ORIGINAL ARTICLE

Evaluation of microbial contamination of air in two haematology departments equipped with ventilation systems with different filtration devices

P. CRIMI, M. VALGIUSTI, G. MACRINA[*], A. GRIECO[*], L. MASSONE[*], A. CIUCCI[*], F. ANSALDI, L. STICCHI, L. SASSO[**], S. DEL BUONO[*], P. DURANDO
Department of Health Sciences, San Martino Hospital, University of Genoa; [*] Health Management Service, "G. Gaslini" Children Hospital, Genoa; [**] Department of Health Sciences, University of Genoa, Italy

Key words

Ventilation systems • Airborne infections • Immunocompromised patients

Summary

Background. *Nosocomial infections (NI) are above all due to health-care workers practices, but also the contamination of the environment could lead to their rise in health-care facilities.*
Introduction. *In the last years, the incidence of NI has increased due to a substantial rise in the number of immuno-compromised patients. These patients are often gathered in hospital areas declared at "high risk" of infection such as Hematology and Bone Marrow Transplant ward. In this study, we evaluated microbial contamination of the air in two divisions with high risk patients, focusing on the validity of the air system with correlation to the presence or not of the HEPA absolute filters.*
Methods. *An environmental surveillance study has been carried out in two Divisions of Haematology, in two different Hospitals. Investigations have been performed by sampling air and by analyzing bacterial and fungal growth on microbiology plates after an incubation period.*
Results. *Unit A, without HEPA filters in the ventilation systems, showed a gradual increase in the bacterial load 20 and 60 days after cleaning of the ventilation system. Mycetes and Aspergilli were not present in basal conditions, at 20 or 60 days after decontamination. Unit B, equipped with HEPA filters placed at the inlet vents, showed extremely low values of the bacterial load either in basal conditions or upon inspection 60 days after cleaning. No mycetes were present.*
Discussion. *From the results obtained, it was evident that following the cleaning operation, the quality of the air is excellent in both types of equipment, since no mycetes were present and the bacterial load was < 20 CFU/mc in all the sites tested. However, although in subsequent controls mycetes were absent in both types of equipment, a great difference in the suspended bacterial load was found: Unit B was close to sterility whereas in Unit A a progressive increase was observed.*

Introduction

Nosocomial infections (NI) refer to infections occurring during hospitalization or, in some cases, after the patient has been discharged, and are due to conditions which were not clinically manifest or in incubation at the time of admission [1].
NI are, by definition, an important problem in the public health system. This not only for the serious repercussions upon the patient and the community, with additional costs to safeguard, treat and restore the patient's health, but also, and above all, for the maintenance of the quality of healthcare provided by the medical profession [2-5].
Over the last few years, this phenomenon has markedly increased, also on account of the considerable rise in the number of patients whose general and immune defences are extremely low. This might be due both to the disease from which they are suffering and to the particularly aggressive diagnostic and therapeutic procedures to which they are submitted [6-9]. These patients are often admitted in hospitalization units defined at "high risk" of infection, including Haematology and Bone Marrow Transplant Centres. In these Units, the microbiological contamination of the air may be important in the spreading of microbes responsible for infections. Often, in many Italian hospital environments, there are ventilation systems which were built only to ensure a good comfortable microclimate but totally lack adequate air turnover and filtering systems able to block the majority of micro-organisms [10-14].
It appeared worthwhile, therefore, in the present study, to evaluate microbial contamination of the air in two divisions reserved for the hospitalization of patients at high risk of nosocomial infections. In particular, attention was focused on the validity of the air system, also in correlation to the presence and the positioning of the HEPA (High Efficiency Particulate Air) absolute filters.

Material and methods

An environmental surveillance study has been carried out in two Divisions of Haematology, in two different Hospitals: Unit A which was equipped with "pocket"

filters at the Air Treatment Unit (UTA) level but without HEPA filters, either centralized or in the peripheral areas, and Unit B equipped with HEPA filters placed at the inlet vents.
Both Units have completed the decontamination of the ducts on the same day, considered as the beginning of the study. Unit A (about 320 m^2) was studied immediately after having completed the decontamination, after 20 days and after 60 days; Unit B (about 300 m^2) was studied immediately after having terminated the decontamination and after 60 days.
Air sampling was carried out in proximity of the inlet vent of the ventilation equipment: in Unit A, samples of air were collected in two wards (Ward 1A, Ward 2A) and in 3 areas of the corridor of the Unit (Beginning, Centre, End); in Unit B two series of air collection were carried out, in 2 wards (Ward 1B, Ward 2B) and in just one area of the corridor (Centre), in the Division.
Sampling was carried out using *SAS* (Surface Air System) equipment programmed to sample 200 litres of air per minute, prolonging aspiration for 5 minutes in order to evaluate an overall volume of 1000 litres of air [15].
The instrument was prepared placing a Rodac plate (PCA2001) 55 mm in diameter (equivalent to a 24 cm^2 surface) containing 16.5 ml PCA culture medium which is suitable for the growth of bacteria and then a Rodac plate containing the same quantity of Sabouraud medium, suitable for the growth of mycetes; the instrument was then placed very close to the position previously selected for collection of the sample, but not actually in contact to avoid contamination by environmental material.
Once the previously defined exposure time to the air flow had been reached, the plate was removed and immediately incubated to prevent alteration of the microbiological characteristics of the sample.

