International Journal of Research in Medical Sciences
Deb A et al. Int J Res Med Sci. 2016 May;4(5):1583-1589
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20161231

Research Article

Is fumigation enough for air conditioning units in operation theatres and Intensive care units?

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Received: 02 March 2016
Accepted: 07 April 2016

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ABSTRACT

Background: Strict asepsis is necessary in operating theatres (OT) and intensive care units (ICU) as the patients undergo invasive procedures. The filters of contaminated air conditioning (AC) units provide a niche for proliferation of fungi and production of fungal spores.
Methods: The routine procedure for maintenance of sterile atmosphere in our hospital, i.e. fumigation and mopping walls with disinfectants often fail to address these fungal spores of the AC filters. We therefore carried out a surveillance of the ACs in ICUs and OTs to find the level of contamination with fungal spores and also to improvise on intervention strategies to tackle the problem. Over 3 months period, 34 ACs from 7 OTs and 2 ICUs were screened by taking 2 swabs from each AC which were then tested for the presence of fungal spores as per standard methods.
Results: The contamination rate was 88.2% before fumigation and 76.9% after fumigation. The fungal spore contamination rate was reduced to 20% (1 out of 5 ACs) after servicing of the ACs was done. Aspergillus spp. was the most common fungal isolate.
Conclusion: Based on the observations, we recommend regular servicing of the ACs as well as wet mopping of the ducts with sporicidal solution at regular intervals.

Keywords: Fumigation, AC, ICU, OT

INTRODUCTION

Strict asepsis is necessary during surgical procedures to prevent post-operative infections, like surgical site infections, which contributes to significant cause of morbidity and mortality.[1] Likewise, Intensive Care Unit (ICU) environments also mandates strict sterile environment as the patient population admitted there are compromised in immunity. Moreover, the patients undergo invasive procedures in the form of surgery or insertion of intravenous or central venous catheters, urinary catheters, and endotracheal tubes which makes them further vulnerable to acquiring infection by strains persisting in the hospital environment.[2] It has been seen that the duration of ICU stay in hospitals is significantly associated with acquiring infections in the hospital, which further highlights ICUs as an important source of nosocomial infections and highlights the necessity to practice strict infection control practices.

Fungi are ubiquitous in distribution, being present on surfaces, air and water, in the hospital environment as well as in the community. Fungi reproduce mostly by spores, which are light in weight and are able to remain suspended in air by means of buoyancy. The indoor air in enclosed spaces within hospital, especially poses an increased risk to infection with such fungal spores, mostly because it allows the aerosols to be confined and their concentration build up to an infectious level.[3] Studies have shown that there is a correlation between

hospital indoor air contamination with fungal spores and the incidence of invasive aspergillosis.[4]

Bio aerosols of fungal spores present in hospital environment are often transmitted through air, surfaces of articles, visitors, healthcare workers, cross-infection from other patients as well as ejected through the vents of contaminated air conditioners (AC).[5,6] The filters of contaminated AC units provide a niche for proliferation of fungi and production of fungal spores.[5,7] Such fungal spores which often includes opportunistic pathogenic fungi can get inoculated at the surgical wounds resulting in post-operative infections or be inhaled resulting in hospital acquired infections (HAI). Cluster of HAI in immune-compromised patients have been reported to be associated with increased levels of the atmospheric dust and fungal spores.[8,9] Fluctuation of air fungal load, as well as the predominant fungal species in the hospital environment influence the incidence of hospital-acquired fungal infections.[10,11]

In our setup, fumigation of rooms with formaldehyde and mopping of the floors and walls with disinfectants is the routine procedure for maintenance of sterile atmosphere in the operating rooms as well as in ICUs. As a part of the Infection Control practices, routine surveillance of the efficacy of fumigation procedure is carried out by monitoring the air bacterial load and by assessment of swabs for anaerobic bacterial culture. However, studies have shown that routine sterilization practices in OTs often fail to address the organisms that persists in the filters of the AC units especially the fungal spores.[7] We therefore planned and executed a surveillance study of air conditioners in various OTs and ICUs in our hospital in order to estimate the level of fungal contamination of the AC units and also to improvise on intervention strategies.

