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Decontamination with vaporized hydrogen peroxide is effective against *Mycobacterium tuberculosis*

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ABSTRACT

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Aims: To determine the efficacy of room fumigation with vaporized hydrogen peroxide (VHP) in decontamination of viable *Mycobacterium tuberculosis*.

Methods and Results: About 8×10^4–$2{\cdot}3 \times 10^6$ CFU of *M. tuberculosis* H37Rv and *M. tuberculosis* Beijing were dried in 10-μl drops in tissue culture plates, placed in steam-permeable Tyvek pouches and distributed on laboratory surfaces. The room was exposed to VHP delivered by air conditioning. Different exposure conditions were tested. Exposure to VHP resulted in sterilization of the bacterial samples in three different test runs.

Conclusions: VHP treatment is an effective means of reducing and eliminating room contaminations of *M. tuberculosis*.

Significance and Impact of the Study: Fumigation with VHP represents an alternative to formaldehyde fumigation, particularly for decontamination of animal rooms in tuberculosis research laboratories.

Keywords: vaporized hydrogen peroxide, disinfection, animal room, room decontamination, inactivation.

INTRODUCTION

Mycobacterium tuberculosis is a major cause of morbidity and mortality worldwide, and is becoming a greater concern due to the development of multidrug resistant strains (World Health Organization 2004). The organism is primarily transmitted by aerosols (e.g. produced by patient coughing) and inhalation. *Mycobacterium* species present a unique challenge to biodecontamination in hospitals and research facilities, because of their intrinsic resistance to disinfection and fumigation processes. This is primarily because of their unique, lipophilic cell wall structure (Besra and Chatterjee 1994; Brennan and Nikaido 1995; Daffé and Draper 1998).

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Potentially contaminated clinical and research laboratories must therefore be equipped with suitable room decontamination systems to complement wipe-down techniques for surface and material disinfection. Traditional room fumigation with formaldehyde, however, is disrupting, toxic, carcinogenic and cost-intensive. Automated vaporized hydrogen peroxide (VHP) fumigation currently provides an alternative to formaldehyde sterilization increasingly applied for decontamination of enclosed environments such as animal rooms (Krause *et al.* 2001). As hydrogen peroxide readily decomposes to form water and oxygen, VHP possesses none of the environmental risks associated with formaldehyde. The broad spectrum efficacy of VHP has been shown against a wide range of micro-organisms over the last decade, including bacteria, viruses, fungi and bacterial spores (Klapes and Vesley 1990; Block 1991; Heckert *et al.* 1997; Kokubo *et al.* 1998). The objective of this study was to determine the efficacy of VHP fumigation in eliminating contaminations of the laboratory strain *M. tuberculosis* H37Rv and a clinical isolate of the *M. tuberculosis* Beijing/W genotype family in a model animal room.

MATERIALS AND METHODS

Sample preparation and enumeration

Mycobacterium tuberculosis Beijing/W was kindly provided by Dr D. van Soolingen, National Mycobacteria Reference Laboratory, Bilthoven, the Netherlands. Two derivatives of the widely used laboratory strain genotype H37Rv were included: the bacteria used in our laboratory (MPIIB) and H37Rv from the laboratory of Prof. W. Jacobs, Albert Einstein College of Medicine, Bronx, New York, USA. Bacteria were grown to stationary phase in Middlebrook 7H9 broth (Difco, Detroit, MI, USA), containing 0·05% Tween-80 and supplemented with albumin-dextrose-catalase. For each genotype variant, 10 μl drops of bacterial culture containing 6×10^4–1×10^7 CFU were applied in duplicate into 24-well tissue culture plates (Becton Dickinson, Oxford, UK), and left with open lids in the safety cabinet for approx. 30 min until the drops appeared dry. Initial bacterial seeding density was determined by plating dilutions of the droplet resuspended in sterile phosphate-buffered saline (PBS) onto 7H11 agar plates immediately after drying. Control plates were sealed with parafilm to prevent excessive drying, left in the safety cabinet (in a different room) throughout the duration of sample exposure and plated simultaneously to the exposed samples. For exposure, the tissue plates were placed into Tyvek pouches (Vis-U-All II*reg*: Steris Ltd/DuPont, Mentor, OH, USA), distributed in the test room and the lid was removed to permit access of the VHP to the wells through the steam-permeable Tyvek material. After exposure, the exposed samples were diluted in PBS supplied with 0·05% Tween-80, plated on Middlebrook 7H11 agar plates supplemented with 10% albumin-dextrose-catalase (Difco) and CFU were counted after incubation at 37°C for 28 d.

