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A STUDY OF MICROBIAL AIR QUALITY BEFORE AND AFTER HYDROGEN PEROXIDE FUMIGATION OF OPHTHALMIC OPERATIVE THEATRE IN THE RESEARCH INSTITUTE OF OPHTHALMOLOGY IN EGYPT

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ABSTRACT

This study aimed to assess air quality in operating rooms (OR) , expressed as colony forming units (CFU)/m^3, during ophthalmic surgeries; exploring the effect of hydrogen peroxide vapor HPV fogging of OR and number of attending personnel on air contamination in the vicinity of the operated eye. Data collection by active air sampling and observations was performed during 452 ophthalmic procedures. The results showed median total viable count (TVC) at rest was 27.5 CFU/m^3 range (0-275) and 30 CFU/m^3 range (0-170) pre HPV and post HPV samplings respectively. The median TVC in operational was 60 CFU/m^3 (range = 0-500) pre-HPV and 75 CFU/m^3 (range = 20-270) post HPV. Results showed a non-significant correlation between the total CFU/m^3 per operation and prior application of HPV (P = 0.077, n = 452). However, air samples exceeding the maximum CFU/m^3 acceptable levels pre- and post-fogging was decreased from 42% to 40.3% (P= 0.8) at rest and from 15.5% to 12.8% (P= 0.6) at operation. A significant weak positive correlation was also found between TVC in CFU/m^3 and the number of persons attending the operation (r = 0.159, P = 0.006, n = 296). Conclusion: Air fumigation with HPV disinfectant and traffic flow has a positive impact on the OR environment.

Key words: Air sampler, Non-touch surface disinfection, Hydrogen peroxide vapor.

INTRODUCTION

Endophthalmitis is the most dreaded complication of any intraocular surgery. The source of these infections can be endogenous or exogenous. Major part of such exogenous infections can be controlled by sterile environment in operation theatres[1]. Surgical site infection (SSI) is the most frequent type of HAI in low-and middle-income countries affecting an average of 11% of patients who undergo a surgical procedure, and the second or third most frequent type of HAI in the United States and Europe[2]. Microorganisms that cause infections in healthcare facilities include bacteria, fungi and viruses and are commonly found in patient's own endogenous flora, but can also originate from health care personnel and from environmental sources. In particular, the environmental matrices (water, air and surfaces) play a leading role as reservoirs of microorganisms[1]. Bacteria as *Legionella* spp. and *Pseudomonas aeruginosa* are usually isolated from water samples in hospital facilities. *Influenza* A virus and other viruses can be isolated from air, while spores of filamentous fungi are found on surfaces in operating theatres[3]. Contaminated surfaces are often a source of airborne microbes, and airborne microbes often produce surface contamination[4]. For this reason, careful cleaning and disinfection of environmental surfaces are essential elements of effective infection prevention programs[2,5]. This is particularly true in high risk healthcare departments, or in operating theatres because of tissue exposure to air[6]. However, traditional manual cleaning and disinfection practices in hospitals are often suboptimal. Newer "no-touch"(automated) decontamination technologies include aerosol and vaporized hydrogen peroxide HPV and mobile devices that emit continuous ultraviolet (UV-C) light; have been shown to reduce bacterial contamination of surfaces[7]. Surface sanitization or disinfection by wiping with chemicals is time-consuming and intricate to validate. Alternatively, fumigation overcomes many critical aspects of wiping in both procedure and validation. Moreover, the use of nebulized hydrogen peroxide (H_2O_2) of moderate concentration resolves remaining reservations as to toxicity, corrosion, and persistence. H_2O_2, H_2O_2 with silver nitrate, peracitic acid and other chemical compounds has been recently demonstrated to be a good fumigant. It is safer, less irritating and requires shorter exposure times than formaldehyde[3,8]. Microbial air contamination monitoring is a key process in facilities with special air cleanliness needs. Through air sampling, it is possible to evaluate microbial contamination in environments at high risk of infection. At the moment, the only effective means of quantifying airborne microbes is limited to the count of colony forming unit (CFU). The CFU count is the most important parameter, as it measures live micro-organisms which can multiply. Air samples can be collected in two ways: by active air samplers or by passive air sampling (the settle plates). Active air sampler collects a known volume of air blown onto a nutrient media by different techniques, thus measures air contamination by counting the CFU/m^3 of air. This system is applicable when the concentration of microorganisms is not very high, such as in an operating theatre and other hospital controlled environments. In fact, international standards offer different techniques (active or passive sampling) and different kinds of samplers, thus leaving the choice of system open [9,10]. The primary objective of this study was to determine the efficacy of the bio-disinfectant, based on hydrogen peroxide stabilized by a colloidal silver complex (1 ppm) fumigation method on air decontamination of operating theatre, and to explore if the number of personnel present in the operating room (OR) affects the air contamination rate in the vicinity of surgical wounds.

