Determining the Efficacy of IonatorEXP™ Activated Tap Water on Growth of *Staphyloccocus aures and Escherichia coli*.

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ABSTRACT

The efficacy in growth inhibition of *Staphylococcus aureus* and *Escherichia coli* of the IonatorEXP™ activated tap water was compared to plain tap water, Quatsyl™, and Butchers Neutral Disinfectant™. Treatment with Quatsyl™ or Butchers Morning Mist™ Neutral Disinfectant resulted in complete inhibition of growth for both *S. aureus* and *E.coli*. Treatments with IonatorEXP™ activated tap water, performed as directed by the manufacturer, or plain tap water, resulted in no observable inhibition of growth for either of the bacterial strains tested. This observation is not unexpected given that analysis of pH, conductivity and H_2O_2 on IonatorEXP™ activated tap water were statistically the same values as those determined for plain tap water that was not activated by the IonatorEXP™.

I. INTRODUCTION

The bacterium *Staphylococcus aureus* is a Gram-positive coccus able to ferment mannitol. *S. aureus* is normally found in the nasal cavities of humans where it's been estimated that 20% of the population are persistent carriers [1]. *Staphylococcus aureus* generally causes mild skin infections such as boils and the common stye. However, if the bacteria spread from the skin lesions to the bloodstream it can result in much more serious infections of the bones, joints and organs. Recently there have been concerns about the spread of antibiotic resistant bacteria, more specifically methicillin resistant *S. aureus* (MRSA). MRSA was first reported in 1961, shortly after the introduction of methicillin and has become increasingly more prevalent in recent years [2]. There are two general strains of MRSA, a strain acquired by nosocomial infections (hospital acquired) and a strain acquired from the community outside of the hospital setting, referred to as the community acquired strain (CA-MRSA).

In 2005 it was estimated, that there were 94,000 MRSA cases in the United States, and of those cases 19,000 resulted in death [3]. Approximately 85% of MRSA cases in 2005 were the result of nosocomial infections while the remaining 15% were as a result of CA-MRSA [3]. Because of the

dissemination of MRSA to the community, there has been increased awareness of MRSA in the general population as well as in hospital settings. There are essentially two ways in which *S. aureus* is transmitted [4]. One method is by direct physical contact with an infected person. Transmission in this case can be prevented by thoroughly washing hands with soap or hand sanitizer, by avoiding contact with people's open sores and by avoiding sharing personal items. The second mode of transmission is by physical contact with any object that has been touched by an infected person. Prevention of transmission in this case involves the maintenance of a clean environment through the use of disinfectants and/or sanitizers. Disinfectants are used to accomplish disinfection, which by definition, is the application of a substance to inanimate objects to destroy microorganisms living on the object [5]. Sanitizers, in contrast, are substances that reduce, but may not eliminate, microorganisms to levels considered to be safe [5]. It is therefore very important to choose the appropriate chemical disinfectant/sanitizer to achieve the desired outcome.

There are numerous products on the market that claim to make the environment safe from microorganisms such as MRSA. One such product is the IonatorEXP™, manufactured by the Activeion Cleaning Solutions, LLC, of Minneapolis, MN. The manufacturer claims [6] that when the IonatorEXP™ is used as directed, not only is it a multi-surface cleaner but it also kills more than 99.9% of most harmful bacteria, including MRSA, *Escherichia coli* and staphylococci. According to the manufacturer's website [6], this is accomplished by applying an electric charge to tap water and as the charged water passes through an ion-exchange membrane the ionized water is separated into an oxygenated mixture of positively and negatively charged nano-bubbles that kill more than 99.999% of harmful bacteria residing on the surface. On the same website, the manufacturer cites a study by an independent, certified laboratory (ATS Labs, Eagan, MN) to validate their claims. However, there is a certain amount of apprehension to the results reported because, according to the ATS Labs report, the test sprayers were filled with sponsor filled tap water before the testing. The laboratory did not perform an analysis on the tap water found in the IonatorEXP™ and water after the treatment with the IonatorEXP™.

The purpose of this study is to determine the validity of the claims by the manufacturer of the IonatorEXP™. To accomplish this we analyzed both the biological and chemical effects of the IonatorEXP™ treated tap water. For the biological analysis we tested the IonatorEXP™ treated water on the viability of bacteria *E. coli* and *S. aureus* after treatment according to the manufacturer's instructions. We analyzed for pH, conductivity and the presence of hydrogen peroxide as part of the chemical analysis of the tap water treated through the IonatorEXP™. To our knowledge this is the first such study of this product.

