Effect of Combination Low Voltage Electricity and Meropenem to Kill *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii is a pathogen that have been causing nosocomial infections worldwide, especially in the hospital settings. In 2017, WHO released a priority pathogen list that is in need of new antibiotics, this included Carbapenem-resistent Acinetobacter baumannii. Physical agents are being scouted as alternatives in this "post-antibiotic era". Low voltage electricity as a physical agent has been found to be effective to eradicate bacteria and biofilm.

This study used a true experimental method, using Acinetobacter baumannii solution with four types of treatment. One only treated with 0.5ml meropenem solution (8 mg/L), one only with low voltage electricity (3 volts for 15 minutes), one with both meropenem and low voltage electricity treatment with the last one being the control.

The result of this study was that meropenem alone is not enough to eradicate Acinetobacter baumannii. Instead, the bacterial colony growth of the ones that received the meropenem treatment surpassed the amount of bacterial colony that the control has with -7.19% eradication rate. The one that only received low voltage electricity has 99.76% eradication rate. The one that received both low voltage electricity and meropenem has 100% eradication rate.

The conclusion of this study is that the synergy of meropenem and low voltage electricity is effective to eradicate Acinetobacter baumannii. The researcher's suggestion for further research is to study more about the potential of low voltage electricity to increase the efficacy of antibiotics. The researcher also suggests for further research to use other types of antibiotics with different mechanism of actions.

Keywords: Acinetobacter baumannii; meropenem; low voltage electricity

1. Introduction

Acinetobacter baumannii is a pathogen that have caused nosocomial infections worldwide, especially in intensive care units (ICUs). Acinetobacter baumannii in the hospital setting have been found to on beds, curtains, medical devices, and even the belongings of medical personnel.[1] *Acinetobacter baumannii* stick to biological and abiotic surfaces to form biofilms.[2] The ability to form biofilm, especially on abiotic surfaces, plays a major role for Acinetobacter baumannii to cause nosocomial infection.[3] Beside biofilm, Aci*netobacter baumannii* have developed multidrug resistance. MDR strain of *Acinetobacter baumannii* have been found to resist carbapenem, the drug of choice to treat this bacteria.[4]

The indication to use carbapenem is to treat hospital-acquired infections when primary antibiotic regimen has failed or when there is suspicion of resistance. Increased usage of carbapenem to treat *Acinetobacter baumannii* has been correlated with increased resistance.[5] In 2017, WHO released a priority pathogens list that is in need of new antibiotics. The list is divided into 3 categories or priorities: critical, high, and medium. Carbapenem-resistent Acinetobacter baumannii is put in the critical priority category alongside carbapenem-resistent Pseudomonas aeruginosa and carbapenem-resistent ESBL-producing enterobacteria. Mortality rate for infections due to Acinetobacter range from 33% to 60% in Africa alone. For the US it ranges from 22% to 49.6% meanwhile in Europe the range is 29% to 71.6%.[6] Worldwide, antibiotics are overprescribed despite warnings regarding overuse. Overuse of antibiotics incite more antibiotic resistance. Development of new antibiotics also has been stalled. New antibiotics are used as the last choice of drug to treat serious illnesses, and yet the resistance of new available agents is inevitable. In 2013, CDC declared that the human race is currently in the "post-antibiotic era" with the emergence of resistant bacteria.[7] To counter the age of "post-antibiotic era", physical agents are being searched as alternatives for antibiotics. One of the physical agents is electricity. Low voltage electricity had been found to be effective eradicate bacteria and biofilm.[8] It has been shown that *Acinetobacter baumannii,* both MDRO and Non-MDRO types, can be sterlized with 1.8 V FOR 2 hours.[9]

There is a need to implement a better way to eradicate *Acinetobacter baumannii.* This research will explore the combination of carbapenem and low voltage electricity to eradicate *Acinetobacter baumannii.* Worldwide, antibiotics are overprescribed despite warnings regarding overuse. Overuse of antibiotics incite more antibiotic resistance. Development of new antibiotics also has been stalled. New antibiotics are used as the last choice of drug to treat serious illnesses, and yet the resistance of new available agents is inevitable. In 2013, CDC declared that the human race is currently in the "post-antibiotic era" with the emergence of resistant bacteria.[7] To counter the age of "post-antibiotic era", physical agents are being searched as alternatives for antibiotics. One of the physical agents is electricity. Low voltage electricity had been found to be effective eradicate bacteria and biofilm. [10] It has been shown that *Acinetobacter baumannii,* both MDRO and Non-MDRO types, can be sterlized with 1.8 V FOR 2 hours.[7]

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2. Methods

2.1 Study Design

This design of the research is experimental with *Acinetobacter baumannii* solution, meropenem solution, and power supply GPS-3030D 90W.

