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Anolyte Water as a Biocide Agent for Microorganisms Isolated From an Egyptian Swimming Pool.

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ABSTRACT

Anolyte water is an environmentally safe oxidizing solution that can be used as a biocidal agent. The aim of this work was to evaluate the inactivation effect of Anolyte water (PH 7-7.5; oxidation-reduction potential 800-850mV) on a predominant isolate of *Acanthamoeba* genotype T4 and other microbes (*Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas aeruginosa,* spore formers and *Candida* spp.) isolated from an Egyptian swimming pool. Different concentrations from the Anolyte water at different contact times were separately applied to cysts and trophozoites of *Acanthamoeba* genotype T4 and microbes persistently isolated from the examined swimming pool. *Acanthamoeba* viability was tested by culturing on non-nutrient agar and by staining with trypan blue vital stain. The obtained results showed that the minimum amoebicidal effect of Anolyte water for *Acanthamoeba* genotype T4 cysts reached 40 % after 6 hours contact time. In contrast, a lower concentration of Anolyte water (7%) was efficient for complete inactivation of *Acanthamoeba* trophozoites after 45 minutes contact time. Anolyte water in concentration 0.1% produced 100% inactivation for *Streptococcus* spp., *Staphylococcus* spp. and *Pseudomonas aeruginosa* after 60 minutes exposure time. While, the same Anolyte concentration produced complete inactivation for spore formers and *Candida* spp. after 120 minutes exposure time. In general, spore formers and *Candida* spp. more resistant than *Streptococcus* spp., *Staphylococcus* spp. and *Pseudomonas aeruginosa* after exposure to different concentrations from Anolyte water. All tested microbes other than *Acanthamoeba* were completely inactivated after exposure to 0.7% Anolyte concentration for 30 min. In conclusion Anolyte water is useful for inactivation of both *Acanthamoeba* and other examined microbes.

Keywords: Anolyte water, *Acanthamoeba,* Bacteria, *Candida*, Swimming pool

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INTRODUCTION

Anolyte is generated using salt and electricity. Salt is added to water and passed through double chamber membrane electrolytic cell. A high-strength active biocide Anolyte is created in the form of HCLO. Anolyte water is characterized by the ability to kill all potential pathogens; is safe, easy, and inexpensive to use; Meets current and upcoming regulations; adds no toxic compounds to the water [1].

Waterborne pathogens enter human hosts through intact or compromised skin, inhalation, ingestion, aspiration, and direct contact with the mucous membranes of the eye, ear, nose, mouth, and genitals. Members of the genus *Acanthamoeba* are opportunistic amphizoic protozoa belonging to sarcodines [2]**.** *Acanthamoeba* are normally found in soil or water and occur worldwide. They are not parasitic per se but can be pathogenic to humans. The most common mode of infection from *Acanthamoeba* for healthy individuals is swimming or diving into water inhabited by these amoebae and their accidental introduction into the nasal passages. Some species of *Acanthamoeba* have an association with human disease, such as granulomatous amoebic encephalitis (GAE), cutaneous lesions, nasopharyngeal, pulmonary and kidney infections, primarily in immunocompromised patients; they also cause amoebic keratitis in immunocompetent persons [3].

Acanthamoeba can act as hosts for a large number of pathogenic bacteria including *Legionella* spp., *Vibrio cholerae*, *Burkholderia cepacia, Listeria monocytogenes, Escherichia coli O*157, *Mycobacterium bovis*, and *Mycobacterium avium* [4,5]. Approximately, 20 to 24% of clinical and environmental isolates of *Acanthamoeba* harbor intracellular bacteria [6]. Moreover, *Acanthamoeba* can harbor echo-virus [7]. Recently, a mimivirus was discovered in *A. polyphaga* [8]. In addition, it is known that Coxsackie virus and adenovirus can infect *Acanthamoeba* [9].The intracellular localizations of these microorganisms protect them from adverse conditions and allow bacteria to evade host defenses, to resist antibiotic actions and to increase its virulence [10]. *Acanthamoeba* cannot be achieved by current disinfection practices of drinking water [11]. Cysts of *Acanthamoeba* were found to be able to survive a 2 hours exposure to chlorine at 100 mg/L at 20 ◦C. *Pseudomonas aeruginosa* has a reputation for being resistant to disinfection, and it does not exhibit any marked resistance to the disinfectants used to treat water such as chlorine, chloramines, ozone, or iodine [12]. Routine monitoring and disinfection of swimming pools which frequently contaminated with the opportunistic pathogen *Pseudomonas aeruginosa*, which can represent a significant public health threat [13]. So in this work we aim to test the disinfection of *Acanthamoeba* and other microbes isolated from a swimming pool by Anolyte water.