METHODS

Study setup

The study was carried out in Indira Gandhi Government Medical College and Mayo Hospital, Nagpur, India, a high-burden tertiary care public hospital in central India. Surveillance of OTs and ICUs were carried over a period of four months (July to October 2015). Overall ACs of seven operation theatres viz. Emergency OT, ENT OT, Gynaecology OT, Ophthalmology OT, Orthopaedics OT, Surgery OT, Septic OT, and two Intensive Care Units viz. Medical ICU (MICU), and Surgical ICU (SICU) were screened in the study. As the study did not involve human or animal subjects, waiver was obtained from the institutional ethical committee.

Sterilization procedure of OTs and ICUs

The routine procedure for sterilization of the OT in our hospital consists of high-level disinfection once a week using formaldehyde gas generated by addition of potassium permanganate and 40% liquid formalin.[12] The quantity of formaldehyde is calculated based on the volume of the air in OT; for every 1000 cubic feet of air volume 280 mL formalin and 150 milligram potassium permanganate is needed. The formalin and potassium permanganate mixture is kept in four enamel bowls placed at four corners of the operating room and allowed to react to generate formaldehyde gas. The OT temperature is maintained at about 20-30 0C and humidity maintained at about 60% for effective fumigation.[13] Water was sprinkled in the OT floor in order to maintain the necessary humid condition. The fumigation procedure is carried out after properly sealing the OT rooms and allowing a contact time of about 12 hours for routine cases, and of 24 hours if any gross contamination occurs during the procedure or operation of septic cases. If any construction work had been carried out in the operating room, a contact time of 48 hours is allowed. After the necessary contact period, ammonia solution is used to neutralize the irritant effect of formaldehyde gas, about 2 hours prior to surgery. For every 280 ml of formalin used, 70 ml of liquor ammonia is used, and is taken either in a basin or is soaked in a gauze piece and kept in the OT for about 2 hours, keeping the exhaust fan on to facilitate the exit of formalin vapours. For the ICUs, fumigation with formaldehyde gas is done approximately once in 3 months depending on patient load.

Apart from fumigation, the floor, surfaces and walls in the OT are wiped with a disinfectant solution after the day's procedures are over and/or about an hour before commencement of surgical procedures the next day. The floors are wiped with 1% solution of sodium hypochlorite. For carbonization of the walls, 2% solution of Bacillocid is wiped up to the head level and allowed to dry.

Routine monitoring procedure of fumigation efficacy

For evaluating the efficacy of the fumigation procedure, we routinely perform monitoring of the air Bacteria Carrying Particle (BCP) load in the OT environment and examination of the swabs taken from various points in the OT. BCP is assessed by sedimentation method, once a week, immediately following fumigation with formaldehyde and before commencement of surgery. For this, a blood agar plate of diameter 9 cm is kept in the operating room on the operating table, allowing contact with the OT air for 30 minutes. During this period, adequate precautions are taken to prevent generation air

currents in the OT under evaluation. The blood agar settle plate is then incubated overnight at 37°C and then examined for BCP load, which is calculated based on the colony count on the blood agar plate after incubation, area of the plate exposed, and the duration of exposure. The operating rooms were said to be conducive for carrying out operative procedures only when the bacterial load was less than 180 per cubic meter.[5] Approximately 180 bacteria per cubic meter of air correspond to 10 colonies settling on a plate. Qualitative analysis of the colonies was done using standard bacteriological methods to rule out the presence of Staphylococcus aureus and Pseudomonas aeruginosa. Detection of even a single colony of S.aureus or P. aeruginosa is considered a risk for infection.[13]

