

An Investigation into the *in-vitro* Effectiveness of Electrolyzed Water against Various Microorganisms

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Abstract

Aim: The aim of this study was to investigate the *in-vitro* antimicrobial activity of usage and normal concentrations of electrolyzed water in hospital.

Material methods: In our study, the effects of different concentrations of electrolyzed water named Enviolyte® (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 Enviolyte® electrolyzed water on cited strains were researched through method of qualitative suspension tests. Petri dishes 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 control cultures were made by using neutralizing agents.

Results: 1/1, 1/2, and 1/10 concentrations of Enviolyte® electrolyzed water were found to be effective on the bacteria that lead to hospital infections used during all test times. Consequently, above mentioned disinfectant which is produced by using water, salt and electric current via Enviolyte® generator has many advantages such as its simple production, being economic, user friendly and non-toxic and having high-efficiency.

Conclusion: Based upon the results we acquired, it was observed that Enviolyte® electrolyzed water of 100% concentration would be convenient to be used for disinfection when diluted to a usage concentration of 1/10.

Key words: Electrolyzed Oxidizing Water, Disinfectant, Bacteria

Introduction

Disinfection means eliminating infecting microorganisms excluding the spores on non-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. However, disinfectant products having antimicrobial effectiveness, are patent medicines including chemical substances that can cause damage to human health due to their physicochemical 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 useage. All research conducted by taking all these disadvantages into account aim at providing the most suitable, 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.² 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 oxyde radikals etc.) has a relative strong oxidation potential and shows considerably high antimicrobic 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.³ The aim of our research is to reveal the in-vitro effect of electrolyzed water against some 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 Envirolyte® (Envirolyte® Industries International Ltd., Estonia) equipment used in the University Hospital of Ondokuz Mayıs is examined against different kinds of bacteria. Electrolyzed water is highly effective at pH between 6,5 to 7,5 Envirolyte® ® 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 monitorized by pH test kit based on a color scale.

Electrolyzed water obtained by mixing water, salt and electricity in the Envirolyte® 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 mult-drug 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.^{4,5,6} The bacteria to be used for the test were passaged at the Tryptin Soya Agar (TSA) (Oxoid, UK) medium and the production was completed after a 24 hour incubation under 37° C in a disinfectant. After a 24 hour-long culturization of the

microorganisms, the bacteria suspension is prepared by means of Tryptin Soy Broth (TSB) and 0.5 McFarland haze (10^8 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 μ l. 10 μ l of bacteria suspensions were poured in every tube of electrolyzed water. After every 1, 2, 5, 10 and 30 minutes, 100 μ l is taken from every bacteria and disinfection mixture and they are put into tubes including 900 μ l neutralizer substance (Dey-Engley Neutralizing Broth-Sigma-Aldrich, USA), (casein enzymatic hydrolysate 5 g/l, yeast extract 2.5 g/l, dextrose 10 g/l, sodium thiosulfate 1 g/l, sodium bisulfite 2.5 g/l, lecithin 7 g/l, polysorbate 80 5 g/l and bromocresol purple 0.02 g/l). After a neutralization process of 5 minutes, 10 μ l 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 disinfectants were prepared in order to check the production of bacteria and controls are performed by using neutralizing substances.

Results

It was observed that 1/1, 1/2, and 1/10 dilutions of electrolysed water produced through the equipment Envirolite® was effective on the standard strains in all the durations performed. In addition, 1 min, 2 min, and 5 min administrations of 1/20 dilution of electrolysed water weren't effective on the *E. coli* ATCC 25922 strain, however, 10 min and 30 min administrations were effective. 1 min and 2 min administrations of this dilution weren't found to be effective on all ATCC strains, however, it was effective in 5 min, 10 min, and 30 min administrations. No effects of 1/50 and 1/100 dilutions of electrolysed water were observed on the standard strains (Table 1).

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.⁵ 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 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 hospital 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 Envirolite® has an immediate bactericidal effect on standard strains and clinical isolates of 1/1, 1/2, 1/10 concentration. Deza and etc. 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*. 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 and etc. conducted a study that revealed electrolyzed water is more effective than 7.5% povidone iodine on hand disinfection.⁸ The study of Landa and etc. 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 5 minutes on *Bacillus atrophaeus*.⁹ Sakurai etc. compared the efficiency of electrolyzed water and Glutaraldehyde on *P. aeruginosa* through an endoscopic disinfection method. This bacteria is put into electrolyzed water for 10 seconds and into glutaraldehyde and electrolyzed water for 5 and 10 minutes, respectively. Consequently, it was found out that electrolyzed water is a more reliable and effective disinfectant on endoscopes.¹⁰ The use of electrolyzed water has become quite popular in Japan recently. The research conducted by Tanaka and etc. 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.¹¹ Nakae H and etc. performed a study on burned injuries and it is found out that irrigation and disinfection as well as super oxydized water is quite effective on preventing the related sepsis.¹² Vorobjeva NV and etc. revealed that super oxydized 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.¹³ The study by Fener and etc. at the University of Zurich the microbial efficiency of electrolyzed water called Medilox® is examined by taking the Standards of the German Veterinary Association into account and the efficiency was tested on *E. faecium*, *P. aeruginosa*, and *S.aureus*. The efficiency tests revealed that electrolyzed water is effective in 30 minutes on all kinds of bacteria.¹⁴ Venkitanarayanan and etc. evaluated the effect of electrolyzed water on *E. coli*. On the other hand VRE can lead hospital epidemics by contaminating medical devices. Fast active 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 inactivated VRE in one minute. *Acinetobacter spp.* have also become an important problem to their ability to survive long time on inanimate surfaces 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.¹⁵

On the other hand Nishimura et al. have reported that we can use super-oxidized water for hand disinfection instead of povidone iodine.¹⁶ 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.¹⁷ So electrolyzed water maybe be a better alternative than both of them according to its antibacterial activity on bacteria. Nakae, et al. in their study reported that using super-oxidized water may be helpful to prevent sepsis associated with burn injury.¹⁸ Sterilox is another electrolyzed water which has been suggested for disinfection of dental water lines and endoscopes.^{19,20} It has not been studied as a surface disinfectant against hospital microorganisms. Moreover, Rossi-Fedele et al. have reported that the stability of this product was effected by the storage conditions and exposure to the sun.²¹

There are some studies about antibacterial activity of electrolyzed water. But in this study we use a wide group of bacteria that cause of nosocomial infections, different kinds of electrolyzed water concentrations and part of times. We used qualitative suspension test

which is one of the first step tests has been used to evaluate efficacy of super-oxidized water in this study. The results of this study proving the efficiency on wide variety of microorganisms causing hospital infections and will ease the second, and third step studies. Also concentration of chloride is very important for antimicrobial activity and toxicity. So it must be checked very carefully. Envirolyte® electrolyzed water chlorine concentration can be easily measured.

In conclusion, super-oxidized water is on-site easily producible disinfectant, which is effective on broad spectrum of bacteria and fungi. 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. This advantageous disinfectant being easily produced in the equipment Envirolyte® by mixing water, salt and electricity is an easy to use, non-toxic, effective, and economic substance. Within the framework of the efficiency results that we obtained in our study, we believe that electrolyzed water can be regarded as a cost effective disinfectant effective on controlling the hospital infections, as it has effect on bacteria showing different resistance patterns. We are in the opinion that this data can be validated through other efficiency papers.

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TABLES

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> ATCC25922	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

Table Envirolyte® electrolyzed water activity againstclinical 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