REVIEW

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Effect of low voltage electric currents on the decrease of *Klebsiella pneumoniae* ESBL and non-ESBL colonies

Dhia Lintang Setya Wijoyo[1], Eko Budi Koendhori[2*], Imam Susilo [3], Puspa Wardhani[4]

[1]Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia;
[2]Department of Clinical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia;
[3]Department of Anatomy Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia;
[4]Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia;

*Corresponding to:
Eko Budi Koendhori;
Department of Clinical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia;
dr_eko@fk.unair.ac.id

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ABSTRACT

Introduction: *Klebsiella pneumoniae* is a gram-negative bacteria and one of the most common causes of nosocomial infections, especially in the intensive care units. The use of liberal and irrational antibiotics is shown the emergence of antibiotic-resistant *Klebsiella pneumoniae.* This research was conducted to evaluate if low voltage electric current on three kinds of solvent media could have an eradication effect.

Methods: This was an experimental study, which was done at the Microbiology Laboratory of Harapan Kita Women and Children Hospital-Jakarta. This study used to isolate bacteria non-ESBL and ESBL *Klebsiella pneumoniae*, thereupon will be dissolved in saline, Aqua destillata, and Ringer Lactate, each consisting of 8 samples. Each sample received a 0.5V and 10mA DC electric current; reduction of colonies was observed at 30, 60, 120 and 240 minutes using DensiCHEK.

Result: There was a decrease in the colony number of 2 bacterial groups in the first 30 minutes in all three media ($p < 0.01$). The reduction was higher in the non-ESBL *K. pneumoniae* group. The decrease of bacterial colonies was higher in the *Klebsiella pneumoniae* group non ESBL in ringer lactate medium during 240 minutes observation compared to saline and Aqua destillata ($p < 0.001$; $p < 0.001$, respectively). The saline solution showed no different effect compared to aquadestillata.

Conclusion: A direct current of 10 mA and 0.5 V intervention, on Ringer lactate media, could have a bacterial killing effect to *Klebsiella pneumoniae* non ESBL started from 30 minutes. *Klebsiella pneumoniae* ESBL needed a longer duration than non ESBL.

Keywords: bioelectric effect, nosocomial infection, *Klebsiella pneumoniae*.
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INTRODUCTION

According to the World Health Organization (WHO), bacterial resistance is a global problem that is increasingly threatening people and must be tackled together. In Indonesia, due to inadequate hand washing facilities and awareness of handwashing, infections due to Gram-negative bacteria, such as *Klebsiella pneumoniae*, *Pseudomonas sp*, *Serratia sp*, and *Enterobacteriaceae sp* are increasing.[1,2]

K. pneumoniae is a Gram-negative bacteria belonging to the *Enterobacteriaceae* family, found 100 years ago in the case of Freidlander's pneumoniae, which is pneumonia acquired in patients with low immunity. *Klebsiella pneumoniae* is a pathogenic bacteria that can potentially live in humans, and often found in patients hospitalized. Recently, there has been increased resistance to *K. pneumoniae* and associated with biofilm formation in patients undergoing invasive procedures. *K. pneumoniae* biofilm form on medical devices is more difficult to be eradicated because of the potential for resistance to antibiotics.[2] The prevalence of biofilm-related infections is estimated to occupy two-thirds of the incidence of bacterial infection.[3–5]

Costerton *et al* (1994) reported microbial eradication by application of electric fields (bioelectric) on colonies of *P. aeruginosa* will increase if the duration of electricity is given more than 24 hours.[6] The application of this bioelectric uses solvent media and often uses liquids such as saline, Aqua destillata and Ringer's lactate.[6-8] There is no studies yet that has observed the effect of low voltage electruc current on colonies of *K. pneumoniae.*

This study evaluates whether the effect of low voltage electric current can reduce the number of *K. pneumoniae* colonies for both extended-spectrum beta-lactamases (ESBL) and non-ESBL and whether the decrease in *K pneumoniae* colonies correlates with the solution medium and the duration of electrical exposure.