MATERIALS AND METHODS

Hydrogen peroxide (H_2O_2) fogging of operative rooms

The HPV in this study was generated using a Nocospray mobile bio-decontamination generator loaded with H_2O_2 product (6% H_2O_2, 1 ppm silver nitrate, polyoxyethylene 0.03%, (S) – p – Mentha - 1.8 - diene 0.03%) cartridge. The operative theatre in RIO includes six operative rooms; from 1 - 4 in the 2nd floor (renewed) air is HEPA - filtered with positive pressure and in the 4th floor (not renewed yet) air is regularly filtered (non-HEPA) without positive pressure. The mean room volume is 72.76 m^3 (range = 77.22 - 70.47). According to the manufacturer using instructions[11], H_2O_2 product 6% was used by injecting 75 ml/room in less than 10 minutes. After decontamination, the recirculation flow of dry HEPA-filtered air continued. No aeration phase was required. Within 8 minutes, 98% of OH ions converts to water vapor and oxygen, and 95% of dry fog settles. Human admission and work started in the rooms within 8 minutes for maximum safety. Fogging with H_2O_2 was done twice per week to all rooms as a routine.

Air sampler

An impactor (sieve type) IUR basic air sampler was used[12], with air volume (10-9900L) and air flow (100l/m-60l/m). The device meets the following requirements: sufficient flow rate to collect 1m^3 in a reasonable time, without significant drying of the sample medium and appropriate air impact speed to the culture medium[13].

Microbiological air sampling

The study was performed in 6 operating rooms at the Research Institute of Ophthalmology in Giza, Egypt. Active sampling was carried out using air sampler. The Total Viable Count (TVC) was evaluated *at rest* (in the morning before the beginning of surgical activity) and *in-operational* (during surgery). The work has been carried out along 10 months period. During the study period, we obtained 452 environmental samples. Air sampling took place twice a week, pre- and post-fogging. A 90 mm Petri dish of blood agar was inserted and the device's lid was screwed in place. Next, accurate volumes of 200 L of air were sampled in two interrupted minutes in fixed sites, one as near as possible to the head side of operative table and the second close to the sterile set trolley by forcing air through the cover sieves towards the Petri plate's blood agar surface. Sealed Blood agar plates were incubated at 37°C for 2 days. The plates were examined for microbial growth and CFU enumeration per plate enables to evaluate microbial air quality. In addition, the number of personnel present *in operational* was recorded to assess the association between the number of people in the room and the value of TVC.

Statistical Analysis

CFU counts during the period of research were compared using the Mann–Whitney *U* test. In 100 L/min sampling capacity, if 1 m^3 of air is tested, then it would require an exposure time of 15 minutes [9]. Since we use 2 minutes sampling time to withdraw 200 L, therefore, to calculate CFU per 1000L or m^3 we multiply results of CFU/plate by 5. Maximum acceptable levels were taken as the standards determined by ISPESL in 2009 for air microbial contamination in operating theatres with turbulent air flow: ≤ 35 CFU/m^3 at rest, and ≤ 180 CFU/m^3 when operational[3,14].

RESULTS

The results of microbial air test in OR pre- and post-H_2O_2 fogging are demonstrated in **(Table 1), (Table 2)** and in **(Figure 1).** The median TVC at rest was 27.5 CFU/m^3 range (0-275) and 30 CFU/m^3 range (0-170) pre-HPV and post-HPV samplings respectively. The median TVC in operational was 60 CFU/m^3 (range = 0-500) pre-HPV and 75 CFU/m^3 (range = 20-270) post-HPV. The data showed a positive non-significant correlation between the total CFU/m^3 per operation and previous application of HPV. (P = 0.077, n = 452). A significant weak positive correlation was also found between TVC CFU/m^3 and the number of persons present in the OR at operation (r = 0.159, P = 0.006, n = 296). At rest, 38 out of 90 (42%) air samples exceeded the recommended level of <35 CFU/m^3 pre-HPV fogging and 29 out of 72 (40.3%) post-fogging, **(Table 3).** At operation, 18 out of 116 (15.5%) air samples exceeded the recommended level of <180 CFU/m^3 pre-HPV fogging and 10 out of 78 (12.8%) post-fogging **(Table 4)**, i.e. air samples exceeding the maximum CFU/m^3 acceptable levels pre- and post-fogging was decreased from 42% to 40.3% (P= 0.8) at rest and from 15.5% to 12.8% (P= 0.6) at operation **(Figure 2)**.