The swabs collected from representative areas in the operation theatre, like the operating table at the head end, floor beneath the operating table, the overhead shadow less lamps, anesthesia trolley and the instrument trolley were inoculated in Robertson's cooked meat (RCM) media and incubated at 37°C for 5 days for detection of anaerobic organisms. After incubation, if turbidity, foul smell or blackening of meat particles was noticed in the RCM, anaerobic cultures were subsequently done on blood agar plates. Presence of Clostridium in these cultures were taken as marker for inadequate sterilization of the operating theatre.[14]

Monitoring of fungal load of ACs in OTs and ICUs

For monitoring the AC units and the impact of fumigation on the fungal spores persisting on the AC filters, two swabs each were collected from the various AC units for fungal culture. The sterile swab sticks were rolled over various sites of the vents of the ACs, both before and after fumigation of the OTs and ICUs. Both the swabs were inoculated on Sabouraud's Dextrose Agar (SDA) slopes and incubated at 37 0C and at room temperature (250C). The slants of SDA were examined every day for one week and weekly thereafter for four weeks for the presence of any fungal growth. Standard mycological methods were used to identify the fungus based on macroscopic and microscopic examination of the growth, nature of mycelium, conidial and hyphal structure.[15] Microscopic examination was done by preparing tease-mount of the fungal growth in Lacto Phenol Cotton Blue and also from slide cultures.

Intervention strategies implemented

Based on the preliminary findings, we suggested intervention in the form of surface cleaning of the vents and maintenance of the ACs which was implemented for 5 AC units. Servicing of the ACs included manual cleaning of the AC filters using detergent solution and water followed by sun-drying of the filters before re-installation. For surface cleaning of the AC vents, wet mopping was done using disinfectant solution Bacillocid® (Raman and Weil private limited, Bombay, India) which consists of 1,6-Dihydroxy 2,5-Dioxy hexane (a chemically bound form of formaldehyde), Glutaraldehyde, Benzalkonium chloride, and alkyl urea derivative. Due to the lack of resources, servicing of the ACs could be implemented for only 5 out of the 34 ACs. After this intervention, swabs from the vents of these 5 ACs were again collected and followed up for fungal growth like the previous swabs.

Statistical analysis

The data obtained was analyzed using Microsoft Xcel ® for determining the rates of infection of the ACs before and after fumigation as well as after servicing. Students' T test was done to determine the significance taking $p < 0.05$ as the cutoff level.

RESULTS

Analysis of the blood agar plate showed that the bacterial air particles were significantly reduced after fumigation as compared to the pre-fumigation status and the bacterial load of the air was within the permissible limit in all the instances of fumigation. Repeated settle plates collected over the week till next fumigation cycle also yielded permissible level of bacterial load in the air, suggesting that fumigation was effective for bacteria. We did not find any Staphylococcus aureus or Pseudomonas aeruginosa in the BCP load of the air in the operating room.

Table 1 shows the rate of isolation of fungi from the swabs obtained from the vents of ACs of the various OTs and ICUs before and after fumigation as well as after intervention of some of the ACs, i.e. after their servicing and wet mopping of the vents and filters. The rate of fungal colonization of the AC filters was 88.2% (60 out of 69 swabs) before fumigation, which slightly reduced to 76.9% (40 out of 52 swabs) after fumigation. However, this decreases in the fungal contamination rate after fumigation was not statistically significant ($p > 0.05$). For majority of the OTs like the Emergency OT, Gynaecology OT, septic OT, Surgery OT and ophthalmology OT, there was no effect of fumigation on the rate of contamination of ACs with fungal spores. Such a comparison could not be done for MICU as fumigation was not possible in our study duration due to continuous patient load.

