# The Bactericidal Effects of Electrolyzed Oxidizing Water on Bacterial Strains Involved in Hospital Infections

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**Abstract:** The study is designed to investigate bactericidal actions of electrolyzed oxidizing water on hospital infections. Ten of the most common opportunistic pathogens are used for this study. Cultures are inoculated in 4.5 mL of electrolyzed oxidizing (EO) water or 4.5 mL of sterile deionized water (control), and incubated for 0, 0.5, and 5 min at room temperature. At the exposure time of 30 s the EO water completely inactivates all of the bacterial strains, with the exception of vegetative cells and spores of bacilli which need 5 min to be killed. The results indicate that electrolyzed oxidizing water may be a useful disinfectant for hospital infections, but its clinical application has still to be evaluated. **Key Words:** Electrolyzed oxidizing water—Bactericidal effect—Hospital infections—Oxidation-reduction potential—Reactive oxygen species.

Hospital infections are a serious medical, social, and economic problem for public health services all over the world. Such pathogens are typically characterized by a wide variety of sources, ways, and factors of transmission, appearing in different types of clinics and preventive hospitals. In this context, the methods of asepsis and active chemical antisepsis are currently becoming increasingly important in terms of the prevention of hospital infections.

There have been a number of reports on the antimicrobial activity of electrolyzed oxidizing (EO) water produced by electrolysis of aqueous sodium chloride solutions using a device in which the anode and cathode are separated by a membrane to form two compartments (1–4). Such a device, named the ROX-20TA electrolyzer, was kindly provided to Lomonosov Moscow State University by the Hoshizaki Electric Company (Aichi, Japan) for use in this study. The investigation of the bactericidal properties of EO water obtained in the ROX-20TA electrolyzer was especially interesting for us because the physical and chemical parameters of tap water in Moscow (Russia) differ from those in Japan and some other countries.

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The objective of this study was to evaluate the bactericidal effect of EO water obtained in the ROX-20TA electrolysis device on common hospital bacterial strains under in vitro conditions.

## MATERIALS AND METHODS

### Bacteria and culture

The test bacteria *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus* (vegetative cells and spores), *Citrobacter freundii*, *Flavobacter* sp., *Proteus vulgaris*, *Alcaligenes faecalis*, and *Aeromonas liquefaciens* were obtained from the Department of Microbiology, Lomonosov Moscow State University.

All bacterial strains were cultured individually on the surface of nutrient broth (Oxoid, no. 2, Unipath, Ltd., Basingstoke, Hampshire, U.K.) containing 1.5% bacto-agar (Difco, Detroit, MI, U.S.A.) for 24 h aerobically at 37°C, with the exception of bacilli which were grown at 30°C. Following the incubation, the bacteria of each strain were collected, washed twice with 0.85% sodium chloride solution, and resuspended in 1 mL of the same solution. The optical density of the suspensions was adjusted to 0.15 at 540 nm (corresponding to approximately $10^9$ CFU/mL). Each suspension was divided into two parts: a control part (treatment with deionized water) and an experimental part (treatment with EO water).

### Preparing spores

One colony of *Bacillus cereus* was plated on the surface of agarized nutrient broth in a tube, incubated for 48 h at 30°C, and then reinoculated on the surface of agarized potato medium in the tube. Following 48 h of incubation on the potato medium, all vegetative cells transformed into spores. The spore transformation was tested by plating cell suspensions on Petri dishes and proved if no growth was detected during 48 h.

### Test solutions

EO water was generated in an ROX-20TA electrolysis device. The current passing through the electrolytic cell and the voltage between the electrodes were set at 19.8 A and 10 V, respectively. At these conditions the mean pH, the oxidation-reduction potential (ORP), the available chlorine concentration (ACC), and the conductivity of the tested EO water were $2.84 \pm 0.01$, $1125 \pm 3$ mV, $43 \pm 0.3$ ppm, and $4.0 \pm 0.02$ mS/cm, respectively. The mean pH, ORP, ACC, and conductivity of tap water were $7.43 \pm 0.1$, $380 \pm 12$ mV, $3 \pm 0.01$ ppm, and $0.43 \pm 0.02$ mS/cm, respectively.