Analysis of the Environmental Samples

The plates with a PCA culture medium (aimed at bacterial growth) were incubated in a thermostat at a temperature of 37°C ± 1°C for 3 days and the plates with Agar Sabouraud medium (used for fungal growth) at 30°C ± 1°C for 7 days. Morphology of the mycelial threads, the conidia, and the spore were examined in blucotton lactophenol (LPCB)(BCLP) in dry conditions, both at low (100x) and high magnification (430x).
The number of colonies growing on the selective Agar was related to the cubic meter of air collected, in order to obtain the colony forming unit per cubic meter of air = CFU/mc.
Bacterial and mycetes load values > 0 CFU/mc were considered as positive [16, 17].

Statistical analysis

Statistical analysis, related to the bacterial load revealed at the various times, was carried out using the *paired T test* with JMP statystical analysis software. Data are expressed as mean ± SD and were considered statistically significant when $p < 0.05$.

Results

Results related to Unit A (Tab. I) show that, immediately after cleaning of the ventilation conduits, the bacterial load was very low in the air collected at the inlet vent.
However, 20 days after cleaning of the ventilation conduits, the bacterial load had increased in all 5 positions checked (the mean value was increased 5-fold with respect to the values recorded immediately after cleaning of the conduits).
At 60 days after cleaning of the ventilation conduits another consistent increase in bacterial load was observed in all 5 positions checked (the mean value was, in fact,

Tab. I. Results concerning ward A ventilation system.

Sampling Site	Ward A ventilation system without hepa filter					
	Air sampling to the inlet vent after decontamination		Air sampling to the inlet vent after 20 days		Air sampling to the inlet vent after 60 days	
	Bacterial load CFU/mc	Mycetes CFU/mc	Bacterial load CFU/mc	Mycetes CFU/mc	Bacterial load CFU/mc	Mycetes CFU/mc
Ward 1A	15	0	40	0	180	0
Ward 2A	0	0	15	0	100	0
Corridor (beginning)	10	0	25	0	70	0
Corridor (centre)	5	0	25	0	80	0
Corridor (end)	0	0	45	0	74	0
mean	6	0	30	0	100.8	0
s.d.	6,51	0	12.24	0	45.75	0
T- test paired			T = 4.311 P = 0.013			T = 3.619 P = 0.022

s.d. = standard deviation

Tab. II. Results concerning Ward B ventilation system.

Sampling site	Ward B ventilation system With hepa filter					
	Air sampling to the inlet vent after decontamination			Air sampling to the inlet vent after 60 days		
	Bacterial load CFU/mc	Aspergillus CFU/mc	Other Mycetes CFU/mc	Bacterial load CFU/mc	Aspergillus CFU/mc	Other Mycetes CFU/mc
Ward 1B	0	0	0	4	0	0
Ward 2B	10	10	0	2	0	0
Corridor (centre)	0	0	0	0	0	0
mean	3,3	3,3	0	2	0	0
s.d.	5,7	5,7	0	2	0	0
T- test paired	T = 4.311 P = 0.013					

s.d. = standard deviation

increased 15-fold with respect to the values recorded immediately after cleaning of the conduits).
Mycetes and Aspergilli were not present in basal conditions, either at 20 days or 60 days after decontamination
Results related to the B Unit (Tab. II) show that, in the first sampling, performed immediately after cleaning of the devices, the bacterial load was extremely low; in the second sampling performed 60 days after cleaning, the bacterial load was still maintained within *very low values*.
As far as concerns mycetes, the Aspergillus load exceeded recommended values in basal conditions, whilst 60 days after cleaning values had returned within the normal range.
No other mycetes were present, either in basal conditions, or upon inspection 130 days after cleaning.