METHODS

Sample and study design

This research was an experimental study, conducted in the Microbiology laboratory of Women and Children Harapan Kita Hospital, Jakarta-Indonesia, for 4 months (April-July 2020). The study population was the isolates of ESBL and non-ESBL *K. pneumoniae*. The subject population was isolated from *K pneumoniae* bacteria grown in BACTEC media, differentiation ESBL from non-ESBL by based on the pattern of bacterial resistance to the beta-lactam ring. The criteria of inclusion

is colonies of bacteries identified as *K. pneumoniae* both ESBL and non-ESBL which are still viable.

Sample preparation

The bacteria were planted on a MacConkey agar plate, incubated for 24 hours at 37 ° C, inoculated into a tube containing the solution medium to be used (saline, Aqua destillata, Ringer's lactate). Each tube was filled with 3 ml of solution. Furthermore, the bacterial suspension solution was measured until the turbidity reached 1 McFarland, which is equivalent to 3 x 10^8 CFU / ml.[9] The sample size for each group and each solvent medium for every 8 samples, based on Federer's formula, so that the total sample size needed from the 2 groups of *K. pneumoniae* bacteria for all 3 solvent media is 48 bacterial suspensions.

Electric current intervention

The bacterial suspension in the solvent medium was inserted into a glass tube, and 2 stainless steel electrodes with a diameter of 3 mm and a length of 12 cm had been sterilized first (Figure 1). The electrodes are connected to a power source, using a GPS 3030D tool that has been made 6 parallel (Figure 2). Electric current uses a voltage of 0.5 V and an electric current of 10mA. The intervention was carried out for 240 minutes. Changes in the number of colonies were counted serially at 4-time points, namely 30, 60, 120, and 240 minutes, using the DensiCHEKTM tool. The method of using the tube using stainless steel electrodes cites the research of Setiawan (2013).[10]

Statistical analysis

We used SPSS 12.0 for data tabulation and compilation. Bonferroni and t-test were done.

RESULTS

The effect of electric currents between media and four serial times

From this study was found that low voltage electric currents can influence and inhibit the growth of K. *pneumoniae* both non-ESBL and ESBL. There was a decline in colony number in both groups between before and all four serial times (30, 60, 120, and 240 minutes, respectively) electric current intervention in all three solvents media (saline, aquadestillata and ringer lactate) as shown in Table 1, Table 2, and Table 3. Both saline and aquadestillata showed the same slower response in decline colonies effect compared to ringer lactate. The effect of electric current on colonies in saline and aquadestillata media began to appear after a minute of 120. The fastest decline of the colony was shown in the ringer lactate group. Only in 30 minutes the colonies rapidly down then sustainably low within the observation period (240 minutes) compared to both saline and aquadestillata group. The colony decline of the ESBL group was lower than the non-ESBL group and these patterns from each media within the group were not different as shown in Figure 3 and Figure 4.

The different patterns of colonies decline between non-ESBL and ESBL groups

This study observed the different effects from low voltage electric currents (10mA and 0.5V) to non-ESBL and ESBL *K. pneumoniae* showed that in saline media, a significant decrease in colony number of non-ESBL K. *pneumoniae* bacteria compared to ESBL starting from the 120 and 240 minutes. There was an overlap of data between the 2 bacterial groups before 120 minutes (Figure 5A).

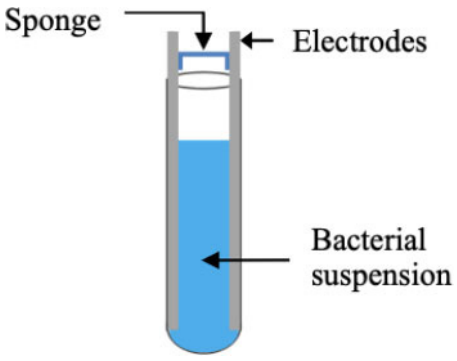

Figure 1. Tube with media and 2 electrodes (anode and cathode) connected to an electric current (DC).[10]

