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Research Note

Effect of a Reactive Oxygen Species–Generating System for Control of Airborne Microorganisms in a Meat-Processing Environment†

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ABSTRACT

The effectiveness of reactive oxygen species (ROS)–generating AMS equipment on the reduction of airborne bacteria in a meat-processing environment was determined. *Serratia marcescens* and lactic acid bacteria (*Lactococcus lactis* subsp. *lactis* and *Lactobacillus plantarum*) were used to artificially contaminate the air via a six-jet Collison nebulizer. Air in the meat-processing room was sampled immediately after aerosol generation and at various predetermined times at multiple locations by using a Staplex 6 stage air sampler. Approximately a 4-log reduction of the aerial *S. marcescens* population was observed within 2 h of treatment ($P < 0.05$) compared to a 1-log reduction in control samples. The *S. marcescens* populations reduced further by ~4.5 log after 24 h of exposure to ROS treatment. Approximately 3-log CFU/m^3 reductions in lactic acid bacteria were observed following 2-h ROS exposure. Further ROS exposure reduced lactic acid bacteria in the air; however, the difference in their survival after 24 h of exposure was not significantly different from that observed with the control treatment. *S. marcescens* bacteria were more sensitive to ROS treatment than the lactic acid bacteria. These findings reveal that ROS treatment using the AMS unit significantly reduces airborne *S. marcescens* and lactic acid bacteria in meat- processing environments within 2 h.

Contamination of meat products by microorganisms is a major economic problem in the meat industry. Contamination can occur at various stages of animal processing and meat fabrication *(22).* Prevention and/or reduction of such contamination is a major objective of hazard analysis critical control point systems and related in-house food safety programs such as good manufacturing practices. There are several strategies available to reduce the populations of microorganisms on carcasses. These include carcass trimming, carcass washing, organic acid treatments, and combinations of the above *(4).* In addition, programs such as cleaning and maintenance of plant and equipment and plant sanitation are included but may not involve steps to prevent air as a source of contamination of food and equipment.

Air has long been recognized as a source of microbial contamination in a range of food processing plants including dairy *(12, 20),* beef *(3, 9, 22),* pork *(13, 17),* and poultry products *(15, 25).* Rahkio and Korkeala *(19)* reported a strong association between aerobic viable counts on beef carcasses and the levels of airborne microbial contamination in the processing environment. It is essential to monitor and reduce the airborne microbial levels in the meat-processing environment.

Various technologies have been developed for the reduction of airborne microorganisms in food processing environments. UV light is widely used to inactivate airborne bacteria and mold in food-processing areas *(7).* The use of filtration along with electrostatic precipitation is used to capture airborne particles *(23)* that harbor bacteria. The electrostatic space charge system has been shown to be highly effective in reducing dust and pathogens in the air and on surfaces *(1).* This system reduced airborne *Salmonella enterica* serovar Enteritidis contamination by 95% in caged layer rooms *(10).* The effect of electrostatic space charge on bacteria in biofilms was studied by Arnold and Mitchell *(2).* They found a 99.8% reduction of mixed populations of bacteria from stainless steel surfaces in a poultry plant. A patented air-cleaning system (Oxyion AMS) utilizes a high-frequency controlled pulse of electric current in a series of reaction chambers inside the unit to convert part of the oxygen in the air into various reactive oxygen species (ROS). These ROS are believed to be capable of oxidizing molds, mildew, bacteria, and viruses. The objec- tive of this study was to determine the efficacy of ROS- generating equipment in reducing airborne bacteria in a meat-processing environment.

MATERIALS AND METHODS

Bacterial cultures. *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactobacillus plantarum* NCDO 955, and *Serratia marcescens* (USDA 2772) strains were from the Food Safety Labo-

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† Mention of trade names or commercial products does not imply recommendation or endorsement to the exclusion of other products by the U.S. Department of Agriculture.

ratory culture collection. Lactic acid bacteria were maintained individually in lactobacilli deMan Rogosa Sharpe broth (MRS; Acumedia, Lansing, Mich.) containing 10% glycerol and were stored at −80°C, whereas tryptic soy broth (TSB; Difco Laboratories, Detroit, Mich.) with 10% glycerol was used for the *S. marcescens* strain. Frozen cultures were partially thawed at room temperature (20°C) for 15 min; *S. marcescens* was streaked on tryptic soy agar slants (Difco), and lactic acid bacterial cultures were streaked on lactobacilli MRS agar slants (Acumedia) and incubated at 35°C for 24 h. Actively growing lactic acid bacterial strains were individually transferred to 100 ml of MRS broth, and the *S. marcescens* culture was transferred to 100 ml of TSB broth followed by 24-h incubation at 35°C. Cells were harvested by centrifugation at 5,000 × *g* for 10 min and resuspended in phosphate-buffered saline (PBS; 50 mM). Each individual bacterial culture was enumerated by serially diluting the cultures in sterile 0.1% peptone water (Difco) and spiral plating (WASP; Microbiology International, Frederick, Md.) onto duplicate R_2A agar (Difco) and MRS agar plates. The average bacterial population of strains used in aerosol formation was ~9 log CFU/ml.