Discussion

The quality of air in the operating theatre is guaranteed by constant checking and, in this respect, several countries, including Italy, have prepared Guidelines (ISPESL) focusing on the microbiological parameters in suspended air**.** On the contrary, no detailed and conclusive evaluation has ever been elaborated and accepted in what concerns the inpatient wards [18].
To date, the quality of air in these units has been evaluated primarily using the Air Duct Cleaners Association system (NADCA), proposed, however, for all indoor environments [19].
Furthermore, some units are now considered more at risk of nosocomial infections than the operating theatre used for "routine" surgical interventions [20]. Of these, it is worthwhile mentioning the Haematology Unit, upon which we have focused our attention. In fact, in these Units the most important problems from an aereogenic point of view are related to Aspergillus infection. For this reason, explicative Guidelines have been elaborated ad hoc [21-23] and assert the efficacy of the ventilation system equipped with HEPA filters to control Aspergillus infection [24-27]. However, the structural modifications needed to follow the Guidelines require considerable economic investments which cannot always be afforded by many Italian hospitals**.**
The aim of the present investigation was to conduct a comparative evaluation of the air system in use in the Haematology Unit, in which the Guidelines have been followed, and the one in use in the Haematology Unit in which the ventilation system has a traditional "pocket" filter".
This comparison has been made and we did not limit our efforts to evaluating only the mycetic load, but also studied the general bacterial load.
Since this ventilation equipment naturally faces a certain degree of particulate as well as dust contamination while in use [28, 29], numerous systems aimed at decontamination, sanification and disinfection have focused on these aspects [30, 31].
Both our ventilation systems have been evaluated immediately after the cleaning operation. For this evaluation we considered it worthwhile measuring the quality of the air according to standardized techniques using the *SAS* (Surface Air System) equipment.
From the results obtained, it was evident that following the cleaning operation, the quality of the air is excellent in both types of equipment since no Mycetes were present and the bacterial load was < 20 CFU/mc in all the sites tested.
Subsequent controls, both for Mycetes and for Aspergillus loads, showed that these microorganisms were absent in both types of equipment, a result expected for the equipment in Unit B and also found in Unit A. This unit, therefore, in the somewhat short period considered, had emitted air of sufficiently good quality to prevent the possibility of Aspergillus infection in the patients.
However, we found a great difference in the suspended bacterial load for the two types of equipment: in Unit B, the suspended bacterial load at the duct remained close to sterility, whereas in Unit A, a progressive increase was observed which, already after two months, had reached a considerable degree.

Conclusion

Our data demonstrate that, in spite of all expectations, the Units with ventilation equipment without HEPA filters are able to maintain emission of air that is not contaminated by Aspergillus for relatively long periods (2 months). Therefore, in our opinion, if these structures need structural changes to install equipment with HEPA filters, they may maintain the old equipment on the condition that the ventilation ducts are frequently cleaned. In other words, since the bacterial load increases considerably in Unit A and, therefore, the quality of the air deteriorates, it is imperative that strict cleaning and disinfection of the surfaces and instruments protocols are applied.

References

[1] Sokalski SJ, Berry M. *Nosocomial infections*. J Am Osteopath Assoc 1977;76:591-4.

[2] Sheng WH, Wang JT, Lu DC, Chie WC, Chen YC, Chang SC. *Comparative impact of hospital-acquired infections on medical costs, length of hospital stay and outcome between community hospitals and medical centres*. J Hosp Infect 2005;59:205-14.

[3] Chen YY, Chou YC, Chou P. *Impact of nosocomial infection on cost of illness and length of stay in intensive care units*. Infect Control Hosp Epidemiol 2005;26:281-7.

[4] Esatoglu AE, Agirbas I, Onder OR, Celik Y. *Additional cost of hospital-acquired infection to the patient: a case study in Turkey*. Health Serv Manage Res 2006;19:137-43.

[5] Peterson AM, Walker PH. *Hospital-acquired infections as patient safety indicators*. Annu Rev Nurs Res 2006;24:75-99.

[6] Darouiche RO. *Device-associated infections: a macroproblem that starts with microadherence*. Clin Infect Dis 2001;33:1567-72.

[7] Von Eiff C, Jansen B, Kohnen W, Becker K. *Infections associated with medical devices: pathogenesis, management and prophylaxis*. Drugs 2005;65:179-214.

[8] Rosenthal VD, Maki DG, Salomao R, Moreno CA, Mehta Y, Higuera Fet et al.; International Nosocomial Infection Control Consortium. *Device-associated nosocomial infections in 55 intensive care units of 8 developing countries*. Ann Intern Med 2006;145:582-91.

[9] Vonberg RP, Behnke M, Geffers C, Sohr D, Ruden H, Dettenkofer M, et al. *Device-associated infection rates for non-intensive care unit patients*. Infect Control Hosp Epidemiol 2006;27:357-61.

[10] Oren I, Haddad N, Finkelstein R, Rowe JM. *Invasive pulmonary aspergillosis in neutropenic patients during hospital construction: before and after chemoprophylaxis and institution of HEPA filters*. Am J Hematol 2001;66:257-62.