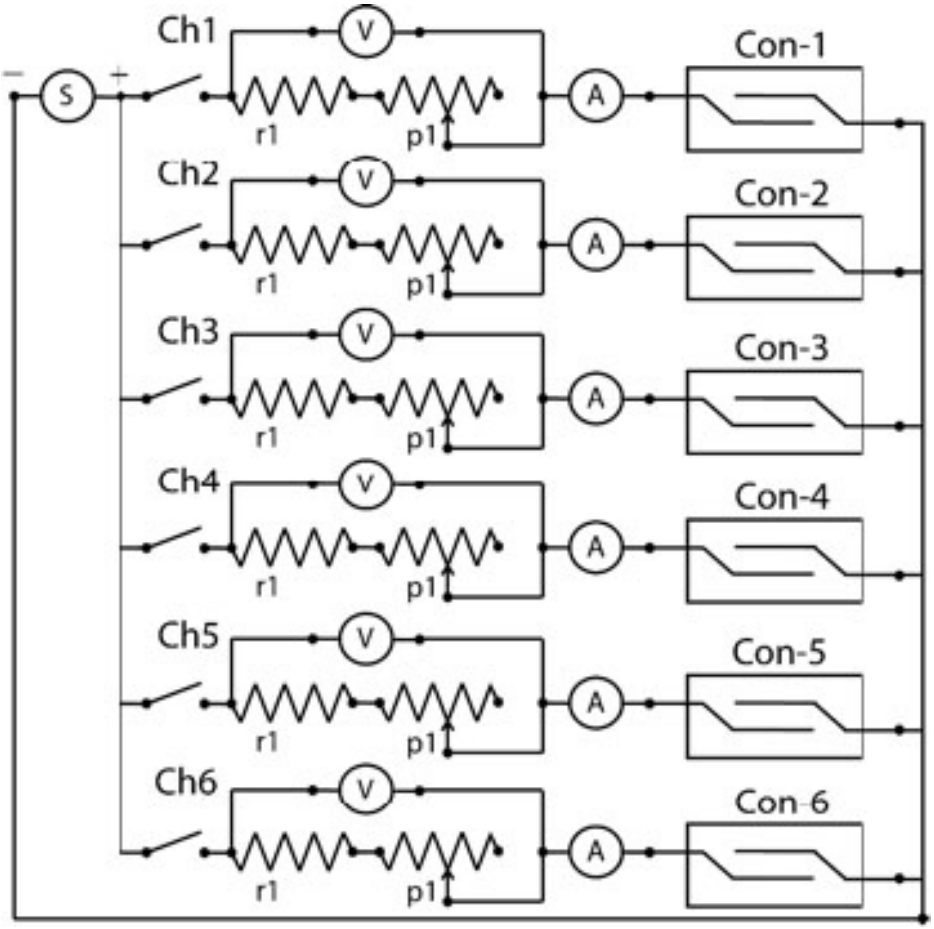

Figure 2. Electric circuit with 6 channels.
S =DC power source; V =voltmeter; A =current meter. r1 = 2 ohms, p1 = 30 Ohm rheostat potentiometer 30 watts, Con-1- 6 (conductors 1 to 6).

In ringer lactate media, compared to those other two media, showed the earliest response of colony decline in the non-ESBL *K. pneumoniae* group. It was started at first 30 minutes, but the declining colony seemed steady, however, the colony number at 240 minutes was still lower compared to aquadestillata and saline media (2.078 vs 2.216 vs 2.261, respectively) (Table I, II, and III). There was no overlapping data between the 2 groups in all four serial times. In all three media decline colony of ESBL *K. pneumoniae* were at first 30 minutes observation but there after were no significant response (Figure 5B).

In aquadestillata media, a decrease in the number of non-ESBL *K. pneumoniae* colonies was promptly seen starting at 60 until 240 minutes and lower compared to ESBL *K. pneumoniae*. Compared to saline media, aquadestilata media showed a similar pattern, but a faster influence on non-ESBL *K. pneumoniae*. However, at 120 and 240 minutes they showed no different colony number (Figure 5C).