DISCUSSION

Disinfectant (spray-fog techniques) for antimicrobial control in hospital rooms has been used. H_2O_2 solutions have been used as chemical sterilants for many years. However, the H_2O_2 Vapor HPV® was not developed for sterilization of medical equipment until the mid-1980s[15]. H_2O_2 fog has recently been demonstrated to be a good fumigant. Not only effective for room air disinfection, but also an excellent surface disinfectant especially for furniture and other articles[16]. Taneja et al[17], has found H_2O_2 fogging highly effective for disinfection of room air, and decontaminated the air-conditioning ducts effectively. Published studies reported that HPV-decontamination has been found to be

Table 1: Microbial Air Test in OR Pre and Post H_2O_2 fogging

	Pre-fogging		Post-fogging		
	Median	**Range**	**Median**	**Range**	**P value**
OR 1 **at rest**	30	(0 220)	25	(0 - 65)	0.271
OR 1 at **operation**	47.5	(0 - 390)	75	(20 - 210)	0.153
OR 2 **at rest**	32.5	(0 - 80)	17.5	(0 -140)	0.798
OR 2 **at operation**	65	(5 - 300)	70	(20 - 270)	0.792
OR 3 **at rest**	25	(0 - 85)	45	(5 - 170)	0.064
OR 3 **at operation**	57.5	(5 - 360)	75	(25 - 225)	0.156
OR 4 **at rest**	25	(0 - 110)	25	(0 - 130)	0.57
OR 4 **at operation**	70	(10 - 500)	67.5	(20 - 230)	0.693
OR 5 **at rest**	15	(10 - 20)	42.5	(35 - 50)	0.333
OR 5 **at operation**	15	(15 - 15)	225	(190 - 260)	0.667
OR 6 **at rest**	157.5	(40 - 275)	70	(50 - 90)	1
OR 6 **at operation**	200	(25 - 215)	195	(170 - 220)	0.8

Table 2: Microbial Air Test in OR Pre and Post H2O2 fogging for all room collectively

.	Pre-fogging		Post-fogging		P value
	Median	**Range**	**Median**	**Range**	
At rest	27.5	(0 - 275)	30	(0 - 170)	0.497
In operation	60	(0 - 500)	75	(20 - 270)	0.077

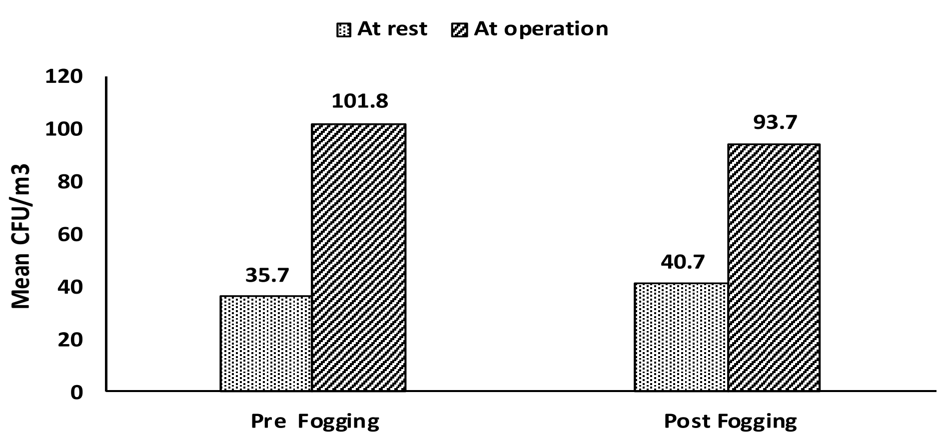

Figure 1. The mean CFU/m^3 at rest and at operation pre- and post-fogging with H_2O

Table 3. The number of air samples with CFU/m3 exceeding the maximum acceptable levels pre- and post-fogging at rest

		Pre fogging		Post fogging			
		N	**%**	**N**	**%**	**Total**	**P value**
Exceeding limit	Yes	38	42.3	29	40.3	67	0.802
	No	52	57.7	43	59.7	95	
	Total	90	100	72	100	162	