MATERIALS AND METHODS

Twelve water samples were collected from swimming pool located at Cairo, Egypt. Four samples proved to be morphologically positive for *Acanthamoeba*. These samples were subjected to molecular identification.

Figure 1: Gel electrophoresis of DNA amplification products from different isolates of *Acanthamoeba* spp. M: 100 plus bp DNA ladder, +ve: positive control, –ve: negative control. 1, 2, 3 and 4: positive samples for *Acanthamoeba* spp.

Acanthamoeba isolates were identified by PCR using genus specific primers; AcantF900 (5'-CCC AGA TCG TTT ACC GTG AA-3') and AcantR1100 (5'-TAA ATA TTA ATG CCC CCA ACT ATC C-3') to amplify 18S rRNA gene fragment of approximately 180 bp (differing by a few bases depending on the species) [14] (Figure 1). The amplification of the respective fragment was visualized by ethidium bromide staining of an agarose electrophoresis gel and compared to DNA 100 plus bp (Gene Direx, China). Amplicons were analyzed by direct sequencing of the PCR product using the ABI 3730XL automated sequencer (Applied Biosystems). Sequences were obtained from both strands and data were processed with the BioEdit and Lasergene7 sequence editor. Multiple sequence alignment was performed by stepwise pairwise alignment using the CLUSTAL_W application. The alignments were assessed by eye and revised manually. Primer sites were excluded from the analysis. The sequences were compared to published sequences from other *Acanthamoebae*. For the *Acanthamoeba* strains the genotype was assessed with the model assumption of 5 % sequence dissimilarity within one genotype as established by Gast et al. [15]. All isolates of *Acanthamoeba* after sequencing processes were identified as *Acanthamoeba* genotype T4. One isolate from the isolated *Acanthamoeba* genotype T4 was used to carry out the following experiment.

The first part of the experiment was to evaluate the effectiveness of Anolyte on *Acanthamoeba* genotype T4 cysts. *Acanthamoeba* genotype T4 was subcultured on non-nutrient agar (NNA) plate seeded with heat-killed *E. coli* and incubated at 30^{o}C. The plate was used for collection of *Acanthamoeba* cysts after one week incubation. After incubation period, the agar surfaces of the plate were flooded with 5 ml of Page's amoebic saline and amoebae cysts were gently scraped with an inoculating loop. *Acanthamoeba* cysts were harvested from the suspension by centrifugation at 350 *g* for 10 min. The supernatant was aspirated, and the sediments were washed twice with Page's amoebic saline in order to eliminate most of the remaining bacteria. The subjected *Acanthamoeba* cysts were adjusted at concentration 10^4 using Sedgewick Rafter counting cell slide. Control samples cysts were comparatively used without any treatment to assess the effect of the Anolyte water. The active biocide component of Anolyte water is HOCl which represent 0.05% from the Anolyte. Anolyte water obtained from Envirolyte W.P.C. Ltd. was applied to *Acanthamoeba* cysts in concentrations 10, 15, 20, 25, 30, 35, 40, 45 and 50 % and exposure time from 1 to 7 hours. Amoebicidal effect of the tested Anolyte water was evaluated by checking the viability of cysts through cultivation on fresh NNA plates and staining the tested cysts with trypan blue stain [16,17].

The second part of the experiment was to evaluate the effectiveness of Anolyte on *Acanthamoeba* genotype T4 trophozoites. The isolated *Acanthamoeba* genotype T4 strain was subcultured on NNA plates seeded with heat-killed *E. coli* and incubated at 30^{o}C. The inoculated plates were used for collection of *Acanthamoeba* genotype T4 trophozoites after 48 hours incubation. *Acanthamoeba* genotype T4 trophozoites were suspended in the plates by Page's amoebic saline then harvested from the suspension by centrifugation at 350 *g* for 10 min. The supernatant was aspirated, and the sediments were washed twice with Page's amoebic saline in order to eliminate most of the remaining bacteria. *Acanthamoeba* genotype T4 trophozoites were maintained in PYG media in tissue culture well plates to evaluate the amoebicidal effect of Anolyte water. Anolyte water in concentrations 1, 3, 5, 7 and 10% were applied to *Acanthamoeba* genotype T4 trophozoites with exposure time 1, 15, 30, 45 and 60 minutes. Amoebicidal effect of the tested Anolyte water was evaluated by checking the viability of the trophozoites through cultivation on fresh NNA plates and staining the tested trophozoites with trypan blue vital stain [16,17].