II. MATERIALS AND METHODS

a. Bacterial Strains, Media and Test Solutions

Bacterial strains used for this study were laboratory strains of *Staphylococcus aureus* and *Escherichia coli*. All the cultures were grown in Todd-Hewitt broth (THB; Difco Laboratories) at 37˚C with shaking at 200rpm. All spread plating of cultures was performed on Columbia Blood Agar base (CBA; Difco Laboratories) and incubated overnight at 37˚C before observations for growth were made. For all the spread plating we used 100µl of an overnight culture unless indicated otherwise. The IonatorEXP™ (Activeion Cleaning Solutions LLC) and Butchers Morning Mist™ Neutral Disinfectant (The Butcher Co., Marlborough, MA) were obtained from the University of Wisconsin – Eau Claire custodial services. The disinfectant Quatsyl™ (Sterling Winthrop Inc. New York) is a laboratory disinfectant. All disinfectants were diluted to working concentrations as directed by the manufactures before testing. As directed by the manufacturer, the IonatorEXP™ was sprayed away from the surface for 3 s to activate it then for 6 s before a sample was taken for analysis. The same procedure was used when spraying CBA plates. The Butchers Morning Mist™ Neutral Disinfectant was sampled or sprayed without

the 10 s delay. Tap water, the same tap water that was used in the IonatorEXP™, was obtained from the city source (lab faucets). Distilled water is essentially tap water that had many of the impurities removed from it by an in-house purification system. Deionized water was in-house distilled water that went through a NANOpure II™ purification system (Barnstead/Thermolyne, Dubuque, IA). All spraying on CBA plates was performed at a distance of 8 cm.

b. Conductivity Analysis

Conductivity was used to test for possible ion formation in the ionator treated tap water against plain tap water and deionized water. The water used in the Ionator EXP™ was tap water as directed for its use by the manufacturer. Water sample from the IonatorEXP™ was sprayed into sterile beakers then poured into new polyethylene vials for tests. All sample vials tested were kept at the same volume of water (14ml). The conductivity probe (Vernier conductivity probe CON-BTA, Vernier Software and Technology) integrated with the Vernier LabPro Kit and logger Pro 3.7 software was used; it was calibrated with a standard conductivity solution (NaCl, 500 mg/L of Total Dissolved Solids, TDS) which measured at 1,000 μS/cm (μS, microsiemens) at room temperature. The conductivity probe has an accuracy of ±1%; the mid-range with resolution of 1 μS/cm was used. After each measurement the probe was rinsed with distilled water. During the measurement the water was agitated with the probe, then held up still until the conductivity reading was stabile (25 s).

c. pH Analysis

pH measurement was carried out with the Vernier pH sensor (pH-BTA) using the Vernier LabPro Kit integrated with logger Pro 3.7 software at 21 °C. The water samples tested were collected in the same manner as those in the conductivity measurements. Water sample from the IonatorEXP™ was sprayed into sterile beakers, then poured into new polyethylene vials for tests. The pH electrode was rinsed after each measurement and calibrated with standard pH buffers at pH 4.00 and 7.00. pH readings were recorded after the readings were stabile (25 s).

d. Determination of Hydrogen Peroxide Formation

To determine the possibility of hydrogen peroxide (H_2O_2) formation, we used the Ferric thiocyanate method (FTC-method), in which Fe^{2+} would react with H_2O_2 according to the Fenton reaction [7, 8] producing Fe^{3+} which in the presence of SCN^- a ferric thiocyanate complex is formed.

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + OH^-$$

$$Fe^{3+} + SCN^- \longrightarrow Fe(SCN)^{2+}$$

In short, the reaction mixture consists of 4.775 ml of 75% (v/v) ethanol/water, 0.1000 ml of 30% (w/v) NH_4SCN, 0.025 ml of sample, and 0.1000 ml of 0.02 M $FeCl_2$ in 3.5% HCl, in the order given. The sample and solvent volumes were adjusted as necessary to 5.000 ml for each mixture. The reference solution contained the same compositions, except the sample volume was replaced with solvent (75% ethanol). After addition of Fe^{2+}, the mixture was vortexed and its absorbance measured within 3 min, using a Cary-50 BIO UV/VIS spectrophotometer. The absorbancy of λ_{max} at 480 nm (ε, 3860 M^{-1} cm^{-1}) was used to estimate the percent of hydrogen peroxide formed. The percent of H_2O_2 of the water sample tested was calculated from a calibration curve (0 – 1.2 ppm range) obtained by the same procedure described with a correlation coefficient $R^2 = 0.99981$.