DISCUSSION

The bioelectric effect is an attempt to kill bacteria by destroying the biofilm layer mechanically through the intervention of low-intensity DC electric fields on certain media. Bioelectric studies that have been often done, always accompanied by anti-microbial administration. Electrical intervention with antibiotics will make the biofilm layer open easily, thus antibiotics can easily penetrate and kill especially resistant bacteria.[11,12] The antibacterial

Table 1. *K. pneumoniae* colonies in 4 series observation times in saline.

Non ESBL group

Time (minutes)	CFU/ml (x10^8)								Mean (SD)
	1	2	3	4	5	6	7	8	
0	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00 (0.0)
30	2.76	2.64	2.64	2.58	2.85	2.97	2.58	2.64	2.708 (0.140)
60	2.76	2.58	2.64	2.55	2.79	2.97	2.52	2.55	2.670 (0.157)
120	2.34	2.4	2.04	2.4	2.7	2.7	2.34	2.19	2.389 (0.227)
240	2.1	2.25	1.8	2.31	2.7	2.7	2.19	2.04	2.261 (0.311)

ESBL group

Time (minutes)	CFU/ml (x 10^8)								Mean (SD)
	1	2	3	4	5	6	7	8	
0	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00 (0.0)
30	2.67	2.55	2.67	2.79	2.64	2.67	2.76	2.61	2.670 (0.077)
60	2.61	2.58	2.64	2.64	2.64	2.55	2.64	2.64	2.618 (0.035
120	2.61	2.7	2.73	2.7	2.61	2.58	2.58	2.61	2.640 (0.060)
240	2.67	2.64	2.67	2.7	2.64	2.55	2.58	2.61	2.632 (0.050)

Table 2. *K. pneumoniae* colonies in 4 series observation times in aquadestillata.

Non ESBL group

Time (minutes)	CFU/ml (x 10^8)								Mean (SD)
	1	2	3	4	5	6	7	8	
0	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00 (0.0)
30	2.85	2.67	2.67	2.61	2.76	2.7	2.64	2.76	2.708 (0.078)
60	2.73	2.46	2.55	2.4	2.64	2.52	2.46	2.64	2.550 (0.112)
120	2.52	2.25	2.34	2.25	2.61	2.25	2.31	2.61	2.392 (0.161)
240	2.43	2.1	2.25	2.1	2.34	2.1	2.1	2.31	2.216 (0.134)

ESBL group

Time (minutes)	CFU/ml (x108)								Mean (SD)
	1	2	3	4	5	6	7	8	
0	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00(0.0)
30	2.82	2.85	2.94	2.85	2.97	2.85	2.82	2.88	2.872 (0.055)
60	2.7	2.76	2.94	2.94	2.97	2.85	2.73	2.76	2.831 (0.108)
120	2.64	2.67	2.91	2.91	2.85	2.7	2.58	2.7	2.745 (0.127)
240	2.55	2.64	2.82	2.55	2.64	2.55	2.55	2.64	2.618 (0.093)

Table 3. ***K. pneumoniae*** **colonies in 4 series observation times in ringer lactate.**

Non ESBL

Time (minutes)	CFU/ml (x10^8)								Mean (SD)
	1	2	3	4	5	6	7	8	
0	3.00	3.00	3.00	3.00	300	3.00	3.00	3.00	3.00(0.0)
30	2.07	2.16	2.1	2.13	2.34	2.22	2.13	2.19	2.168 (0.084)
60	1.95	2.13	2.07	2.07	2.16	2.07	2.07	1.95	2.059 (0.075)
120	1.98	2.1	2.28	2.07	2.16	2.16	2.04	1.98	2.096 (0.102)
240	1.98	2.1	2.1	2.07	2.22	2.1	2.07	1.98	2.078 (0.076)

ESBL group

Time (minutes)	CFU/ml (x10^8)								Mean (SD)
	1	2	3	4	5	6	7	8	
0	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.0	3.00 (0.0)
30	2.55	2.67	2.55	2.61	2.64	2.61	2.64	2.61	2.610 (0.042)
60	2.67	2.58	2.61	2.55	2.61	2.55	2.55	2.61	2.591 (0.042)
120	2.49	2.58	2.88	2.55	2.58	2.49	2.49	2.58	2.580 (0.128)
240	2.46	2.64	2.55	2.52	2.55	2.46	2.46	2.55	2.524 (0.063)

