Use of atmospheric non-thermal plasma as a disinfectant for objects contaminated with methicillin-resistant *Staphylococcus aureus*

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Background: Health care-associated infections because of methicillin-resistant strains of *Staphylococcus aureus* (MRSA) are increasing worldwide despite current infection control measures. Novel methods for disinfection of MRSA would be useful.
Methods: We tested the effectiveness of atmospheric, non-thermal plasma discharge at killing *S aureus*, including USA300 strains, and at disinfecting experimentally contaminated hospital pagers.
Results: Exposure of *S aureus* to plasma at different concentrations and for varying lengths of time resulted in up to a 4- to 5-$\log_{10}$ kill on tryptic soy agar plates within 10 minutes and was not toxic to epithelial cells. USA300 strains of MRSA were more resistant to plasma-based killing than other tested strains. Disinfection of hospital pagers experimentally coated with clinically relevant amounts of MRSA could be achieved in as little as 30 seconds.
Conclusion: Generation of plasma is a promising method for disinfection of objects or surfaces that warrants further study in hospital settings. The USA300 strains of *S aureus* may be more resistant to disinfection than other strains.
Key Words: MRSA; *Staphylococcus aureus*; infection control; plasma; disinfection.

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Staphylococcus aureus is the leading cause of health care-associated infections (HAI).[1] Infections with methicillin-resistant forms of *S aureus* (MRSA) increased approximately 10-fold in the decade between 1995 and 2005 such that an estimated 278,000 to 368,000 hospitalizations for treatment of this organism now occur annually.[1,2] This trend is linked to the emergence of the USA300 and USA400 clones of MRSA. Because the scope and seriousness of this problem have increased despite implementation of numerous infection control measures, there is interest in new approaches and technologies.

Infection control for prevention of MRSA HAIs is typically a layered approach encompassing surveillance, handwashing, barrier precautions, and disinfection of contaminated surfaces and objects.[3] Transmission of MRSA from health care workers to patients is generally considered to occur via contamination of the hands; good hand hygiene practices are considered the cornerstone of any infection control program. Weaker evidence suggests that bacterial contamination of objects in the environment plays a role in transmission, probably through an indirect mechanism by contributing to colonization of health care workers.[4] Disinfection of potentially contaminated surfaces and objects is recommended to reduce this potential risk.[5] Few studies have been done linking contamination of inanimate objects carried by or worn on the health care worker to nosocomial transmission. Concern over the role of clothing, including neckties,[6] in nosocomial transmission has led to calls for changes in dress code for health care workers.[7] The potential for involvement of other commonly carried items, such as hospital badges, beepers, and cell phones, has received relatively little attention.[8]

Most sterilization and disinfection techniques involve exposure to chemical compounds or intense heat for prolonged periods of time.[5] These treatments are not ideal for a number of potential vectors of transmission because of potential damage to the objects being treated (eg, electronics, health care workers' hands) and are impractical for casual treatment of common items. Recently, increased interest in alternative methods of disinfection and sterilization has led to development of techniques using exposure to plasmas to kill bacteria, viruses, and bacterial spores.[9] Plasmas

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are generated by inducing gases to enter an ionized state.[10,11] These plasmas contain short-lived active oxygen species such as ozone, hydroxyl, superoxide, and nitrogen oxides, which can have antimicrobial effects. Because plasmas can be generated by application of an electric current at atmospheric pressure and at room temperature, they are being increasingly considered for disinfection or inactivation of bacteria in situations in which typical sterilization techniques would cause damage such as with fresh produce.[12,13] This technique has been shown to be active against a number of gram-negative and gram-positive organisms including biofilm-forming agents.[9,12-14] We conducted the current study to determine whether sterilization of *S aureus* including clinically relevant MRSA strains was possible using an atmospheric non-thermal plasma discharge apparatus. Furthermore, we tested its efficacy at disinfecting common hospital items such as beepers, which might become contaminated with MRSA.

