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Bacterias Isolated from Commercial Avian Clinical Cases Inactivated with a Long Acting Electro Oxidized Water (LEOW)

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ABSTRACT

In this work isolated bacteria from avian clinical cases, the antibacterial effect of a long acting Electro Oxidized Water (LEOW) (90 days after fabrication), LEOW was diluted with distilled or/and tap hard water and tested against suspensions of bacterial strains isolated from avian clinical cases (*E. coli, Candida albicans, Staphylococcus aureus, and Bacillus subtilis)* (in agreement with Mexican sanitary regulations). After fifteen minutes contact with LEOW, the bactericidal effect of LEOW was 99.99% effective. It was concluded that the bactericidal effect a Long acting EOW was not affected by different water qualities and pH, therefore a long acting pH 7.0 LEOW should be considered as an emergent option for the control of pathogens present in food production and animal husbandry. When comparing antibacterial properties with phenol it was observed that LEOW was efficient as/or better that phenol.

KEYWORDS: Long acting pH 7 EO water, bactericidal, hard water, distilled water

INTRODUCTION

Antiseptics and antibiotics are important drugs to diminish bacterial contamination and infections in both animal husbandry and all animal and agricultural food products, since the beggining of antimicrobial drugs, it was observed that bacteria and fungi that affect human, animal and plant health; become resistant to the antiseptic and antibacterial effects of many biocides (Kossiakoff, 1887; Thornley and Yudkin, 1959).

The presence of pathogenic bacteria in food and animal products represent a challenge to medical sciences; obligating for the continuous search for new antiseptic and antibacterial drugs.

The technology for the production of electrolyzed water was developed in Japan, and it was first used around the 1900´s for the disinfection of bottles in commercial drinking water and in beverages sold for human consumption, at the same time sodium hypochlorite was also introduced as a sanitizer. Electrolyzed water was also introduced as sanitary aid for stored water and in automatic water dispensers.

Electrolysis technology has since, been applied in different fields and is now a promising non-thermal treatment method for hygiene control in different fields of animal, agricultural produce and in the fishing industry (Al-Haq et al., 2003; Al-Haq et al., 2003).

The characteristics of EOW have evolved continually, and due to its antibacterial and antiseptic properties it has being certified for medical use in Japanese hospitals (Iwasawa et al., 1993). Chier et al., (2000) report that it is effective against mycobacterium and many types of spores.

Electrolyzed Oxidizing water (EOW), is produced by adding a very small amount of NaCl (around 0.1%) to pure water, a small voltage (10 to 20 V DC) electrical current is passed trough an anode and cathode. With a single cell chamber or a two-cell chamber, separated by a diaphragm, with this treatment a high concentration of hypochlorous acid is produced with a high oxidation-reduction potential (Koseki et al., 2003).

The above described procedure produces Acidic EO water, the latter has useful properties as a disinfectant for surfaces that are in contact with food (Venkitanarayanan et al., 1999), similar use has being reported for agricultural products (Fabrizio et al., 2002; Kim et al., 2000;Park et al. 2001), and also for animal products such as poultry meat and eggs

(Fasenko et al., 2009; Fenner et al 2006; Park et al., 2001; Russel, 2003). In humans, electro oxidized water has being introduced for the prevention of hospital infections (Vorbjeva et al., 2004; Yagai et al., 2000), at present, EOW has being slowly accepted and considered a novelty because it is not corrosive and it is effective against bacteria and fungi (Novak et al., 2008). In order to be universally accepted as an anti-microbial option for the prevention of bacterial contamination in food and animal management areas, EOW needs to be evaluated and compared with current antiseptics against different bacterial strains of bacteria's and fungi isolated from food from animal and plant origins (Cher et al., 2000). The first EOWaters used were acidic and had a very short duration of action; some are corrosive and unstable with a very short shelf life, factors that limited its use (Fabrizio and Cutter, 2003).

At present there are new methods for the formulation of EOW with a neutral pH and high oxi reduction potential (0RP) with a long lasting shelf life. In Mexico Steripharma Laboratories have produced a pH 7.0 EOW with more than 800 ORP. During the fabrication procedure of LEOW, distilled water and NaCl is used, applying a patented electrical wave current to produce a LEOW with >800 ORP and very low concentrations of sodium and chlorine, characteristics that extend the shelf life of this biocide.