[11] Thio CL, Smith D, Merz WG, Streifel AJ, Bova G, Gay L, et al. *Refinements of environmental assessment during an outbreak investigation of invasive aspergillosis in a leukemia and bone marrow transplant unit*. Infect Control Hosp Epidemiol 2000;21:18-23.

[12] Engelhart S, Glasmacher A, Exner M, Kramer MH. *Surveillance for nosocomial infections and fever of unknown origin among adult hematology-oncology patients*. Infect Control Hosp Epidemiol 2002;23:244-8.

[13] Muhlemann K, Wenger C, Zenhausern R, Tauber MG. *Risk factors for invasive aspergillosis in neutropenic patients with hematologic malignancies*. Leukemia 2005;19:545-50.

[14] Eckmanns T, Ruden H, Gastmeier P. *The influence of high-efficiency particulate air filtration on mortality and fungal infection among highly immunosuppressed patients: a systematic review*. J Infect Dis 2006;193:1408-18.

[15] AA.VV. SAS-SUPER 100. International p.b.i. S.p.a. Milano, 1999.

[16] NCCLS-National Committee for Clinical Laboratory Standards. Approved Standard: M2-A7. Performance Standards for Antimicrobial Disk Susceptibility Tests, 7th edn. Wayne, Pa: NCCLS 2000.

[17] NHS Estates. Health Tecnical Memorandum 2025: Ventilation in Healthcare Premises. London: HMSO 1994.

[18] Linee guida per la definizione degli standard di sicurezza e di igiene ambientale dei reparti operatori. Roma: ISPESL 1999.

[19] NADCA (National Air Cleaners Association): ACR Standard 2006 Edition: Assessment Cleaning & Restoration of HVAC Systems.

[20] Angeles-Garay U, Velazquez-Chavez Y, Anaya-Flores VE, Valencia-Martinez JC, Lopez-Guerrero ME. *Nosocomial infections in a high specialty hospital. Factors associated to mortality in the infected patient*. Rev Med Inst;43:381-91.

[21] 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(RR-10):1-42.

[22] AIA, The American Institute of Architects. Guidelines for Design and Construction of Health Care Facilities. July 2006.

[23] CDC. Guidelines for preventing opportunistic infections among hemopoietic stem cell. MMWR Morb Mortal Wkly Rep 49, 2000, pp. 1-28.

[24] Lortholary J, Barquins S, Coureul MH, Deliere E, Zittoun R, Brucker G, et al. *Efficacy of prevention by high-efficiency particulate air filtration or laminar airflow against Aspergillus airborne contamination during hospital renovation*. Infect Control Hosp Epidemiol 1999;20:508-13.

[25] Cornet M, Levy V, Fleury L, Lortholary J, Barquins S, Coureul MH, et al. *Efficacy of prevention by high-efficiency particulate air filtration or laminar airflow against Aspergillus airborne contamination during hospital renovation*. Infect Control Hosp Epidemiol 1999;20:508-13.

[26] Hahn T, Cummings KM, Michalek AM, Lipman BJ, Segal BH, McCarthy PL Jr. *Efficacy of high-efficiency particulate air filtration in preventing aspergillosis in immunocompromised patients with hematologic malignancies*. Infect Control Hosp Epidemiol 2002;23:525-31.

[27] Berthelot P, Loulergue P, Raberin H, Turco M, Mounier C, Tran Manh Sung R, et al. *Efficacy of environmental measures to decrease the risk of hospital-acquired aspergillosis in patients hospitalised in haematology wards*. Clin Microbiol Infect 2006;12:738-44.

[28] Crimi P, Argellati F, Macrina G, Tinteri C, Copello L, Rebora D, et al. *Microbiological surveillance of hospital ventilation systems in departments at high risk of nosocomial infections*. J Prev Med Hyg 2006;47:105-9.

[29] Perdelli F, Sartini M, Spagnolo AM, Dallera M, Lombardi R, Cristina ML. *A problem of hospital hygiene: the presence of aspergilli in hospital wards with different air-conditioning features*. Am J Infect Control 2006;34:264-8.

[30] Ahmad I, Tansel B, Mitrani JD. *Effectiveness of HVAC duct cleaning procedures in improving indoor air quality*. Environ Monit Assess 2001;72:265-76.

[31] Holopainen R, Asikainen V, Tuomainen M, Bjorkroth M, Pasanen P, Seppanen O. *Effectiveness of duct cleaning methods on newly installed duct surfaces*. Indoor Air 2003;13:212-22.

■ Received on July 15, 2008. Accepted on January 8, 2009.

■ Correspondence: Prof. Paolo Crimi, Department of Health Sciences, "San Martino" Hospital, University of Genoa, via Pastore 1, 16132 Genoa, Italy - E-mail: paolo.crimi@hsanmartino.liguria.it