with p value = 0.008. Meanwhile, the MDRO shows statistically insignificant

results even though it gives the lowest results compared to other amperes. The results table also shows that all tubes decreased at each monitoring time of 30 minutes, 2 hours, and 4 hours. 5 mA showed no significant result between the comparison of the time difference between MDRO and Non-MDRO with the best result at 4 hours (240 minutes) with $p = 0.093$.

It can be seen in the 10-mA table for non-MDRO that the p value shows $p = 0.096$ and although it does not show a significant result, it is still the second-best result after 5 mA whose results provide statistically significant results. *Acinetobacter baumannii* MDRO gave an insignificant value and gave the worst p value. Many tubes gave a bacteriostatic or stagnant effect with the same number of bacterial colonies without any changes. It was also given that 30 minutes of monitoring gave the best results even though it was not statistically significant with p value = 0.061.

From all the results obtained, it can be seen that *Acinetobacter baumannii*, with low voltage treatment on a milliamper scale, gave the most optimal results with 5 mA. *Acinetobacter baumannii* non-MDRO showed statistically significant results, but not with MDRO. The best time from the results of the comparison of MDRO and Non-MDRO was found at 30 minutes; the bacteria had started to go through eradication so that the optimal time was obtained at 30 minutes.

This research still has weaknesses such as only one species of bacteria that we did the experiment so that the results obtained still cannot be generalized to all bacteria. There is still a need for further research to determine how effective this electrical current is for killing and inhibiting the growth of bacteria that can potentially cause nosocomial infections.

CONCLUSION

It can be concluded that low voltage electricity can kill *Acinetobacter baumannii* MDRO and non-MDRO bacteria with an optimal electric current of 5 mA and an optimal time of 30 minutes. The research method can be used as a reference if similar research is implemented on sterilizing medical devices in health facilities.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest regarding this study.

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None.

AUTHOR CONTRIBUTION

All of the authors contributed equally to this study.

ETHICAL CONSIDERATION

This research has received ethical approval from the Ethics Committee of Universitas Airlangga and the Institutional Review Board of Harapan Kita Mother and Child Hospital, Jakarta.

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