Experimental facility. The establishment was one of the oldest animal-slaughtering facilities (>80 years old) in the United States and, therefore, did not have the most modern air-handling system. Further, the establishment was not used regularly for animal slaughter. ROS-generating equipment (model MDS 202BS, Oxyion) was installed inside the meat-processing room. The room (30 ft long by 16 ft wide by 10 ft high) was primarily used for further processing of meats. It was equipped with further processing machinery such as a band saw, brine pump, vacuum tumbler, meat grinder, bowl chopper, mixer, and vacuum stuffer, which are used to divide meat carcasses into primal cuts, brine inject solid muscles, mince or grind meat for sausage formulation, and also emulsify meat batters for frank- furters.

ROS-generating console unit. The ROS-generating console unit (Oxyion AMS) was installed on the wall with a discharge pipe in a room. The unit is based on a system of tubular arrays and a very specific electrical field configuration to generate steady-state cold plasma. As air circulates through the unit's reaction chambers, part of the oxygen is electrically excited and converted to various ROS on a temporary basis. Given the difference in electrical potential across the glass walls of the tubular arrays inside the reaction chambers, several reactions involving oxygen occur. ROS include low levels of ozone (O_3), vapor hydrogen peroxide, and other oxidative compounds. A monitoring device regulates low levels of O_3 as the marker to determine contamination and adjusts according to the requirement of the application. Since the room air is circulated through the AMS unit, the short-lived ROS are able to carry on their oxidizing benefits.

Aerosol generation and sampling. Natural microbial load in the meat-processing room was evaluated using a Staplex 6-stage air sampler (Staplex Inc., Brooklyn, N.Y.) and R_2A agar media, using the procedure described below. Low levels of natural contamination (<1.5 log CFU/m^3) were reduced to nondetectable levels within 2 h of ROS exposure. Therefore, aerosols were generated to increase the microbial contamination in the meat-processing room.

Equal volumes (25 ml) of *S. marcescens, L. lactis,* and *L. plantarum* suspensions were mixed and transferred to a six-jet Collison nebulizer (model CN-25, BGI, Waltham, Mass.). The nebulizer was placed 12 in. above the predetermined sampling sites, and bacterial suspensions were aerosolized for 15 min at each site, using 20-lb/ft^2 air pressure. The initial population of aerosolized bacteria (0 h) was determined at three locations by using a Staplex 6-stage air sampler prior to turning the ROS-generating unit on. Sampling locations 1, 2, and 3 were 4, 12, and 18 ft away from the ROS console, respectively. Air samples were taken at 2, 4, 8, and 24 h during the treatment. The air sampler was kept about 36 in. above the floor at each location. The sampler was calibrated with an RC-50 totometer (Staplex) to maintain a flow rate of 28.3 liters/min during the sampling period. MRS and R_2A agar plates were used in the Staplex sampler for detecting mixtures of lactic acid bacteria and *S. marcescens,* respectively, in the airborne environment. After pulling air samples for 15 min, the plates were incubated at 35°C for 48 h. The control experiment was carried out using the same procedure as the treatment exposure; however, under the control conditions the ROS-generating unit was turned off. Each experiment was started on Monday morning and completed within a 24-h period. The experiment was replicated three times.

Statistical analysis. The total colony counts of four plates obtained at each sampling period and each location were converted to log CFU per cubic meter. The data obtained from three replicates were analyzed by two-way analysis of variance using a ''Proc Mixed'' statement (SAS 8.2, Cary, N.C.) for effects of treatment, location, sampling time, and interaction. For each response, covariance analysis, which allows correlation among levels of location, time, or both, was utilized. In all cases, the level of statistical significance used was set at P values of <0.05.