Bacteriologically, also twelve water samples were collected from the same swimming pool for microbiological examination. Water samples were separately collected in 1 liter sterile glass bottle containing sodium thiosulphate crystals (18 mg/L) to eliminate chlorine residual. The collected samples were sent to the laboratory and processed at the same day (during 2 hours) of collection. *Streptococcus* spp*., Staphylococcus* spp*., Pseudomonas aeruginosa,* spore formers and *Candida* spp*.* were detected according to APHA (2012) [18]. The detected methods were plate count agar using poured plates for spore formers. The Most Probable Number (MPN) values for *Pseudomonas aeruginosa, Staphylococcus* spp. and *Streptococcus* spp. While, *Candida* spp. detection was carried out by the membrane filter technique using specific chromogenic media. All isolated microbes were confirmed by using Himedia [18].

Microbial isolates (*Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas aeruginosa,* spore formers and *Candida* spp.) were used to evaluate the biocidal effect of Anolyte water. These microbial isolates were prepared as follow, under sterilization condition one colony was taken from each microorganism growing on specific media was separately inoculated in sterile 5 ml tripticase soy broth and incubated at 37°C for 24 hours.

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After incubation, the inoculated broth was centrifuged at 5000 rpm for 15 minutes, then the pellets were transferred to 5 ml sterile saline water. The centrifugation step was repeated three times. The obtained counts from saline water were adjusted at $2.1x10^8$, $5.5x10^8$, $3.2x10^8$, $2.2x10^8$ and $4.1x10^8$ cfu/ml for *Streptococcus* spp*., Staphylococcus* spp*., Pseudomonas aeruginosa,* spore formers and *Candida* spp*.*, respectively by used poured plate count agar and dilution test tubes [18]. One ml of saline water microbes was separately inoculated in an autoclaved swimming pool water (1000 ml) to evaluate the biocidal effect of Anolyte water. Anolyte water was applied to *Streptococcus spp., Staphylococcus* spp*., Pseudomonas aeruginosa,* spore formers and *Candida* spp*.* in concentrations 0.1, 0.3, 0.5 and 0.7% with exposure time from 10, 30, 60, 120 minutes [19]. After the exposure to the Anolyte water the viability of microorganisms were estimated according to APHA (2012) [18].

Statistical analysis

The statistical analyses (Percentages) were carried out according to procedure of Snedcor and Chochran [20]. The mean data values were using (MSTAT) computer software package version 2.1.

RESULTS

It was found that the Anolyte water in concentrations 10 % and 15% had no amoebicidal effect on *Acanthamoeba* genotype T4 cysts after 1 to 7 hr exposure time. On the other hand, Anolyte water began to be effective against 5% of *Acanthamoeba* cysts at a concentration 20 % and 7 hours contact time. Consequently, the amoebicidal effect of Anolyte water increased with the increase in concentration and contact time against *Acanthamoeba* cysts. The minimum amoebicidal concentration of Anolyte water that produced complete death of *Acanthamoeba* cysts reached 40% with contact time 6 hours. The *Acanthamoeba* cysts showed no growth at 45 % Anolyte water concentration after 2 hours contact time. *Acanthamoeba* cysts were inactivated with 50% Anolyte water concentration after exposure time 1 hour (Table 1, Figure 2, 3).

Table 1: Inactivation of *Acanthamoeba* genotype T4 cysts by Anolyte water.

Anolyte concentration	Contact time (hours) for *Acanthamoeba* genotype T4 cysts													
	1		2		3		4		5		6		7	
	Death (%)	Cult.•	Death (%)	Cult.	Death (%)	Cult.	Death (%)	Cult.	Death (%)	Cult.	Death (%)	Cult.	Death (%)	Cult.
10%	0	+ve	0	+ve	0	+ve	0	+ve	0	+ve	0	+ve	0	+ve
15%	0	+ve	0	+ve	0	+ve	0	+ve	0	+ve	0	+ve	0	+ve
20%	0	+ve	0	+ve	0	+ve	0	+ve	0	+ve	0	+ve	5	+ve
25%	7	+ve	10	+ve	15	+ve	17	+ve	20	+ve	25	+ve	35	+ve
30%	20	+ve	30	+ve	50	+ve	55	+ve	55	+ve	60	+ve	65	+ve
35%	55	+ve	60	+ve	70	+ve	75	+ve	80	+ve	87	+ve	93	+ve
40%	78	+ve	85	+ve	85	+ve	90	+ve	95	+ve	100	-ve	100	-ve
45%	90	+ve	100	-ve	100	-ve	100	-ve	100	-ve	100	-ve	100	-ve
50%	100	-ve	100	-ve	100	-ve	100	-ve	100	-ve	100	-ve	100	-ve

Cult.: Culture

It was found that Anolyte water in concentrations 1%, 3% and 5% did not produce 100% amoebicidal effect on *Acanthamoeba* genotype T4 trophozoites after 1, 15, 30, 45 and 60 minutes exposure time. Consequently, the amoebicidal effect of the Anolyte water increased with the increase in concentration and contact time against *Acanthamoeba* trophozoites. *Acanthamoeba* trophozoites lose thier viability completely at 7% Anolyte water concentration and 45 minutes contact time (Table 2, Figure 3).

