Original article

Antibacterial effect of alternating current against Staphylococcus aureus and Pseudomonas aeroginosa

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Abstract:
Background — The use of physical means as an aid for modern medicine in the champion against pathogenic microorganisms holds new approach that recently have begun to be widely recognized. The use of an additional physical means, alternating currents, introduced to inhibit bacterial growth and enhance disinfectant potency. The present study aimed to determine the best frequency of alternating currents in prevention of bacterial growth and to detect the efficacy of alternating currents on disinfectant bactericidal potency.
Material and Methods — Electric field strength of 6 and 10 V/cm^2 at 50 KHz, 1 MHz, 10 MHz and 20 MHz was applied continuously during lag phases of staphylococcus aureus and pseudomonas aeroginosa. Then Changes in bacterial growth were investigated by the time kill method. Efficacy alternating currents on the current disinfectants bactericidal potency (microzed, deconex, Dettol and glutaraldehyde) were evaluated by MIC and MBC.
Results — Alternating current at the low voltage and high frequency (10 V/cm^2 at 20 MHz) reduced the growth of S. aureus and P. aeruginosa effectively. Electric field strength of 10 V/cm^2 at 20 MHz showed a better effect on the low level disinfectants such as Dettol and Deconex compared to the high level disinfectants such as Glutaraldehyde, Microzed GP-H and Aniosyme.
Conclusion — Detection of a suitable form of alternating current is necessary in the future. This method may be applied as a complementary for eliminating of conductive and semi-conductive surfaces of hospital and increase disinfectant bactericidal potency.

Keywords: alternating current, antibacterial effect, disinfectant

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Introduction

The use of physical means in modern medicine as a new approach in the fight against microorganisms has recently been used. Ultrasound waves are used in dentistry [1] and, in combination with antibiotics, for the in vivo and in vitro eradication of bacterial biofilms [2-4]. Photodynamic therapy is currently being used in dental and dermal infections [5, 6]. In addition, thermotherapy that has originally been introduced as a tool in treatment of tumors was found to be effective against cutaneous leishmaniasis [7]. Electrical stimulation as a physical method has been used for decades for different medical purposes including muscle strength training and wound healing [8]. Inhibitory effect of electric currents on bacterial growth was reported firstly by Rowley et al. [9] over 40 years ago. Also bioelectrical effect of different types of electric currents on bacterial growth has been reported by other researchers [10-12]. The antibacterial effects of direct electric current have been studied for several decades, however, there has been little research on the effects of alternating current (AC) on the bacterial growth and biofilm potency [13-15].

Exposing bacteria to an electric current causes environmental stresses on the bacterial cells and therefore a physiological reaction to these stresses by bacteria can result in alteration of surface properties or even bacterial cell deformity [16, 17]. However, it is not comprehensively clear how the cell structural surface properties change during exposure to electric currents.

Electric current has recently been targeted for the development of novel techniques to disinfect of environment [18, 19] but its application is still far from being fixed. Many experiments in this field have been described, but standardizing of the processes has incurred difficulties related to the nonhomogeneous experimental conditions and different parameters such as voltage, current intensity, possible electrode use and duration of treatment that must be taken into consideration simultaneously.

The present study aimed to determine the best frequency of alternating currents (AC) in prevention of bacterial growth and to detect the efficacy of alternating currents on disinfectant bactericidal potency.

Material and Methods

Test strains and growth conditions

Pure cultures of *S. aureus* (ATCC29213) and *Pseudomonas aeroginosa* strain PAO1 were used. Laboratory evaluations were performed in trypticase soy broth medium (Conda, Hispanlab, Spain). Bacteria were grown in broth at 37°C and 150 rpm until the concentration of bacteria was 0.5 on the McFarland Scale. Half a McFarland standard was prepared by spectrometer measuring the absorbance at 600 nm was confirmed.

Electric device

The electric power was produced by the functional generator (Rigol, China). Technical characteristics of the unit includes the input voltage used for 220 volts, the output voltage 6 V to10 V, current intensity; 10mA and the frequency up to 20 MHz. The tests were carried out in cylindrical glass tanks (20 cm diameter, 10 cm high), and each test was performed using the copper electrodes and two voltages (6 V/cm and 10 V/cm) and different Frequency (50 KHz, 1 MHz, 10 MHz, 20 MHz). The separation distance between electrodes was 6cm. The covered culture size was 6x6 cm and 4.5 cm deep.

