Universal Journal of Microbiology Research 2(3): 50-55, 2014 http://www.hrpub.org
DOI: 10.13189/ujmr.2014.020302

Analysis of in Vitro Efficiency of Electrolyzed Water Against Fungi Species Frequently Detected in Nosocomial Infections

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Abstract Fungi are the factors causing nosocomial infections but they are not considered in the first hand still, their importance is gradually increased as they are frequently detected microorganisms. We aimed to determine in vitro efficiency of electrolyzed water on different molds and yeasts. Materials and Methods-In School of Medicine Hospital of Ondokuz Mayıs University, Samsun, Turkey, the effect of electrolyzed water produced in Envirolyte (Envirolyte Industries International Ltd., Estonia) device on the yeast fungi and mold fungi in different concentrations and contact period isolated from clinical samples with qualitative suspension test method. As media Sabouraud dextrose agar, and as Dey-Engley Neutralizing broth (Sigma-Aldrich, USA) were used. Results-Envirolyte electrolyzed water was detected to be effective against all yeasts used in the study as well as *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida krusei, Candida lusitaniae,Trichosporon* spp. and mold; *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* isolates in the concentrations of 1/1, 1/2, 1/5, 1/10 in 1 minute and other testing durations.Based upon the results we acquired, we believe that Envirolyte electrolyzed water is a cheap and easy to obtain and natural disinfectant for controlling the nosocomial infections.

Keywords *Aspergillus*, *Candida*, Electrolyzed Water

1. Introduction

Micro-organisms that cause nosocomial infections show periodic changes depending on various factors. In the last 25-30 years, rates of infections caused by fungi are remarkably increased [1-3]. Fungal pathogens that mostly cause hospital infections are listed as *Candida albicans*, other *Candida* species and *Aspergillus* species [4-6]. Nosocomial infections may be endogenous as well as they may be exogenous infections caused by eco-borne micro-organisms [7]. It is already known that disinfection failure may lead to additional treatment costs and even morbidity or mortality of patients, furthermore, unnecessary operations about disinfections may increase hospital expenses and also may cause selection of resistant micro-organisms [8].The subjects such as efficacy spectru m of disinfectant, the suitability with the area where it has been needed to be used, required time to demonstrate the expected effectiveness, possible after-use damages on environment and the equipment and cost are taken into consideration in the selection of the disinfectant at hospitals. Yet, disinfectant products which have antimicrobial effects are substances and chemicals that form danger to human health and the environment due to their physicochemical properties in a way that the employees working in spaces where these products have been used, on patients who can possibly touch the equipments and surfaces containing these product and also they are post-consumer wastes. The studies have been done considering all these disadvantages are intended to provide accurate, inexpensive, easily applicable and reliable disinfectants which can be used against hospital disinfections [8]. To this end, electrolyzed water which has been begun to be widely used in recent years has many advantages such as not to compose toxic product, low transaction costs, to be harmless on human tissue and it is also considered to be safe for patients and staff during the hospital disinfection processes [9]. Electrolyzed water which is obtained by applying electric current too salty water is used for disinfection and strelilization procedures due to its broad sprectrum of activity against micro-organisms. Electrolyzed water (hypoclorous acid, hypochlorite ions, dissolved oxygen, ozone, superoxide radicals and etc.) formed after electrolysis shows a high degree of antimicrobial activity and strong oxidation potential depending on electrolysis process.

It is more efficient, low cost, non toxic and vide spectrum

of usage. Addition it kills bacterias, viruses, fungi and parasites quickly and can be used for disinfection of hard surfaces and water systems [10]. The aim of this study is to analyze the in-vitro activity of electrolyzed water – has a very few number of studies about its disinfectant effectiveness - against various yeasts and molds by using different concentrations.

This study The 5th Eurasian Congress of Infectious Diseases (EACID) in the (15-18 May 2013, Tirana - Albania) is presented as a poster written.

