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# Effect of static electric field treatment on multiple antibiotic-resistant pathogenic strains of *Escherichia coli* and *Staphylococcus aureus*

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This study evaluated the effect of a (4.5 kV/cm, 50 Hz) static electric field (SEF) on pathogenic strains of *Escherichia coli* and *Staphylococcus aureus* with multiple antibiotic resistance. The bacteria were grown overnight at 37°C in a nutrient broth medium, then inoculated in 5 mL fresh nutrient broth medium and incubated for 2 h at 25°C with continuous shaking at 190 rpm. 10 × colony-forming units/mL of these bacteria were subjected to a 4.5 kV/cm, 50 Hz, SEF for various time periods. The effects of 5 different SEF exposure times (30, 60, 90, 120 and 150 min) on the bacteria were evaluated by the plate count agar method. The growth percentages of SEF treatment groups were significantly less than that of the control group. Inactivation significantly increased with the duration of SEF exposure. The results indicate that growth inhibition by SEF in the Gram-negative bacteria, *E. coli,* was greater than that in the Gram-positive bacteria, *S. aureus*. This study has demonstrated the antimicrobial effects of SEF treatment on 2 important pathogens, suggesting its potential for application as a method for controlling microbial population growth within in a variety of environments.

**Key words:** Electromagnetic fields, *Escherichia coli*, multiple drug resistance, radiation effects, *Staphylococcus aureus*

Electric fields produced by electric power systems and other sources use frequencies in the power frequency range. Electric fields are produced by electric charges and exert forces on other charges. If in motion, these charges will produce magnetic forces. Power generation depends on the frequency at which electric and magnetic fields induce electric current in conducting bodies, which may include living organisms [1].

The effects of a static electric field (SEF) on living systems remain controversial. Investigations of how magnetic and electric fields affect living organisms at the molecular level have revealed impacts on the biologic functions of organisms via changes in the concentration of hormones, activity of enzymes, transport of ions by the cell membrane, or changes in the synthesis or transcription of DNA [2-4]. However, data are limited on the response of membrane transport and cell processes to electromagnetic treatment with the 3 "windows" of frequency, amplitude, and duration. Previous studies have also neglected the effects of environmental conditions such as temperature, conductivity, osmolarity, nutrition medium and special additives in studying these effects [5-7]. Preliminary studies have suggested that the application of electric fields is a potentially useful method of non-thermal decontamination and that it can replace, at least in part, thermal processes [8].

In the non-thermal pasteurization technique of pulsed electric fields (PEF), irreversible structural changes in the membranes of inactive microorganisms were effected, resulting in pore formation and loss of selective permeability in the cell membranes [9-11]. Various bacteria have shown different responses to electric field treatments. While membrane permeability was the main factor involved in the mechanism of inactivation, the growth phase and the acidity of the environment also had influences. An apparent affect of morphology on membrane permeability was also reported, with larger cells more easily permeabilized than smaller cells [12].

This study investigated the effects of SEF on growth inhibition of hospital strains of multiply resistant *Esherichia coli* and *Staphylococcus aureus*.

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## Materials and Methods

### SEF Treatment

An SEF treatment system was developed using a direct current high voltage power supply unit, with a voltage range of 0 to 10 kV (Leybold Co., Germany), and 2 flat aluminum electrodes ($25 \times 25 cm^2$) with a 2 cm gap between electrodes, which were tightly fitted on glass tubes with the same inner diameter.

The applied electric field strength was 4.5 kV/cm at the location of growth exposure and incubation of cells. The assessment of exposure at a 4.5 kV/cm SEF intensity was based on calculation using the equation: E = V/d, where E = SEF intensity (kV/cm); V = difference in potential between electrodes (kV; an input voltage of 4.5 kV was used throughout this study); and d = the distance between the electrodes (cm).

In all cases, the temperature during treatment was maintained at 25 ± 2°C in order to rule out thermal effects.

### Bacterial strains and culture

The bacterial strains used in this study were multiple-resistant *E. coli* and *S. aureus*, including 38 hospital strains of *E. coli* and 38 hospital strains of *S. aureus*. These bacteria were identified and isolated from patients with various types of infections [13,14]. The resistance patterns of these bacteria had been previously determined to beta-lactam antibiotics, chemical agents such as cetrimide and heavy metals, and physical agents such as ultraviolet and gamma rays [15,16]. The available strains with the highest minimum inhibitory concentrations to the tested antibiotics and conditions were selected for use in this study [17].

