Efficacy of HOCl fogging treatment in environmental decontamination

Clark J, Barrett S, Stapleton R, Selkon J.

Abstract

We assessed the efficacy of decontamination using HOCl fog against methicillin resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*. Ceramic tiles were inoculated with the test organisms and, once dried, were subjected to HOCl fogging treatment using a stationary vaporising machine sited at a distance of 3 meters for ten minutes and then left for a further hour. In a second experiment using the same organisms, the first fogging (ten minutes at 3 metres distance) was followed by a directed, second fogging of 30 seconds at a distance of 1 meter. Post fogging organisms were cultured from the horizontally and vertically placed tiles, plated onto tryptone soya agar and incubated for 48 hours. Initial counts of approximately 10^9 cfu/ml for both organisms, were reduced approximately 10^4 fold for MRSA and $10^{5.8}$ fold for *A. baumanii* when using a single fogging. The second fogging resulted in $10^{6.8}$ fold reductions for both organisms. HOCl 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 activity against a variety of organisms on different surfaces.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA), and the means of controlling it, continue to be of major interest to the health-care community[1,2,3] Various methods of transmission of these organisms have been identified and a wide variety of infection control measures, including the use of disinfectants, has been tried with different degrees of success[2,4,5, 6,7]. .

Decontaminating the clinical environment after a patient has been infected with MRSA, or with a multi-resistant Gram negative bacterium, is thought to be a sensible precaution in stopping nosocomial transmission of these organisms. Although a recent report by French et al (2004) reported the use of a vaporised disinfectant system reduced environmental MRSA contamination, further work is required to determine the effect of environmental decontamination on MRSA infection rates. However, work performed by Wilcox and co-workers (2004) reported that using specific disinfectants in decontaminating hospital wards reduced the incidence of *Clostridium difficile* infection.

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 cocco-bacillus that is frequently resistant to virtually all antibiotics[8]. It has been found resident in intensive care units with reservoirs such as curtains being identified[9], and can be a particular problem to treat due to its broad antibiotic resistance profile. The financial impact of healthcare associated infections is well recognised with an increase of inpatient stay, morbidity and mortality, and an

estimated cost to the UK National Health Service in the region of £1 billion annually[10].

HOCl is an established disinfectant with a broad range of applications, the most recognisable in the UK being the disinfection of heat labile flexible endoscopes.[11] HOCl has a broad spectrum of activity against mycobacteria, fungi, viruses, bacterial endospores and Gram positive and Gram negative bacteria. HOCl is sometimes termed super-oxidised water and its principal ingredient is pH neutral hypochlorous acid, which is safe to use and not harmful to the environment. The manufacturers of HOCl recommend a two stage fogging treatment[12] as part of a biohazard decontamination protocol. The disinfection stage of the treatment uses a portable handheld fogger and consists of a 10 minute general fog of the environment followed by a shorter targeted fog of all surfaces. The present study examined the decontamination efficacy HOCl of fog against MRSA and Acinetobacter dried onto environmental surfaces.

Materials and Methods

This study was carried out using two strains of MRSA and two strains of *Acinetobacter baumannii*. The MRSA strains comprised a clinical isolate (sensitive to fusidic acid, vancomycin, teicoplanin, linezolid, rifampicin, 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 (sensitive to colistin only) 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) and incubated at 30-35 ^{0}C. A bacterial suspension to 5 McFarland was prepared and a serial tenfold dilution to 10^{-7} was made in maximum recovery diluent (MRD) bioMerieux (Part no. 42076). The 10^{-5} to 10^{-7} concentrations were plated on to TSA and incubated for 48 hours. These plates were counted and the initial suspension concentration (cfu/ml) calculated. 10cm x 10cm ceramic tiles were cleaned using detergent followed by 70% isopropyl alcohol. These were wrapped in aluminium foil and autoclaved at 121^{0}C for 15 minutes. Ten drops (100µl per drop) of bacterial suspension (10^{9} cfu/ml) were evenly distributed onto 13 tiles (Positive Tiles), and 10 droplets (100µl per drop) of sterile MRD on to 2 tiles (Negative Tiles) as controls. They were left to dry at room temperature for two hours.

A Dyna-Fog® Model 2739 Hurricane "Cold Fog" ULV/Mister (supplier Dynafog, Indianapolis USA) was used for this experiment (capacity 3.8 litres, maximum output of 19 litres/hour). Five tiles were positioned horizontally on a laboratory workbench and five tiles positioned vertically. Additionally, three positive tiles and the two negative tiles were sealed inside in a Laminar Flow cabinet that was not switched on, to create positive and negative controls within a sealed environment within the laboratory. The Dyna-fog® was positioned three metres away with an obstruction-free path to the tiles. The fogging machine was run for ten minutes on maximum output. Afterwards the laboratory was left for one hour 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. At the end of the ten-minute initial fogging, the Dyna-fog® was held approximately one metre from the tiles and a further thirty second fogging 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 the laboratory floor near the Dynafog® machine was dried, due to the accumulation of liquid caused by the fogging process.