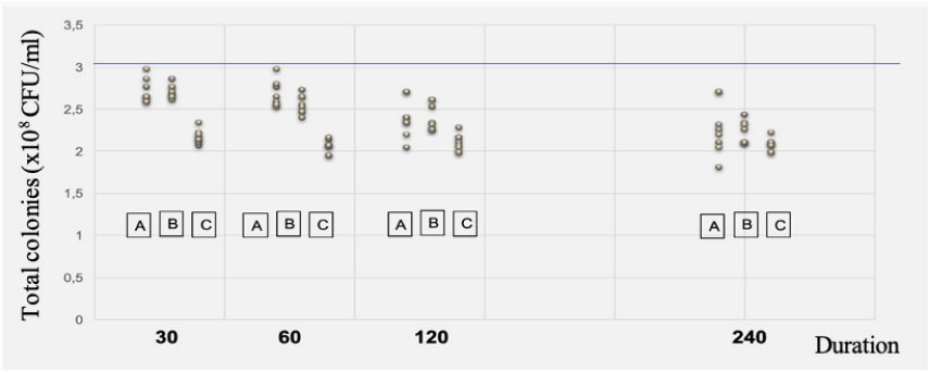

Figure 3. Patterns of declination non-ESBL K. pneumoniae colonies within 4 serial times on 3 media (A= saline media; B=aquadestillata; C= ringer lactate).

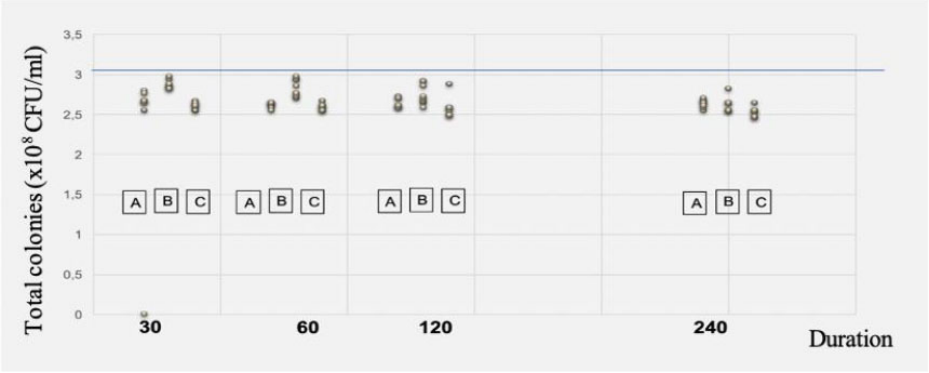

Figure 4. Patterns of declination ESBL K. pneumoniae colonies within 4 serial times on 3 media (A= saline media; B=aquadestillata; C= ringer lactate).

mechanism of electric currents results from electrolysis (for example, H_2O_2, oxidizing radicals, chlorine molecules), enzyme and coenzyme oxidation, changes in pH which are thought to cause bacterial membrane damage, and a decrease in bacterial respiration rate.[4,13,14] There are many methods of bioelectric studies. In this study, using a simple method, namely by using a tube given two electrodes (anode and cathode) and connected to a DC power source.[10]

Previous studies mentioned a significant reduction in colony number (CFU / ml) between electric current only and a combination of electric current and antibiotics.[6,15,16] This study used a low voltage and electric current without antibiotics, with the aim of knowing how far the effect of electric current alone in eradicating bacterial colonies, especially for *K. pneumoniae*. As far as we know, only one study evaluated the effect of low voltage electric current to *K. pneumoniae*. We considered these bacteria based on the high rate of hospital-acquired infection due to *K. pneumoniae* and increasing the emergence of ESBL *K. pneumoniae*. The selection of the amount of current and voltage cited from research by Kim *et al* which suggested that the electric voltage should be in less than 0.9V to avoid massive electrolysis. The use of an electric current of 10mA based on recommendations from Wellman *et al* who examined the effect of electric current on *K. pneumoniae* biofilms. As well as the consideration that 10mA of electric current is safe for humans.[15]

