

Original Article

An investigation into the *in-vitro* effectiveness of electrolyzed water against various microorganisms

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Abstract: The aim of this study was to investigate the *in-vitro* antimicrobial activity of usage and normal concentrations of electrolyzed water in hospital. In our study, the effects of different concentrations of electrolyzed water named Envirolyte® (Industries International Ltd., Estonia) on two gram positive, four gram negative standard strains and clinical isolates of four gram negative, two gram positive, one spore-forming bacillus and *Myroides* spp strains that lead to hospital infections were researched. The effects of different concentrations and different contact times of Envirolyte® electrolyzed water on cited strains were researched through method of qualitative suspension tests. Petri dishes fo bacteria have been incubated at 37 °C 48 hours. Bactericidal disinfectant was interpreted to be effective at the end of the period due to the lack of growth. Solutions to which disinfectant were not added were prepared with an eye to control reproduction and controlcultures were made by using neutralizing agents. 1/1, 1/2, and 1/10 concentrations of Envirolyte® electrolyzed water were found to be effective on the bacteria that lead to hospital infections used during all test times. As a conclusion, based upon the results we acquired, it was observed that Envirolyte® electrolyzed water of 100% concentration would be convenient to be used for disinfection when diluted to a usage concentration of 1/10.

Keywords: Electrolyzed water, disinfectant, bacteria

Introduction

Disinfection means eliminating infecting microorganisms excluding the spores on no-viable medical equipment and this method is mostly used by means of chemical substances known generally as sanitisers. When selecting the type of sanitisers to be used at hospitals, different kinds of criterion such as the effect spectrum of the sanitiser, the convenience of the sanitiser with regard to the area of use, the period required to see the desired effect, any damages to occur potentially on any atmosphere or equipment and the cost of the relevant sanitiser shall be taken into account [1]. However, disinfectant products having antimicrobial effectiveness, are patent medicines including chemical substances that can cause damage to human health due to their physico-chemical features, to the employees working in that atmosphere due to their hazardous effect

on the working environment, to the patients due to the surfaces and equipments where they are applied and to the environment due to their waste substances that come out after usage [1, 2]. All research conducted by taking all these disadvantages into account aim at providing the most suitable, toxic effects, cost-effective, easy to use and reliable disinfectants to be used to cure infections at hospitals [1]. To this end, it is revealed that the electrolyzed water being used widely in the recent years has many advantages such as the elimination of toxic substances, the reduction of the cost, the environmentally acceptable reliability with regard to the safety of the employees and patients taking place in the hospitals and the structure that does not cause any damage to human tissue [3]. Also super-oxidized water, has many advantages such as not having toxic products, not harming human tissue, safety to the patients, the staff, and the environment [4, 5]. But it is

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corrosive when it used at high doses and prolonged contact with surgical instruments endoscopes [2, 5]. Electrolyzed water is obtained by applying electricity on salty water and it is used during the disinfection and sterilization processes, as it has a wide spectrum effect against microorganisms. The electrolyzed water obtained after the electrolyze process (hypochlorous acid, hypochloride ions, melted oxygen, ozone, super oxide radicals etc.) has a relative strong oxidation potential and shows considerably high antimicrobial activity. To this end, this water kills the bacteria, virus, fungi and parasites in a fast manner. It can be used to disinfect robust surfaces and the water systems [2]. But the effects of the Envirolite® on microbes is not long. It depends on the half-life of metabolites especially chloride. By the way persence of organic matter reduces efficacy of Envirolite®. Therefore stronger concentrations and longer contact time should be considered [2, 5]. Envirolite® may combined with other substances that make it more effective [5].

The aim of our research is to reveal the in-vitro effect of electrolyzed water against same nosocomial bacteria under different concentrations, which is an issue not researched in depth until now.