Table 1. Effects of Electrolyzed water on the growth of yeast

Yeast	Time (minute)	Dilution rate of Envirolyte electrolyzed water					
		1/1	1/2	1/10	1/20	1/50	1/100
C. albicans	1	-	-	-	-	+	+
	2	-	-	-	-	+	+
	5	-	-	-	-	+	+
	10	-	-	-	-	+	+
	30	-	-	-	-	+	+
C. tropicalis	1	-	-	-	+	+	+
	2	-	-	-	+	+	+
	5	-	-	-	+	+	+
	10	-	-	-	+	+	+
	30	-	-	-	-	+	+
C. parapsilosis	1	-	-	-	+	+	+
	2	-	-	-	+	+	+
	5	-	-	-	+	+	+
	10	-	-	-	-	+	+
	30	-	-	-	-	+	+
C. glabrata	1	-	-	-	-	+	+
	2	-	-	-	-	+	+
	5	-	-	-	-	+	+
	10	-	-	-	-	-	+
	30	-	-	-	-	-	-
C. krusei	1	-	-	-	+	+	+
	2	-	-	-	+	+	+
	5	-	-	-	-	+	+
	10	-	-	-	-	+	+
	30	-	-	-	-	+	+
C. lusitaniae	1	-	-	-	+	+	+
	2	-	-	-	+	+	+
	5	-	-	-	+	+	+
	10	-	-	-	+	+	+
	30	-	-	-	-	+	+
Trichosporon spp.	1	-	-	-	+	+	+
	2	-	-	-	+	+	+
	5	-	-	-	+	+	+
	10	-	-	-	+	+	+
	30	-	-	-	-	+	+

(-): Growth was not observed, (+): Growth was observed

2. Material and Methods

In our study, efficacy of electrolyzed water produced via Envirolyte (Envirolyte Industries International Ltd., Estonia) device already using in Ondokuz Mayıs University Hospital has been investigated on various fungal species. Thence, electrolyzed oxidizing water is used after it has been obtained in Envirolyte device via electrolysis method by using water, salt and electric current. The dosing for water disinfection is generally 0,1% but may slightly vary depending on COD and BOD of the source water

The efficacy of the oxidising water above mentioned has been analyzed with the method of qualitative suspension on seven different yeast (*Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata, Candida krusei, Candida lusitaniae, Trichosporon spp.*) and three fungi (*Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger*) species isolated from clinical specimens [11,12]. The fungal species used for the test have been passaged to Sabouraud dextrose agar (SDA) culture media and yeast have been produced with incubation at 37° C for 24 hours and molds at 25ºC for 72 hours [13]. 4 McFarland tubidity ($12x10^6$ CFU/ml) suspension of micro-organisms has been prepared by using 24 hours cultures of fungi [14,15]. Various concentrations of electrolyzed oxidising water such as 1/1, 1/2, 1/10, 1/20, 1/50 and 1/100 have been prepared and 1000 µl tubes have been used for each concentration. 10 µl of yeast suspensions have been added to each of these tubes. After 1, 2, 5 , 10 and 30 minutes duration of contact, 100 µls of fungal and disinfectant mixtures have been taken and transfered into the tubes which contain 900 µl neutralising agent (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, polysorbate 80 5 g/l and bromacresol purple 0.02 g/l), (Dey-Engley Neutralizing Broth - Sigma-Aldrich, USA). At the end of 5 minutes of neutralization period, 10 µls of mixtures have been taken to inoculate in SDA. Petri dishes have been incubated at 37° C - 48 hours for yeast fungi and 25° C – 72 hours for mold fungi. The absence of growth is interpreted as disinfectant has been fungicidally effective. Disinfectant added solutions have been prepared in order to control reproduction and check plantings have been done by using neutralizing agents.