Alternating current effect on bacterial growth curve

10 ml of a 1.5×10^8 cells per milliliter broth culture (monitored spectro-photometrically at 600 nm) were used as inoculum in 90 ml fresh sterile medium. The number of bacteria in the test and control samples were counted. Then AC current was delivered with biphasic electrical stimulation (sine wave) at different frequencies (50 KHz, 10 MHz, 20 MHz) and for a period of 2 hours (*S. aureus*) and 2.5 hours (*P. aeroginosa*). After this step bacterial suspension of test and control were counted at the 2, 4 and 6 h via Time Kill method. During the whole process both test and control samples were incubated for 6 h at 25°C with continuous shaking at 150 rpm in a shaker incubator (GFL, Germany). Bacterial growth curve of the test and control samples were then compared. The process was repeated again in the next day for each bacterium.

Evaluation of alternating current on the disinfectants potency

10 ml of a 1.5×10^8 cells per milliliter broth culture (monitored spectro-photometrically at 600 nm) were used as inoculum in 90 ml fresh sterile trypticase soy broth. Samples were tested under alternating current. AC current was delivered with biphasic electrical stimulation (sine wave) at the different frequency and voltage for a period of 2 hours (*S. aureus*) and 2.5 hours (*P. aeroginosa*) and then was added to the test and control tubes. Finally bactericidal effect of the test and control samples was evaluated by MBC method (Minimum Bactericidal Concentration).

Statistical analysis

Data was analyzed using the SPSS version 16 (SPSS Inc, Chicago, USA). Mixed between within ANOVA was used to assess the effect of alternating current on *S. aureus* and *P. aeroginosa*.

Results

The results of the present study indicated that the alternating current effected on the growth curves of both *S. aureus* and *P. aeruginosa*, reducing the bacterial population via a bacteriostatic effectiveness. Mixed between-within subjects ANOVA (repeated measures ANOVA with three independent factors of voltage, frequency and group) was used to evaluate the effect of voltage and frequency interventions on *P. aeruginosa* and *S. aureus* in the test and control groups at four time points (pre-intervention, 2, 4 and 6 h after intervention). The interactions between time and frequency as well as time and voltage were significantly different at all frequencies and voltages (PV: 0.014 and PV: 0.001). The highest antibacterial effects for either *S. aureus* or *P. aeruginosa* were observed in the frequency of 20 MHz and the voltage of 10 V (PV: 0.001 and PV: 0.005). The antibacterial effect of the alternating current on *S. aureus* compared to *P. aeruginosa* (PV: 0.005) was higher (*Figures* 1 and 2). The lowest antibacterial effect was observed in the alternating current with the frequency of 1 MHz and voltage of 6 V (*Figures* 3 and 4) (PV: 0.005 and PV: 0.005).

Figure 5 shows the decrease curve of the number of viable (*S. aureus* and *P. aeruginosa*) in abase 10 logarithmic scale in the presence of common disinfectants (Deconex, Microzed, Dettol and Glutaraldehyde) in health care centers along with their controls.

According to the results of this study, the greatest decrease in the number of *S. aureus* was observed in Dettol and Deconex-Silosept (0.8 and 1.0 /log10, respectively); however, the smallest decrease in the number of bacteria was observed in Microzed GP-H and Aniosyme (0.4 and 0.4 /log10, respectively). Comparing the number of viable bacteria (*P. aeruginosa*), in the presence of common disinfectants (Deconex, Microzed, Dettol and Glutaraldehyde), between health centers and their controls, the greatest decrease was revealed in Dettol and Silosept (0.9 and 0.8 /log10, respectively) and the smallest decrease was seen in Microzed GP-H and Aniosyme (0.3 and 0.3 /log10, respectively).