Table 4. The number of air samples with CFU/m3 exceeding the maximum acceptable levels pre- and post-fogging at operation

		Pre fogging		Post fogging			
		N	**%**	**N**	**%**	**Total**	**P value**
Exceeding limit	Yes	18	15.5	10	12.8	28	0.6
	No	98	84.5	68	87.2	166	
	Total	116	100	78	100	194	

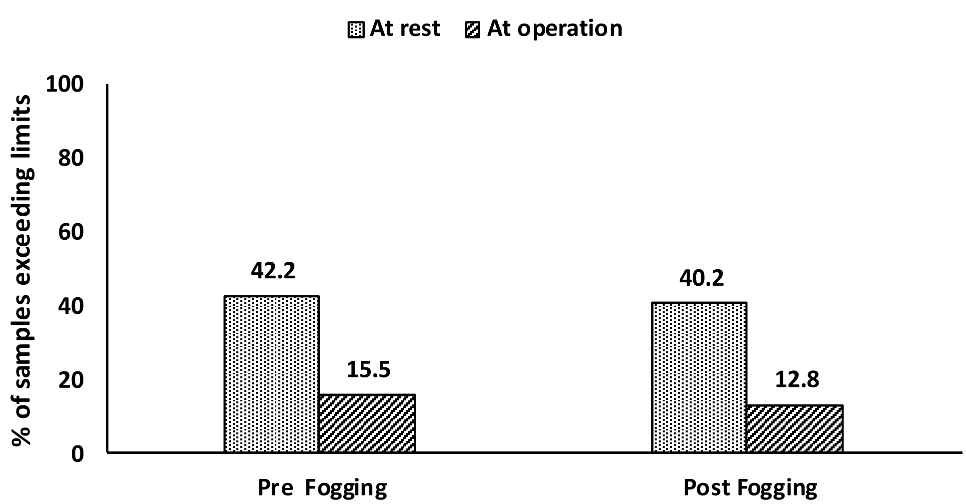

Figure 2. The percentage of air samples exceeding the maximum acceptable levels of CFU/m^3 pre- and post-fogging at rest and at operation.