RESULTS

The effect of ROS generated by the AMS system on airborne *S. marcescens* population is shown in Table 1. Airborne *S. marcescens* populations varied from 3.99 to 4.88 log CFU/m^3 immediately following aerosolization at different sampling locations. Gradual reduction of *S. marcescens* populations was observed in the control samples. The reduction of airborne *S. marcescens* was from 0.08 log (site 3) to 0.81 log (site 1) 2 h following aerosolization in the control treatment. In contrast, a >4-log reduction ($P < 0.05$) in the *S. marcescens* population was observed within 2 h of ROS treatment. Extended ROS exposure of up to 24 h resulted in nondetectable *S. marcescens* level at site 2. *S. marcescens* populations after ROS treatments were significantly lower than those of the corresponding control samples for 4, 8, and 24 h. While *S. marcescens* populations decreased with time in control samples, the reduction was ca. 2 log CFU/m^3 after 24 h, compared with a ca. 5-log CFU/m^3 reduction of this bacterium after 24 h of ROS treatment. Sampling sites did not have a significant effect on the recovery of airborne *S. marcescens* populations, as the populations detected at different sampling sites were similar ($P > 0.05$) at specific sampling times of ROS treatment.

Initial populations of aerosolized lactic acid bacteria varied from 4.23 log CFU/m^3 at site 3 to 4.97 log CFU/m^3 at site 1 (Table 2). Initial lactic acid bacterial populations following ROS exposure were higher than the initial lactic acid bacterial populations of control samples; however, the difference was not significant. Approximately 3-log CFU/m^3 reductions ($P < 0.05$) in lactic acid bacteria were observed at each location following 2 h of ROS treatment.

TABLE 1. Serratia marcescens *populations in meat-processing room following treatment with ROS-generating system*

Sampling site[a]	Treatment	Bacterial populations (log CFU/m^3) at time point[b]: 0 h	2 h	4 h	8 h	24 h
1	Control	4.62 AX	3.81 AXY	2.90 AYZ	2.09 AZ	2.22 AZ
	AMS	4.88 AX	0.87 BY	0.28 BY	0.28 BY	0.38 BY
2	Control	4.13 AX	3.97 AX	2.82 AY	2.48 AY	2.22 AY
	AMS	4.77 AX	0.28 BY	0.57 BY	ND BY	ND BY
3	Control	3.99 AX	3.91 AX	2.71 AY	2.47 AY	2.19 AY
	AMS	4.69 BX	ND BY	ND BY	ND BY	0.28 BY

[a] 1, mixer by the kill room entry door; 2, weighing scale; 3, cutting table.

[b] For each site, means in the same column with different letters (A, B) are significantly different ($P < 0.05$); means in the same row with different letters (X, Y, Z) are significantly different ($P < 0.05$). ND, nondetectable.

While extended ROS exposure further reduced lactic acid bacteria at each location over 8- and 24-h treatments, the reduction during these periods was not significant. Lactic acid bacterial populations obtained from ROS-treated sites after 2 and 4 h were significantly lower than the lactic acid bacterial populations from corresponding control samples. As with *S. marcescens,* a gradual reduction of lactic acid bacteria was also found in untreated samples. At each sampling period of 8 or 24 h, airborne lactic acid bacterial populations obtained from control and ROS-treated samples were similar, except for sampling site 1, where lactic acid bacterial populations in ROS-treated samples were significantly lower than those populations obtained after 8 h of treatment.

DISCUSSION

Initial air sampling at three sites in the meat-processing room revealed very low levels of background airborne bacterial loads (<1.5 log CFU/m^3). These results were comparable to ~1.5 log CFU/m^3 airborne microbial counts in commercial food-processing facilities that were reported by Cundith et al. *(4),* but lower than other reported airborne bacterial loads, such as 2.0 to 2.3 log CFU/m^3 *(19)* and 2.4 to 3.4 log CFU/m^3 *(18)* in beef slaughterhouses, and 2.5 to 3.6 *(14)* and 2.3 to 3.1 log CFU/m^3 *(17)* in pork processing plants. Since ROS treatment of low levels of airborne bacteria resulted in nondetectable levels at each sampling site within 2 h (data not shown), we increased airborne microbial load by using aerosolization. *S. marcescens* and lactic acid bacteria were used in this study because these bacteria are known to occur in meat products *(6, 11)* and are also found in meat-processing environments *(8).* In the meat industry, large concentrations of *S. marcescens* cause spoilage of fresh and ready-to-eat meats, resulting in off-flavor, color changes, rancidity, and slime *(5, 16, 24).*