Each tile was placed in a plastic bag containing 100ml of MRD with 1% sodium thiosulphate for HOCl neutralisation. The tiles were then agitated by hand within the bag for one minute and serial 10-fold dilutions in MRD from each bag were plated onto TSA to a sensitivity of <100 cfu/tile. This process was repeated for the positive controls, and the negative controls were plated using 0.5ml of neat dilution aliquots only. The plates were then incubated at 35ºC for two days and colonies were counted. A neutralisation validation was carried out to determine the ability of sodium thiosulphate to neutralise HOCl residue. This involved adding 10ml of fogging solution to 100ml of MRD containing 1% sodium thiosulphate with a control that replaced HOCl with 10ml of MRD. Both solutions were inoculated with 1ml of 10^4 bacterial suspension of the test organisms and the suspensions were then plated onto TSA and incubated for 48hrs.

Results

Two runs of fogging were undertaken and the tile elutes were plated onto TSA. Recovery of the organisms from the positive controls demonstrated that the organisms remained viable once dried onto the ceramic tiles. Mean recovery from the MRSA controls were 10^9 cfu/tile on the first fogging run and $1.6x10^9$ cfu/tile on the second run for the type strain, and $2.1x10^9$ cfu/tile on the first run and $1.37x10^9$ cfu/tile on the second run for the clinical isolate. Mean recovery from the *Acinetobacter* controls were $9.5x10^8$ cfu/tile and $8.5x10^8$ cfu/tile for the first and second runs, and the type strain yielded $4.8x10^8$ cfu/tile and $1.4x10^9$ cfu/tile for the clinical isolate on its respective fogging runs. The negative controls for all organisms grew zero colonies.

The MRSA type strain yielded a mean colony count of $3.8x10^4$ CFU from the horizontal tiles and $1.8x10^5$ cfu from the vertical tiles on the first fogging run. The second fogging run showed horizontal and vertical yields of 520 cfu/tile and 260 cfu/tile respectively. The clinical isolate of MRSA had mean counts of $2.52x10^5$ cfu/tile for the horizontal tiles and $5.64x10^5$ cfu/tile for the vertical tiles on the first fogging run, and mean counts of 140 cfu/tile and 140 cfu/tile on the second run. The type strain of acinetobacter had a mean colony count of $4.6x10^2$ cfu/tile from the horizontal tiles from the first run and $1.1x10^4$ cfu/tile from the vertical tiles. The second fogging run demonstrated counts of 100 cfu/tile for both horizontal and vertical tiles. The clinical isolate of Acinetobacter showed a mean count of 340 cfu/tile for the horizontal tiles and $1.7x10^3$ cfu/tile for the vertical tiles on the first run. The second fogging run yielded mean counts of 380 cfu/tile from the vertical tiles and counts of 320 cfu/tile from the five horizontal tiles. The mean number of colony forming units per tile into Log_{10} results is shown in Table 1.

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 for the first fogging run and 6.99 for the second, for the acinetobacter clinical isolate, values were 5.85 and 6.57 respectively.

Table 1: Log_{10} mean results for the colony counts of the four organisms

		MRSA Type Strain	MRSA Clinical Isolate	Acinetobacter Type Strain	Acinetobacter Clinical Isolate
	Unfogged control	9	9.32	9	8.7
First fogging	Horizontal tiles	4.6	5.4	2.7	2.5
	Vertical tiles	5.3	5.75	4	3.2
	Unfogged control	9.2	9.14	8.9	9.12
Second fogging	Horizontal tiles	2.72	2.15	2	2.58
	Vertical tiles	2.41	2.15	2	2.51

Note: log 2 was the limit of sensitivity and a result of 2 signifies that no organisms were recovered.

Discussion

The use of HOCl in the fogging studies resulted in a 4 log decrease of the MRSA type strain and a 3.75 log reduction of the MRSA clinical isolate after a single treatment and >log6.5 reduction of both the type strain and clinical isolate after the two stage treatment. Acinetobacter strains showed greater reductions after one fogging compared with MRSA, with a 6.65 and 5.85 log reductions observed for the type and clinical strain respectively. After a second fogging, reductions similar to the MRSA results were observed (Table 1). The use of the recommended two stage fogging treatment, whilst improving efficacy, does require more user interaction in a clinical setting.