**Sample treatments**

A volume of 4.5 mL of EO water (treatment) or sterile deionized water (control) was transferred to separate sterile tubes. To each tube 0.5 mL (equivalent to $0.5 \times 10^9$ CFU) of bacterial suspension was added, and samples were incubated in a water bath (23°C) for 0, 0.5, and 5 min. After the incubation, the number of viable cells in each sample was determined by plating 0.1-mL aliquots directly or after serial (1 : 10) dilutions on duplicate agarized nutrient broth plates. Colonies of the inoculated bacteria were counted on the plates after incubation at 37°C or 30°C for 48 h. Plate count data are expressed as mean ± SD.

## RESULTS

To obtain EO water with the strongest bactericidal properties, we plotted the curves of the applied current (amperage/A) vs. pH (Fig. 1a), ACC (Fig. 1b), conductivity, and ORP. Using the resulting curves, and taking into account that for lower pH and higher chlorine concentration and ORP, the bactericidal effect of EO water is stronger, the following parameters were selected: a pH 2.84, an ACC of 43 ppm, a conductivity of 4.0 mS/cm, and an ORP of 1125 mV at the applied 19.8 A and 10 V.

The bactericidal effects of this EO water are shown in Table 1. It can be seen that the counts of the majority of the bacterial strains in the treatment samples were reduced to zero after 0.5 min of treatment, whereas the population of *B. cereus* was 3.76 log CFU/mL. After 5 min of treatment the counts of the vegetative cells and spores of *B. cereus* were reduced to zero. No differences in bacterial counts were observed in the control samples throughout the study (not shown). The sensitivity of the bacteria to EO water was not correlated with the Gram staining data under these conditions.

**TABLE 1.** *A comparison of bactericidal effects on bacterial strains treated with electrolyzed oxidizing water*

| Bacterial strain | Viable counts after treatment for (mean log CFU/mL)* | | |
|---|---|---|---|
| | 0 min | 0.5 min | 5 min |
| **Gram-negative** | | | |
| *Pseudomonas aeruginosa* | 8.04 ± 0.07 | 0 | — |
| *Escherichia coli* | 8.21 ± 0.04 | 0 | — |
| *Citrobacter freundii* | 7.63 ± 0.06 | 0 | — |
| *Flavobacter* sp. | 8.12 ± 0.02 | 0 | — |
| *Proteus vulgaris* | 8.01 ± 0.04 | 0 | — |
| *Alcaligenes faecalis* | 7.80 ± 0.03 | 0 | — |
| *Aeromonas liquefaciens* | 7.90 ± 0.04 | 0 | — |
| **Gram-positive** | | | |
| *Enterococcus faecalis* | 8.23 ± 0.03 | 0 | — |
| *Staphylococcus aureus* | 8.36 ± 0.08 | 0 | — |
| *Bacillus cereus* | 6.72 ± 0.02 | 3.76 ± 0.02 | 0 |
| *Bacillus cereus* (spores) | 7.98 ± 0.06 | — | 0 |

0, not detected in dilutions of $10^{-1}$–$10^{-4}$; —, not measured.

The physical and chemical properties of the tested EO water are as follows: the ORP, 1125 mV; the pH, 2.84; the ACC, 43 ppm.

*Results are mean ± SD.