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Table 2: Inactivation of *Acanthamoeba* genotype T4 trophozoites by Anolyte water

Conc. of Anolyte water	Contact time (minutes) for *Acanthamoeba* genotype T4									
	1		15		30		45		60	
	Death (%)	Cult.•	Death (%)	Cult.	Death (%)	Cult.	Death (%)	Cult.	Death (%)	Cult.
1%	0	+ve	0	+ve	0	+ve	0	+ve	5	+ve
3%	7	+ve	12	+ve	15	+ve	20	+ve	20	+ve
5%	35	+ve	50	+ve	60	+ve	80	-ve	95	-ve
7%	85	+ve	90	+ve	95	+ve	100	-ve	100	-ve
10%	95	+ve	100	-ve	100	-ve	100	-ve	100	-ve

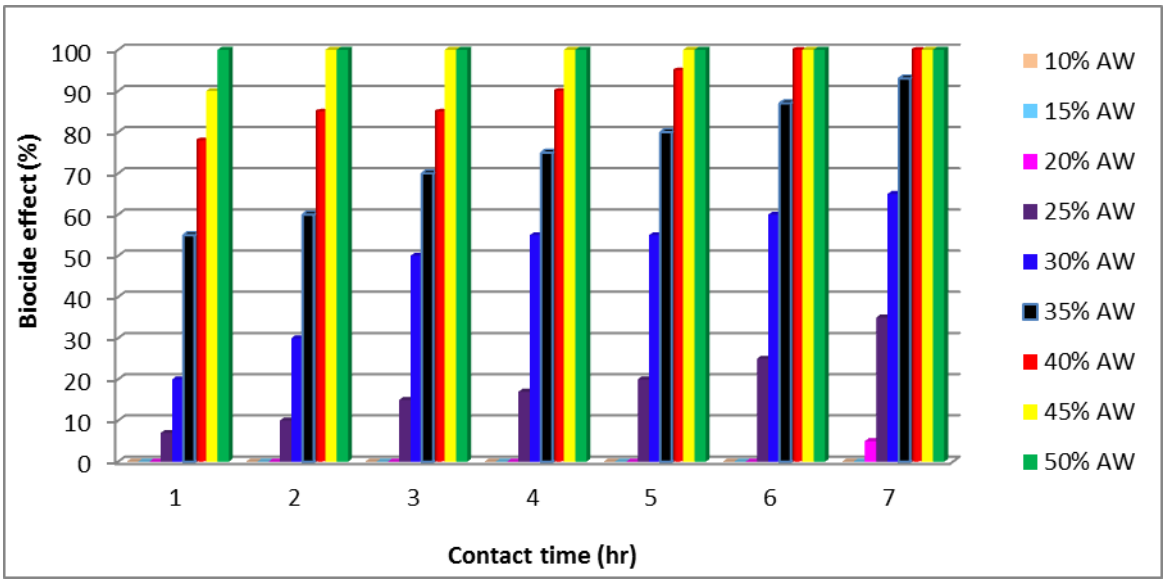

Figure 2: Effects of different Anolyte water doses and contact times on *Acanthamoeba* genotype T4 cysts