e. Disc Diffusion Analysis

To test the efficacy of various products on *E. coli* and *S. aureus*, we used the disc diffusion technique. The procedure was repeated three times on three different occasions**.** Briefly, overnight cultures of *E. coli* and *S. aureus* were spread plated onto separate CBA plates. Filter paper discs (Whatman International Ltd.; 1.5 cm) were soaked in each sample prior to placement onto CBA plates. As directed, the IonatorEXP™ was sprayed for 3 s prior to spraying the disks for 6 s. Zone of inhibition

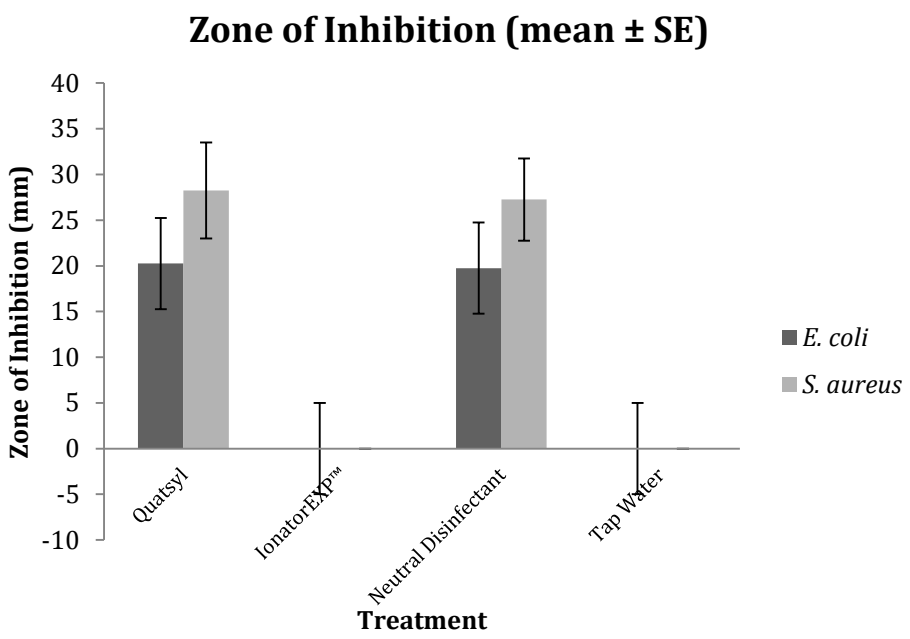

Figure 1. Disc diffusion analysis for various treatments.

for each sample was measured as the diameter of the area around the disks that inhibited the growth of the test strains.

f. Qualitative Analysis by the Spray Test

We performed a qualitative test using spray bottles to determine the efficacy of the samples. Tap water, distilled water, Butchers Morning Mist™Neutral Disinfectan**t** and Quatsyl were placed in an aerosolizing spray bottle for this test. Plates of CBA were spread plated with overnight cultures of *E. coli* or *S. aureus*. The plates were dried at room temperature for 15 min. After the plates dried, half of the plate was covered with cardboard. Samples to be tested were dispersed as a fine mist via the spray bottles from a distance of 8 cm. For the IonatorEXP™, the sample was sprayed directly from the IonatorEXP™ onto the plates as directed. The result was that half of the plate was sprayed with the sample and the other half served as a control that was not sprayed. All the plates were incubated overnight at 37˚C. To determine the efficacy of the samples we performed qualitative observations for the amount of inhibition of bacterial growth for the treated sample compared to the untreated one.

g. Quantitative Analysis of Sample Efficacy

Serial ten-fold dilutions of overnight *E. coli* or *S. aureus* cultures were performed in THB. Each dilution was sampled by plating 100µl onto CBA. After an overnight incubation we were able to determine the dilution that produced a concentration of approximately 1000 bacteria/ml. Once the bacterial solutions were identified, they were used as sources for a 1/10 dilution into the test samples. Specifically, all the test samples were dispensed in a sterile 10ml glass beaker according to the instructions of the manufacturer. Immediately 900µl was transferred from the beaker to a 1.5ml eppendorf microcentrifuge tube that contained 100µl of the diluted bacterial sample. The sample was mixed for 10 s by vortexing. After mixing the solution, a 100µl sample was plated onto dry CBA agar plates. The number of colonies was recorded for each treatment after an overnight incubation at 37˚C. Dilutions with THB were performed in the same manner and served as controls for determining the bacterial concentrations before treatment with one of the test samples. The analysis was performed in duplicate on three different occasions.