After the initial phase of the study we suggested interventions to tackle this problem of contamination of the ACs, mostly in the form of servicing of the ACs

whenever practically feasible including the cleaning of filters before re-installation and also surface cleaning of the vents with a fungal sporicidal solution. Due to the lack of resources, this intervention could be implemented in only 5 ACs, which included 1 AC in Orthopedics OT, 3 ACs in MICU and 1 AC in the MICU. We collected 2 swabs each from the vents of these 5 ACs (total 10 swabs), 5 days after the intervention was done. We observed that there was persistence of fungal spores in only 1 out of the 5 ACs (20%) after the servicing and cleaning procedure that we suggested. Only 1 AC in the MICU showed persistence of fungal spores after the recommended cleaning procedure. Interestingly, the load of fungal spores in this particular AC in MICU was visibly less as compared to that obtained from the pre-fumigation and post-fumigation swabs. A statistical test of significance could not be done to compare the effect of servicing due to the limited number of samples.

Table 2 shows the fungal isolates obtained from the swabs before and after fumigation as well as after servicing. We observed that Aspergillus spp, followed by Rhizopus, Penicillium and Chaetomium were the most commonly isolated fungi from the ACs both before as well as after fumigation with formaldehyde. Yeasts like Candida and Rhodotorula were also isolated occasionally.

Table 1: Results of culture of swab from ACS obtained before and after fumigation and after intervention.

Name of OT / ICU	No. of AC	No. of swab	Growth from swabs before fumigation n (%)	Growth from swabs after fumigation n (%)	Growth from swabs after servicingn (%)
Emergency OT	2	4	4 (100)	4 (100)	NA
ENT OT	3	6	6 (100)	4 (66.7)	NA
Gynecology OT	4	8	6 (75)	6 (75)	NA
Septic OT	2	4	4 (100)	4 (100)	NA
Surgery OT	4	8	6 (75)	6 (75)	NA
Ophthalmology OT	1	2	2 (100)	2 (100)	NA
Orthopedics OT	4	8	8 (100)	6 (75)	0/2 (0)
MICU	8	16	14 (87.5)	NA*	2/6 (33.3)**
SICU	6	12	10 (83.3)	8 (66.7)	0/2 (0)
Total	34	68	60 (88.2)	40/52 (76.9)	2/10 (20)

NA: Not Available,*Fumigation was not possible due to admitted patient load, **Fungal colony count was significantly reduced post-servicing.

Table 2: Fungal isolates obtained from culture of the swab from ACs obtained before and after fumigation and after intervention.

Name of OT / ICU	Isolates before fumigation	Isolates after fumigation	Isolates after servicing
Emergency OT	A. niger, A. flavus, Rhizopus	A. niger, A. flavus, Rhizopus	NA
ENT OT	A. flavus, Rhizopus, Penicillium, Chaetomium	A. flavus, Rhizopus, Penicillium	NA
Gynecology OT	A. niger, A. flavus, Rhizopus, Penicillium, Candida spp.	A. niger, A. flavus, Penicillium, Candida spp.	NA
Septic OT	A. niger, A. flavus, Rhizopus	A. niger, A. flavus, Rhizopus	NA
Surgery OT	A. niger, A. flavus, Rhizopus, Chaetomium	A. niger, A. flavus, Rhizopus	NA
Ophthalmology OT	A. niger, Rhizopus	A. niger, Rhizopus	NA
Orthopedics OT	A. flavus, A. niger, Penicillium	A. flavus, Penicillium	-
MICU	A. niger, A. flavus, Rhizopus, Penicillium, Rhodotorula, Candida	A. niger, A. flavus, Rhizopus, Rhodotorula, Candida	A. niger, A. flavus, Rhizopus
SICU	A. niger, A. flavus, A. fumigatus, Candida, Rhizopus, Rhodotorula, Chaetomium	A. niger, A. flavus, A. fumigatus, Rhizopus, Rhodotorula, Candida	-

Albeit reduced by a significant amount, the spores of Aspergillus niger, Aspergillus flavus and Rhizopus could withstand the effects of cleaning with Bacillocid, a disinfectant solution that was used for cleaning the ACs, and were subsequently recovered from one of the ACs in MICU.