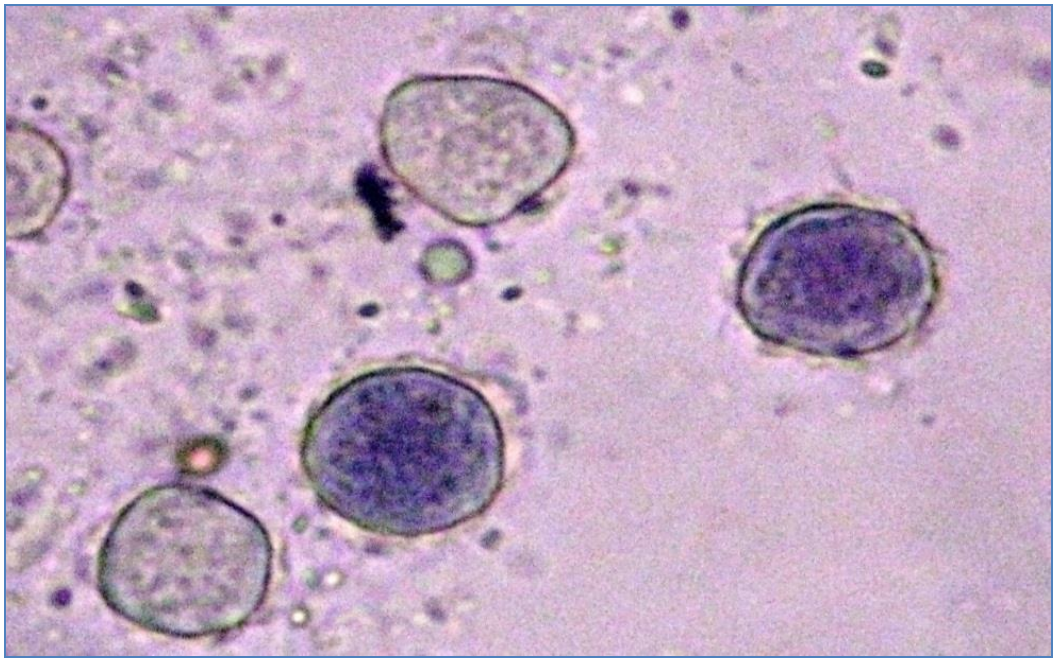

Figure 3: Photomicrograph for viable (unstained) and unviable (stained) *Acanthamoeba.*

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Anolyte water in a concentration of 0.1 % and contact time 10 min had biocidal effect in percentages 42.2, 33.3, 59.4, 24.8 and 34.6 on *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas aeruginosa,* spore formers and *Candida* spp., respectively. Consequently, the microbicidal effect of Anolyte water increased with the increase in contact time against microbes. Anolyte water in concentration 0.1% produced 100% inactivation for *Streptococcus* spp., *Staphylococcus* spp. and *Pseudomonas aeruginosa* after 60 min exposure time. While, the same concentration produced complete inactivation for spore formers and *Candida* spp. after 120 min exposure time. After 10 min and 0.3% Anolyte water concentration, *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas aeruginosa,* spore formers and *Candida* spp. were 46.2, 58.7, 78.8, 24.4 and 49.6%, respectively. By increasing the Anolyte water concentration and contact time, the microbicidal effect of Anolyte against microbes increase. On the other hand, *Streptococcus* spp., *Staphylococcus* spp. spore formers and *Candida* spp. were still viable at the same treatment conditions. *Streptococcus* spp., *Staphylococcus* spp. were inactivated completely at 0.3% Anolyte water concentration after contact time 60 min. While, spore formers and *Candida* spp. lose their viability at 0.3% Anolyte water concentration after 120 min contact time. After 10 min contact time, 0.5% Anolyte concentration had biocidal effect in percentages 77.4, 79.5, 95.4, 58.7 and 66.6% on *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas aeruginosa,* spore formers and *Candida* spp., respectively. *Pseudomonas aeruginosa* did not grow at 0.5% Anolyte concentration after 30 min exposure time. *Streptococcus* spp. and *Staphylococcus* spp. lose their viability at 0.5% Anolyte concentration after 60 min contact time. On the other hand, the same concentration value had biocidal effect on 100% of spore formers and *Candida* spp. after 120 min exposure time. At 10 min contact time with Anolyte water concentration was 0.7%, *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas aeruginosa,* spore formers and *Candida* spp. lose their viability in percentages 96.6, 93.3, 97.8, 83.4 and 91.1%, respectively. While, All tested microbes did not survive after 30 min exposure time when the concentration of Anolyte water was 0.7% (Table 3 and figure 4, 5, 6 and 7).

Table 3: Percentages of inactivation of the tested microbes

Conc. / 100 ml	Time / min.	% of inactivation of microbes				
		Strep. spp.	***Staph. spp.***	***Ps. aerug***	**S. formers**	***Cand spp.***
0.1%	0	0	0	0	0	0
	10	42.4	33.3	59.4	24.8	34.6
	30	77.8	75.4	84.2	59.2	68.4
	60	100	100	100	79.2	85.6
	120	100	100	100	100	100
0.3%	0	0	0	0	0	0
	10	46.2	58.7	78.8	24.4	49.6
	30	88.8	89.2	100	65.4	66.3
	60	100	100	100	82.2	76.7
	120	100	100	100	100	100
0.5%	0	0	0	0	0	0
	10	77.4	79.5	95.4	58.7	66.6
	30	92.2	95.2	100	74.2	84.4
	60	100	100	100	94.2	99.1
	120	100	100	100	100	100
0.7%	0	0	0	0	0	0
	10	96.6	93.3	97.8	83.4	91.1
	30	100	100	100	100	100
	60	100	100	100	100	100
	120	100	100	100	100	100