Materials and methods

The effectiveness of the electrolyzed water produced by the Envirolite® (Envirolite® Industries International Ltd., Estonia) equipment used in the microbiology laboratory of University Hospital is examined against different kinds of bacteria. Electrolyzed water is highly effective at pH between 6.5 to 7.5 Envirolite® device is calibrated according to the instructions of the producer to produce electrolyzed water at pH 6.5 including 500-700 ppm chlorine. End product is monitored by pH test kit based on a color scale.

Electrolyzed water obtained by mixing water, salt and electricity in the Envirolite® equipment is used for this research. The electrolyzed water is analyzed on six American Type Culture Collection (ATCC) strains (*Acinetobacter baumannii* 19606, *Escherichia coli* 25922, *Enterococcus faecalis* 29212, *Klebsiella pneumoniae* 254988, *Pseudomonas aeruginosa* 27853, *Staphylococcus aureus* 29213) and eight different multidrug resistant bacteria (A.

baumannii, *E.coli*, vancomycin resistant *Enterococcus faecium*, *K. pneumoniae*, *P. aeruginosa*, methicillin resistant *S. aureus*, *Bacillus subtilis*, *Myroides spp.*) with the method of qualitative suspension tests [6-8]. The bacteria to be used for the test were passaged at the Triptin Soya Agar (TSA) (Oxoid, UK) medium and the production was completed after a 24 hour incubation under 37°C. After a 24 hour-long culturization of the microorganisms, the bacteria suspension is prepared by means of Triptin Soy Broth (TSB) and 0.5 McFarland haze (10⁸ CFU/ml). Thereafter, different kinds of electrolyzed water concentrations (1/1, 1/2, 1/10, 1/20, 1/50, 1/100) are put into tubes of 1000 µl. 10 µl of bacteria suspensions were poured in every tube of electrolyzed water. After every 1, 2, 5, 10 and 30 minutes, 100 µl is taken from every bacteria and disinfection mixture and they are put into tubes including 900 µl neutralizer substance (Dey-Engley Neutralizing Broth-Sigma-Aldrich, USA), (casein enzymatic hydrolysate 5 g/l, yeast extract 2.5 g/l, dexrose 10 g/l, sodium thiosulfate 1 g/l, sodium bisulfite 2.5 g/l, lecithin 7 g/l, polysorbate80 5 g/l and bromacresol purple 0.02 g/l). After a neutralization process of 5 minutes, 10 µl is taken and TSA is poured in the tubes. The petris are incubated for 48 hours under 37°C. As there was no extension in the volume of bacteria, this can be interpreted as a bactericidal effect of the disinfectant. New solutions not including disinfecants were prepared in order to check the production of bacteria and controls are performed by using neutralizing substances.

Results

It was observed that the 1/1, 1/2, and 1/10 dilutions of electrolyzed water produced through the Envirolite® equipment was effective on the standard strains for all intervals of time. The one, two, and five minute administrations of the 1/20 dilution of electrolyzed water were not effective on the *E. coli* ATCC 25922 strain; however, the ten minute and thirty minute administrations were effective. The one and two minute administrations of 1/20 dilution [1] were not found to be effective on all ATCC strains; however, the five, ten, and thirty minute administrations were effective. No effects were observed on the standard strains from the 1/50 and 1/100 dilutions of electrolyzed water (**Table 1**).

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Table 1. Envirolyte® electrolyzed water activity against strains ATCC

		Envirolyte® (dilution rate)					
		1/1	1/2	1/10	1/20	1/50	1/100
<i>Acinetobacter baumannii</i> ATCC 19606	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
<i>E. coli</i> ATCC 25922	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	+	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
<i>Enterococcus faecalis</i> ATCC 29212	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
<i>Klebsiella pneumoniae</i> ATCC 254988	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
<i>Pseudomonas aeruginosa</i> ATCC 27853	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
<i>Staphylococcus aureus</i> ATCC 29213	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+

min: minute, (-): no growth on culture, (+): bacteria growth on culture.