3. Results

Electrolyzed water produced in Envirolyte device is seen to be effective on all dilutions of yeasts and molds used in our study within one minute for 1/1, 1/2, 1/10 rates and 1/20 for *C. albicans* ve *C. glabrata* dilutions. In addition, it has been found to be effective in thirty minutes for *C. tropicalis,C. lusitaniae* yeasts and 1/20 concentration of mold fungi *Trichosporon* spp., effective in ten minutes for 1/20 concentration of *C. parapsilosis* and in five minutes for 1/20 concentration of *C. krusei*. It has been determined that it is effective on 1/20 concentration of *A. fumigatus* mold fungi within two minutes, in five minutes for 1/50 concentration; within two minutes for 1/20 concentration of *A. flavus* and in five minutes for 1/50 concentration whereas in ten minutes for 1/100; within five minutes for 1/20 concentration of *A. niger*. The efficacy results have been presented in table 1 and 2 for each micro-organism according to the variety of time and concentration.

Table 2. Effects of Electrolyzed water on the growth of molds

Molds	Time (minute)	Dilution rate of Envirolyte electrolyzed					
		1/1	1/2	1/10	1/20	1/50	1/100
A. fumigatus	1	-	-	-	+	+	+
	2	-	-	-	-	+	+
	5	-	-	-	-	-	+
	10	-	-	-	-	-	+
	30	-	-	-	-	-	+
A. flavus	1	-	-	-	+	+	+
	2	-	-	-	-	+	+
	5	-	-	-	-	-	+
	10	-	-	-	-	-	-
	30	-	-	-	-	-	-
A. niger	1	-	-	-	+	+	+
	2	-	-	-	+	+	+
	5	-	-	-	-	+	+
	10	-	-	-	-	+	+
	30	-	-	-	-	+	+

(-): Growth was not observed, (+): Growth was observed

4. Conclusions

Disinfection procedures are important factors in the prevention of nosocomial infections. In the mean time, it will be able to select the accurate disinfectant which has already shows that it has been determined by right and reliable tests which indicate its efficacy on micro-organisms possible found in hospital environment and setting the accurate application method and concentration [16]. If the use of electrolyzed water in our country has been examined for a recent period, it is posibble to see applications in a variety of industries. There are plenty of international studies which have been done to determine the efficacy of electolyzed water. However, this study is one of the limited number of works which has been presented to examine the efficacy of electrolyzed water against fungi cause nosocomial infections and it will guide for the future studies. This study shows that the electrolyzed water produced in Envirolyte device is quickly fungasidial effective on 1/1, 1/2 and 1/10 dilutions of clinical fungal isolates. In recent years, incidence of *Candida species* is seriously increased and is settled in fourth place by constituting the 8-10% among all nosocomial bloodstream infections agents [17]. The most commonly isolated fungal pathogen is *C. albicans* (59.8%). It has been followed by other *Candida* species (18.6%) and *Aspergillus* species (1.3%) [4]. The sensitivity of fungi isolated from different hospital environmets against frequently used disinfectants can differ because of their changing development of resistance. Therefore, in the control of nosocomial infections, it is wise to choose material by determining the effective disinfectant against existing micro-organisms for each hospital [18]. There are several studies presenting disinfectant effects of neutral electrolyzed water against bacterias and yeasts [19-24]. Venkitanarayanan et al. evaluated the effects of electrolyzed oxidising water on *Escherichia coli*, *Salmonella enteritidis* and *Listeria monocytogenes* with their study. At the end of their work, they said that electrolyzed oxidising water might suggested to be a convenient disinfectant in case it had been validated [19]. Electrolyzed oxidising water was tested in vitro medium by Landa et al. to understand its efficacy of antimicrobial, fungicidal and antiviral. In this study, it had been found to be effective on *C. albicans* together with some bacterial pathogens within 30 seconds [20]. However, Fenner et al. tested the efficacy of electrolyzed water by considering the standards of German Veterinary Association (DVG). Besides some bacterial pathogens, starains of *C. albicans* were used in their analysis. Electrolyzed oxidising water was ascertained to be effective on all micro-organisms after 30 minutes of contact duration [22]. There was not any evidence in the study about the effect of neutral electrolyzed water on molds. In our study, 1/1, 1/2 and 1/10 concentrations of electrolyzed water produced via Electrolyte device have been found to be effective on all yeasts and molds used within one minutes. Moreover, it has been seen to be effective within one minute against 1/20 concentration of *C. albicans* which is most commonly isolated from fungal pathogens in hospitals. In addition, it was effective on 1/100 degree concentration of *C. glabrata* within thirty minutes and in ten minutes on 1/100 *A. flavus* concentration. Except these two micro-organisms, there was not detected an effect on 1/100 concentrations of any yeasts and molds. Within the light of these information, it was thought that electrolyzed water could be used as a surface disinfectant in order to prevent nosocomial fungal infections. As soon as the work intensity in the hospitals is considered, it is known that it is an advantage to use a disinfectant which can provide a fast disinfection [25]. From this point of view, it is quite remarkable that the electrolyzed water has been effective on 1/1, 1/2 and 1/10 concentrations of all fungi species used in the study within as little as one minute.

