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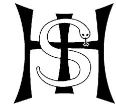

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Efficacy of super-oxidized water fogging in environmental decontamination

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Summary The efficacy of decontamination using Sterilox fog was assessed against meticillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*. Ceramic tiles were inoculated with the test organisms and, once dried, were subjected to Sterilox fogging using a stationary vaporizing machine sited at a distance of 3 m for 10 min and then left for a further hour. In a second experiment using the same organisms, the first 10-min fogging period was followed by a directed fogging period of 30 s at a distance of 1 m. Organisms were cultured from the tiles, plated on to tryptone soya agar and incubated for 48 h. Initial counts of approximately 10^9 colony-forming units/mL for both organisms were reduced approximately 10^4 fold for MRSA and $10^{5.8}$ fold for *A. baumannii* when using a single fogging. The second fogging resulted in $10^{6.8}$-fold reductions for both organisms. Sterilox fog is safe and simple to use, and can reduce levels of nosocomial pathogens by a factor of almost 10^7. It is worthy of clinical evaluation in clinical settings to determine whether it maintains its microbicidal effects against a variety of organisms on different surfaces.
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Introduction

Meticillin-resistant *Staphylococcus aureus* (MRSA), and the means of controlling it, continue to be of major interest to the healthcare community.[1–3] The use of disinfectants to decontaminate hospitals has mixed success in eliminating organisms from the environment, and novel methods of cleaning have been explored previously.[4,5] Various methods of transmission of these organisms have been identified, and many infection control measures have been tried with different degrees of success.[2,6,7]

Decontaminating the clinical environment after a patient has been infected with MRSA, or with

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a multi-resistant Gram-negative bacterium, is thought to be a sensible precaution in stopping nosocomial transmission of these organisms. French *et al.* reported the use of a vaporized disinfectant system that reduced environmental MRSA contamination, although further work is required to determine the effect of environmental decontamination on MRSA infection rates.[4] However, work performed by Wilcox *et al.* indicated that using specific disinfectants when decontaminating hospital wards reduced the incidence of *Clostridium difficile* infection.[8] Whilst the interest of the popular press has focused on MRSA, multi-resistant Gram-negative bacilli have attracted far less interest. *Acinetobacter baumannii* is a Gram-negative coccobacillus that is frequently resistant to virtually all antibiotics.[9] It has been found resident on intensive care units, with reservoirs such as curtains being identified, and can be a particular problem to treat due to its broad antibiotic resistance profile.[10] The financial impact of healthcare-associated infections is well recognized with an increase of inpatient stay and morbidity and mortality, and an estimated cost to the UK National Health Service in the region of £1 billion annually.[11]

Sterilox® is an established disinfectant for heat-labile flexible endoscopes, and has a broad spectrum of activity against mycobacteria, fungi, viruses, bacterial endospores, and Gram-positive and Gram-negative bacteria.[12] Sterilox is sometimes termed 'super-oxidized water' and its principal ingredient is hypochlorous acid, which is safe to use and not harmful to the environment. The present study examined the decontamination efficacy of Sterilox fog against MRSA and acinetobacter dried on to environmental surfaces.

Materials and methods

This study was carried out using two strains of MRSA and two strains of *A. baumannii*. The MRSA strains comprised a clinical isolate (sensitive to fusidic acid, vancomycin, teicoplanin, linezolid, rifampicin and mupirocin, and resistant to erythromycin, trimethoprim and tetracycline) and a type strain (National Collection of Industrial and Marine Bacteria, NCIMB 50143). The acinetobacter strains comprised a clinical isolate (resistant to all commonly used antibiotics except colistin) and a type strain (NCIMB 12457). Two days prior to the study, a pure culture of bacteria was plated on to tryptone soya agar (TSA, bioMérieux, Basingstoke, UK) and incubated at 30–35 °C. A bacterial suspension to 5 McFarland units (equivalent to 10^9 organisms/mL) was prepared and a serial 10-fold dilution to 10^{-7} was made in maximum recovery diluent [MRD, (peptone water), bioMérieux part no. 42076]. The 10^5 to 10^7 dilutions were plated on to TSA, incubated for 48 h, colonies were counted and the initial bacterial concentration was calculated. Ceramic tiles measuring 10 cm × 10 cm were cleaned using detergent followed by 70% isopropyl alcohol, wrapped in aluminium foil and autoclaved at 121 °C for 15 min. Ten drops (100 μL/drop) of bacterial suspension [10^9 colony-forming units (CFU)/mL] were evenly distributed on to 13 tiles ('positive tiles'), and 10 drops (100 μL/drop) of sterile MRD were evenly distributed on to two tiles ('negative tiles') as controls. They were left to dry at room temperature for 2 h.

