

## NOTES

# Survival of Nosocomial Bacteria and Spores on Surfaces and Inactivation by Hydrogen Peroxide Vapor<sup>∇</sup>

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**With inocula of 6 to 7 log<sub>10</sub> CFU, most vegetative bacteria and spores tested survived on surfaces for more than 5 weeks, but all were inactivated within 90 min of exposure to hydrogen peroxide vapor in a 100-m<sup>3</sup> test room even in the presence of 0.3% bovine serum albumin to simulate biological soiling.**

Certain nosocomial pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) (4), vancomycin-resistant enterococci (VRE) (3), *Clostridium difficile* (1), and *Acinetobacter* sp. (5) can contaminate hospital surfaces, can survive for extended periods (13), and may not be eradicated by conventional cleaning (1, 4), and surface contamination can contribute to transmission (2, 3, 8, 15). Hydrogen peroxide vapor (HPV) is a sporicidal and mycobactericidal (7, 12) vapor-phase method for the decontamination of surfaces and medical equipment (1, 4). HPV has been shown to inactivate several nosocomial pathogens in situ (1, 4), but no in vitro efficacy data are available for common nosocomial pathogens. We investigated the surface survival of common nosocomial pathogens and the in vitro effectiveness of HPV.

(This work was presented in part at the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, October 2004 [4a].)

Five strains of MRSA and three strains each of VRE, *Acinetobacter* sp., *Klebsiella pneumoniae*, and *C. difficile* were tested. *C. difficile* spore suspensions were prepared by 48-h anaerobic culture of seven anaerobe blood agar plates (Oxoid, Basingstoke, Hampshire, United Kingdom), which were then held aerobically for 7 days before being inoculated into 5 ml of sterile distilled water (SDW). Staining with malachite green indicated the presence of >90% spores. Five milliliters of absolute ethanol was added, and the spore suspension was stored at room temperature. To prepare test discs, overnight broth cultures for vegetative bacteria or *C. difficile* spore suspensions were washed in SDW, and 10- $\mu$ l volumes were air dried on stainless steel discs (Apex Laboratories Inc., Sanford, NC) overnight to achieve recoverable inocula of 6 to 7 log<sub>10</sub> CFU per disc. To investigate the impact of biological soiling, suspensions were air dried for 4 h in 0.3% bovine serum albumin (BSA). To investigate the impact of the reduced inoculum,

suspensions were air dried in SDW for 4 h to achieve recoverable inocula of 5 log<sub>10</sub> CFU per disc. Inoculated discs were sonicated at 60 Hz for 20 min (FS200b ultrasonic bath; Decon Laboratories, Hove, East Sussex, United Kingdom) in 1 ml SDW and enumerated by serial 10-fold dilutions using 10- $\mu$ l volumes; the remaining 990  $\mu$ l of the original volume was poured to detect low concentrations.

To test desiccation resistance, inoculated discs were stored at ambient room temperature and humidity in a laboratory, and viable counts were performed using three discs per strain weekly over 6 weeks. Viable counts were additionally performed for *Enterococcus faecium* NCTC 12204 and a *C. difficile* clinical isolate after 12 weeks of drying.

To test HPV resistance, inoculated discs were placed inside a purpose-built 100-m<sup>3</sup> room sized to simulate a large hospital room or small bay. HPV decontamination using a Clarus R suite (Bioquell UK Ltd., Andover, Hampshire, United Kingdom) was conducted as described previously (7). Discs were removed via an air lock for viable counting after 0, 10, 20, 30, 40, 50, 60, 75, and 90 min. One disc for each organism was removed at each time point, and cycles were repeated three times for each strain.

Relative resistance to drying at ambient temperature (21°C to 27°C) and humidity (40 to 63%) was as follows: *C. difficile* > VRE > MRSA = *Acinetobacter* sp. > *K. pneumoniae* (Fig. 1). *E. faecium* NCTC 12204 and the *C. difficile* clinical isolate had a <3-log reduction in concentration after 12 weeks of drying. There were species and strain differences in survival, but there was no consistent difference between reference and clinical strains of the same species. Other studies previously reported extended survival times for nosocomial bacteria (13, 16). Variation in reported survival times is due partly to species and strain variation but also to differences in experimental conditions including inoculum size, humidity, the suspending medium, and the substrate (11, 18).