Information related to the effect of long acting pH 7.0 EOWaters, against bacterial species isolated from avian clinical cases and subjected to different environments and diluted in different water qualities is limited, therefore in this work we studied the bactericidal effect a long acting EOW with a pH of 7.0, diluted in different concentrations of distilled and tap water against known bacterial species. The hypothesis was that a long acting EOW, 90 days after formulation, will destroy 99.99% of all bacteria isolated from Avian Clinical cases, using 1 X 10^{12} of bacterial inoculums of E. coli, Candida albicans, Staphylococcus aureus, and Bacillus subtilis *in vitro* when diluted 1:10 and up to 1:50 with distilled or/and tap water with different degrees of hardness.

MATERIALS AND METHODS

Bacterial strains were isolated from avian clinical cases referred by intensive poultry producers to the Department of Microbiology, Department of Avian Animal Production, of the Faculty of Veterinary Medicine of the National Autonomous University of Mexico. Prior to testing, the isolated bacteria were sub cultured at least twice onto suitable media to ensure purity and viability. When sufficient growth was observed after 3 weeks, were harvested in sterile saline (0.9 m NaCl) and vigorously vortexed.

Candida albicans was subcultured at least twice onto TSA supplemented with 2.0% dextrose. The yeast suspension was clarified from massive clumps of yeast cells and agar residues by filtration through columns consisting of 10-ml syringes filled with sterile glass wool and adjusted to the turbidity of a 2 McFarland standard (108–109 cells/ml).

The long acting EOW (90 days after formulated) was obtained from Steripharma SA CV laboratories, with a 6.5 pH and >800 Oxy Reduction potential (ORP) 90 days after being formulated.

An aliquot (10 µL) of the stock solution containing (108–109 cells/ml) of each of the following pathogens: E. coli, Candida albicans, Staphylococcus aureus, and Bacillus subtilis (in agreement with Mexican sanitary regulatorions NMX-BB-O4O-SCFI-1993) was transferred to a 10 mL test tube.

The Minimum Inhibitory Concentration (MIC) of the EO solution *in vitro* the macro dilution technique was used. Inoculums of the mentioned bacteria were used, and dilutions were made in accordance to table 1, using double distilled water and tap water (pH 8 – 10.0) and allowing 15 minutes for the EO solution to take effect.

The bactericidal effect of EOW was also tested using hard water with pH 8 following the same procedure outlined in table 2.

EO water was furthermore tested for bactericidal potency using hard water with pH 10 as outlined in table 3.

After 15 minutes a 1 mL aliquot of the test solution was transferred to agar agar petri dishes and incubated for 24 hours. Bacterial colonies if present were counted and bactericide efficiency was noted.

Inhibition of bacterial growth was compared using aqueous phenol at different concentrations (1.0, 0.50, 0.25, 0.10, and 0.01%). The fungi static efficacy of both EOWs was determined accordingly using C. albicans as the test strain, and an incubation of 96 h at 37_C; 3.0% formalin and its dilutions in WSH as mentioned above were used as inhibition controls of C. albicans. All tests were performed at least in duplicate.

The identity of growing microorganisms was confirmed by random checks using standard laboratory procedures.

Growth controls were incorporated for each test and consisted of a 1:1 mixture of sterile WSH with double concentrated trypticase soy broth (TSB) and bacterial inoculums.

Analysis of variance was used to compare results.

RESULTS

In table 1 we can observe that the long acting pH 7.0 EOW was 99% effective to kill the bacteria obtained from avian clinical cases, after 15 minutes of contact, with dilutions (1:1 up to 1:30) of EOW in double distilled water. When comparing with the effect of phenol bacterial colonies were more evident in both antiseptic solutions, in dilutions 1:20 and up to 1:40.

In table 2 using hard water pH 8.0 and pH 10 to dilute the long acting EOW a similar result was observed, showing that hard water did not interfere with the antibacterial properties of this long acting EOW, the EOW used in this study was formulated 90 days before the experiment was carried out

DISCUSSION

Electrolyzed water (EWO) is gaining popularity as a sanitizer in the food industries of many countries, but it must be noted that not all EOWaters are the same, some are acidic and some are corrosive and with a very short duration of their biocidal effect, and the others are EOW with a long duration of their biocidal effect, because they have a high ORP value and a neutral pH (Sampson and Muir, 2002). The one here tested is a long acting EOW with a high ORP VALUE (>880) and a neutral pH. In our study it was observed that the bactericidal effect of the long acting EO water was 99.99% effective when diluted in distilled or/and tap water. Statistical analysis showed a high significance due to the fact that EO water was 100% effective against the bacterial strains tested.

Observations in vitro reported in this work are similar to the ones reported by Issa-Zacharia, (2010). When acid EOW was used as a sanitizer in broiler carcasses (Hinton et al 2007), and in egg shells, microbial load an effective antibacterial effect was reduced using acid EOW (Fenner et al., 2006).