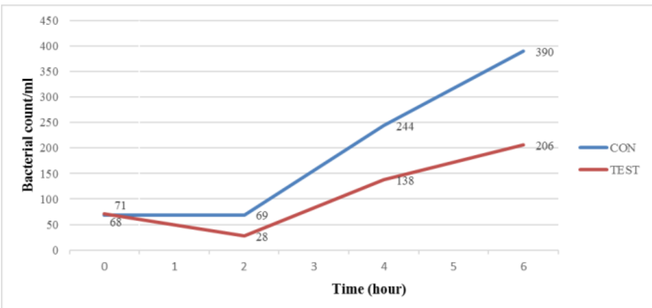

Figure 1. The growth curve of *S. aureus* after effects of alternating current with 10 V voltage and 20 MHz frequency

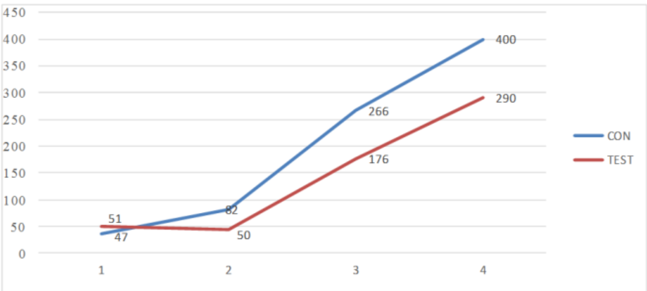

Figure 2. The growth curve of *P. aeroginosa* after effects of alternating current with 6 V voltage and 1 MHz frequency

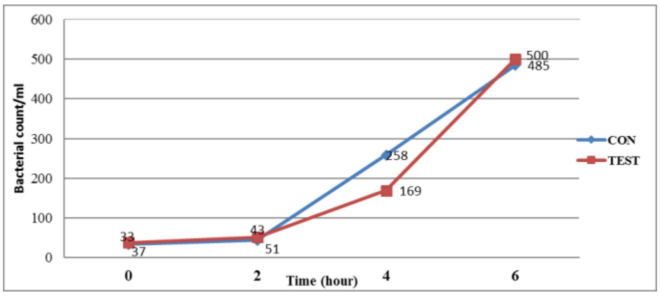

Figure 3. The growth curve of *S. aureus* after effects of alternating current with 6 V voltage and 1 MHz frequency

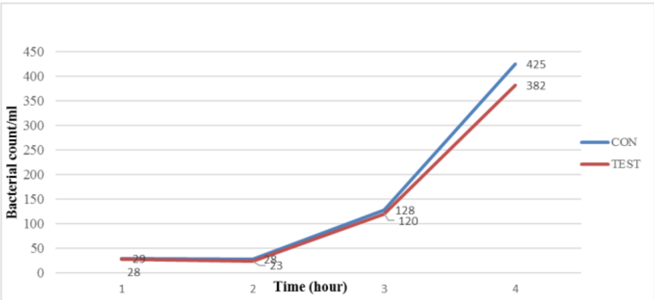

Figure 4. The growth curve of *P. aeroginosa* after effects of alternating current with 6 V voltage and 1 MHz frequency

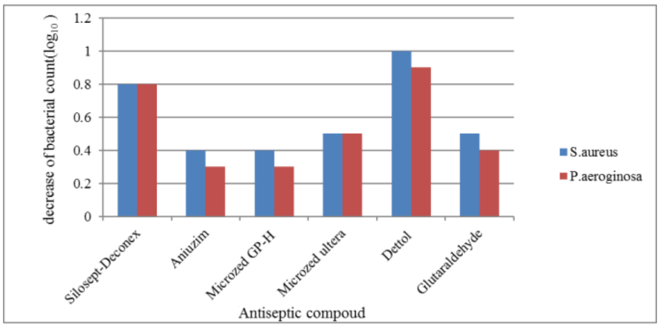

Figure 5. Reduction in the number of bacteria by disinfectants supplemented with alternating current

Discussion

The present study showed that an alternating electric current at low voltage and high frequency effectively reduced the growth of *S. aureus* and *P. aeruginosa* compared to their controls. However, its inhibitory effect was higher on *S. aureus*. It seems that there was a device limitation on the use of higher frequencies. To confirm the test repeatability, each experiment was repeated in three consecutive days. In this study high frequency and low voltage alternating current showed a better effect on the low level disinfectants such as Dettol(phenol base) and Deconex (ammonium compound base) compared to the high level disinfectants such as Glutaraldehyde, Microzed GP-H and Aniosyme. Considering that the alternating current does not produce free radicals and electroporation in the bacteria, it is likely that it inhibits the bacterial growth via effect on the bacterial division. Therefore, it has a higher synergistic effect on the low level disinfectants.