The starting temperature and relative humidity ranged from 18°C to 24°C and 30 to 50%, respectively. HPV concentration and relative humidity peaked at levels consistent with the onset of "microcondensation" on surfaces, which is critical for rapid inactivation (7, 17). The relative resistance to HPV was as

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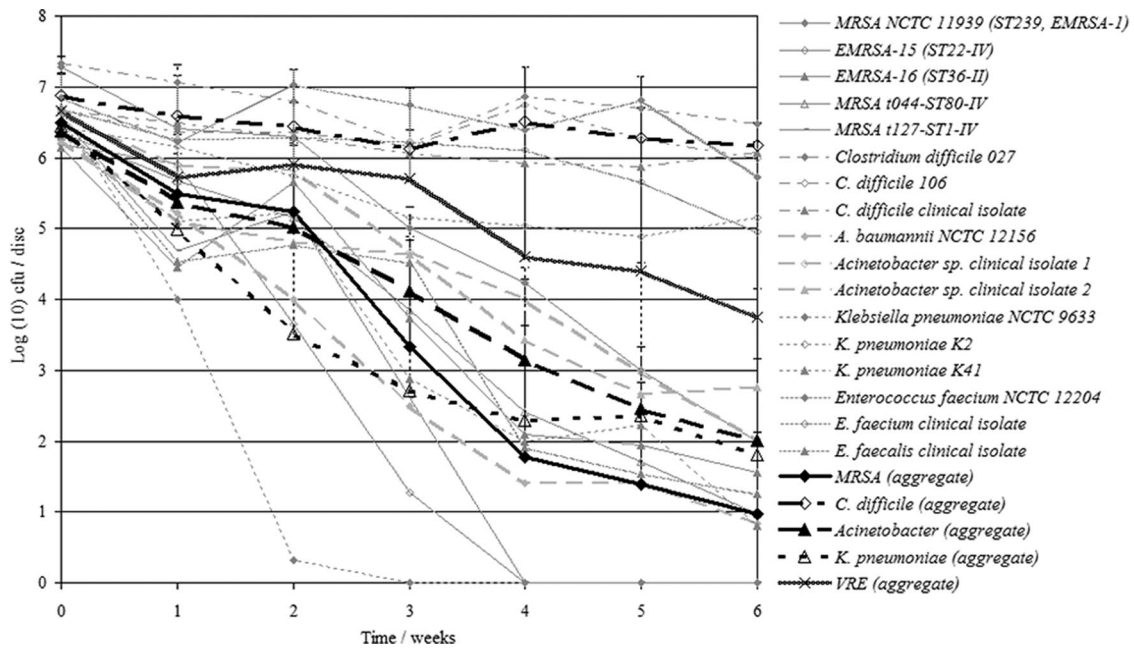


FIG. 1. Resistance of nosocomial bacteria and spores to drying over a 6-week sampling period when exposed to room air at ambient relative temperature and humidity in a laboratory. Black lines indicate aggregates of all data for each species; error bars represent +1 standard deviation. For gray lines, each data point represents a mean of the results obtained with three replicate discs for each strain. EMRSA, epidemic MRSA.

follows: *Acinetobacter* > MRSA = *K. pneumoniae* > *C. difficile* > VRE. All organisms were inactivated by 90 min of exposure to HPV (Fig. 2). Differences in the starting temperature, relative humidity, and inoculum resulted in large standard deviations between cycles (17, 19). VRE, which lack catalase, were inactivated most rapidly, with no organisms being recoverable after

10 min of exposure to HPV, representing a >6-log reduction (data not shown). *C. difficile* spores, which are metabolically inert, were more susceptible to HPV than the catalase-positive bacteria. Hydrogen peroxide is an oxidizing agent, and catalase-peroxidase systems are known to play a key role in bacterial defense against oxidative stress (6, 9, 10). Therefore, the

