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] **Dr. James Marsden Kansas State University - August 6, 2008**

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 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 detectable 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 healthcare 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 to 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 have placed a greater burden on health care facilities to find ways to remediate contaminants with limited resources (Chotani et al., 2004). Older and poorly designed buildings may harbor contaminants 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 healthcare 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 that 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 resuspension in 0.1% peptone water (PW). The microbial cultures were combined by species 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°C 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

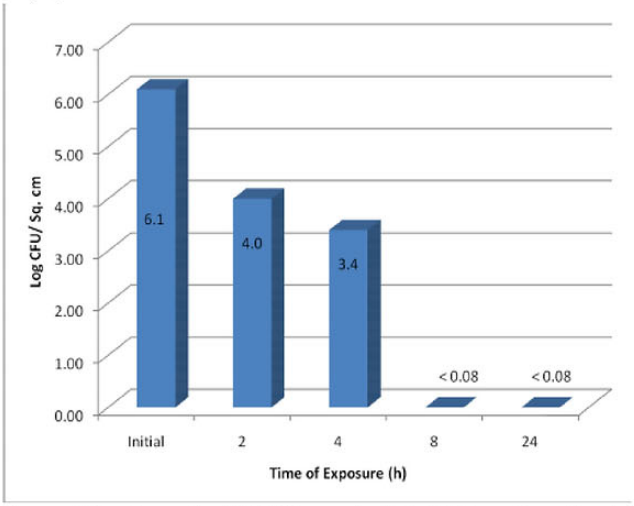

Figure 1: Population (log10 CFU/ sq. cm) of Methicillin resistant *Staphylococcus aureus* on Stainless Steel surfaces observed after 0, 2, 4, 8, and 24 h of exposure to Reactive Oxygen Species produced by ROS Reactor.

Listeria monocytogenes

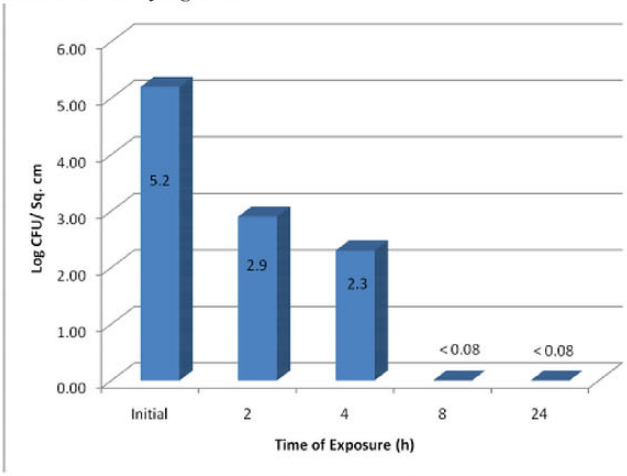

Figure 2: Population (log10 CFU/ sq. cm) of *Listeria monocytogenes* on Stainless Steel surfaces observed after 0, 2, 4, 8, and 24 h of exposure to Reactive Oxygen Species produced by ROS Reactor.

Acinetobacter baumannii

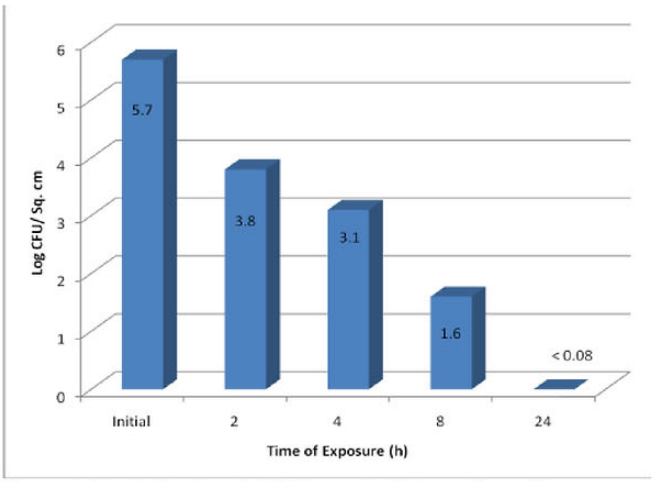

Figure 3: Population (log10 CFU/ sq. cm) of *Acinetobacter baumannii* on Stainless Steel surfaces observed after 0, 2, 4, 8, and 24 h of exposure to Reactive Oxygen Species produced by ROS Reactor.

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 healthcare and other indoor air environments.

Conclusion

Levels of MRSA, Listeria moncytogenes, and Acinetobacter baumannii were brought to non-detectable levels after 24 hours .08 cfg/m2 and considered clean and acceptable.

REFERENCES

BAHNFELTH, W. P. & KOWALSKI, W. J. 2005. Indoor-air Quality: Issues and resolutions. HPAC Engineering: 6-16.

CHOTANI, R. A., ROGHMANN, M., & PERL, T. M. 2004. Nosocomial infections. In N.M.H.Graham, C. Masters, &. K.E.Nelson, (Eds.). Infectious disease epidemiology: Theory and practice. (pp655-673). London: Jones and Bartlett Publishers.

HOTA, B. 2004. Contamination, disinfection, and cross-colonization: Are hospital surfaces reservoirs for nosocomial infection? Clinical Infectious Diseases. 39: 1182-1189.

KOHN, L., CORRIGAN, J., & DONALDSON, M. (1999). To err is human: building a safer health system. Washington, DC: Institute of Medicine, National Academy Press, retrieved May 20, 2005, from http://www.nap.edu/books/0309068371/html/

TILTON, D. 2003. Nosocomial infections: diseases from within our doors. Retrieved May 15, 2005, from http://www.nursingceu.com/NCEU/courses/nosocomial/