Exner *et al*[13] showed transmission of *Staphylococcus aureus* within the environment could result from inadequate cleaning. A suspension of *Staphylococcus aureus* of 0.05ml ($3x10^7$ cfu/ml) was inoculated onto a 5cm by 5cm square of floor and then mopped in a U-shaped direction using a variety of cleaning agents. After drying, swabs were taken from the initial square of floor and three additional squares of identical size 7cm apart and plated to determine the presence of *S. aureus.* Mops soaked in water, quaternary ammonium compounds, or alkylamines showed incomplete killing of the *Staphylococcus aureus* and caused dissemination throughout the non-inoculated tiles. Only aldehydes and peroxides showed complete killing of the *Staphylococcus aureus* with no dissemination. Environmental contamination with *Acinetobacter baumannii* in an intensive care setting suggested that poor cleaning was associated with more patient colonisation[14]. In view of the environment being a potential source of patient contamination, and since the conventional "mop and bucket" technique appears to run the risk of leaving residual contamination of surfaces, then the use of a fogging treatment, which is able to permeate into all the various recesses that can be found within most clinical settings (such as behind drawers, within the bed frame etc) becomes attractive. The manufacturers of HOCl

currently recommend a room decontamination protocol that combines a cleaning phase along with a two stage disinfectant fogging treatment.

This study has shown that HOCl when fogged is able to significantly reduce the burden of MRSA and *acinetobacter* on environmental surfaces. It is recommended that further work is undertaken on the activity of HOCl against other common clinical environmental organisms such as Clostridium and Enterococcus species. Additionally, the use of HOCl fog should be evaluated in the clinical setting to discover any potential challenges of using the fog in a functioning clinical area.

With the UK Government publishing 'Winning Ways'[15], it is clear that infection control is a major public concern and the cleaning and cleanliness of hospitals remains high on the clinical and political agenda. The reductions observed in this study compare favourably to the use of alkylamine compounds[13] and the safety profile of HOCl means that it is a good candidate for decontaminating the hospital environment.

References

1. Barrett SP, Simmons NA. Controlling MRSA: What is the way forward? *J Hosp Infect* 2005;**59**:170-171.
2. Cooper BS, Stone SP, Kibbler CC, Cookson BD, Roberts JA, Medley GF, Duckworth G, Lai R, Ebrahim S. Isolation measures in the hospital management of methicillin resistant Staphylococcus aureus (MRSA): systematic review of the literature. *BMJ* 2004;**329**:533-540.
3. Farrington M, Redpath C, Trundle C, Brown N. Controlling MRSA. *J Hosp Infect* 1999;**41**:251-4.
4. French GL,Otter JA, Shannon KP, Adams NMT, Watling D, Parks MJ. Tackling contamination of the hospital environment by methicillin-resistant *Staphylococcus aureus* (MRSA): a comparison between conventional terminal cleaning and hydrogen peroxide vapour decontamination. *J Hosp Infect* 2004;**57**:31-37.
5. Johnston MD, Lawson S, Otter JA. Evaluation of hydrogen peroxide vapour as a method for the decontamination of surfaces contaminated with Clostridium botulinum spores. *J Microbiol Methods* 2005;**60**:403-11.
6. Ribner BS, Landry MN, Gholson GL. Strict versus modified isolation for prevention of nosocomial transmission of methicillin-resistant Staphylococcus aureus. *Infect Control* 1986;**7**:317-320.
7. Rayner D. MRSA: an infection control overview. *Nurs Stand* 2003 Jul 23-29;**17**:47-53.
8. Livermore DM. The threat from the pink corner. *Ann Med* 2003;**35**(4):226-34.
9. Das I, Lambert P, Hill D, Noy M, Bion J, Elliott T. Carbapenem-resistant Acinetobacter and role of curtains in an outbreak in intensive care units. *J Hosp Infect* 2002;**50**:110-114.

10. Public Health Laboratory Service: Plowman, Rosalind et el. The socio-economic burden of hospital acquired infection: executive summary. Dept of Health; 1999.
11. Selkon JB, Babb JR, Morris R. Evaluation of the antimicrobial activity of a new super-oxidized water, Sterilox®, for the disinfection of endoscopes. *J Hosp Infect* 1999;**41**:59-70.
12. Sampson M, Rogers M. Sterilox Room Remediation Fogging Protocol. Company Technical Note Ref Nos Food M0003.
13. Exner M, Vacata V, Hornei B, Dietlein E, Gebel J. Household cleaning and surface disinfection: new insights and strategies. *J Hosp Infect* 2004;**56** Suppl 2:S70-75.
14. Denton M, Wilcox MH, Parnell P, Green D, Keer V, Hawkey PM, Evans I, Murphy P. Role of environmental cleaning in controlling an outbreak of Acinetobacter baumannii on a neurosurgical intensive care unit. *J Hosp Infect* 2004;**56**:106-110.
15. Chief Medical Officer. Winning Ways: working together to reduce healthcare associated infection in England. London: Department of Health; 2003