Effect of electrolysed water on the clinical isolates was found similar with the effect on the same standard species. Its efficacy on *Myroides* spp. was found to be similar with the effect on the clinical isolates other than *E. coli* and *B. subtilis*. 1/1, 1/2, and 1/10 dilutions of electrolysed water were found to be effective on *B. subtilis* in each durations performed, however, 1/20 dilution was effective only in 30 min administration, and 1/50 and 1/100 dilutions had no effects (**Table 2**). It was found that 1/1, 1/2, and 1/10 dilutions of electrolysed water were effective either on the standard strains or on the clinical isolates in each durations, however, that dilutions of 1/20 and over had similar

effects on the standard strains and the clinical isolates except *B. subtilis*.

Discussion

Disinfection processes are strictly important on preventing the infections in hospitals. The reliability of a disinfectant should be tested on the microorganisms available in the hospital and the methodology and the concentration shall be described properly before selecting the right disinfectant [6]. When the recent use of electrolyzed water is examined in our country, it is possible to come across with different kinds of examples from different industries. There are

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Table 2. Envirolyte® electrolyzed water activity against clinical strains

		Envirolyte® (dilution rate)					
		1/1	1/2	1/10	1/20	1/50	1/100
<i>Acinetobacter baumannii</i>	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
<i>E. coli</i>	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	+	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
Vancomycin resistant <i>Enterococcus faecium</i>	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
<i>Klebsiella pneumoniae</i>	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
<i>Pseudomonas aeruginosa</i>	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
Methicillin resistant <i>Staphylococcus aureus</i>	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+
<i>Bacillus subtilis</i>	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	+	+	+
	10 min	-	-	-	+	+	+
	30 min	-	-	-	-	+	+
<i>Myroides spp.</i>	1 min	-	-	-	+	+	+
	2 min	-	-	-	+	+	+
	5 min	-	-	-	-	+	+
	10 min	-	-	-	-	+	+
	30 min	-	-	-	-	+	+

min: minute, (-): no growth on culture, (+): bacteria growth on culture.

lots of international papers proving the efficiency of electrolyzed water. However, this paper is among the few papers that examine the electrolyzed water on active microorganisms in hos-

pital infections and hence, we believe that this will be among the leading papers in this field. This study reveals that the electrolyzed water produced through the equipment named

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Enviolyte® has an immediate bactericidal effect on standard strains and clinical isolates of 1/1, 1/2, 1/10 concentration. Deza and et al. conducted a study at the University of Santiago and efficiency tests are conducted in this study on glass and stainless surfaces in order to determine the effect of electrolyzed water on *E. coli*, *P. aeruginosa* and *S. aureus* [9]. The effect of electrolyzed water is compared with the effect of sodium hypochloride in this study. The analyses revealed that electrolyzed water was more effective than sodium hypochloride on pathogen bacteria placed on glass and stainless surfaces [7]. When compared to chlor, electrolyzed water has some advantages such as requiring low cost and being non-toxic. Although it has a very fast antiseptic activity on hands, it has a major disadvantage on alcoholic hand rubs due to its long drying time. Nishimura et al. conducted a study that revealed electrolyzed water is more effective than 7.5% povidone iodine on hand disinfection [10]. The study of Landa et al. at the University of California based on in vitro antimicrobial and antiviral efficiency tests revealed that electrolyzed water is effective in 30 seconds on *S. aureus*, *E. coli*, and *P. aeruginosa* and in five minutes on *Bacillus atrophaeus* [11]. Sakurai et al. compared the efficiency of electrolyzed water and Glutaraldehyde on *P. aeruginosa* through an endoscopical disinfection method. This bacteria is put into electrolyzed water for 10 seconds and into glutaraldehyde and electrolyzed water for five and 10 minutes, respectively. Consequently, it was found out that electrolyzed water is a more reliable and effective disinfectant on endoscopes [12]. The use of electrolyzed water has become quite popular in Japan recently. The research conducted by Tanaka et al. revealed that according to the tests on electrolyzed water and 2% Dialox-cj and 3.8% formaline, electrolyzed water is much more effective than the other disinfectants available [13]. Nakae et al. performed a study on burned injuries and it is found out that irrigation and disinfection as well as super oxidized water is quite effective on preventing the related sepsis [14]. Vorobjeva et al. reported that super-oxidized water on hospital effective microorganisms does have an effect on gram positive and negative as well as the vegetative cells of spore bacillus and their spores [15]. In addition, a remarkable study was done by Gunaydin et al., which found that Medilox® super-oxidized water was effective against all