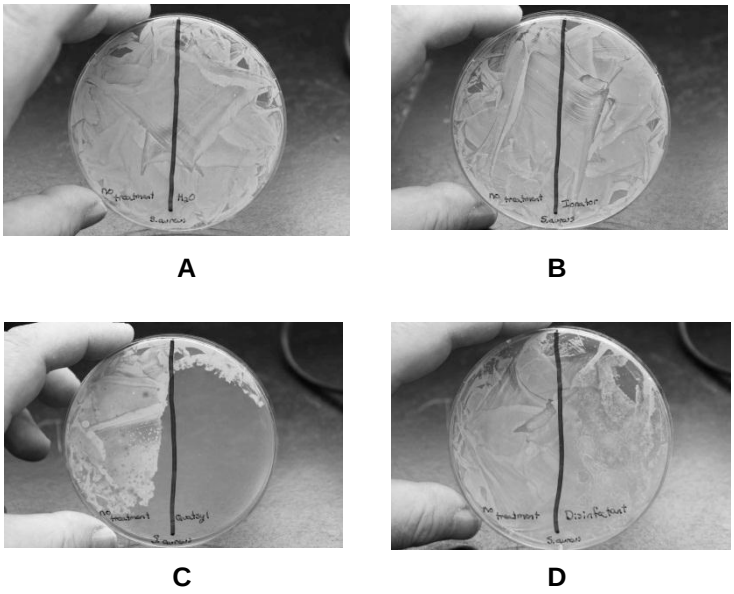

Figure 2. Qualitative analysis by the spray test. The efficacy of samples was compared by spraying one half of the CBA with the sample while the other half was not treated. The results represent effects of treatment on *S. aureus*. Panel A represents treatment with tap water; panel B represents treatment with IonatorEXP™ spray; panel C represents treatment with Butchers Morning Mist™ Neutral Disinfectant; and panel D represents treatment with Quatsyl™ spray.

III. RESULTS

a. Conductivity Analysis

To find out if the IonatorEXP™ could ionize and create permanent charged species in the activated water (sprayed water), we tested the conductivity of the activated water and water in the IonatorEXP™ container (ionator water) that had not passed through the electrical system against control waters (tap water and deionized water). The significant formation of charged species of the sprayed water would explain the ability of the IonatorEXP™ to destroy microbes. The data obtained for the conductivity analysis is presented in Table 1. The conductivity of tap water and ionator water were essentially the same, at 278 and 269.8 μS/cm, and higher than the conductivity of deionized water (2 μS/cm) due to small amount of dissolved mineral species in the tap water. The activated water showed a conductivity of 331.6 μS/cm, a value that is higher, but not significantly different as compared to ionator tap water and tap water ($p>0.05$). This value indicated more charged species present in the activated water, however, it is not clear if this level of conductivity alone is significant to cause microcidal activity. Two main parameters established as responsible for killing microbes in the application of electrical sterilization are the electrical field strength (e.g., high voltage) and treatment time [9-11].

b. pH analysis

The results of pH analysis on water samples from the IonatorEXP™, activated water, tap water and deionized water are presented in Table 1. The pH of the ionatorEXP™ water, tap water and deionized water was 6.43, 6.21 and 6.83, respectively. Both tap water and IonatorEXP™ water were the same water and their pH's were slightly on the acidic side as typical of tap water due to dissolved carbon dioxide in the water ($p>0.05$). The deionized water has a neutral pH 6.83 as expected. The activated water had a more acidic pH at 6.13. The difference between the activated tap water and the tap water in the IonatorEXP™ was not found to be significant ($p>0.05$).

