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Cost-Effective Decontamination of Conventional Laboratory Animal Rooms Using Hydrogen Peroxide Aerosols

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Abstract

Conventional animal facilities lack HVAC systems with HEPA filters and therefore pose great hurdles in maintaining the pathogen-free status of macro-environment of the animal rooms. Even in places where HVAC has no HEPA filters, maintaining clean air in the animal room is a challenge. Production of healthy animals for biomedical research is a key objective of any laboratory animal facility. There are several factors which can contribute to achieve this goal, and macro-enviornment is one of them. Decontamination of animal rooms eliminates the pathogens from the room. Chemical liquids and vapours are mainly used as decontaminating agents. Vaporised Hydrogen Peroxide (VHP) technology is being widely used for this purpose. For VHP technology, motorised equimpents are required which are very expensive. Small animal facilities can not afford to buy expensive VHP equipments. Therefore, the present study was planned to find out cost effective alternative for expensive equipments. Fine mist of working solution Huwa-San- TR50 was sprayed over the walls using a domestic Vacuum Cleaner. Effectnivness of decontamination procedure was conducted by microbiological examination of air and surface monitoring with swab methods after 15 mins. of contact time. Our results suggested that after decontamination, there was significant reduction in microbiological load from animal rooms. Hence, alternatively, facilities with tight annual budget can use this technique to limit the microbiological load in animal facilities in cost effective manner.

Introduction

Production of healthy laboratory animals depends on several imperative factors such as macro- and micro-environmental conditions, nutrition, water quality, managemental practices, etc. Room environmental conditions, also called macro-environmental conditions, play a significant role in producing healthy animals. Macro-environment is controlled by the Heating, Ventilation, and Air Conditioning (HVAC) system. For the production of Specific Pathogen Free (SPF) animals, many facilities install High Efficiency Particulate Air (HEPA) filters at the terminal end of HVAC systems which blow 99.9% clean air inside the animal rooms. However, recurring cost of replacement of HEPA filters is very high. Alternatively, facilities adopt barrier housing systems using positive pressure isolators or Individually Ventilated Caging (IVC) systems to reduce this cost burden.

Non-filtered air through the HVAC system without HEPA filters increases the overall microbial load inside the animal rooms, compromising the health status of laboratory animals. To overcome this issue, facilities carry out regular decontamination of the animal rooms using chemical liquids and vapors as a decontaminating agent. A wide range of liquid-based detergents and disinfectants are currently being used for environmental cleaning, which includes Chlorine based compounds like sodium hypochlorite, quaternary ammonium compounds, alcohol-based compounds, Glutaraldehyde, Iodine/ phenol-based products, proxygene compounds like Hydrogen peroxide (Devan et al., 2018). Efficacy of each disinfectant varies as per the activities. Majorly of them are bactericidal, virucidal, sporicidal and fungicidal in action.

Room fumigation is conventionally being conducted by gas produced in combination of formaldehyde and potassium permanganate. However, it cannot be used in the animal rooms where animals are housed in the proximity. Moreover, formaldehyde has been classified as toxic and carcinogenic for rodents and humans (Swenberg et al., 2013).

Peroxygen compound, Hydrogen Peroxide (H^2O^2) provides an alternative to formaldehyde fumigation because of its high biological efficacy against various microorganisms (Fichet et al., 2004; Quilez et al., 2005). Hydrogen peroxide (H^2O^2) is a

transparent, colourless and odourless disinfectant liquid which is missible in any ratio with water. H^2O^2 easily decomposes to form water and oxygen. H^2O^2 is environmentally friendly and relatively low in toxicity which acts as a strong oxidizer causing cell death or making them inactive within a short contact time (Ingraham and Fleischer, 2003). Hydrogen peroxide vaporizor system produces vapours of hydrogen peroxide that is dispersed throughout the area to be disinfected (Otter et al., 2011). For vaporization of H^2O^2, dedicated automated systems are being widely used. There are many automated systems available in the market. The cost of procurement of an automated system is very high. Facilities whose annual budget does not allow buying such expensive automated vaporizers face great difficulty in decontamination of animal rooms. It is an urgent need for such facilities to find out other ways of cost-effective decontamination of animal rooms.