highly effective in eradicating methicillin-resistant *Staphylococcus aureus* (*MRSA), Serratia marcescens*, *Clostridium botulinum* spores and *Clostridium difficile* from rooms, furniture, surfaces and/or equipment[18,19,20]. In the present study the feasibility of utilizing vapor-phase silver H_2O_2 as environmental decontaminant was evaluated. A weak, yet still positive correlation between TVC pre- and post-HPV fogging has been found (P = 0.077, n = 452). **A**ir samples exceeding the maximum CFU/m^3 acceptable levels pre- and post-fogging was decreased from 42% to 40.3% (P= 0.8) at rest and from 15.5% to 12.8% (P= 0.6) at operation. In our study, HPV was applied at the end of the day and the post-fogging samples were not taken at the same day but the following day in the morning. A study published by Otter and co workers[16] assessed the biological efficacy and rate of recontamination following HPV-decontamination. The authors reported that recontamination was encountered within a week post fogging in a room occupied by a patient colonized with MRSA and gentamicin-resistant Gram-negative rods. Our results showed that TVC in operational (working) samples was in general bigger than that at rest (empty room) for both pre- and post-HPV fogging. In empty theatres, median bacterial values of 30 CFU/m^3 (range 0 – 170) post-fogging and 27.5 CFU/m^3 (range 0 – 275) pre-fogging (P= 0.49) were recorded. In working theatres, these values increased to 75 CFU/m^3 (range 20 - 270) post-fogging and 60 CFU/m^3 (range 5 – 500) pre-fogging (P=0.07). Maximum recorded values were 275 CFU/m^3 for empty theatres, and 500 CFU/m^3 for working theatres**.** Pasquarella and coworkers[10] reported that in empty theatres, median bacterial values of 12 CFU/m^3 [interquartile range (IQR) 4-32] and 1 index of microbial air contamination (IMA) (IQR 0-3) were recorded. In working theatres, these values increased significantly ($P < 0.001$) to 80 CFU/m^3 (IQR 42-176) and 7 IMA (IQR 4-13). Maximum recorded values were 166 CFU/m^3 and 8 IMA for empty theatres, and 798 CFU/m^3 and 42 IMA for working theatres. Napoli and co-authors[3], also reported in their study that, *in-operational* sampling showed higher values of TVC than *at rest* with both active and passive methods (93.8 vs 12.4 CFU/m^3 and 10496.5 vs 722.5 CFU/m^2/h respectively). This would be expected due to the inevitable microbial shedding and dispersion from personnel in operation[3,10]. A significant weak positive correlation has been found in our study between air contamination and the number of personnel in OR (r = 0.159, P = 0.006, n = 296). These results are in accordance with a study made by Andersson et al [21], whose data showed a strongly positive correlation between the total CFU/m^3 per operation and total traffic flow per operation ($r = 0.74$; $P = .001$; n = 24) then after controlling for duration of surgery, a weaker, yet still positive correlation between CFU/m^3 and the number of persons present in the OR (r = 0.22; P = .04; n = 82). However the results of Napoli and coworkers[3] showed a significant association between the number of people in OR and the TVC ($R^2 = 0.608$; $F = 26.6$; $p < 0.01$). The mean number of people present in the operating theatre during the 19 *in-operational* samplings was high at 7.4 (SD = 3.1; range = 3-13). That was typical of university hospitals in Italy where teaching is done directly in the theatre. In 2017 CDC[22], had reported that new technologies involving fogging for room decontamination (e.g., ozone mists, vaporized H_2O_2) have become available since the 2003 and 2008 CDC recommendations were made. These newer technologies were assessed by CDC and HICPAC in the 2011 Guideline. CDC does not yet make a recommendation regarding these newer technologies. Stating that "more research is required to clarify the effectiveness and reliability of fogging to reduce environmental contamination (no recommendation / unresolved issue)". Microbial air monitoring in operating theatres has been a subject of interest and debate. No generally accepted sampling methods and threshold values are available[10]. Moreover, each active sampler gives different results in the same place at the same time, showing a high variability[9]. According to Napoli et al[3], there are no specific indications with regard to the protocol to be used in air sampling. This has created a strange situation in that there are recommended target limits, but no precise guidelines on how to obtain the TVC value. Moreover, previous studies have not given consistent results due to the different samplers used, the different places sampled (OR, dental clinics, pharmaceutical clean-rooms etc.), and / or the different parameters applied (volume of air sampled, sampling time protocol, point of sampling, etc.). In a most recent study in 2017 by Poongodi and his coworkers[23], surveillance methods using settle plate, air sampler and surface swabs, they concluded that air sampler calculates suspended particles thus measures the microbial burden more accurately whereas settle plate calculates the settling large bacteria carrying particles, so it has more practical application in reflecting the risk of infection. The microbiological quality of the air in operating theatres is a significant parameter to control healthcare associated infections, and regular microbial monitoring can represent a useful tool to assess environmental quality and to identify critical situations which require corrective intervention[3]. However, restriction of personnel traffic, closing of OR doors and good ventilation system using special air flow pattern (filtered and purified air circulates and contaminated air is removed continuously), standard cleaning, disinfection and sterilization, good theater practice and discipline can provide a microbiologically safe environment.

CONCLUSION

Decontamination using vaporized H_2O_2 (VHP) offers several appealing features that include environmentally safe by-products (H_2O and oxygen), good material compatibility and ease of operation. Microbiological monitoring is a useful tool for assessment of the contamination of operating theatres in order to improve air quality. Fogging cannot replace manual cleaning. Since human activity plays a major role in microbial air quality, meticulous cleaning and strict adherence to operating theatre protocol are essential. Fumigation may in fact cause a false sense of security leading to the abandonment of more effective infection control measures. We recommend fogging in OR when newly constructed, any remodeling, reconstruction or renovation alterations are done, after outbreak or air-conditioning system maintenance, and for rooms that have housed patients infected / colonized with multidrug-resistant organisms. Further work is needed to determine the decontamination and residual effect of HPV on OR surfaces and air quality through other approach like sampling swaps to be done in an expanded manner for sufficient duration before a set of guidelines are established.