Compared to previous studies, except no use antibiotics, also observed the influence of solvent media. We considered three media (saline, aquadestillata, and ringer lactate) was because those often used in clinical practice and easy to obtain. In addition, observations were carried

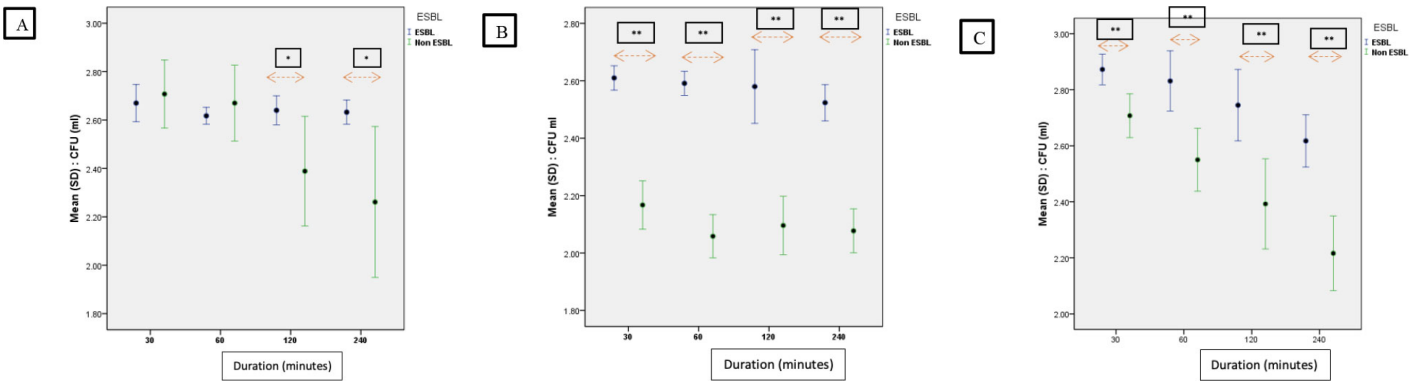

Figure 5. Patterns of decreasing colonies (CFU / ml x108) for 4 serial times between 2 groups on 3 media. A=saline; B=ringer lactate; C=aquadestillata. *t-test *: p<0.05; **: p<0.001.*

out for only 4 hours and unlike most other bioelectric studies that examined a minimum of 24 hours, this was based on the consideration that the intervention of electric current was given directly to the isolated bacteria, and not direct to biofilms. Thus, it was expected that the electric current alone was sufficient.[3,15]

This study showed a decrease in the number of colonies before and after the intervention of electric currents in all solvent media, which proves that the intervention of low voltage electric currents and without antibiotics can have a bacterial killing effect. The colony decline was found to differ with the duration of electrical intervention and the media used. In accordance with the results of research by Costerton (1994) regarding the effects of electric current on MRSA colonies and biofilms.[6]

During the experimental study, one of the confounding factor that may impact on the outcome is the temperature of the room during the experiment, thus the room temperature is always set to 27°C to minimalize it from affecting the result. The limitation of this study is the duration of the intervention with electric current on is only up to 4 hours, so it is not known whether it is possible that electric current intervention can fully eradicate *K. pneumoniae* bacteria colonies on a longer period of time without the use of antibiotics, thus it is recommended to do further research.

CONCLUSION

An electric current (10mA and 0.5V) without adding antibiotics has a bacterial killing effect started from 30 minutes when using a ringer lactate medium. We suggested, in low resources health facilities, using low voltage direct currents might be an alternative method in sterilization medical equipment.

DISCLOSURE

Funding

All costs incurred by the authors without any other source of funds.

Conflict of Interest

All authors declare that they have no conflicts of interest.

Author Contribution

All authors have been contributed in this study.

ETHICAL CLEARANCE

This research has been approved by the Ethical Committee Medical Faculty of Airlangga University, Surabaya and Institutional Review Board Women and Children Harapan Kita Hospital, Jakarta.

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