The current study was conducted in the Laboratory Animal Facility, Advanced Centre of Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai to assess the cost-effective way of decontamination of animal room wall surfaces using dry and wet vacuum cleaner machine.

Materials and methods

Animal vivarium overview

Laboratory Animal Facility (LAF) of ACTREC https://actrec.gov.in/index.php/cri-research-support-facility-detail/70 has over 15,000 Square feet (1394 Square meter) area spread over two floors. First floor is occupied exclusively by laboratory animals of different species housed in individually ventilated cages as well as conventional housing system. Twenty-one rooms are available on the first floor, out of which laboratory animals occupy 15 rooms, 2 rooms for *in vivo* imaging, 1 Assisted Reproductive Technology (ART) laboratory, 1 procudure room, 1 water bottle washing/ re-filling room and 1 clean material storage room. Animal room walls are made up of cement concrete which are painted with antifungal washable paint. The entry and exit are strictly followed through clean and service corridors, respectively. The size of the animal room is ~150 sq. feet. All animal rooms are environmentally controlled with a dedicated HVAC system with positive pressure but without HEPA filters. Temperature and humidity are maintained between 22-25^{o}C and 40-70%, respectively, with 12-15 air changes per hour. Light and dark cycle of 12:12 hrs each is maintained using digital timers. Quarantine room is located at the ground floor entrance.

Environmental Monitoring

ACTREC Animal Facility routinely conducts environmental monitoring for the presence of microbial load inside the animal rooms. The microbial monitoring is conducted according to Standard Operating Procedure (SOP) laid down for airborne microbial examination and surface monitoring with the swab method. In brief, for checking the airborne microbial load, two 60 mm Nutrient agar (NA) plates were exposed near the front (Entry) and backside (Exit) doors of the rooms for minimum of 30 mins. The Surface monitoring with the swab method is conducted by wiping the sterile normal saline wet cotton swab over two randomly selected walls of the room. Later, direct plating was done by streaking the cotton swab over nutrient agar plates under the laminar hood. The agar plates were observed for growth of total number of colonies after 48 hrs of incubation at 37°C. The microbial load was graded as per the number of colonies formed on the agar plates. Considering nature of our Animal Facility, the grades were classified into '*no contamination'* (0 colonies), '*mild*' (0-10 nos.), '*moderate*' (10-20 nos.) and '*severe*'/ '*too numerous to count*' (TNTC) (>20 nos.).

Decontamination of Animal Rooms

We used Huwa-San- TR50 (Roam Technology, Belgium), fig. 1, a commercial disinfectant containing an active substance, Hydrogen Peroxide, 49.0-49.9% (w/w) and colloidal silver (0.026-0.033%) as a stabilizer. It is effective bactericidal, fungicidal, viricidal and algaecidal. We used 1% working solution after dilution as per manufacturer's instructions. The concentration was decided based upon our previous standardization studies carried out using various concentrations. Animal rooms were vacated by transferring the animal in another environmentally controlled backup room. Fine mist of working solution was sprayed over the walls using a domestic vacuum cleaner (Model Euroclean, wet and dry, Eureka Forbes Ltd., Mumbai), fig. 2. Working solution was sprayed by housekeeping personnel after taking all the precautionary measures like wearing safety goggles, face cover, hand gloves, N95 mask, personal protective equipment, etc. Dampers of the inlet and exhaust air duct were closed before spraying. We used 2 litres of solution (20 ml Huwa-San + 1980 ml water) per room of an average area of 150 square feet. The average time required for spraying was 15 minutes. The mist was sprayed in such a way that the solution was drained along the walls. The average contact time was given 15 minutes. Post contact time, the surfaces were wiped out using a clean cloth. The validation of the decontamination procedure was done by airborne microbial examination and swab method. All the animal were shifted after aeration time of three hours post spraying.