Air is an important vehicle for bacterial contamination in slaughterhouses and meat-processing facilities *(17, 19, 25).* Effective reduction of airborne pathogenic and spoilage bacteria should be an integral part of the concerted efforts to reduce or prevent potential cross-contamination in meat and meat products. Cundith et al. *(4)* used germicidal air-cleaning console units and found 1- to 1.5-log reductions in airborne bacteria and molds in different areas of a meat-processing facility. Similar results were reported by St. Georges and Feddes *(23),* who used air filtration and electrostatic precipitation. Electrostatic space charge systems reduced total aerobic bacteria in broiler breeder facility by 76%, resulting in fewer *Salmonella* Enteritidis–positive hens and chicks *(21).* Burfoot et al. *(3)* studied the effect of HEPA filter ultraclean air in a beef slaughterhouse. They observed reduced contamination on beef carcasses via airborne routes. This study showed the significant effect of ROS exposure in reducing airborne microorganisms in a meat-processing environment. ROS are moderate to strong oxidizing agents. They inactivate bacteria by rupturing the cell wall. The ROS level (monitored by an O_3 marker) of 0.0389 ppm used in this study is well below the permissible (0.1 ppm) 8-h exposure limit for a worker. Because of its

TABLE 2. *Populations of lactic acid bacteria in meat-processing room following treatment with ROS-generating system*

Sampling site[a]	Treatment	Bacterial populations (log CFU/m^3) at time point[b]: 0 h	2 h	4 h	8 h	24 h
1	Control	4.54 AW	3.76 AX	2.68 AY	2.08 AYZ	1.90 AZ
	AMS	4.97 AX	2.05 BY	1.94 BY	1.53 BY	1.80 AY
2	Control	4.41 AW	3.80 AX	2.64 AY	2.05 AYZ	1.74 AZ
	AMS	4.90 AX	2.04 BY	1.72 BY	1.68 AY	1.68 AY
3	Control	4.23 AX	3.65 AX	2.22 AY	2.22 AY	1.40 AY
	AMS	4.81 BX	1.92 BY	1.37 BY	1.70 AY	1.53 AY

[a] 1, mixer by the kill room entry door; 2, weighing scale; 3, cutting table.

[b] For each site, means in the same column with different letters (A, B) are significantly different ($P < 0.05$); means in the same row with different letters (W, X, Y, Z) are significantly different ($P < 0.05$).

short life, undesirable effects of ROS treatment are considered to be minimal, if not inconsequential. The reduction of airborne microorganisms using the ROS-generating system seemed to be more efficient than those reported with other approaches, such as electrostatic precipitation *(23)* and germicidal air purification console units *(4)*.

Based on these findings, our study reveals that active oxygen treatment using the ROS-generating AMS unit significantly reduces airborne *S. marcescens* and lactic acid bacteria in a meat-processing environment within 2 h. The treatment is more inhibitory to *S. marcescens* than to lactic acid bacteria. The difference in sensitivity of these bacteria to ROS could be attributed to differences in cell wall structure. The unit has an application for controlling airborne contamination in meat-processing facilities. Air with fewer bacteria in meat-processing environments could help improve meat shelf life and reduce cross-contamination in meats.

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Evaluation of the Efficacy of ROS reactor at Reducing Populations of Methicillin Resistant *Staphylococcus* aureus, *Listeria monocytogenes* and *Acinetobacter baumannii* on Stainless Steel Surfaces[1]

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SUMMARY

Stainless steel coupons were inoculated with Methicillin resistant *Staphylococcus aureus*, *Listeria monocytogenes* and *Acinetobacter baumannii*, placed in a controlled environmental chamber and exposed to Reactive Oxygen Species produced by the AMS technology. The initial inoculum was 6.1 $\log_{10}$ CFU/cm ^{2} for Methicillin resistant *Staphylococcus*, 5.2 $\log_{10}$ CFU/cm^2 for *Listeria monocytogenes*, and 5.7 $\log_{10}$ CFU/cm^2 for *Acinetobacter baumannii*. The exposure times were 0, 2, 4, 8, and 24 h. Background/ambient ozone levels were measured in the chamber prior to and after activating the ROS system.

The exposure to Reactive Oxygen Species for a 2h period resulted in reductions in Methicillin resistant *Staphylococcus aureus* of 2.1 $\log_{10}$ CFU/cm^2. Populations of

[1] A companion report on the same reactor measuring gas phase hydrogen peroxide output has been issued.

L. monocytogenes and *Acinetobacter baumannii* were reduced by 2.3 and 1.9 $\log_{10}$ CFU/cm^2, respectively.

Four hours of exposure resulted in log reductions for 2.7 $\log_{10}$ CFU/cm^2 for Methicillin resistant S*taphylococcus aureus*, 2.9 $\log_{10}$ CFU/cm^2 for *Listeria monocytogenes* and 2.6 $\log_{10}$ CFU/cm^2 for *Acinetobacter baumannii*.