*E. coli* and *S. aureus* were grown overnight at 37°C in nutrient broth medium (Difco, Detroit, MI, USA). Next, suspensions of the pretreated bacteria were prepared by pouring aliquots into test tubes containing 5 mL fresh nutrient broth medium, which were then incubated for 2 h at 25°C with continuous shaking at 190 rpm in an orbital shaker (MSB–33 ZZA L–GS; Blue Electronics, Blue Island, IL, USA). The bacteria were cultured at the early logarithmic phase. Stock bacterial culture was maintained in agar slants at 4°C.

### Effect of SEF treatment on bacteria

The concentrations of viable cells, expressed as colony-forming units (CFU) per mL, were determined before and after SEF treatment using plate count agar (Merck Armstadt, Germany). The plates were incubated aerobically at 37°C for 24 h. Fresh bacterial cultures were used throughout the experiments, and approximately $10^2$ CFU/mL of the bacteria were used as the initial cell concentration, which was exposed to an SEF intensity of 4.5 kV/cm. Five different exposure times (30, 60, 90, 120 and 150 min) were selected in order to evaluate the effects of SEF treatment on the cells. Each experiment was repeated 4 times alongside control groups, which were kept under identical conditions except for exposure to SEF.

### Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA). All reported values were expressed as mean ± standard deviation (SD). Analysis of variance of the effect of SEF treatment on *E. coli* and *S. aureus* populations was performed using the general linear model procedure. The effects of the 5 different durations of exposure to SEF on bacteria were analyzed using Duncan's multiple range test (DMRT). Error bar graphs were expressed as mean ± SD with a 95% confidence interval.

## Results

SEF (4.5 kV/cm, 50 Hz) at different durations of exposure resulted in a decrease in the populations of *E. coli* and *S. aureus*. The effect of SEF at an intensity of 4.5 kV/cm on the number of CFU per mL in *E. coli* and *S. aureus* cultures after various durations of exposure is shown in Table 1. The colony numbers of *E. coli* and *S. aureus* after various SEF exposure times were all significantly less than the control group ($p<0.05$). Growth increase percentages of bacteria treated with SEF at different exposure durations are shown in Table 2. The growth increase percentages of *E. coli* and *S. aureus* after SEF treatment were significantly ($p<0.05$) less than those of the control groups.

The effect of SEF on *S. aureus*, a Gram-positive bacterium and *E. coli*, a Gram-negative bacterium, were also investigated (Fig. 1). Growth inhibition resulting from SEF exposure was greater for *E. coli* than for *S. aureus* (Fig. 1). Analysis of the effect of the duration of exposure to SEF on *E. coli* and *S. aureus* revealed that inactivation of bacteria significantly increased with increasing duration of exposure to SEF (Fig. 2 and Fig. 3). Analysis using DMRT revealed no significant difference in the effects of SEF exposure for 0, 30, 60 and 90 min between the 2 species, but the colony

**Table 1.** Number of colony-forming units (CFU) per mL of *Escherichia coli* and *Staphylococcus aureus* versus control after various durations of exposure to static electric field (SEF)[a]

| Bacteria | SEF exposure time (min) | Control | Treatment |
|---|---|---|---|
| *E. coli* | 0 | 1.330 ± 0.266 | 1.330 ± 0.266 |
| | 30 | 4.625 ± 0.125 | 3.000 ± 0.081[b] |
| | 60 | 9.975 ± 0.100 | 6.925 ± 0.150[b] |
| | 90 | 22.225 ± 0.262 | 9.625 ± 0.150[b] |
| | 120 | 48.850 ± 0.238 | 24.675 ± 0.221[b] |
| | 150 | 110.825 ± 0.221 | 49.300 ± 0.245[b] |
| *S. aureus* | 0 | 1.605 ± 0.251 | 1.605 ± 0.251 |
| | 30 | 3.512 ± 0.132 | 2.412 ± 0.175[b] |
| | 60 | 7.525 ± 0.125 | 3.595 ± 0.132[b] |
| | 90 | 13.350 ± 0.238 | 5.600 ± 0.090[b] |
| | 120 | 29.755 ± 0.238 | 8.775 ± 0.170[b] |
| | 150 | 66.475 ± 0.330 | 30.475 ± 0.340[b] |

[a]Values are mean ± standard deviation from 4 experiments (x $10^2$) CFU/mL of *E. coli* and *S. aureus* bacteria after treatment by 4.5 kV/cm SEF in nutrient broth medium at 25°C for various exposure times.

[b]Significant difference from control $p<0.05$.

numbers of *E. coli* after SEF treatment for 120 and 150 min were significantly decreased compared to all other exposure durations (Table 3). The colony numbers of *S. aureus* exposed to SEF for 120 and 150 min were also significantly reduced compared to 0, 30 and 60 min exposure times, but there was no significant difference between the colony numbers of *S. aureus* subjected to SEF treatment for 90 and 120 min.