standard and clinical isolates except VRE in a dilution of 1/2 within 1 minute and the other durations of exposure. Medilox® super-oxidized water was found to be most effective on *E. Coli* isolates in a dilution of 1/5 [16]. The concentration of chloride in Medilox® is lower than in Enviolyte®; therefore Enviolyte® has a greater effect on isolates at a 1/10 concentration. However, VRE can lead hospital epidemics by contaminating medical devices. Fast-acting surface disinfectants are necessary to be able to remove these microorganisms from the environment. In the present study, it has been proven that super-oxidized water (= electrolyzed water) inactivated VRE in one minute. *Acinetobacter spp.* has also become a problem, due to their ability to survive on inanimate surfaces for a long time and ineffective disinfection procedures in hospitals. Our results have proved that super-oxidized water inactivated *A. baumannii* in one minute, even at a dilution of 1/10. This study revealed that electrolyzed water is a beneficial disinfectant in case it is validated [16].

On the other hand Nishimura et al. have reported that we can use super-oxidized water for hand disinfection instead of povidone iodine [17]. Also alcoholic disinfectants have very fast antiseptic activity on hands, it has a major disadvantage on hand rubs due to its long drying time. Choi et al. reported in the light of their study evaluating its activity on 25 bacterial strains that super-oxidized water can be used for disinfection of skin, instruments and surfaces [18]. However, electrolyzed water maybe be a better alternative than both of them (skin, instruments, and surfaces) based on its antibacterial effects on bacteria. Nakae et al. reported that using super-oxidized water may be helpful to prevent sepsis associated with burn injury [2, 19]. Sterilox is another electrolyzed water but it has been suggested for only disinfection of dental water lines and endoscopes [20, 21]. Moreover, Rossi-Fedele et al. have reported that the stability of this product was effected by the storage conditions and exposure to the sun [22].

There are some studies about the antibacterial effects of electrolyzed water, but it has not been studied as a surface disinfectant against hospital microorganisms. In this study, we used a wide range of bacteria that cause nosocomial infections, different kinds of electrolyzed water

concentrations, and various intervals of time. We used a qualitative suspension test as one of the first-steps in evaluating the efficacy of super-oxidized water in this study. The results of this study prove the efficiency of super-oxidized water on a wide variety of microorganisms that cause hospital infections, and these results will assist the second- and third-step studies. Envirolyte® is a surface disinfectant and is used at room temperature, so this study was performed at room temperature. The effects of Envirolyte® on microbes at different temperatures was not tested, however. Also, the concentration of chloride is very important for antimicrobial activity and toxicity, so it must be checked very carefully in future studies. The Envirolyte® electrolyzed water chlorine concentration can be easily measured.

In conclusion, super-oxidized water is effective on a broad spectrum of bacteria. It was observed that Envirolyte® electrolyzed water of 100% concentration would be convenient to use for disinfection when diluted to a concentration of 1/10. Within the framework of the results of our study, we believe that electrolyzed water can be regarded as a cost-effective disinfectant that is successful in controlling hospital infections, as it had an effect on bacteria showing various resistance patterns. This data can and will be reproduced and validated through other efficiency studies.

Disclosure of conflict of interest

None.

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