Eight hours of exposure reduced Methicillin resistant *Staphylococcus aureus* and *Listeria monocytogenes* to levels below the detection limit. *Acinetobacter baumannii* was reduced by 4.1 $\log_{10}$ CFU/cm^2. After 24 hours of exposure, all of the pathogens tested were reduced below detectible levels (< 0.08 CFU/ Sq. cm).

INTRODUCTION

Microbial contamination of indoor air and affected surfaces represents a major public health problem and a potential source for sick-building-syndrome. For example, certain species of mold and bacteria may cause health concerns in homes, schools, offices, and health care facilities (Hota, 2004). In addition to being unattractive to see and smell, mold also gives off spores and mycotoxins that cause irritation, allergic reactions, or disease in immune-compromised individuals (Bahnfleth et al., 2005).

The term nosocomial infection refers to an infection that is acquired in the hospital or a health care facility (Chotani et al., 2004). Environmental contamination has produced devastating consequences in these facilities, resulting in the morbidity and mortality of tens of thousands of patients every year. Persons who visit hospitals, nursing homes, or health clinics have a risk of acquiring an infection as a result of their stay (Tilton, 2003). It is estimated that approximately one patient in ten acquires an infection as a result of an extended visit in one of these health care facilities (Tilton, 2003). Nosocomial acquired

infections are responsible for approximately 100,000 deaths with an annual cost approaching $29 billion (Kohn et al., 1999).

Nosocomial infections have a number of potential causes that promote the spread of disease. Common health care surfaces such as countertops, bedding, bedpans, and medical devices can all be used to transmit and spread disease from one person to another (Hota, 2004). Under hectic and stressful conditions, these surfaces can become easily contaminated, often by overworked employees. Cutbacks in staffing at health care facilities due to budget constraints, has placed a greater burden on health care facilities to find ways to remediate contaminates with limited resources (Chotani et al., 2004). Older and poorly designed buildings may harbor contaminates that are not easily eliminated using conventional disinfection methods. Studies have shown that microorganisms such as *Staphylococcus aureus* and *Candida albicans* survive in environmental reservoirs found in health care facilities (Hota, 2004). The World Health Organization reported that 40% of all commercial buildings pose a serious health hazard due to indoor air pollution.

Historically, UV light has been used in health care and other indoor air environments to provide continuous decontamination. UV light is a “line of sight” technology and does not provide the most effective means of control. Ideally, a system for continuous decontamination would produce antimicrobials which reduce contamination on surfaces and in the air. The ROS Reaction Chamber produces Reactive Oxygen Species (ROS) which are in the form of antimicrobial gases that inactivate microorganisms in the air and on surfaces. These gases can reach all surfaces in health care and related environments.

The purpose of this study is to evaluate the efficacy of which is designed to produce gas phase hydrogen peroxide and very low levels of ozone in reducing populations of

Methicillin Resistant *Staphylococcus aureus*, *Listeria monocytogenes* and *Acinetobacter baumannii* on stainless steel surfaces.

MATERIALS AND METHODS

Preparation of Cultures:

Methicillin-resistant *Staphylococcus aureus* (ATCC # 33591): *Acinetobacter baumannii* (ATCC # 11171) and *Listeria monocytogenes* (KSU # 56 and 70) were used for this study. Bacterial species were independently grown in Tryptic Soy Broth (TSB; Difco Laboratories, Detroit, MI) and YM broth (Difco Laboratories, Detroit, MI) respectively to mid-exponential phase followed by a wash and re-suspension in 0.1% peptone water (PW). The microbial cultures were combined by specie type to ca. 10^8 CFU/ml.

Preparation of environmental surfaces:

Environmental surfaces were simulated using coupons made of stainless steel (6.4 x 1.9 cm). Before treatment and inoculation, all coupons were cleaned using Fisherbrand Sparkleen* detergent (pH 9.5 - 10 in solution; Fisher Scientific). Stainless steel coupons were sterilized by autoclaving.

Preparation of Samples and ROS Treatment:

The coupons tested were dipped per microbial inoculum and vortex 15 sec optimizing microbial dispersion. Sterile binder clips were used to hang each coupon from a cooling rack for 1 h until dryness in a laminar flow biohazard air hood. The initial microbial population attached to the stainless steel coupons was in the range of 10^5 to 10^6 CFU/ sq. cm. The inoculated stainless steel coupons were transferred to a controlled airflow Biological Safety Cabinet (Nuaire) at 26°C, 46 % relative humidity (ambient conditions),

and exposed to ROS produced by the ROS Reaction Chamber for periods of 2, 4, 8 and 24 hours. Inoculated controls were prepared and placed in the test cabinet for 2, 4, 8 and 24 hours without ROS treatment. Ozone levels in the test cabinet were monitored throughout the study (Model 500, Aeroqual, New Zealand).