Types of Water*	pH (mean±SE)	Conductivity, μS/cm (mean±SE)	ppb** of H_2O_2 (mean±SE)
Tap	**6.2 ± 0.1**	**278.2 ± 1.0**	**2.9 ± 1.3**
Deionized	**6.8 ± 0.1**	**2 ± 0**	**1.9 ± 1.2**
IonatorEXP™ Tap	**6.4 ± 0.1**	**269.8 ± 0.3**	**2.0 ± 2.5**
Activated Tap	**6.1 ± 0.1**	**331.6 ± 87.3**	**9.3 ± 2.2**

* Tap water = water taken from city water supply, Deionized = deionized water, IonatorEXP™ Tap = tap water in IonatorEXP™ prior to spraying through the IonatorEXP™, Activated Tap = tap water sprayed through the IonatorEXP™ system. ** ppb = ppm x 10^3.

Table 1. pH, conductivity and level of H_2O_2 measured for ionator water and activated water compared to controls.

c. Determination of Hydrogen Peroxide Formation

The level of H_2O_2 in parts per billion (ppb) in the activated water (9.33 ppb) is higher than those in the tap water (2.88 ppb), in ionator water (2.02 ppb) and in deionized water (1.87 ppb). The difference between the activated water and both tap water and ionator water were not found to be significant ($p>0.05$). This level of H_2O_2 is far below the known level used in disinfection, sterilization, sanitation and antiseptics, which is typically in the range of 1 – 3% [8, 12]. The level of H_2O_2 present would not be effective against microbes.

d. Disc Diffusion Analysis

The data from the disc diffusion analysis are presented in Fig. 1. Only Quatsyl and Butchers Morning Mist™ Neutral disinfectant treated discs resulted in zones of inhibition for both *E. coli* and *S. aureus*. The differences between the zones of inhibition for those samples compared to tap water or IonatorEXP™ activated tap water were statistically significant ($p < 0.05$). Discs treated with tap water or IonatorEXP™ activated water did not demonstrate inhibition of *E. coli* or *S. aureus* under these conditions.

e. Qualitative Analysis by the Spray Tests

The results for the spray test for *S. aureus* are presented in Fig. 2. When we compared the treatment of *S. aureus* with tap water or IonatorEXP™ activated tap water to untreated areas we observed that there was no observable difference between the treated and untreated areas (Fig. 2 panels a and b), there was no reduction in microbial growth. Quatsyl™ and Butchers Morning Mist™ Neutral disinfectant treated areas, however, demonstrated inhibition of cell growth against *S. aureus* (Fig. 2 panels c and d). Quatsyl™ exhibited total inhibition of growth while the Butchers Morning Mist™ Neutral disinfectant was less inhibitory. These observations were the same for *E. coli* (data not presented) with the only difference being that the Butchers Morning Mist™ Neutral disinfectant had a greater inhibitory effect on *E. coli* than on *S. aureus*. This difference could be due to the fact that the cell wall structure of these two strains is different.

f. Quantitative Analysis of Sample Efficacy

The data for the quantitative analysis is presented in Fig. 3**.** Treatment of *S. aureus* or *E. coli* with Quatsyl™ or Butchers Morning Mist™ Neutral disinfectant resulted in total inhibition of growth. For both strains, treatment with tap water or IonatorEXP™ activated tap water resulted in colony counts that were statistically indifferent from each other ($p>0.005$) and, more importantly, those colony counts were statistically no different than the counts observed for control samples which had no treatment ($p>0.005$).