Results

During our routine schedule of microbial load assessment in animal rooms, mild to severe microbial load was observed in few animal rooms. The microbial load was maximum (>20 colonies, fig. 3) in conventional rooms than Nude/ SCID mice rooms. Table 1 shows the room-wise status of the microbial load and efficacy of decontamination. In order to check the efficacy of decontamination, we had exposed the NA plates in those rooms whose colony count were more than 20 colonies (severe microbial load). Plates were explosed after giving contact time of ~15 minutes. Post decontamination of the room, we have observed that the colony count was significantly reduced in the surface monitoring with swab method (Fig. 4). There were few colonies observed in airborne microbiological examination in comparison with swab method. (Fig. 5). However, the bacterial count was reduced from severe to mild which was within our set grades/ limit

Discussion

The study was planned to assess the cost-effectiveness of decontamination of animal rooms using hydrogen peroxide aerosol spray. Decontamination of enclosed areas such as isolation units and rooms is important in many industrial, research, and healthcare facilities. Vaporized Hydrogen Peroxide (VHP) Technology has been used for over 10 years as an alternative to formaldehyde or other liquid/ gaseous methods for isolator decontamination (Meszaros et al., 2005). This technology is widely being used in clean rooms research areas (Krause et al., 2001). Likewise, aqueous Hydrogen peroxide (H^2O^2) is a potent and relatively safe antimicrobial agent. Its mode of action is proposed to be due to direct interaction with cellular components, including proteins (McDonnell & Russell, 1999). H^2O^2 is a strong oxidizing agent having multiple targets within the cell. It can react strongly with thiol groups in enzymes, proteins, DNA and bacterial cell membrane. (Linleyet al.,2012). Strong oxidizing agents can cause the formation of free radicals such as ferryal radicals which are formed from DNA associated iron and has role in DNA oxidation. (Linleyet al, 2012; Fichet et al,. 2004)

In general, for decontamination of the animal rooms, a dry-mist dispensing device that delivers aerosolized disinfectant like H^2O^2 is crucial. Such a device generates an aerosol that uses evaporation to quickly disperse H202 vapor and increasing concentrated micro-droplets. However, the cost of procurement of such devices is relatively high. The small animal facilities can not always offer to buy such an expensive device. We have found an alternate to such type of device. We demonstrated that the use of a domestic vacuum cleaner (Dry and wet model) is a suitable option. Our results revealed that the decontamination done by vacuum cleaner aerosol spray significantly reduces the number of colonies on nutrient agar plates. The one time cost of procurement of vacuum cleaner may be less than Rs. ~15000/-. Each of the room having average area of ~ 150sq. ft can be covered within 15 minutes, which requires 20 ml (1%) of H^2O^2 liquid. Our results are indicative that the aerosol spray reduces the overall microbial load inside the animal rooms. There was no surface damage, stains or corrosive action noticed in the room. However, after a gap of ~2 weeks, the animal rooms again showed colonies on agar plates. This indicates that the air coming through the HVAC system should be routed through HEPA filter, or else periodic spraying is needed to limit the microbial load to acceptable level. For this reason, irrespective of the number of colonies, we decontaminate all the rooms on a rotational basis every fortnight. Altough, we used Huwa-San- TR50 for our study, any hydrogen peroxide based disinfectant can be used for this purpose.

In conclusion, our study shows that aerosol spray of H^2O^2 disinfectant using dry and wet vacuum cleaner reduces the colony counts and is very cost-effective against the automated dry-mist foggers.

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Fig. 1. Huwa-San- TR50 (Roam Technology, Belgium).

Fig. 2. Euroclean, wet and dry Vacuum Cleaner, Eureka Forbes Ltd.

Fig. 3. > 25 colonies (cfu)/ TNTC, severe microbiological load.