Investigation of the inhibitory effects (bacteriostatic or bactericidal) of SEF during exposure of the culture (Fig. 2 and Fig. 3) revealed that the slope of the dependence of CFU on the duration of exposure dose

**Table 2.** Increase in the percentage growth of *Escherichia coli* and *Staphylococcus aureus* bacteria (x $10^2$) in nutrient broth medium at 25°C after static electric field (SEF; 4.5 kV/cm) exposure for various durations

| Bacteria | SEF exposure time (min) | Growth percentages[a] | |
|---|---|---|---|
| | | Treatment | Control |
| *E. coli* | 30 | 2.50 | 1.25 |
| | 60 | 6.50 | 4.20 |
| | 90 | 15.71 | 6.24 |
| | 120 | 35.73 | 17.55 |
| | 150 | 82.33 | 36.10 |
| *S. aureus* | 30 | 1.18 | 0.50 |
| | 60 | 3.68 | 1.24 |
| | 90 | 7.32 | 2.49 |
| | 120 | 17.53 | 4.46 |
| | 150 | 40.41 | 18.00 |

[a]Values are mean from 4 experiments.

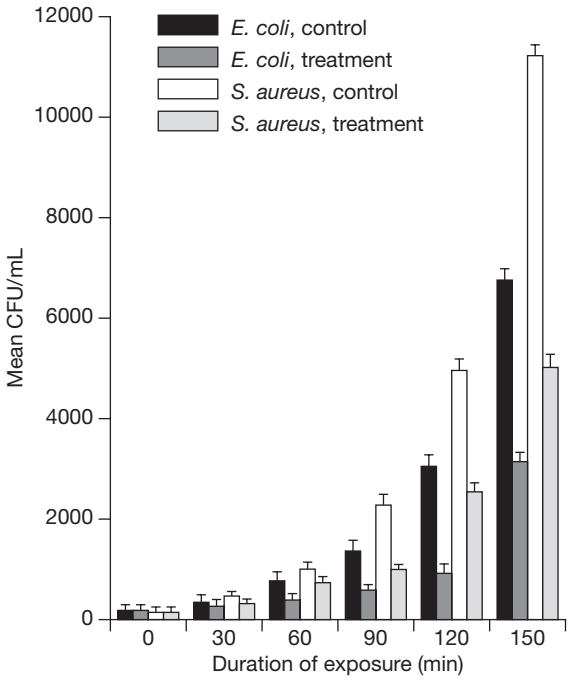


**Fig. 1**. Comparison of effect of 4.5 kV/cm SEF on *Staphylococcus aureus*, *Escherichia coli* and control after exposure for 0, 30, 60, 90, 120 and 150 min in nutrient broth medium at 25°C. Results are mean from 4 experiments; bars indicate standard deviation. CFU = colony-forming units.

was equal to zero, indicating that the effects of SEF are not bacteriostatic.

## Discussion

This study demonstrated that SEF treatment significantly reduced *E.coli* and *S. aureus* growth in culture compared to controls (Fig 2 and Fig. 3). The reproducibility of all data in this study was assured by the use of 4 repeated experiments.

This is the first study to demonstrate a significant effect of SEF treatment on growth inhibition of bacterial cells. Similar effects to those of SEF in this study on bacteria were reported previously with treatments using electromagnetic waves, PEF and other kinds of electric and magnetic fields on various microorganisms [2,8,12, 18,19]. Our results, however, suggest that SEF may be an effective and economical technique for controlling microbial populations in various applications with limited energy consumption.

In the present study, increased duration of exposure to SEF resulted in logarithmic decrease of *E.coli* and *S. aureus* colony numbers in culture. These results are in agreement with those of Pol et al who found that pulsed-electric field treatment enhanced the bactericidal

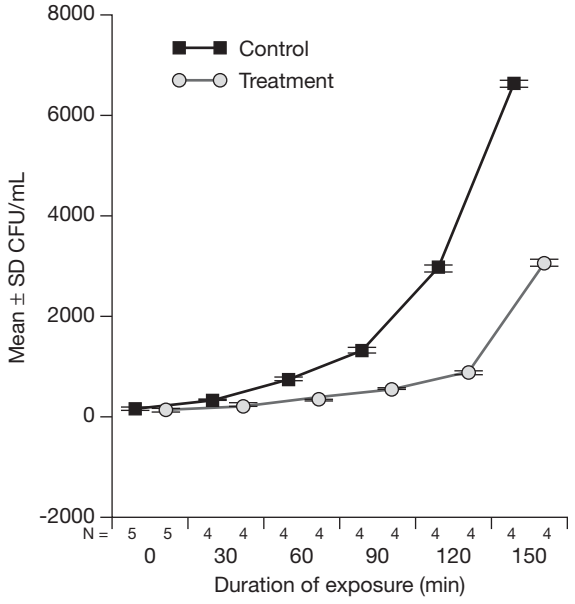


**Fig. 2.** Change in viable cell number of *Staphylococcus aureus* in nutrient broth medium at 25°C under static electric field (4.5 kV/cm) exposure for various durations. Results are mean from 4 experiments; bars indicate standard deviation. SD = standard deviation; CFU = colony-forming units.