Consequently, above mentioned disinfectant which is produced by using water, salt and electric current via Envirolyte generator has many advantages such as its simple production, being economic, user friendly and non-toxic and having high-efficiency. Electrolyzed water does not contain generated in a membrane electrolyzer. Due to isolation of Na through the membrane NaOH is formed in the cathode chamber of the electroyzer. We do not use cathodic fluid for disinfection. So it is not hazardous to human. Absence of NaOH is one of the fundamental differences between electrolyzed water and bleach, i.e removal of Na from the electrolyzed water in order to produce pure HCLO.

Elektrolize water is not corrosive on sensitive equipment. If you want to use bleach in a short time it is cost-effective. But if you want to use for a long time and for some sensitive equipment elektrolize water is cost-effective. But they have same effective microorganism according to some studies11,12. Electrolyzed water and commercial bleach with the same concentration are similar. But in some applications, e .g. Legionella prevention, lacking of a comparison with commercial bleach with the same concentration is not that important since bleach is not effective against Legionella and it is only electrolyzed water which can solve the problem. There are many other similar instances. Moreover, growing bacteria resistance to bleach makes in ineffective even in its traditional applications. Application of electrolyzed water as a hard-surface disinfectant on a daily basis for more than ten years, demonstrated that microorganisms do not develop resistance against it over time. Bleach in concentration of 5% is effective only in disinfection, but not sterilization. Bleach combines Cl2 with caustic soda (lye) to stabilize chlorine. Household bleach and pool chlorinator solutions are typically stabilized by a significant concentration of lye (caustic soda, NaOH) as part of the manufacturing reaction. Skin contact will produce caustic irritation or burns due to defatting and saponification of skin oils and destruction of tissue. The slippery feel of bleach on skin is due to this process. Chlorination of drinking water can oxidize organic contaminants, producing trihalomethanes (also called haloforms), which are carcinogenic. The extent of the hazard thus created is a subject of disagreement. Mixing bleach with some household cleaners can be hazardous. For example, mixing an acid cleaner with Bleach bleach generates chlorine

gas. Mixing with ammonia solutions produces chloramines. Both chlorine gas and chloramine gas are toxic. Bleach can react violently with hydrogen peroxide and produce oxygen gas. It is estimated that there are about 3300 accidents needing hospital treatment caused by Bleach solutions each year in British homes. Bleach is ineffective against cysts (Guardia, Cryptosporidium). Bleach loses its activity during long-term storage. Bleach poses potential danger of gaseous chlorine emission during storage. The formation of hypochlorite (OCl-) during electrolysis (reaction 6) in standard hypochlorite generators has a number of limiting effects on both electrolysis efficiency and the amount of chlorine produced. The major limiting factor is the reduction of OCl- back to chloride (Cl-) at the cathode (26-28)

In accordance with the efficacy results obtained in our study, we think Envirolyte elektorlyzed water can be considered to be a disinfectant as a low cost and in-situ produced material effective on fungi species and in the control of nosocomial infections by validating its use with other efficacy studies.

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