Sampling:

At the end of the designated holding time, coupons were placed into 30 ml of 0.1% peptone water and vortexed for 30 sec; samples were serially diluted and plated onto Tryptic Soy Agar (TSA; Difco Laboratories, Detroit, MI) for bacteria recovery. The colony-forming units per square centimeter (CFU/cm^2) were estimated after incubating at 35^oC for 24h.

RESULTS AND DISCUSSION

Figures 1, 2, and 3 show the log_{10} CFU/ sq. cm. reductions of Methicillin-resistant *Staphylococcus aureus, Acinetobacter baumannii,* and *Listeria monocytogenes* on stainless steel surface respectively.

Staphylococcus aureus

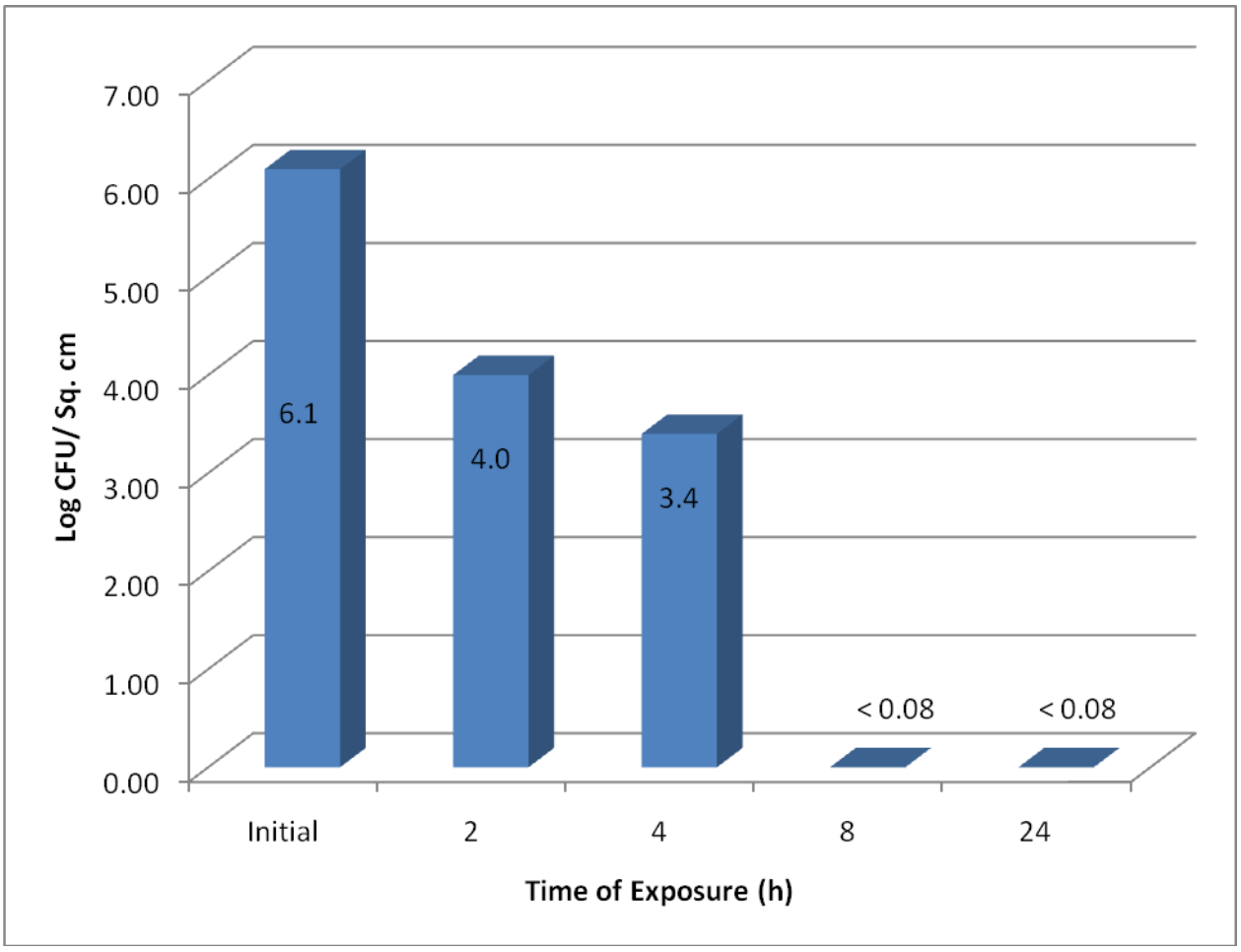

Figure 1: Population (log_{10} CFU/ sq. cm) of Methicillin resistant *Staphylococcus aureus* on Stainless Steel surfaces observed after 0, 2, 4, 8, and 24 h of exposure to Reactive Oxygen Species produced by ROS Reactor.