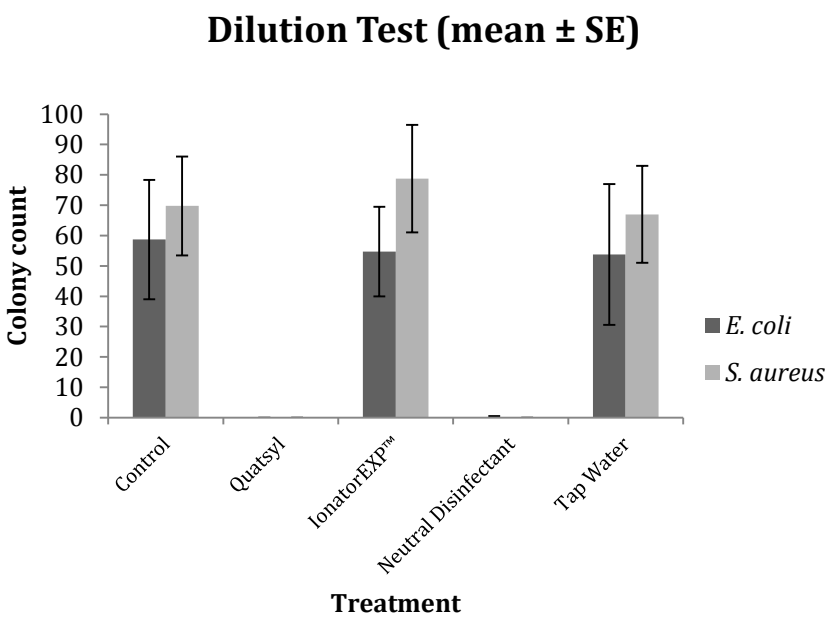

Figure 3. Quantitative analysis of the efficacy of various treatments on bacterial samples.

IV. DISCUSSION

In this report we demonstrated the efficacy of IonatorEXP™ activated tap water compared to tap water, Quatsyl™ and Butchers Morning Mist™ Neutral disinfectant in the reduction of *S. aureus* and *E. coli*. The data indicate that the IonatorEXP™ activated tap water is as effective a sanitizer as plain untreated tap water and not as effective as either Quatsyl™ or Butchers Morning Mist™ Natural Disinfectant. The IonatorEXP™ is marketed by the manufacturer as an environmentally responsible alternative to classical sanitizers. It is claimed by the manufacturer that the IonatorEXP™ transforms tap water into a powerful cleaning agent by introducing a slight electrical charge to the tap water that results in formation of ionized water (activated water). This activated water purportedly carries a low level electrical field to the bacteria residing on surfaces. The exposure of bacteria to the electrical field results in the effective killing of 99.999% of harmful bacteria including *S. aureus* and *E. coli*.

Our conductivity analysis demonstrated that there was slight increase of charged particles when tap water was treated with IonatorEXP™. However, the slight difference between the two samples was determined not to be significant ($p>0.05$). Additionally, the difference was not enough to kill bacterial samples as demonstrated by our qualitative observations and quantitative analysis. When we subjected bacterial samples with different treatments we observed no visual difference in the amount of growth between treatments with IoinatorEXP™ activated tap water and tap water for either *S. aureus* or *E. coli*. Treatment of *S. aureus* and *E. coli* with Quatsyl™ or Butchers Morning Mist™

Neutral Disinfectant resulted in the total elimination of growth for both strains. This observation is further verified by the disc diffusion assay in which we again observed no difference in growth inhibition between IoinatorEXP™ activated tap water and tap water for both bacterial species. We explored the possibility that ionization of tap water by the IonatorEXP™ would change the pH or would produce H_2O_2. When we analyzed for changes in pH and H_2O_2 between IoinatorEXP™ activated tap water and tap water we found the differences to be insignificant in both cases ($p > 0.05$). To further determine if bacterial cells were reduced by various treatments we

performed quantitative analysis for each treatment. Quantitative analysis of bacterial reduction after treatment by various samples demonstrated that Quatsyl™ and Butchers Morning Mist™ Neutral disinfectant significantly reduced the number of *S. aureus* and *E. coli*. We observed total elimination of viable cells by these two treatments. IonatorEXP™ activated tap water and tap water treated samples resulted in colony counts that were statistically no different form each other. Moreover, they were statistically no different than the control (cells with not treatment).

Herein, we demonstrated that Quatsyl™ and Butchers Morning Mist™ Neutral disinfectant are effective sanitizing agents against both *E. coli and S. aureus*. IonatorEXP™ activated tap water, when used as recommended by the manufacturer, had statistically the same reductions in *E. coli* or *S. aureus* as tap water that was not activated by the IonatorEXP™. Moreover, when we compared the pH, H_2O_2 and conductivity for IonatorEXP™ activated tap water to that of tap water, we observed statistically no difference between the values. These data indicate that IonatorEXP™ activated tap water is no more effective in inhibiting *S. aureus* or *E. coli* than regular tap water. More importantly IonatorEXP™ should not be advertised as an effective alternative to traditional sanitizers. The claims by the manufacturer that the IonatorEXP™ kills more than 99.999% of harmful bacteria when used as directed are unsubstantiated. The IonatorEXP™ activated tap water is as ineffective at killing *S. aureus* or *E. coli* as is plain tap water. The claims by the manufacturer could potentially lead to dangerous consequences if the IonatorEXP™ is used with the assumption that it kills 99.999% of harmful bacteria.

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