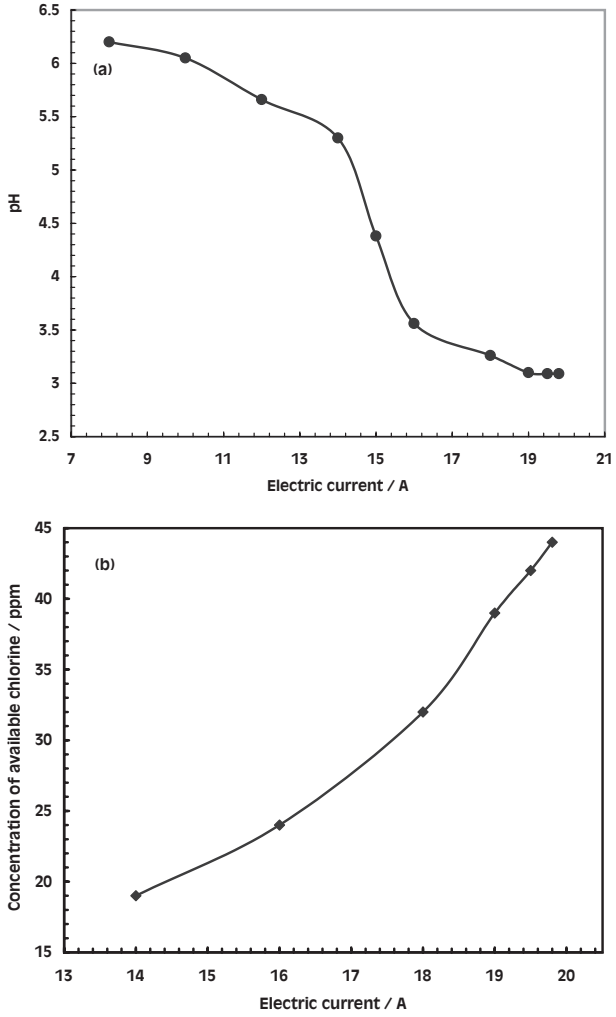


**FIG. 1.** The relationship between (a) the current and pH, and (b) the current and the concentration of available chlorine, in the oxidized water produced by the electrolysis of 0.1% NaCl solution.

## DISCUSSION

The bacterial strains subjected to the treatment by EO water in this work belonged to different genera; however, they were not chosen arbitrarily. Most of them are the opportunistic pathogens and dangerous causative agents of different diseases frequently appearing in hospitals and in disabled and immunocompromized patients.

Methods for the prevention of the dissemination of such pathogens are difficult because of the prob-

lem of their resistance to many antimicrobial drugs. Such a bactericide as EO water overcomes the problem of drug resistance due to its strong nonselective antimicrobial properties. The EO water which was produced for the first time in Russia and applied initially for sanitation (5,6), water disinfection (7), and regeneration (8) has acquired particular importance over recent years in medical practice (1–3).

The bactericidal effects of EO water are due to its physical and chemical properties, such as low pH, high ORP (9), large amounts of dissolved chlorine gas and hypochlorous acid (HClO) (10), hydrogen peroxide ($H_2O_2$), and reactive oxygen species (ROS) including superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), $OCl^{\bullet -}$, etc. The composition of EO water resembles that of the respiratory burst of activated eukaryotic phagocytes. We suggest that the synergistic effect of oxidants and ROS, the low pH, and the high ORP make the anolyte or EO water a strong bactericidal drug.

As was shown in this study, the EO water which was produced in the ROX-20TA electrolyzer under our conditions of including the specific properties of the used tap water (i.e., the ACC corresponding to 3 ppm) renders a strong bactericidal action to both Gram-positive and Gram-negative bacteria as well as to the vegetative cells and spores of bacilli.

This EO water can be recommended for use as a strong disinfectant for the equipment, and diagnostic and medical devices in hospitals. However, future clinical research has to be done under an in vivo system, in order to evaluate the stability of EO water under operating conditions and its safety for medical personnel and patients.

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# Selective Adsorption of Homocysteine Using an HFR-ON LINE Technique

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**Abstract:** HFR-ON LINE (double chamber HDF with reinfusion of ultrafiltrate regenerated through a charcoal-resin cartridge) is a novel method which combines the processes of diffusion, convection, and adsorbance. We have investigated the effect of such a treatment on the homocysteine (Hcy) levels in ten patients with a mean Hcy level of 57.6 µmol/L (range 24.1–119.7 µmol/L). We have measured the Hcy, folate, and vitamin B12 predialysis and postdialysis, and in the ultrafiltrate precartridge and postcartridge at 10, 120, and 240 min. The mean Hcy levels were 57.6 and 35.3 µmol/L (range 9.9–80.3 µmol/L) ($P = 0.005$) predialysis and postdialysis, respectively, while folate and vitamin B12 were unchanged. Precartridge and postcartridge Hcy levels were 11.6 vs. 2.5 µmol/L ($P = 0.005$), 9.3 vs. 3.9 µmol/L ($P = 0.005$), and 7.7 vs. 4.6 µmol/L ($P = 0.012$) at the three time points considered, while folate and vitamin B12 were essentially undetectable.These preliminary data, which need confirmation in a long-term study, seem to indicate that HFR-ON LINE is able to reduce Hcy levels not only through a likely reduction of uremic toxins, but also through an actual removal of Hcy by adsorbance onto the charcoal-resin cartridge. **Key Words:** Homocysteine—HFR-ON LINE—Hemodialysis—Coronary heart disease (CHD) in renal disease.

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