Room decontamination

The experiments were conducted in a 64·5 m^3 (19·5 m^2 surface, 3·30 m height) air-conditioned laboratory room containing a safety cabinet, a bench and a cupboard. Room temperature was within the range of 20–22°C with relative humidity at approx. 40%. VHP fumigation was performed by means of a VHP®1001 decontamination system (Steris), which has been described in detail elsewhere (Krause *et al.* 2001). Briefly, it is a compact mobile unit that produces VHP and delivers it into an enclosed environment in a closed circuit which was established by connecting the VHP decontamination system to the inlet and outlet air lines of the air-conditioning system supplying the test room. The air inlet and outlet ports were provided with ventilation filters (Camfil Farr, Boras, Sweden) located at the ceiling of the room. Aeration was therefore carried out by the room ventilation system (Fig. 1). The decontamination cycle consisted of four phases: dehumidification, conditioning, decontamination and aeration. During dehumidification the relative humidity is reduced to approx. 30% by circulation of the air in a closed loop. During the conditioning and decontamination phases, VHP levels in the room were raised and maintained at the indicated levels by vaporization of 35% liquid hydrogen peroxide (Steris) and introduced into the circulating air system. It was very important to ensure (as controlled by the system) that the VHP concentration be kept below the condensation point; condensation (from the gas to a liquid phase) of hydrogen peroxide can be damaging to surfaces and presents safety risks in particular in room decontamination. Finally, the aeration phase allowed for complete decomposition of the hydrogen peroxide by recirculation through a destroyer. The parameters for each phase were developed specifically for the room, taking into consideration the ambient temperature and humidity. The three cycles and their individual parameters are listed in Table 1. The level of VHP in the room was monitored throughout the duration of the cycle using a Guided wave NIR H_2O_2 sensor, ensuring that VHP levels were maintained at 0·4 mg l^{-1} and dropped during the aeration phase. Two oscillating stand fans (SMC Networks) were placed in the room and switched on during exposure to allow even air distribution. On completion of the cycle, levels of VHP were monitored for safe re-entry (<1 ppm) into the room. Bacterial samples containing either both *M. tuberculosis* H37Rv and *M. tuberculosis* Beijing/W or *M. tuberculosis* H37Rv (MPIIB) only were placed at different sites throughout the room as shown in Fig. 1. The door was sealed with tape to prevent evasion of hydrogen peroxide.

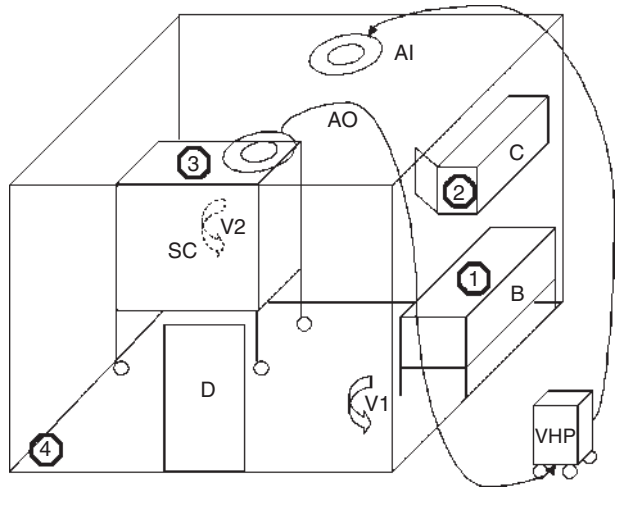

Fig. 1 Room setup for decontamination. AI, air inlet; AO, air outlet; B, bench; C, open cupboard; D, door; SC, safety cabinet; V1, V2, ventilators located in the front right and left rear corner of the room. Sample positions indicated by numbers were as follows: 1, on the bench surface; 2, inside an opened cupboard; 3, on top of the safety cabinet beneath the ceiling; 4, on the floor in a corner of the room

Table 1 Cycle parameters used for the exposure of *Mycobacterium tuberculosis* to vaporized hydrogen peroxide