Notes:- *Strep*. spp. =*Streptococcus* spp.; Cand.spp. = Candida spp.
Staph* spp**.=*Staphylococcus* spp.; ***Ps. aerug =*Pseudomonas aeruginosa*
S. formers = spore formers **Conc**.= concentration

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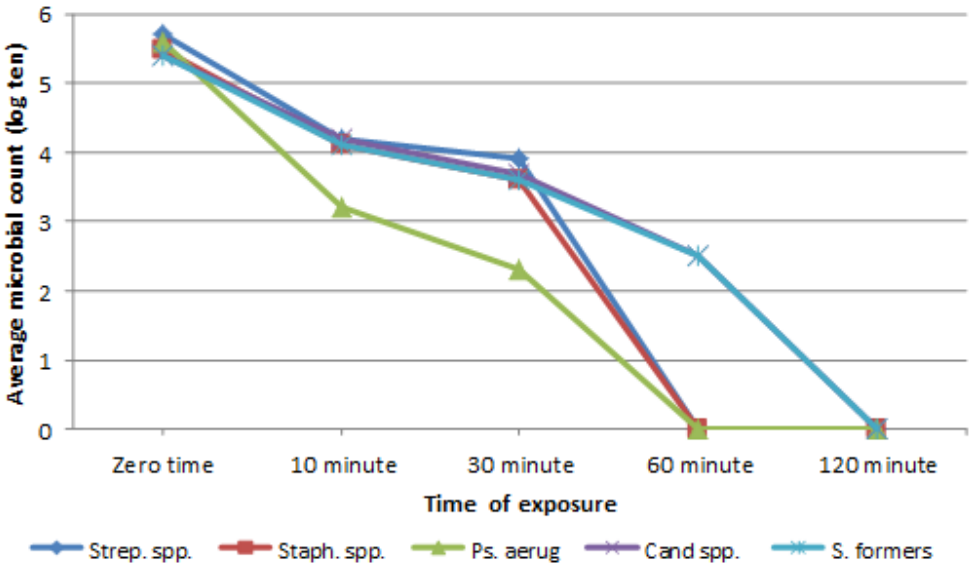