MATERIALS AND METHODS

Bacterial strains and growth conditions

S aureus strains NRS193 (USA400), LAC (USA300), and Newman were obtained through the Network on Antimicrobial Resistance in *S aureus* (NARSA, NIAID). *S aureus* strain Le Bonheur (LB) was obtained from Dr. Steve Buckingham at the Le Bonheur Children Medical Center in Memphis, TN, and is a USA300 type clinical isolate from a patient with necrotizing pneumonia. *Escherichia coli* strain DH5α was obtained from New England Biolabs, Inc. (Ipswich, MA). Cell cultures of *S aureus* and *E coli* cells were grown at 37 °C on tryptic-soy broth (to late-log phase) and agar plates.

Plasma discharge apparatus

The plasma discharge apparatus was built by author I.A. and generates a non-equilibrium-resistive barrier plasma discharge at atmospheric pressure.[9,10] The plasma is generated between 2 planar electrodes using a low-frequency alternative current (120 V, 60 Hz) fed through a step transformer (output voltage: 15 KV across secondary terminals at 60 mA). The experimental design set up of the plasma reactor consists of 2 electrodes: a bottom and top electrode, which rests in a highly resistive wetted unglazed ceramic barrier. The ceramic barrier is cooled and rendered conductive by using either water or hydrogen peroxide (30%), while ensuring no contact with the inoculated agar plates or test items. The working medium in the plasma reactor is air and traces of water vapor or hydrogen peroxide. The resistive barrier has a resistance of 1 MΩ, which prevents the diffuse discharge from contracting into an arc. A non-conducting frame separates the 2 electrodes creating an air gap of 0.25 inch. The electrode arrangement and rotary blower to circulate the generated ozone and other plasma produced species during plasma discharge are housed in the upper glass compartment. The high-voltage circuitry and plasma reactor, which generates up to 1800 cc of air plasma, is housed in the lower compartment.

Plasma discharge parameters and conditions

All bacteria samples were exposed to an atmospheric pressure plasma discharge in a gas medium of air and residue from water or hydrogen peroxide. The discharged products reached the surface of the specimen through diffusion from the region of discharge aided by a rotary fan, which generated air currents across the top of the plates.

Plasma bactericidal activity

Inocula of *E coli* and various *S aureus* strains were prepared by adding 100 μL of bacterial suspension (ranging from 6.0 to 6.3×10^8 to 6.0 to 6.3×10^1 colony-forming units (CFU)/mL) to tryptic soy agar plates prior to exposure to plasma discharge for times varying by 30-second intervals from 30 seconds to 10 minutes. The percentage ratio of viable bacterial CFU from treated samples was compared with untreated samples after a 48-hour incubation at 37 °C. Each data set represents the mean value plus standard deviation of at least 3 exposure experiments.

Plasma bacterial decontamination from objects

Inocula were prepared by adding 100 μL of bacterial suspension (6.0 to 6.3×10^1 to 6.0 to 6.3×10^2 CFU/mL) to the surface of 1-way pagers and either exposed immediately (wet) or dried at 37 °C (for ~20 minutes) prior to exposure. Before and after treatment, surviving bacteria were isolated from different areas on the surface of the pager (~10% of the initial inoculum was recovered by this method at each time point) using a sterile swab inoculated with sterile saline solution and streaked on tryptic-soy agar plates.

Toxicity tests

In vitro cytotoxic activity of plasma discharge sterilization was determined by viability as measured by the trypan blue dye exclusion assay. Briefly, a continuous cell line of Madin-Darby canine kidney (MDCK) cells was maintained as described.[15] Culture media overlaying 80% confluent cells in 6-well tissue culture plates was removed, and cells were treated with plasma discharge for 0 or 10 minutes without liquid media present. After 10 minutes, fresh culture media was added, and cells were incubated at 37 °C in 5% CO_2. The percentage of cytotoxicity was determined by trypan blue exclusion using the