action of nisin against *Bacillus cereus* [9]. Statistical analysis revealed significant difference in colony numbers of *E. coli* and *S. aureus* compared to controls after SEF treatment for all durations of exposure in this

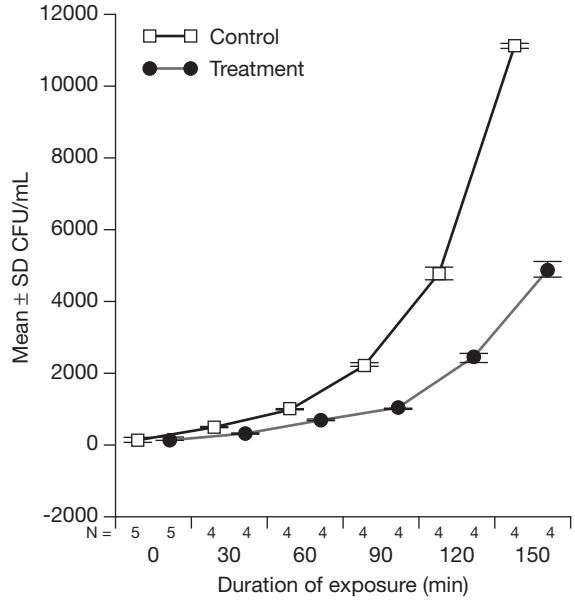


**Fig 3.** Changes in viable cell number of *Escherichia coli* in nutrient broth medium at 25°C under static electric field (4.5 kV/cm) exposure for various durations. Results are mean from 4 experiments; bars indicate standard deviation. SD = standard deviation; CFU = colony-forming units.

**Table 3.** Comparison of colony numbers (colony-forming units; CFU) per mL of *Escherichia coli* and *Staphylococcus aureus* after treatment with 4.5 kV/cm static electric field (SEF) for various durations in nutrient broth medium at 25°C

| SEF exposure time (min) | Colony numbers (CFU/mL) | |
|---|---|---|
| | *E. coli* | *S. aureus* |
| 0 | 133.0 | 160.5 |
| 30 | 300.0 | 241.2 |
| 60 | 692.5 | 359.5 |
| 90 | 962.5 | 560.0 |
| 120 | 2467.5[a] | 877.5[a] |
| 150 | 4930.0[b] | 3047.5[b] |

[a]Significant difference versus all other values for each species; $p$=0.05 by Duncan's multiple range test (DMRT).
[b]Significant difference versus all other values for each species; $p$=0.05 by DMRT.

study (Table 1). These findings suggest that SEF affected the growth inhibition of these bacteria. SEF had a greater impact on the Gram-negative bacteria *E. coli* than Gram-positive bacteria *S. aureus* in this study (Fig. 1, Fig. 2 and Fig. 3). These results are in agreement with the findings of Wouters et al [20]. The mechanism of action of the antimicrobial effects of electric fields remains unclear. Previous researchers have postulated a possible effect of electric fields on the permeability of the ionic channels in the bacterial cell membrane [21-23]. Other possible mechanisms include the formation of free radicals due to SEF exposure and the bactericidal effects of hydroxyl radical formation resulting from electric field effects [24].

Many factors influence the inactivation kinetics of SEF treatment, including process parameters (strength and duration of the electric field) and microbial characteristics such as microorganism, strain or inoculum size as well as product parameters such as nutrients, pH and temperature. Further research is needed to analyze the effects of SEF on the growth of resistant bacteria. There is limited information concerning the resistance of bacteria to electric field treatment and the factors that influence this resistance. Further delineation of these characteristics is necessary for predicting the response of these bacteria under electric field conditions. Analysis of proteins which may be expressed specifically in cells under SEF treatment, as well as gene expression studies, may further explain the mechanisms of effectiveness. Further study employing 2-dimensional gel electrophoresis and DNA arrays of *E. coli* and *S. aureus* genes to compare the expression patterns of cells under SEF conditions may be informative [25].

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