2.2 Population and Sample size

The bacterial sample was taken from the bacteria stock of Microbiology Department in Faculty of Medicine of Universitas Airlangga in NaCl 0,9% PZ

The sample size is calculated using Frederer formula [(t-1) (n-1) ≥ 15] with t as the number of treatments and n as the number of samples. Since there is 4 types of treatments the calculation would be:

$(4-1)(n-1) \geq 15$
$3(n-1) \geq 15$
$n-1 \geq 5$
$n \geq 6$.

Hence the amount of sample needed is at least or equal to 6 each for MDRO *Acinetobacter baumannii* sample.

2.3 Researched Variables

The independent variables are:

1. *Acinetobacter baumannii* solution (0.5 McFarland)
2. Voltage of electricity that will be used and the duration of exposure which is 3 volt[11] for 15 minutes
3. Concentration of meropenem based on resistant *Acinetobacter baumannii* MIC which is 8 mg/L[12]

The dependent variables are:

1. CFU/ml of *Acinetobacter baumannii* after the treatment.

2.4 Research Instrument

The instrument used in this research is a single machine output power supply, Direct Current type 3030D 90W and set in a parallel of 6 rows of electric network. Each network is given a potentiometer rheostat (30 ohm 30 watt) as the manager of the output of the voltage and ampere of the electricity. Each potentiometer

is given is given a resistor to avoid short circuit from the minimal resistance potentiometer rheostat. Each pathway of the network can be monitored for the voltage and the ampere and will be given 4 digit digital voltage-current meter. This makes it possible for a real-time measurement for each pathway and to ensure the voltage and ampere is correct.

2.5 Sampling Procedure and Data Retrieval

Clinical bacteria taken from the stock at bacteria stock of Microbiology Department in Faculty of Medicine of Universitas Airlangga.

Acinetobacter baumannii bacteria solution in NaCl 0,9% solution PZ or aquades in a test tube as much as 3 ml. In this research there would be 4 types of treatment. The control without any intervention, the one given low voltage electricity current, the one with added meropenem solution, and the last one with both low voltage electricity current and meropenem solution. Each treatment lasts for 15 minutes and then note the population size in CFU/ml.

The materials and tools that are needed are prepped, for example the conductors should be sterilized by heating it for 10 seconds with a bunsen burner. The machine should be set at 5mA with 0.5 V. The bacteria solution should be mixed with the NaCl 0,9% PZ solution with the concentration checked with DensiCHECK^{tm} until reaching 0.5 McFarland (1.5 x 108)

2.6 Ethical Aspect of Research

This research started after obtaining the permission from Komite Etik Penelitian Kesehatan Fakultas Kedokteran Universitas Airlangga. This research was conducted in the Laboratory of Microbiology Fakultas Kedokteran Universitas Airlangga. This research's ethical number is: No.70/EC/KEPK/FKUA/2022 .

3. RESULTS AND ANALYSIS

3.1 Research's Result Data

Amount of Bacterial Colony (CFU/ml)							
	Iteration Number						
	1.	2.	3.	4.	5.	6.	Average
Control	48350	89570	156750	115530	5020	88560	83963.33
Meropenem	94150	90080	92120	118580	59550	85500	89996.67
Low voltage electricity	0	0	1220	0	0	0	203.33
Meropenem + low voltage electricity	0	0	10	0	0	0	1.67

Table 1. The table of the research's result data

Across the 6 iterations the results are relatively on a similar range except for the third iteration. The third iteration has a higher amount of bacterial colonies than the other iterations. The third iteration is also the only iteration that has a bacterial colony growth for the treatments that use low voltage electricity. The increased amount of bacterial colonies can be explained through accidental prolonged incubation period or miscalculation when making the bacterial solution at the start.

The amount of bacterial colony for the control is higher compared to other types of treatment except for the one that was only given meropenem. The higher amount of bacterial colony in the meropenem group can be explained due to the fact that Acinetobacter baumannii has antibiotic resistance towards meropenem. The amount of bacterial colony in the control is higher than the ones that got the low voltage electricity treatment. The ones that used low voltage electricity also has a lower amount of bacterial colony than the ones that received meropenem treatment. The lowest amount of bacterial colony belongs to the one that received both meropenem and low voltage electricity, lower than the one only received low voltage electricity treatment.