Many studies have been conducted on using direct and alternating electric currents for the treatment of infections with a high diversity due to using of different forms, frequencies and voltages of alternating current [11, 20, 21]. Most studies in this extent have been performed on the effect of alternating electrical currents on the performance of antibiotics and bacterial biofilm formation [22, 23]. However, very few studies have been conducted on the control of health care environmental contamination and the effects of these currents on the efficacy of hospital disinfectants [19]. In the preliminary studies it was found that the pulsed alternating currents have been able to stop the bacterial growth with different pulses [20, 21]. In the present study, the high sinusoidal frequency of the alternating current showed a greater bacteriostatic effect on *S. aureus* and *P. aeruginosa*. A study conducted by Petrovski et al. [11] reported that the sinusoidal alternating current had a significant inhibitory effect on *P. aeroginosa* but little antibacterial effect on *S. aureus*; however, in our study the inhibitory effect of the alternating current on *S. aureus* was higher compared to *P. aeruginosa*. This discrepancy is probably due to the difference between the voltages and frequencies used in our study (voltage: 300 V and frequency: 20 MHz) and Petrovski et al. (voltage: 300 V and frequency: 30 Hz).

Giladi et al. [24] reported the antibacterial effects of high-frequency and low-voltage sinusoidal alternating current by using coated electrodes instead of conductive electrodes. The only considerable point of this method was inhibition of the production of toxic compounds and oxidative free radicals by preventing the electrolysis process and therefore, it would not pose a problem while using in the body. Those results were in consistent with the results of the present study. However, it did not require the use of coated electrodes because main objective was using this method as a supplement in decontamination of health care environment.

Using electric currents as a prevention agent was reported 40 years ago [15]. Depend on the nature of the current, several mechanisms have been proposed for this inhibitory effect. It is believed that the direct electric current has good bioelectric effects on the biofilm due to changes in the pH and displacement of excess ions induced by electrolysis into biofilms or production of free radical oxidants. However, high frequency and low voltage alternating current does not displace any ions. It does not produce free radical oxidants or excess heat as well as new ions in the liquid medium and also does not have any electroporation effect [25]. The power of the pulsed electric current (electric field over 1000 V/cm) could cause the electroporation [20, 21]. However, it seems that the electric field due the high frequency alternating current prevents the reproduction of bacteria, via effect on the bacterial division during cytokinesis, which may be due to induction of nonhomogeneous electric field near the area of separation of daughter cells. This nonhomogeneous field is able to create one way movements of polar molecules within the bacteria or interfere with the bacterial electrostatic forces, which in both cases affects the integrity of the cell structure. In eukaryotic cells, microtubules with high polarity accept the greatest impact. Alternating electric field interferes with the microtubule spindles and polarization and depolarization processes in chromosome separation process. Although bacteria do not have complex microtubule structures which are present in eukaryotic cells, however, homologous tubules are found at the presence of bacteria [23].

Another problem in the treatment of pathogens by antibiotics is bacterial ability in raising their resistance to antibiotics. There is no evidence so far showing any resistance to the alternating electric current effect by the bacterial strains used in this study. The bacteria should change their physical properties radically to be able to escape from the effects of the electric field, which seems impossible. Moreover, it is necessary to find a new method to improve the effectiveness of disinfectants against resistant microorganisms such as biofilm-producing bacteria.

Conclusion

It seems that application of bioelectric effects especially high frequency and low voltage alternating currents, considering their acceptable antibacterial activity and safety of the method, can be used as a supplement to increase the effectiveness of disinfectants and eliminate contaminations in the conductive and semi-conductive surfaces of hospital.

Conflict of interest: none declared.

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