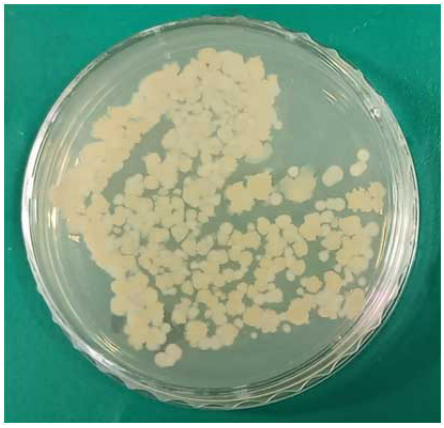

Fig. 4. Efficacy after decontamination by Surface Swab Method.

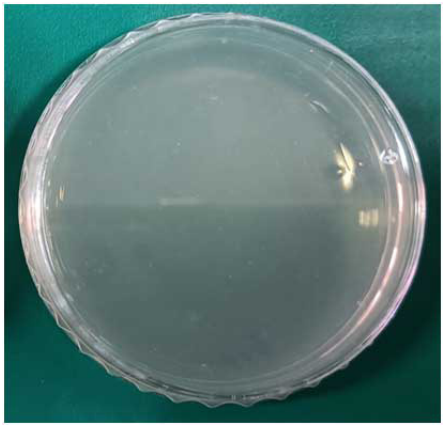

Fig. 5. Efficacy after decontamination by Airborne microbiological method.

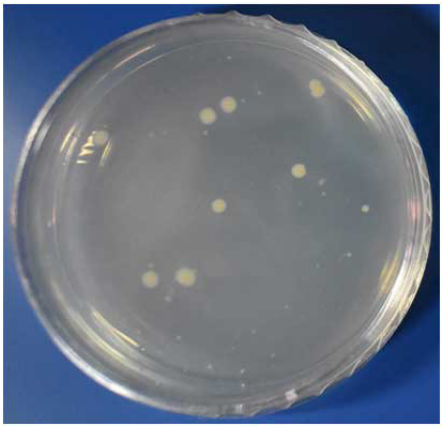

Table 1. Efficacy of pre- & post-Decontamination of animal rooms by 1% Hydrogen Peroxide Aerosol spray.

Type of animal room	Room No.	Number of Colonies Before Decontamination			Efficacy After decontamination of animal rooms		
		Airborne Microbiological examination (Number of Colonies on Nutrient Agar Plate)		Surface Monitoring with Swab method (Number of Colonies on Nutrient agar plate)	Airborne microbiological method (Number of Colonies on Nutrient agar plate)		Surface Monitoring with Swab method (Number of Colonies on Nutrient agar plate)
		Frontside (Entry)	Backside (Exit)		Frontside (Entry)	Backside (Exit)	
Nude/ NOD SCID Mice Rooms	AH/104	NG	2	NG	Plates not exposed*	Plates not exposed*	Plates not exposed*
	AH/106	2	2	1	Plates not exposed*	Plates not exposed*	Plates not exposed*
	AH/108	NG	NG	1	Plates not exposed*	Plates not exposed*	Plates not exposed*
	AH/109	NG	NG	1	Plates not exposed*	Plates not exposed*	Plates not exposed*
	AH/111	NG	NG	2	Plates not exposed*	Plates not exposed*	Plates not exposed*
Conventional Rooms	AH/110	14	12	20+	3	2	1
	AH/112	12	9	20+	1	3	NG
	AH/113	21	16	20+	4	2	NG
	AH/114	12	5	5	Plates not exposed*	Plates not exposed*	Plates not exposed*
	AH/115	20+	20+	20+	13	9	NG
	AH/116	5	1	20+	9	7	NG
	AH/117	20+	17	14	10	12	NG
	AH/119	20+	20+	20+	2	2	NG
	AH/120	5	3	20+	4	6	NG
	AH/121	3	4	20+	3	7	NG
	AH/123	4	5		Plates not exposed*	Plates not exposed*	Plates not exposed*
Common Area	Water Room	3	-	10	Plates not exposed*	Plates not exposed*	Plates not exposed*
	Experimental Room	1	NG	NG	Plates not exposed*	Plates not exposed*	Plates not exposed*

*** Since no. of colonies were within set limit/ grades, plates were not exposed.**