Listeria monocytogenes

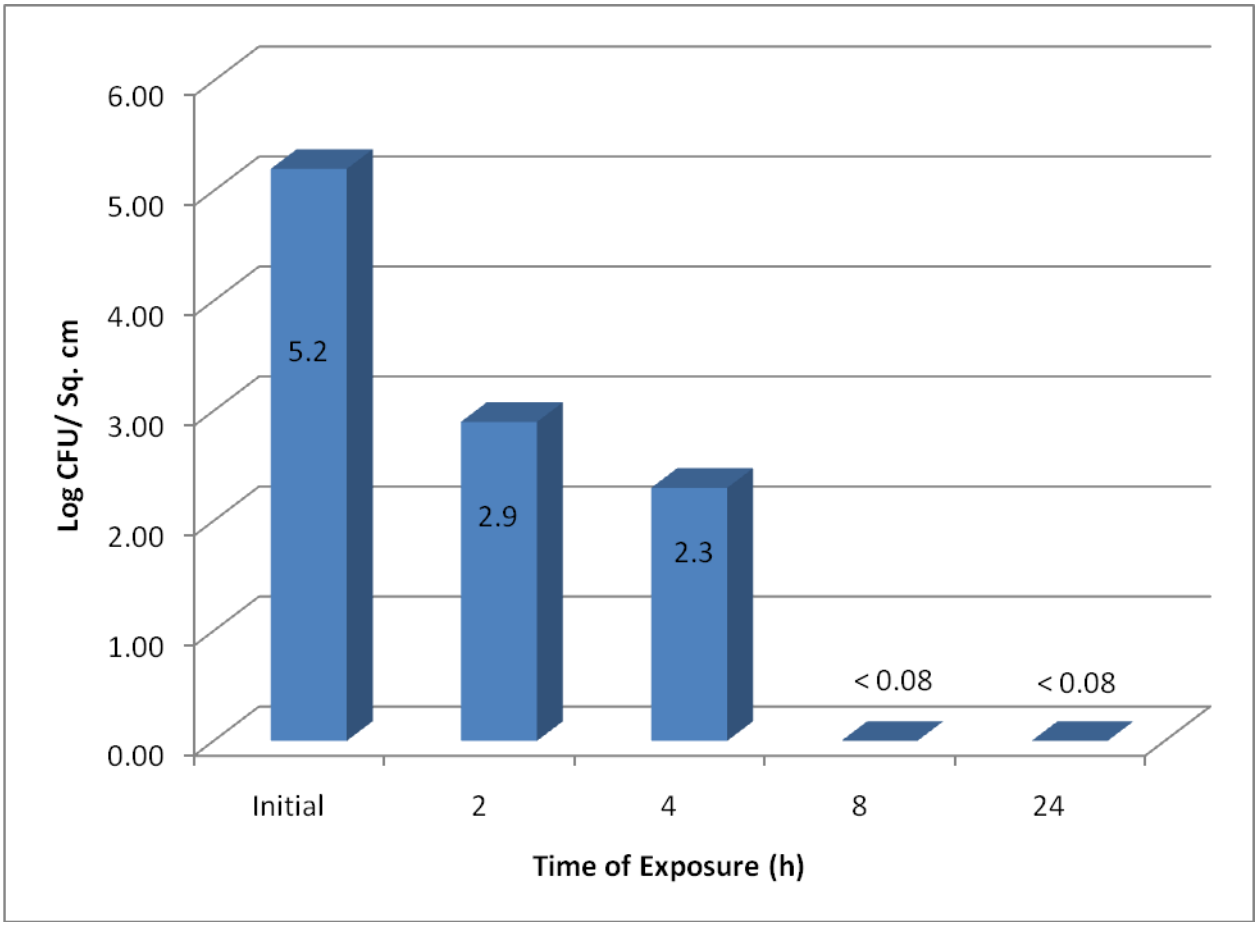

Figure 2: Population (log_{10} CFU/ sq. cm) of *Listeria monocytogenes* on Stainless Steel surfaces observed after 0, 2, 4, 8, and 24 h of exposure to Reactive Oxygen Species produced by ROS Reactor.