	Run 1	Run 2	Run 3
Dehumification			
Air flow (m^3 h^{-1})	32	36	36
Absolute humidity (%)	30	30	30
Time (min)	20	20	20
Conditioning			
Air flow (m^3 h^{-1})	32	36	Conditioning phase
Injection rate (g min^{-1})	8·9	12	was not included
Time (min)	20	15	in this cycle
Decontamination			
Air flow (m^3 h^{-1})	32	36	36
Injection rate (g min^{-1})	7·2	4·7	9
Time (min)	60	90	105
Aeration			
Air flow (m^3 h^{-1})	32	36	36
Time (min)	270	240	240

Chemical and biological indicators

Chemical (VHP indicator, Steris Ltd) and biological indicators consisting of 5×10^5 spores of *Bacillus stearothermophilus* on a metal support (HMV 091, Apex Laboratories, NC, USA) were placed throughout the room and at different levels above the floor to monitor VHP exposure. They were also placed inside the Tyvek pouches with the test plates. On cycle completion chemical indicators were evaluated by colour change, and biological indicators were inoculated in growth media (CASO medium, Merck, Germany) at 55°C for 7 d, and growth was determined visually by observing turbidity. Viability of the biological indicators was confirmed by incubating nonexposed positive control samples.

RESULTS

The resistance of *M. tuberculosis* to VHP fumigation was tested in a model animal room. Bacterial genotypes tested include the clinical isolate and laboratory strains, *M. tuberculosis* Beijing/W and *M. tuberculosis* H37Rv respectively. As phenotypic variability among derivatives of the latter isolate have been observed, we included a variant of H37Rv obtained from another laboratory (NY) in addition to the one used in our laboratory (MPIIB). The results of three VHP fumigation exposure runs are shown in Tables 2–4. No viable mycobacteria were recovered after

Table 2 Total bacterial counts per well after vaporized hydrogen peroxide fumigation run 1

Sample location*	Genotype	Bacterial count (CFU/10 μl)	Average bacterial count (CFU/10 μl)
Unexposed control	H37Rv (MPIIB)	Well 1 = $2·8 \times 10^5$	$2·3 \times 10^6$
		Well 2 = $4·3 \times 10^6$	
	H37Rv (NY)	Well 1 = $6·0 \times 10^5$	$6·0 \times 10^5$
		Well 2 = $6·0 \times 10^5$	
	Beijing/W	Well 1 = $1·0 \times 10^5$	$8·0 \times 10^4$
		Well 2 = $6·0 \times 10^4$	
On bench middle room (1)	H37Rv (MPIIB)	Well 1 = 0	0
		Well 2 = 0	
	H37Rv (NY)	Well 1 = 0	0
		Well 2 = 0	
	Beijing/W	Well 1 = 0	0
		Well 2 = 0	
Top of safety cabinet (3)	H37Rv (MPIIB)	Well 1 = 0	0
		Well 2 = 0	
	H37Rv (NY)	Well 1 = 0	0
		Well 2 = 0	
	Beijing/W	Well 1 = 0	0
		Well 2 = 0	
Bottom left corner (4)	H37Rv (MPIIB)	Well 1 = 0	0
		Well 2 = 0	
	H37Rv (NY)	Well 1 = 0	0
		Well 2 = 0	
	Beijing/W	Well 1 = 0	0
		Well 2 = 0	

*Locations of exposed samples are numbered according to Fig. 1. Controls were left in a safety cabinet in a different room during exposure and plated simultaneously with the corresponding samples.

Table 3 Total bacterial counts per well after vaporized hydrogen peroxide fumigation run 2

Sample location*	Genotype	Bacterial count (CFU/10 μl)	Average bacterial count (CFU/10 μl)
Unexposed control	H37Rv (MPIIB)	Well 1 = $2{\cdot}9 \times 10^5$ Well 2 = $4{\cdot}0 \times 10^5$	$3{\cdot}4 \times 10^5$
	H37Rv (NY)	Well 1 = $1{\cdot}5 \times 10^5$ Well 2 = $1{\cdot}2 \times 10^5$	$1{\cdot}3 \times 10^5$
	Beijing/W	Well 1 = $4{\cdot}0 \times 10^5$ Well 2 = $1{\cdot}4 \times 10^5$	$2{\cdot}7 \times 10^5$
On bench middle room (1)	H37Rv (MPIIB)	Well 1 = 0 Well 2 = 0	0
	H37Rv (NY)	Well 1 = 0 Well 2 = 0	0
	Beijing/W	Well 1 = 0 Well 2 = 0	0
Bottom left corner (4)	H37Rv (MPIIB)	Well 1 = 0 Well 2 = 0	0
	H37Rv (NY)	Well 1 = 0 Well 2 = 0	0
	Beijing/W	Well 1 = 0 Well 2 = 0	0

*Locations of exposed samples are numbered according to Fig. 1. Controls were left in a safety cabinet in a different room during exposure and plated simultaneously with the corresponding samples.