DISCUSSION

Strict asepsis in OTs and ICUs is essential to minimize the chances of nosocomial infections thereby reducing their burden. Infective agent could be transmitted due to inadequately sterilized equipment, presence of shedder of pathogenic organisms amongst the hospital personnel, contaminated environment through air and surfaces.[16] Airborne moulds pose a significant risk to the patients because of the chances of inhalation of conidia.[17] Moreover, products of mold growth such as Microbial volatile organic compounds (MVOC) may contribute to symptoms of illness or discomfort[18,19] not only to the patients but also to the healthcare workers as well. Surgical procedures in particular, expose patients to chances of infective complications. So it is necessary that the operating theater all sources of pollution have to be kept under control. Similarly, patients admitted in ICUs are also at high risk of acquiring infections from hospital pathogens, which makes it necessary to maintain a strict aseptic condition in the ICUs.[20,21]

Most sophisticated operation theatres rely on laminar air flow maintained by air conditioning units attached to HEPA filters, for providing a conducive indoor air with limited number of microorganisms, especially where complicated procedures like implant surgeries take place.[22,23] Similarly, many ICUs use Air Decontaminating Units to filter the air in order to provide a sterile atmosphere besides relying on the ACs for enabling adequate exchange of purified air. In most modern air-conditioning system, the laminar air flow is directed from the ceiling downwards, thereby enabling a proper hourly air exchange rate and a sufficient supply of fresh air. Many operation theatres use a three step filtration of circulating air using integrated filters with the ventilator.[23] These filters usually remove airborne particles ≥5 µm in size with an efficiency of 80±95%.[22] However, this mandates that a constant air flow is maintained through the filters so that the quality of the air is maintained. Contamination of the filter impedes the necessary air flow resulting in reduction of the air exchange. This leads to increase in the number of microorganisms in the rooms and loss of the required class of air cleanness.[23] Moreover, the contaminated filters act as a reservoir for fungal spores, by acting as a niche for multiplication of the fungi.[7]

A study from private hospitals in India had earlier reported a contamination rate of 26% of AC filters[7] which is quite low as compared to our findings of a contamination rate of 88.2% pre-fumigation and 76.9% post-fumigation. This may be explained by the lack of periodic maintenance of equipment in the government hospitals owing to the paucity of financial resources as well as due to the lack of consciousness. Similar study done in the ICUs in Egypt for assessment of air fungal load has also reported persistence of fungi in the environment and all ACs to have persistent fungal spores.[24] A surveillance study from Italy 25 have shown that 25 out of 36 air samples in ophthalmology OT was positive for fungi over a period of 3 years. Moreover, surface samples collected from OTs and controlled environments in ICU in this study showed that 29.1% were positive for molds. The contamination rate in our study was relatively higher than other studies, highlighting the necessity of regular servicing and cleaning of the surfaces of ACs.

In our setup, the ICUs and OTs were fitted with wall mounted ACs for providing filtered air in the rooms. Studies have shown that wall mounted ACs provide better air filtration parameters as opposed to window mounted ACs.[7] However, in the absence of proper servicing, the filters the ACs, both wall mounted and window mounted, start acting as an active nidus for multiplication of fungi and leads to generation of fungal spores. Study by Dettenkofer et al[26] suggest that even the exchange of filters at periodical interval is not a sufficient measure to ensure microbiological sterility of the air in operating rooms; periodical drying the ventilation pipes and elements of equipment is recommended to generate adequately sterile air necessary to maintain a conducive atmosphere in the OT. This may possibly explain why few fungal spores persisted in one of the ACs in MICU even after stringent servicing of the filters. Dettenkofer et al[26] also suggested that shutting the ventilation systems off when they are not in use may not increase microbiological contamination of indoor air.