Acinetobacter baumannii

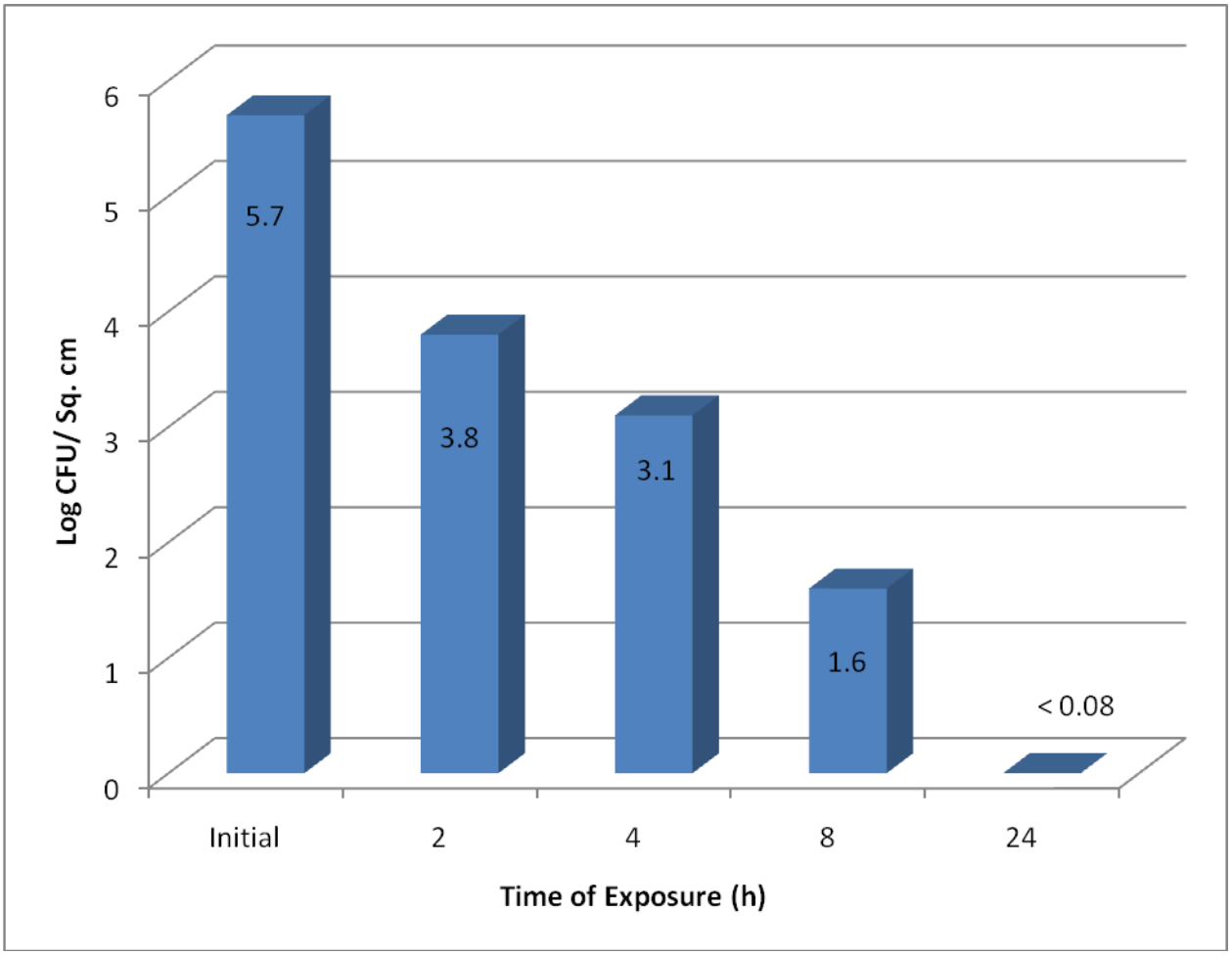

Figure 3: Population (log_{10} CFU/ sq. cm) of *Acinetobacter baumannii* on Stainless Steel surfaces observed after 0, 2, 4, 8, and 24 h of exposure to Reactive Oxygen Species produced by ROS Reactor.

Ozone levels were measured in the test chamber at 0.006 - 0.008 ppm. The ambient level of ozone in the control study was measured at 0.003 ppm. Levels of vaporized Hydrogen Peroxide in the chamber ranged from 0.02 – 0.04 ppm. All of these levels are well below OSHA limits for continuous interaction.

Based on the results of this study, the ROS system and the ROS it produces have the potential to reduce microbial contamination in health care and other indoor air environments.

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