



**Evaluation of the Efficacy of a Reflective Electromagnetic Energy System (REME™
RGF Environmental) at Reducing Populations of Methicillin Resistant
Staphylococcus aureus on Stainless Steel Surfaces**

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SUMMARY

Stainless steel coupons were inoculated with Methicillin resistant *Staphylococcus aureus*, and treated by Reactive Oxygen Species produced by a Reflective Electromagnetic Energy System (REME™ RGF Environmental, West Palm Beach, FL). The initial inoculum was 6.7 log CFU/cm² for the treated samples and 6.9 log CFU/cm² for the control. The exposure times were 0, 2, 4, 8, and 24 h. Background ozone and hydrogen peroxide levels were measured in the chamber prior to and after activating the REME Cell and throughout the 24 hour treatment period.

The exposure to oxidative gases, including ozone and vaporized hydrogen peroxide resulted in reductions in Methicillin resistant *Staphylococcus aureus* of 2.3 log CFU/cm² after two hours, 2.5 log CFU/cm² after 4 hours, 2.9 log CFU/cm² after 8 hours

and 4.7 log CFU/cm² after 24 hours. The reductions in the control samples were less than 0.5 log CFU/cm² after 24 hours in the chamber without the REME cell.

This experiment demonstrated the effectiveness of the REME cell at reducing populations of Methicillin resistant *Staphylococcus aureus* (MRSA) on stainless steel surfaces.

INTRODUCTION

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 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 Methicillin Resistant *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 REME Cell 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 the REME Cell which is designed to produce gas phase hydrogen peroxide and very low levels of ozone in reducing populations of Methicillin Resistant *Staphylococcus aureus* on stainless steel surfaces.

MATERIALS AND METHODS

Preparation of Cultures:

Methicillin-resistant *Staphylococcus aureus* (ATCC # 33591) was used for this study. Bacterial cultures 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 (Nuair) at 26°C, 46 % relative humidity (ambient conditions), and exposed to ROS produced by the REME cell 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). Hydrogen peroxide levels were measured using Drager tubes.

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 35°C for 24h.

RESULTS AND DISCUSSION

Table 1 shows the recovery of Methicillin-resistant *Staphylococcus aureus*, on stainless steel surfaces treatment with the REME cell for 0, 2, 4, 8, and 24 hours. The exposure to oxidative gases, including ozone and vaporized hydrogen peroxide resulted in reductions in Methicillin resistant *Staphylococcus aureus* of 2.3 log CFU/cm² after two hours, 2.5 log CFU/cm² after 4 hours, 2.9 log CFU/cm² after 8 hours and 4.7 log CFU/cm² after 24 hours. The reductions in the control samples were less than 0.5 log CFU/cm² after 24 hours in the chamber without the REME cell.

Table 1. Average recoveries (Log CFU/cm²) of Methicillin Resistant *Staphylococcus aureus* on inoculated stainless coupons after treatment with a REME™ Cell for periods of 0, 2, 4, 8, 12 and 24 hours vs. an untreated control.

Sample (Hours)	Treated Samples Methicillin Resistant Staphylococcus aureus Log CFU/cm²	Control Samples Methicillin Resistant <i>Staphylococcus aureus</i> Log CFU/cm²
0	6.70	6.90
2	4.40	6.70
4	4.20	6.50
8	3.80	6.50
24	2.00	6.45

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 0.035 – 0.045 ppm. All of these levels are well below OSHA limits for continuous interaction.

Based on the results of this study, the REME 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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