A Dyna-Fog® Model 2739 Hurricane 'Cold Fog' ULV/Mister (Dynafog, Indianapolis, USA) fogging machine was used for this experiment (capacity 3.8 L, maximum output 19 L/h). The Sterilox solution contained 180 parts per million of available free chlorine at pH 5.2. Five positive tiles were positioned horizontally on a laboratory workbench and five tiles were positioned vertically. Additionally, three positive tiles and the two negative tiles were sealed inside a laminar flow cabinet in the laboratory, which was not in use, to create positive and negative controls inside a sealed environment. The fogging machine was positioned 3 m away with an unobstructed path to the tiles, and was run for 10 min on maximum output. Afterwards, the laboratory was left for 1 h to allow time for the fog to settle and act upon the exposed tiles. In the second modified procedure, tile preparation and fogging were performed as above, but at the end of the 10-min initial fogging, the fogging machine was held approximately 1 m from the tiles and a further 30-s fogging was performed. The tiles received no physical cleaning action, i.e. the tile surface was not wiped in any way during the fogging process. After this stage, it was necessary to dry the laboratory using a mop due to the accumulation of liquid caused by the fogging process.

Each tile was placed in a plastic bag containing 100 mL MRD with 1% sodium thiosulphate for Sterilox neutralization. The tiles were then agitated manually within the bag for 1 min, and serial 10-fold dilutions were made in MRD. Since each tile was eluted into 100 mL MRD, and 1 mL (i.e. 2 × 0.5 mL aliquots) of this was plated neat on to TSA, the limit of detection was 100 organisms/tile, i.e. 1 CFU/mL neat eluate. This process was repeated for the positive controls, and the negative controls were plated using 0.5 mL of neat dilution aliquots alone. The plates were then incubated at 35 °C for two days and colonies were counted (with no growth being equivalent to <100 colonies on the tile; the limit of sensitivity). A neutralization

validation was performed to determine the ability of sodium thiosulphate to neutralize Sterilox residue. This involved adding either 10 mL fogging solution (test) or 10 mL MRD (control) to 100 mL MRD containing 1% sodium thiosulphate, and inoculating both solutions with 1 mL of the 10^4 dilution of each test organism, plating after 1 min and incubating for 48 h to ensure complete microbial recovery.

Results

The experiment was conducted twice, with the standard method used on the first run and the modified method involving the second hand-held fogging on the second run. After each run, the tile elutes were plated on to TSA. Recovery of the organisms from the positive controls demonstrated that the organism remained viable once dried on to the ceramic tiles. Mean recovery from the MRSA controls was 1.0×10^9 CFU/tile on the first fogging run and 1.6×10^9 CFU/tile on the second run for the type strain, and 2.1×10^9 CFU/tile on the first run and 1.37×10^9 CFU/tile on the second run for the clinical isolate. Mean recovery from the acinetobacter controls was 9.5×10^8 CFU/tile and 8.5×10^8 CFU/tile for the first and second runs, and the type strain yielded 4.8×10^8 CFU/tile and 1.4×10^9 CFU/tile for the clinical isolate on its respective fogging runs. The negative controls for all organisms gave no growth.

On the first fogging run, the MRSA type strain (NCIMB 50143) yielded a mean colony count of 3.8×10^4 CFU/tile from the horizontal tiles and 1.8×10^5 CFU/tile from the vertical tiles. The second fogging run showed horizontal and vertical mean yields of 5.2×10^2 CFU/tile and 2.6×10^2 CFU/tile, respectively. The clinical isolate of MRSA gave mean counts of 2.52×10^5 CFU/tile for the horizontal tiles and 5.64×10^5 CFU/tile for the vertical tiles on the first fogging run, and mean counts of 1.4×10^2 CFU/tile for both horizontal and vertical tiles on the second run. The type strain of acinetobacter (NCIMB 12457) gave a mean colony count of 4.6×10^2 CFU/tile from the horizontal tiles and 1.1×10^4 CFU/tile from the vertical tiles on the first fogging run. The second fogging run gave counts of 1.0×10^2 CFU/tile for both horizontal and vertical tiles. The clinical isolate of acinetobacter showed a mean count of 3.4×10^2 CFU/tile for the horizontal tiles and 1.7×10^3 CFU/tile for the vertical tiles on the first run. The second fogging run yielded mean counts of 3.8×10^2 CFU/tile from the vertical tiles and 3.2×10^2 CFU/tile from the horizontal tiles. The results are shown in Table I.

Pooling the results for horizontal and vertical tiles, the type strain of MRSA gave $\log_{10}$ reduction factors of 4.05 and 6.64 for the first and second fogging experiments, respectively. For the clinical isolate of MRSA, the corresponding figures were 3.75 and 6.99. The acinetobacter type strain gave $\log_{10}$ reduction factors of 5.65 and 6.99 for the first and second fogging runs, respectively, and for the acinetobacter clinical isolate, the corresponding figures were 5.85 and 6.57.