Figure 4: Effect of 0.1% concentration of Anolyte water on the tested microbes

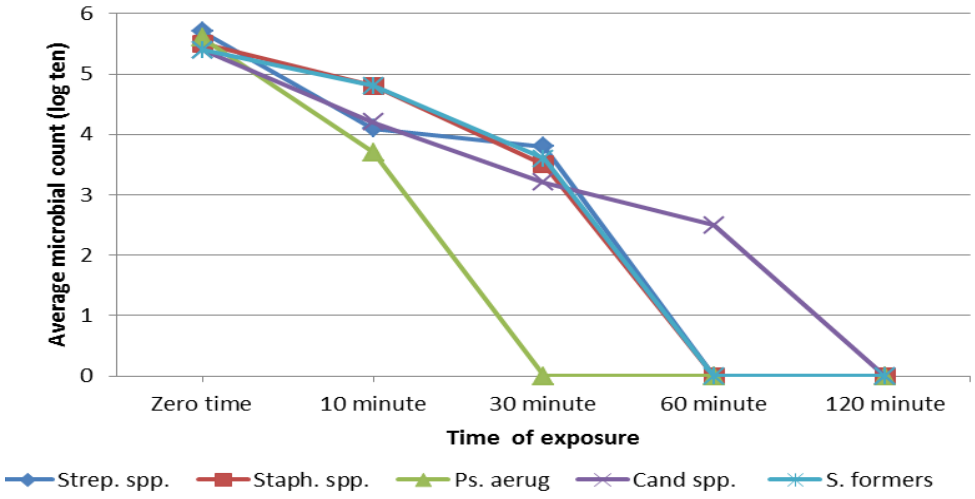

Figure 5: Effect of 0.3% concentration of Anolyte water on the tested microbes

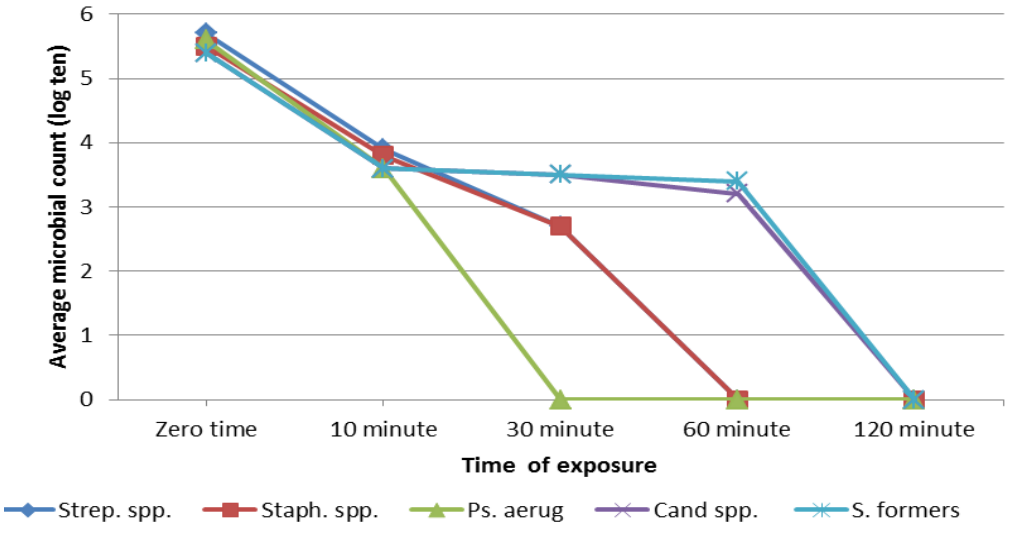

Figure 6: Effect of 0.5% concentration of Anolyte water on the tested microbes

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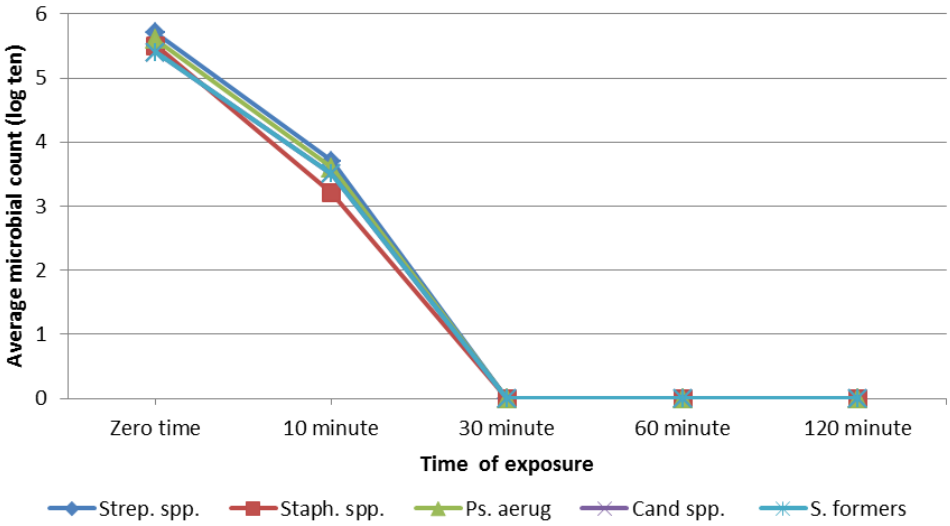