REFERENCES

1. Gupta C, Vanathi M, Tandon R. Current concepts in operative room sterilization Delhi Journal of Ophthalmology *2015*. DOI: http://dx.doi.org/10.7869/djo.106
2. Sehulster L, Chinn RY: Guidelines for environmental infection control in health - care facilities. Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC) MMWR Recomm Rep 2003,52:1.
3. Napoli C, Marcotrigiano V and Montagna MT. Air sampling procedures to evaluate microbial contamination: a comparison between active and passive methods in operating theatres. BMC Public Health 2012;12:594.
4. Kowalski W. Ultraviolet germicidal irradiation handbook. UVGI for air and surface disinfection. Springer-Verlag Berlin Heidelberg 2009. DOI: 10.1007/978-3-642-01999-9.
5. Beldi G, Bisch-Knaden S, Banz V, Mühlemann K, Candinas D: Impact of intraoperative behavior on surgical site infections. Am J Surg 2009;198:157-162.
6. Weiss KD, Osborne SF, Callahan-Lyon P: Prevention of surgical-site infections. N Engl J Med 2010;362: 1541-1542.
7. Boyce JM. Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. Antimicrobial Resistant Infection Control 2016;5:10.
8. Vanhecke P, Sigwarth V, Moirandat C. A potent and safe H_2O_2 fumigation approach. PDA J Pharm Sci Technol 2012;66(4):354-70. DOI: 10.5731 / pdajpst.2012.00870.
9. Pasquarella C, Pitzurra O, Savino A. The index of microbial air

contamination. Journal of Hospital Infection 2000;46:241–256.

10. Pasquarella C, Vitali P, Saccani E, Manotti P, Boccuni C, Ugolotti M, Signorelli C, Mariotti F, Sansebastiano GE, Albertini R Microbial air monitoring in operating theatres: experience at the University Hospital of Parma. J Hosp Infect 2012; 81(1):50-7.
11. Innotec Hygiene Solutions, OXYPHARM. A brief overview as to the Technical function of the Oxypharm system, 2017. http://www.innotechygienesolutions. com/oxypharm.
12. Spin Air - Air Sampler - IUL Instruments: http://www.iul-inst.com/en/air-sampling/spin-air
13. Kelly J. Microbiological Air Samplers and ISO 14698-1/2. Categories of air samplers and factors to consider when choosing one.http://www.cemag.us/article/2005/05/microbiological - air - samplers –and – iso – 14698 - 12.
14. Istituto Superiore per la Prevenzione e la Sicurezza del Lavoro. Linee guida per la definizione degli standard di sicurezza e di igiene ambientale dei repart operatori 2009, available at http://www.ispesl.it/documentazione/comp_sett.asp. Accessed January, 2012.
15. CDC Center for Disease Control and Prevention and Healthcare Infection Control Practices Advisory Committee (HICPAC). Guideline for Disinfection and Sterilization in Healthcare Facilities 2008. https://www.cdc.gov/hicpac/disinfection_sterilization/13_10othersterilizationmethods.html
16. Otter JA, French GL, Adams NM, et al. Hydrogen peroxide vapour decontamination in an overcrowded tertiary care referral center: some practical answers. J Hosp Infect 2006; 62:384-385.
17. Taneja N., Biswal M., Kumar A., Edwin A., Sunita T., Emmanuel R., Gupta A.K., Sharma M. Hydrogen peroxide vapour for decontaminating air-conditioning ducts and rooms of an emergency complex in northern India: time to move on. Journal of Hospital Infection 2011;78:200-203.
18. Jeanes A, Rao G, Osman M, Merrick P. Eradication of persistent environmental MRSA. J. Hosp. Infect 2005;61:85-6.
19. Bates CJ, Pearse R. Use of hydrogen peroxide vapor for environmental control during a Serratia outbreak in a neonatal intensive care unit. J. Hosp. Infect. 2005;61:364-6.
20. Boyce JM, Havill NL, Otter JA, et al. Impact of hydrogen peroxide vapor room bio-decontamination on environmental contamination and nosocomial transmission of Clostridium difficile. The Society of Healthcare Epidemiology of America, 2006; Abstract 155:109.
21. Andersson AE, Bergh I, Karlsson J, Eriksson BI and Nilsson K. Traffic flow in the operating room: An explorative and descriptive study on

air quality during orthopedic trauma implant surgery. American Journal of Infection Control 2012; 40(8):750-755. DOI: http://dx.doi.org/10.1016/j.ajic.2011.09.015

22. CDC - Publications - HICPAC https://www.cdc.gov/hicpac/pubs.html Jan 11, 2017. The Guidelines for Environmental Infection Control in Health-Care Facilities; evidence-based recommendations on the preferred methods for cleaning, disinfection and sterilization of patient-care medical devices and for cleaning and disinfecting the healthcare environment. Update: Environmental Fogging Clarification Statement.

23. Poongodi S @lakshmi, Palaniappan N, Kannan M and Nithya gomatheeswari S. Microbiological surveillance of operation theatre: Why…What…How…Where…Which…?" International Journal of Basic Medical Science 2017;7 (5).