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

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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 *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 *Staphylococcus 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. 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

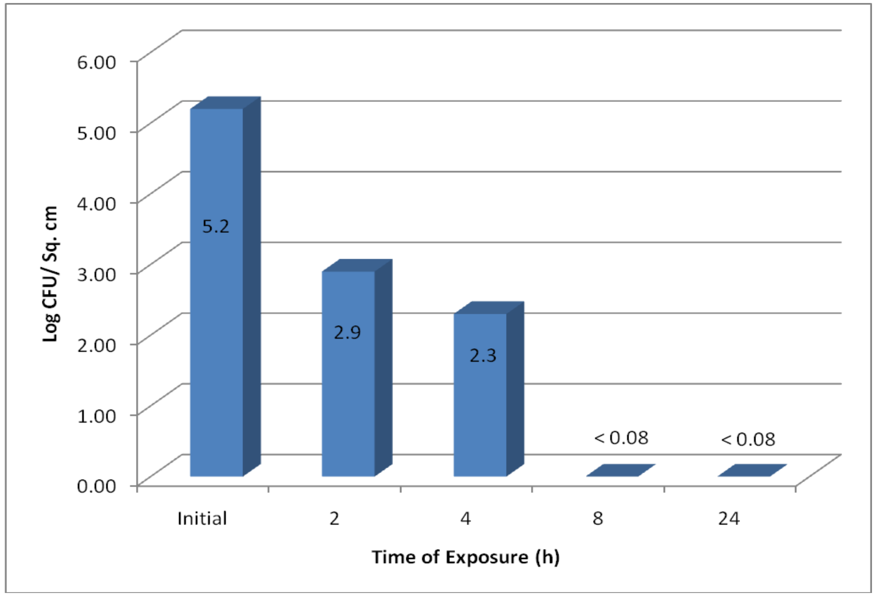
Preparation of Cultures:
Methicillin-resistant Staphylococcus aureus (ATCC # 33591): Acinetobacter baumannii (ATCC # 11171) and Listeria monocytogenes (KSU # 56 and 70) were used for this study. Bacterial species were independently grown in Tryptic Soy Broth (TSB; Difco Laboratories, Detroit, MI) and 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. 108 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 105 to 106 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/cm2) were estimated after incubating at 35oC for 24h.

Listeria monocytogenes control



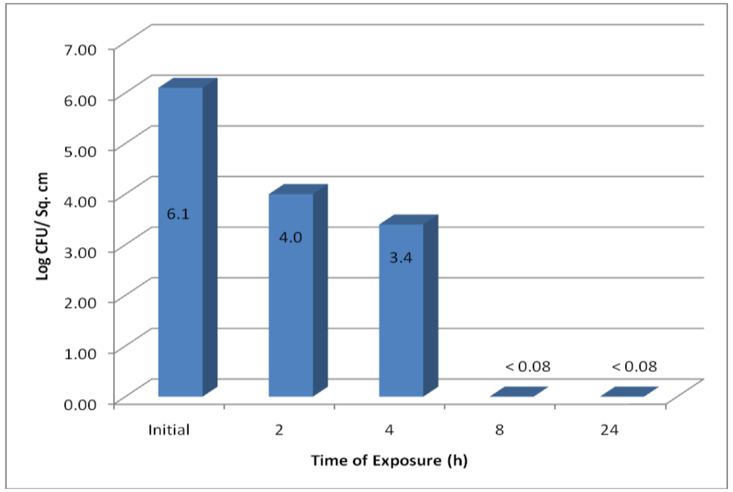
LM Figure: Population (log10 CFU/ sq. cm) of Listeria monocytogenes on Stainless Steel surfaces observed after 0, 2, 4, 8, and 24 h of exposure to Reactive Oxygen Species produced by ROS Reactor.

RESULTS AND DISCUSSION

Figures 1, 2, and 3 show the log10 CFU/ sq. cm. reductions of Methicillin-resistant Staphylococcus aureus, Acinetobacter baumannii, and Listeria monocytogenes on stainless steel surface respectively.

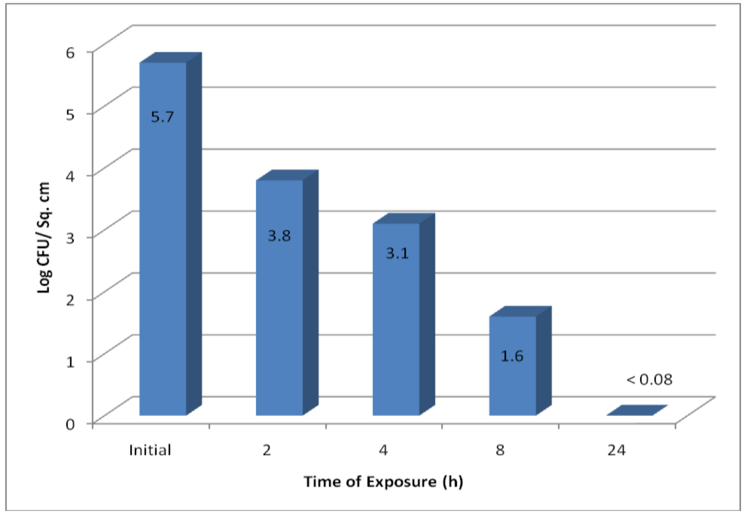
Ozone levels were measured in the test chamber at 0.006 - 0.008 ppm. The ambient level of ozone in the control study was measured at 0.003 ppm. Levels of vaporized Hydrogen Peroxide in the chamber ranged from 0.02 – 0.04 ppm. All of these levels are well below OSHA limits for continuous interaction.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

Staphylococcus aureus control



SA Figure: Population (log10 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.