Discussion

The use of Sterilox in the fogging studies resulted in a 10^4-fold decrease of the MRSA type strain and a $10^{3.75}$-fold reduction of the MRSA clinical isolate after a single treatment, and a $10^{6.64}$-fold decrease and a $10^{6.69}$-fold decrease of the type strain and the clinical isolate after the two-stage treatment. Acinetobacter strains showed greater reductions after one fogging compared with MRSA ($10^{6.65}$-fold and $10^{5.85}$-fold reductions for the type and clinical strain, respectively). After a second fogging, reductions similar to the MRSA results were observed (Table I). The use of the recommended two-stage fogging treatment, whilst improving efficacy, does involve more user interaction in a clinical setting, which may restrict its clinical application.

Table I $\log_{10}$ mean colony counts of the four fogging experiments

		MRSA type strain	MRSA clinical isolate	Acinetobacter type strain	Acinetobacter clinical isolate
First fogging	Unfogged control	9	9.32	9	8.7
	Horizontal tiles	4.6 (4.40)	5.4 (3.92)	2.7 (6.30)	2.5 (6.20)
	Vertical tiles	5.3 (3.70)	5.75 (3.57)	4 (5.00)	3.2 (5.50)
Second fogging	Unfogged control	9.2	9.14	8.9	9.12
	Horizontal tiles	2.72 (6.48)	2.15 (6.99)	2 (6.90)	2.58 (6.52)
	Vertical tiles	2.41 (6.79)	2.15 (6.99)	2 (6.90)	2.51 (6.61)

Note: 100 colony-forming units/mL was the limit of sensitivity and a result of 2 signifies that no organisms were recovered. Figures in parentheses are $\log_{10}$ reduction factors achieved by fogging. MRSA, meticillin-resistant *Staphylococcus aureus*.

Exner *et al*. showed that spread of *S. aureus* within the environment could result from inadequate cleaning.[13] These authors used a suspension of *S. aureus* of 0.05 mL (3×10^7 CFU/mL) inoculated on to a 5 cm × 5 cm square of floor and then mopped in a U-shape using a variety of cleaning agents. After drying, swabs were taken from the initial square of floor and three adjacent squares of identical size, 7 cm apart, and plated to determine the presence of *S. aureus*. Mops soaked in water, quaternary ammonium compounds or alkylamines showed incomplete killing of the *S. aureus* and caused dissemination throughout the non-inoculated tiles. Only aldehydes and peroxides showed complete killing of the *S. aureus* with no dissemination. Environmental contamination with *A. baumannii* in an intensive care setting suggested that poor cleaning was associated with increased patient colonization.[14] In view of the environment being a potential source of patient contamination, and since the conventional 'mop and bucket' technique appears to risk leaving residual contamination of surfaces, the use of a fogging treatment that may be able to permeate into the various recesses that can be found within most clinical settings (such as behind drawers, within the bed frame etc.) becomes attractive.

This study found that Sterilox is able to reduce the burden of MRSA and acinetobacter on environmental surfaces when fogged. It would clearly be of interest to investigate the activity of Sterilox against other nosocomial pathogens that can persist on surfaces in the clinical environment such as clostridia and enterococci. Organic contamination of the environment is an important consideration of any decontamination process, and the manufacturers of Sterilox emphasize that a thorough cleaning of contaminated areas should be carried out prior to the 60-min disinfectant fogging treatment as part of a biohazard decontamination protocol. The microbiocidal activity of Sterilox in the presence of organic load has been demonstrated in previous work. Selkon *et al*. reported that Sterilox, in a suspension test, was rapidly effective in the presence of 1% horse serum against a variety of organisms including *Escherichia coli*, *Pseudomonas aeruginosa* and MRSA with kill rates comparable to 2% glutaraldehyde.[12] Both agents required a longer contact time in the presence of high organic loading (5% calf serum). Shetty *et al*. reported Sterilox to be equally effective under high and low soil conditions (1% and 5% horse serum, respectively) against four *Mycobacteria* spp. and strains of *Helicobacter pylori*, vancomycin-resistant enterococci and *Candida albicans*.[13,15] However, activity against *C. difficile* spores in the presence of 5% horse serum was diminished.[13] As yet, there has been no work examining the impact of organic matter contamination on a fogging system. The manufacturers recommend that the system should only be utilized at the end of a cleaning process, and in such circumstances, any organic contamination should have been removed. However, further work needs to be carried out to investigate any role of organic contamination of surfaces on the efficacy of Sterilox in these situations. Work is also needed on the use of Sterilox fog in the clinical setting to discover any potential problems of using the fog in a functioning clinical area with respect to the technical aspect of using the system, as well as the resultant liquid residue interfering with clinical appliances.

With the UK Government publishing 'Winning ways', it is clear that infection control is a major public concern, and the cleaning and cleanliness of hospitals remains high on the political agenda.[16] The reductions observed in this study compare favourably with the use of alkylamine compounds, and the safety profile of Sterilox means that it is a good candidate for decontaminating the hospital environment.[14]

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