Table 4 Total bacterial counts per well after vaporized hydrogen peroxide fumigation run 3

Sample location*	Genotype	Bacterial count (CFU/10 μl)	Average bacterial count (CFU/10 μl)
Unexposed control	H37Rv (MPIIB)	Well 1 = $3{\cdot}1 \times 10^6$ Well 2 = $1{\cdot}3 \times 10^6$	$2{\cdot}2 \times 10^6$
On bench middle room (1)	H37Rv (MPIIB)	Well 1 = 0 Well 2 = 0	0
Inside cupboard (2)	H37Rv (MPIIB)	Well 1 = 0 Well 2 = 0	0
Top of safety cabinet (3)	H37Rv (MPIIB)	Well 1 = 0 Well 2 = 0	0
Bottom left corner (4)	H37Rv (MPIIB)	Well 1 = 0 Well 2 = 0	0

*Locations of exposed samples are numbered according to Fig. 1. Controls were left in a safety cabinet in a different room during exposure and plated simultaneously with the corresponding samples.

any of the fumigation experiments. Bacterial numbers in nonexposed samples ranged between $6{\cdot}0 \times 10^4$ and $4{\cdot}3 \times 10^6$ bacteria per well. These numbers corresponded to 7–44% of the initial seeding amounts, which were assessed by immediate plating of the 10 μl drops after seeding and drying (data not shown). Thus the viable control bacteria in the safety cabinet were reduced by 0·35–1·1 log values during the exposure time, depending on the duration of the fumigation run; this was most likely due to desiccation. However this reduction was insignificant in comparison with the more than 6 log value reduction observed in the exposed samples. All chemical indicators, including those within Tyvek pouches, had changed colour equally. All exposed biological indicators, including those placed within Tyvek pouches, demonstrated no turbidity after 7 d incubation and therefore 100% kill. The results of the indicators demonstrated the efficiency of VHP dispersal within the room.

DISCUSSION

Hydrogen peroxide (H_2O_2) acts as a disinfectant by producing reactive oxygen species (hydroxyl radicals,

superoxide anions), which attack essential cell components such as DNA, lipids, and proteins. Increased microbial resistance to hydrogen peroxide depends largely on the production of superoxide dismutase, catalase and other peroxidases. *In vitro* studies investigating the resistance of *M. tuberculosis* to oxidative stress demonstrated that low hydrogen peroxide concentrations between 10 and 100 mmol l^{-1}, which corresponds to 0·034–0·34%, can kill the bacteria (Dussurget *et al.* 2001; Edwards *et al.* 2001). Microbial resistance to oxidative stress is overcome by the higher concentrations of oxidative agents used for disinfection and sterilization (McDonnell and Russell 1999). Accordingly, hydrogen peroxide concentration in disinfectants for surface wiping is commonly 3%. Hydrogen peroxide is a more effective antimicrobial agent in the gaseous form in comparison with the liquid (Block 1991). The VHP fumigation system described in this report used a 35% hydrogen peroxide solution to generate and maintain the 'dry' vapour within the room at 0·4 mg l^{-1}. Our finding that VHP efficiently killed surface-exposed dry *M. tuberculosis* indicates that VHP fumigation represents an alternative to formaldehyde fumigation, particularly for decontamination of animal rooms in tuberculosis research laboratories. It should be noted that the typical area that can be decontaminated will depend on the delivery system used; for example, the VHP1000 unit has been used to decontaminate areas up to 230 m^3, but multiple units can be used to decontaminate larger areas. The parameters for each decontamination phase must then be developed accordingly. Ongoing studies will determine whether decontamination levels observed under the parameters defined in this study meet clinical standards for sterilization of *M. tuberculosis* contaminations.

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