Acinetobacter baumannii control



AB Figure: Population (log10 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.

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Gas Phase Hydrogen Peroxide Produced by ROS's Tubular Reactor at Various Levels of Relative Humidity

In this study the gas phase Hydrogen Peroxide levels produced by ROS at various levels of relative humidity were evaluated. The ROS modular system produces Reactive Oxygen Species (ROS), including very low levels of Ozone (O3), Vaporized Hydrogen Peroxide (H2O2) and other oxidative compounds. The measurable ROS are H2O2 and O3. The antimicrobial properties of these ROS, including H2O2 and O3 in the air and on surfaces are well established. There is a demonstrated need in the health care industry and in indoor air environments generally for improved environmental sanitation technologies. Since much contamination and risk is associated with airborne contamination of environmental surfaces, a technology that addresses these risks is needed. Previous findings have reported that ROS technology has applications in health care facilities for control of environmental pathogens. As a continuously operating system, it allows for the continuous decontamination of air and environmental surfaces.

EXPERIMENTAL DESIGN:

Testing was conducted in a controlled environmental chamber (Terra Universal, Anaheim, CA) to measure the amount of gas phase H2O2 and O3 produced at 20, 30, 40, 50, and 60% level of relative humidity. Hydrogen Peroxide was measured using a spectrophotometric detector in the ultraviolet (UV) region between 200-400 nm. Measurements were recorded in the UV region because water vapor does not absorb in the UV region, allowing for direct measurement of gas phase H2O2. Ozone levels were measure using a Model 500 Aeroqual (New Zealand). All measurements were recorded as parts per million (ppm).

The system was activated and remained on for a period of one hour prior to measuring hydrogen peroxide levels. This was done to allow the system to reach equilibrium. The average ambient Ozone level measured prior to activation of Tubular Reactor was 0.0033 ppm in all three replications. Ambient ozone levels were also recorded at each humidity level prior to that test and are reflected on the chart below.

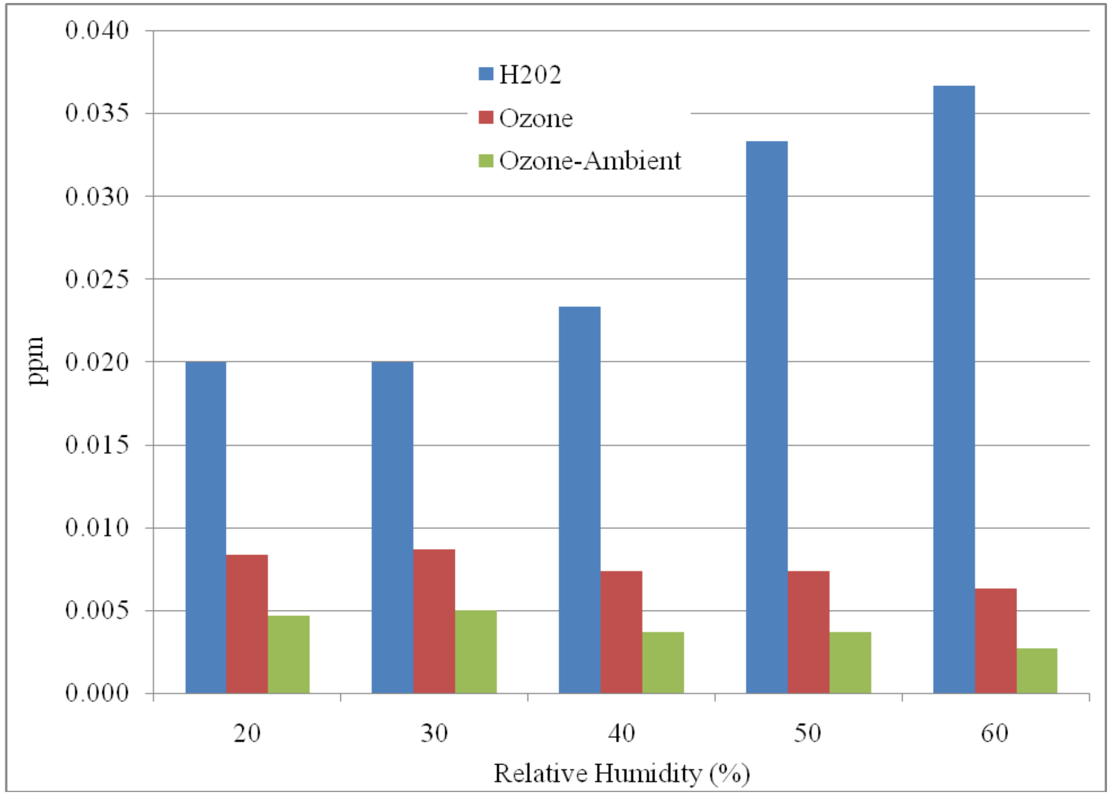

Figure 1: Gas phase Hydrogen Peroxide and Ozone produced (ppm) by ROS's tubular reactor at various levels of Relative Humidity (%).