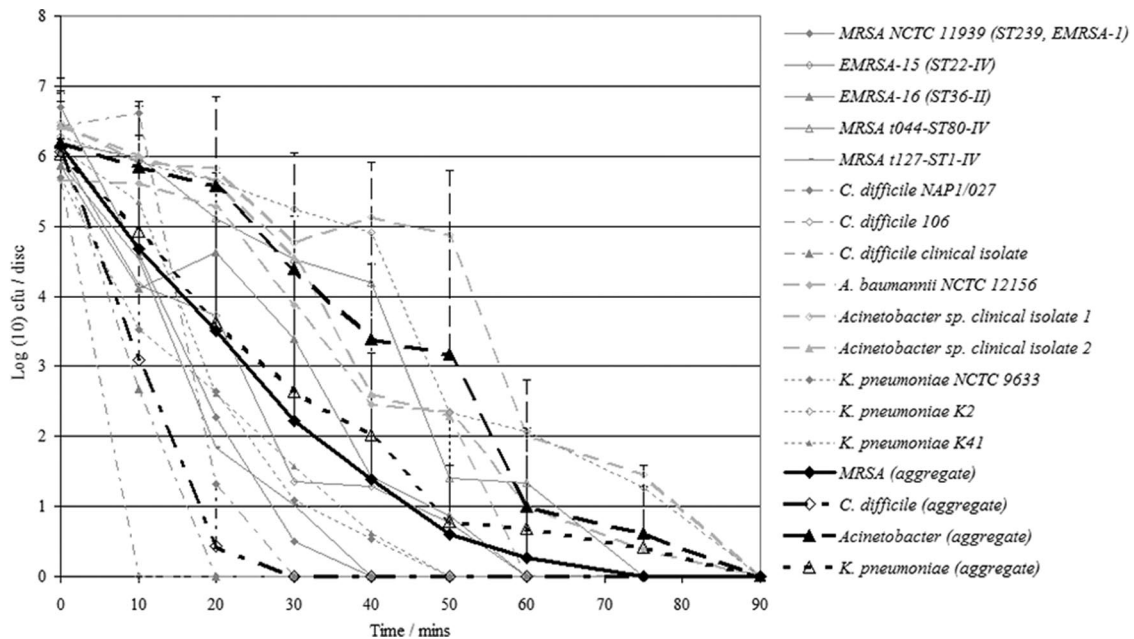


FIG. 2. Resistance of nosocomial bacteria and spores to hydrogen peroxide vapor in a 100-m<sup>3</sup> test room over a 90-min exposure period. Black lines indicate aggregates of all data for each species; error bars represent +1 standard deviation. For gray lines, each data point represents a mean of three replicate runs for each strain. EMRSA, epidemic MRSA.

presence of catalase would appear to account for the relative resistance of these organisms to HPV.

Inocula above  $7 \log_{10}$  CFU per disc were difficult to inactivate, especially for the catalase-positive bacteria (data not shown). In contrast, low inocula of MRSA strain NCTC 19939 ( $5.1 \pm 0.2 \log_{10}$  CFU per disc), *Acinetobacter baumannii* NCTC 12156 ( $5.1 \pm 0.5 \log_{10}$  CFU per disc), and *K. pneumoniae* NCTC 9633 ( $5.0 \pm 0.2 \log_{10}$  CFU per disc) were inactivated within 10 min of exposure to HPV. A concentration of 6 to  $7 \log_{10}$  CFU per disc ( $0.8 \text{ cm}^2$ ) is considerably higher than the concentration of bacteria likely to be encountered in the hospital environment (1, 14).

All strains tested were inactivated within 90 min in the presence of 0.3% BSA: *K. pneumoniae* NCTC 9633 ( $7.4 \pm 0.7 \log_{10}$  CFU per disc) by 90 min, *S. aureus* NCTC 11939 ( $6.9 \pm 0.1 \log_{10}$  CFU per disc) by 50 min, *A. baumannii* NCTC 12156 ( $6.7 \pm 0.4 \log_{10}$  CFU per disc) by 40 min, and *C. difficile* 106 ( $6.4 \pm 0.3 \log_{10}$  CFU per disc) by 30 min. The resistance of organisms dried in BSA and in the low-inoculum experiments cannot be directly compared with those dried in SDW because of differences in the drying times (overnight versus 4 h). As with any other disinfection method, HPV is applied after cleaning, so levels of soiling encountered in the field should be low; nevertheless, HPV has been shown to be effective in rooms that have not been cleaned (4). Further research is required to examine the impact of increased soiling levels on HPV resistance.

In summary, we found that dried inocula of a range of nosocomial pathogens survived on surfaces for several weeks but were rapidly inactivated by HPV in a  $100\text{-m}^3$  room. HPV has a potential role in decontaminating surfaces and equipment contaminated with such organisms.

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