Acidic EOW used against C. albicans was highly effective, observation that was previously reported by Fenner et al., (2006).

When comparing the efficacy of the long acting EOW with freshly made acid electolized water, and with properties similar to the long acting EO water, and tested against different types of foodborne pathogens (*Escherichia coli* O157:H7, *Listeria monocytogenes*, *Bacillus cereus* and *E. coli* O157:H7), it was reported that 30 seconds after the application of acid EOW all bacteria were inactivated (Kim et al 2000; Hung and Brackett 2000; Mandal et al., (2010).

When beef samples were studied at different storage temperatures and treated with acid EO water, the growth rate and lag time of E. coli populations growth was decreased at 4°C, but when temperature increased, an increment in bacterial growth rate and lag time was also observed (Tian et al., 2010). Furthermore, Mukhopadhyay and Ramaswamy, (2012) reported that acid EO water was more effective reducing spoilage and pathogenic microorganisms including *Salmonella* in fresh poultry, fresh-cut fruit and vegetables, seeds and sprouts.

When acidic EO water was formulated with different water flow rates, its biocidal properties were not affected. The latter indicated that pH and dissolved oxygen concentration of the water used for producing EOW did not affect the antimicrobial properties of the produced EOW.

The antimicrobial effect of acid EOW, is related to the ORP and to the presence of HOCL, factors that affect the metabolic processes in the membrane of prokariotic cells, resulting in the death of pathogenic bacterial as stated by Mukhopadhyay and Ramaswamy, (2012) and Shun-Yao (2005). The latter result was caused because anaerobic and aerobic bacteria grow in the range of -200 and up to 800 mV (ORP), therefore, the high ORP of LEOW saturates or sequester ions in the membrane making it unstable and inactivating the bacterial cell (Jay, 2000).

Acid EO water is useful when freshly produced, and can be recomended as a fungicide during postharvest processing of fruits and vegetables, and as a sanitizer for washing the carcasses of meat and poultry. It is cost-effective and environment-friendly.

The long acting EO water treatment, like the one here studied, would be a better alternative because it is possible to generate on site, with a ong shelf life, with a high antibacterial effect and with no residual contamination (Mukhopadhyay and Ramaswamy, 2012)

It was concluded that the use of distilled and/or tap hard water used to dilute a long acting pH 7 EOW, does not affect its antibacterial properties and should be considered as an emergent option for the control of bacterial pathogens such as those present in food production and animal husbandry. Note should be made that the long acting EOW was formulated 90 days prior to its use in this study, supporting the objective of demonstrating the antibacterial effect of the long acting EOW when it is formulated with a 6 to 7 pH range and a high ORP value.

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TABLE 1. BACTERICIDE EFFECT OF EOW USING BIDISTILLED WATER AS DILUENTS AFTER 15 MINUTES OF CONTACT WITH DIFFERENT BACTERIAL STRAINS

Solute:dilution	2	5	10	20	30	40	50
	1:1	1:5	1:10	1:20	1:30	1:40	1:50
EO Solution mL	5	2	1	0.5	0.33	0.25	0.2
Double distilled wáter mL	5	8	9	9.5	9.67	9.75	9.8
Total mL	10	10	10	10	10	10	10
Colonies present with EOW	0	0	0	0	0	1	2
Colonies using phenol	0	0	0	0	1	3	5

Result: The EO Solution diluted in double distilled water had a bactericidal effect of 99.99% ($P<0.001$)

TABLE 2. BACTERICIDAL EFFECT OF EO WATER USING HARD WATER WITH PH 8..

Solute:disolution	2	5	10	20	30	40	50
	1:1	1:5	1:10	1:20	1:30	1:40	1:50
EO water mL	5	2	1	0.5	0.33	0.25	0.2
Hard water pH 8. (mL)	5	8	9	9.5	9.67	9.75	9.8
Total mL	10	10	10	10	10	10	10
Colonies present	0	0	0	0	1	0	1
Colonies using phenol	0	0	0	0	2	3	5

TABLE 3. BACTERICIDAL EFFECT OF EO WATER USING HARD WATER WITH PH 10

Solute:disolution	2	5	10	20	30	40	50
	1:1	1:5	1:10	1:20	1:30	1:40	1:50
EO water mL	5	2	1	0.5	0.33	0.25	0.2
Hard wáter pH 10. (mL)	5	8	9	9.5	9.67	9.75	9.8
Total mL	10	10	10	10	10	10	10
Colonies present	0	0	0	0	0	1	1
Colonies using phenol	0	0	0	0	1	2	5