From the epidemiological point of view, isolation of fungi with documented pathogenicity from the environment is a concern, especially in hospitals. The most frequently isolated fungus in the present study was A. fumigatus, A. flavus and A. niger which is a known opportunistic pathogen. Other opportunistic fungi isolated in this study are also known to cause nosocomial infections.[6,7,27] The spores of Aspergillus have a diameter of 1.5-3.5 µm and a settling velocity of approximately 1m/hour in still air. With this buoyancy, the spores which resist drying can remain in the air indefinitely[7] and get settled into surgical wounds as well as inhaled. The spores are large enough to be eliminated by HEPA filters used in adjunct with ACs as such filters can eliminate particles over 0.3 µm in size. The spores usually get restricted to the surface of a properly functioning filter. However, the abundance of such spores in the

environment under study suggests inefficient filtration resulting from inadequate maintenance of the AC filters.

OTs in most healthcare facilities in India do not comply with the recommended physical parameters necessary for conventionally ventilated OTs.[7] Ophthalmic infections as well as post-operative infections by fungi present in hospital environment have been reported.[28,29] Patients undergoing surgery are exposed for longer duration to organisms suspended in OT air and thus to infective complications. The operating theater is considered a complex habitat in which all sources of pollution have to be kept under control.[30,31] Likewise, in ICUs, patients are usually immune-compromised and are usually exposed to numerous invasive procedures making them vulnerable to acquiring infection from pathogens in the hospital environment. However the minimum number of spores required to initiate infection needs to be investigated.

Following the results of this surveillance study, we recommended basic training of the hospital personnel regarding cleaning and disinfecting the filters and vents of AC units. We also recommended cleaning and disinfection of the AC units once every 3 months, with the help of vacuum suction device followed by wet mopping using sporicidal disinfectants such as chlorine dioxide in adequate concentration. The filters of AC units should be manually cleaned, washed thoroughly with mild detergent and water and then sundried before re-installation.[7] Although formaldehyde is known to be fungicidal, the formaldehyde vapours generated during fumigation often fails to reach the filters in adequate concentration. Moreover, Occupational Safety and Health Administration (OSHA) permits an exposure limit of 8 hours at a concentration of 0.75 ppm and 15 minutes at a concentration of 2 ppm considering its carcinogenic potential.[32] Glutaraldehyde, alcohols, iodophores, and sodium hypochlorite at a working concentration of 4-6% are other effective fungicidal agents which can be readily used in hospitals for surface cleaning of AC vents.[33] Such simple measures like cleaning and disinfecting the AC units at regular intervals will help in the reduction of nosocomial infections in OTs and ICUs.

The present study was undertaken for 4 months. However long term studies involving periodical monitoring of nosocomial fungal infections resulting from fungal colonization of AC units should be done throughout the year to know the fungal isolation pattern and also the clinical correlation of such findings, so as to cleaning and maintenance protocols can be modified accordingly. Also, we had to restrict our intervention strategies to 5 ACs only due to financial constraints. Although, an air-sampler is recommended for obtaining OT air bacterial load, but its cost precluded its use in the current study.

To conclude, we found 88.2% of ACs were contaminated with fungal spores prior to fumigation and the contamination was slightly decreased after fumigation (76.9%). Servicing of the ACs along with cleaning of filters resulted in a significant decrease in contamination rate (20%). However, fumigation resulted in satisfactory air bacterial load in the OTs and ICUs. We recommend regular servicing of the ACs and wet mopping of the ducts with sporicidal solution at periodic intervals.

ACKNOWLEDGEMENTS

Authors would like to thank Hospital Infection Control Committee, Indira Gandhi Government Medical College and Mayo Hospital, Nagpur, India.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: None declared

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Cite this article as: Deb A, Raut S, Gajbhiye S, Patil P, Raut S. Is fumigation enough for air conditioning units in operation theatres and Intensive care units? Int J Res Med Sci 2016;4:1583-9.