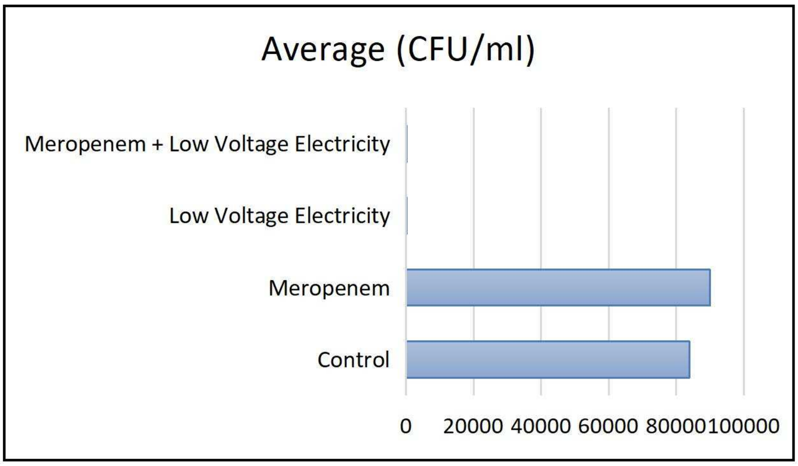

Fig. 1. Average of each type of treatments

There are significance differences of number of colony for the different type of treatments. The average for the one only given meropenem has more bacterial colony than the control's average. On the other hand, the one given low voltage electricity is at the second lowest number of bacterial colony. The treatment that gives both meropenem and low voltage electricity is at the lowest at only 1.67 CFU/ml.

3.2 Analysis of Data

	Treatment Type			
	Meropenem	Low Voltage Electricity	Meropenem + Low Voltage Electricity	Control
CFU/ml	89996.67	203.33	1.67	83963.33
Eradication Rate (%)	-7.19%	99.76%	100.00%	

Fig. 2. Eradication rate of each treatment type

The eradication rate of treatment is showed in percentage, comparing the CFU/ml between the treatment type result and the control. There is a significance difference between the treatments with the using only meropenem is at the lowest -7.19% eradication rate. The negative value indicating a growth in number instead of eradication. This can be caused by the antibiotic resistance of the bacteria. The eradication rate for using low voltage electricity only and for using both meropenem and low voltage electricity is similar at

99.76% and 100.00% respectively.

4. DISCUSSION

In this research it was shown that the combination of Meropenem and Low Voltage Electricity to kill *Acinetobacter baumannii* is more effective compared than only using meropenem or low voltage electricity. Out of the 6 iterations of the experiment, the one that uses both meropenem and *Acinetobacter baumannii* have no bacterial colony left except for one of the iterations. The eradication rate for the combination of meropenem and low voltage electricity is 100.00% (rounded to two decimal places). The research also shows that the usage of meropenem as antibiotic alone is not enough to fully eradicate *Acinetobacter baumannii*. In fact, in most of the iterations the amount of *Acinetobacter baumannii* the bacterial colony is higher than the control's amount of bacterial colony. This can be contributed to the fact that *Acinetobacter baumannii* has been discovered to be resistant towards carbapenems including meropenem.[1] The vast difference between the usage of meropenem alone and with the low voltage electricity indicates that the two do have synergy as antimicrobials.

The mechanism behind the synergy of meropenem and the low voltage electricity in eradicating *Acinetobacter baumannii* is still unclear. This phenomenon can be contributed to how low voltage electricity is able to break the bacterial membrane.[10] This mechanism might be able to help meropenem enter the bacteria through the broken membrane.

The results of this research is consistent with previous studies that uses low voltage electricity to increase the effectiveness of antibiotic against multidrug resistance bacteria. A study used both low voltage electricity with vancomycin against MRSA and low voltage electricity with gentamicin against *Pseuodomonas aeruginosa* and both shows reduced CFUs, compared to the control and the usage of the low voltage electricty and antibiotic alone.[9] Another study demonstrated the increased efficacy of ciprofloxacin and polymyxin B paired with electrical currents against *Pseudomonas aeruginosa* compared to the efficacy of the same dosage of the antibiotics but without the electricity.[13] However, this research can not rule out other possibilities of the death of the bacteria through other means like the electricidal effect or other effects.

The limitations of this research is the inability to examine the media precisely, whether it has formed hydrogen peroxide or oxygen that might have been formed from the electrolysis. The media also doesn't represent real world medical tool surfaces in clinical settings where *Acinetobacter baumannii* is usually found on.[1] The research is also unable to represent the physiological state of the human body.

The usage of the low voltage electricity in tandem with antibiotics can be beneficial in clinical settings. *Acinetobacter baumannii* with multidrug resistance can become the culprit of nosocomial infections. Increasing the efficacy of antibiotics with the help of low voltage electricity can help fight against infections.

5. CONCLUSION

Based on the research's results regarding the effect of combination between meropenem and low voltage electricity to kill *Acinetobacter baumannii*, it can be concluded that:

1.The usage of meropenem alone against *Acinetobacter baumannii* as an antimicrobial agent is not effective, indicated by the lowest eradication rate at -7.19%.

2.The combination of meropenem and low voltage electricity is more effective than treatment using meropenem or low voltage electricity alone with the highest eradication rate amongst all treatment at 100,00%

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