Figure 7: Effect of 0.7% concentration of Anolyte water on the tested microbes

DISCUSSION

Anolyte water in a concentration of 0.1 % and contact time 10 min had biocidal effect in percentages 42.2, 33.3, 59.4, 24.8 and 34.6 on Streptococcus spp., *Staphylococcus* spp., *Pseudomonas aeruginosa*, spore formers and *Candida* spp., respectively. Rare studies have done to test the disinfection of bacteria and fungi by Anolyte water. These results were agreement with that of Guentzel et al. [21], where they found that treatment of pure culture of some organisms, (*E. coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Enterococcus faecalis*) with electrolyzed oxidizing water at concentrations of 20, 50, 100, and 120 ppm of total residual chlorine for 10 min of contact time, resulted in 100% inactivation of all five organisms. Moreover, Abadias et al. [22] demonstrated that bactericidal activity of diluted neutral electrolyzed water (containing approximately 50 ppm of free chlorine, against *E.coli* O157:H7, *Salmonella, Listeria innocua* and *Erwinia carotovora* on lettuce, results were reductions from 1–2 log units for 1 to 3 min contact time. In general, spore formers and *Candida* spp. more resistant than *Streptococcus* spp., *Staphylococcus* spp. and *Pseudomonas aeruginosa* after exposure to different concentrations from Anolyte water. Other investigators found that spore formers more resistant than vegetative bacteria like *Streptococcus* spp., *Staphylococcus* spp. and *Pseudomonas aeruginosa* when exposures to H_2O_2 and active chlorine (500 – 5000 mg/l) [23]. This result was in concordance with the current results. Other researchers compared between the effectiveness of Anolyte water and chlorinated solutions in controlling growth of aerobic bacteria, molds, yeasts and coliform bacteria during the storage of carrots. They found that the Anolyte water reduce and limit the growth of aerobic bacteria, molds, yeasts and coliform bacteria during storage. The researchers concluded that, the Anolyte water treatments had no effect on total soluble solid content, pH value, firmness and overall visual appearance of carrots [24]. Undertaken by Department of Laboratory Medicine and second department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan; Anolyte microbial activity was tested against methicillin-sensitive *Staphylococcus aureus*, *Staphylococcus epermidis*, *Serratia marcencens*, *Escherchia coli*, *Pseudomonas auruginosa* and *Burkholderia cepacia* which are important pathogens. The bactericidal properties of Anolyte were evaluated with three conventional disinfectants, including 0.1% chlorhexidine (Herbitane solution, ICI-pharma, Osaka, Japan), 0.02% povidine iodine (Isodine solution, Meiji Seika, Tokyo) and 80% ethanol (ethanol for disinfection, Maruisha Pharmaseutical Co. Ltd, Osaka). The selected concentrations represent those commonly used in solutions prepared for handwashing. All disinfectant solutions were mixed with sterile distilled water at the time of their use. Sterile distilled water was used as a control. The number of bacteria was reduced below detection limit following incubation in Anolyte for 10s. The bactericidal activity of Anolyte was similar to that of 80% ethanol, but superior to that of 0.1 chlorhexidine and 0.02% povidine iodine. The researchers concluded that Anolyte is a low cost but powerful disinfectant [1]. The merits of chlorination as a means of destroying bacteria in water is beyond dispute and the process is firmly and universally established. In recent years, however, the appearance of resistant strains of bacteria, the discovery of legionella and other problems associated with chlorine compounds have promoted an interest in alternative water purification systems

amongst which Anolyte water is prominent [1]. The main drawbacks of Anolyte water are; electrolysis machines are expensive, the electrolysis process needs to be monitored frequently for the correct potency, electrolyzed water loses its potency fairly quickly, so it cannot be stored for long time [25]. According to our knowledge there was no study on the inactivation of *Acanthamoeba* spp. by Anolyte water. Members of genus *Acanthamoeba* can cause rare, but severe infections of the eye, skin, and central nervous system. The ameba is found worldwide in the environment in water and soil. The ameba can be spread to the eyes through contact lens use, cuts, or skin wounds or by being inhaled into the lungs. Most people will be exposed to *Acanthamoeba* during their lifetime, but very few will become sick from this exposure. To date, isolates belonging to seven genotypes (T2, T3, T4, T5, T6, T11, and T15) were found to be associated with *Acanthamoeba* keratitis (AK) [26,27]. However, the most prevalent genotype in AK is the T4 genotype. The current results showed that lower concentration of Anolyte water (7%) was effective for disinfection of *Acanthamoeba* genotype T4 trophozoites. On the other hand, higher concentration of the same biocidal agent (40%) was enough to produce complete inactivation for *Acanthamoeba* genotype T4 cysts. The disinfection of *Acanthamoeba* is important not only to prevent the transmission of *Acanthamoeba* themselves but also due to the risks associated with a range of microbial pathogens that are found to be associated intracellularly with these microorganisms. Trophozoites are considered relatively sensitive to most chemicals, but cysts have been shown to be more resistant [17]. *Acanthamoeba* cyst wall protects from desiccation, starvation and a variety of chemical and physical agents [28]. Cysts have been known to survive in vitro for greater or equal to 20 years [29]. Free chlorine in concentration of 5040 mg min/L could inactivate *Acanthamoeba* spp. For *Acanthamoeba polyphaga* cysts, chlorine dioxide can also cause 99% amoebacidal effect with Ct value 20 mg min/L. Ozone can be used with Ct value 1.6 mg min/L for a 2-log reduction of *Acanthamoeba* spp. cysts. Two log reduction of *Acanthamoeba* cysts could be achieved by monochloramine with CT value 352 mg min/L. Hydrogen peroxide in concentration 3 % ineffective against *Acanthamoeba* spp. within the recommended contact time of 30 min but it was cysticidal after 4 h [30,31]. Continuously injecting Anolyte water within a swimming pool render the swimming water safe without the adverse effects of chlorine. Anolyte water destroy all bacteria, viral organisms, fungi and algae at a neutral pH level. This is particularly relevant for older skin which is more easily damaged by exposure to chlorine treated water. The disappearance of chlorine smell, absence of burning eyes sensation and negative skin reactions all make the water a more pleasant environment to swim in [1].

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