


ORIGINAL ARTICLE

Impact of negative pressure system on microbiological air quality in a Central Sterile Supply Department

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Abstract

Objective: Guidelines recommend that the cleaning area in a Central Sterile Supply Department (CSSD) maintain a negative pressure of the environmental air, but how much this system can impact the contamination of the air by bioaerosols in the area is not known. The objective of this study was to assess the impact of negative pressure on CSSD by evaluating the microbiological air quality of this sector.

Methods: Microbiological air samples were collected in two CSSD in the same hospital: one with and one without a negative air pressure system. Outdoor air samples were collected as a comparative control. Andersen six-stage air sampler was used to obtain the microbiological air samples.

Results: The concentration of bioaerosols in the CSSD without negative pressure was 273.15 and 206.71 CFU/m³, while in the CSSD with negative pressure the concentration of bioaerosols was 116.96 CFU/m³ and 131.10 CFU/m³. The number of isolated colonies in the negative pressure CSSD was significantly lower ($P = .01541$).

Conclusion: The findings showed that the negative pressure system in the CSSD cleaning area contributed to the quantitative reduction in bioaerosols. However, the concentration of bioaerosols was lower than that established in the guideline for indoor air quality of many countries. Therefore, it cannot be concluded that CSSDs which do not have a negative pressure system in their cleaning area offer occupational risk.

KEYWORDS

aerosols, air microbiology, air pressure, Central Sterile Supply Department, indoor air quality, occupational exposure

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1 | INTRODUCTION

A negative pressure system is intended to maintain an air pressure differential exerted in an environment to prevent air from passing into adjacent spaces such as corridors.¹ In environments considered to be at risk of dispersion of particles from biological source suspended in the air (bioaerosols)²—classically illustrated by the respiratory isolation room—maintaining negative pressure has been strongly indicated. Traditionally, the Central Sterile Supply Department (CSSD), the cleaning room is also included as an environment where there should be negative pressure.^{3–6} The CSSD has the following areas/rooms: reception and cleaning room (dirty sector); inspection room, preparation and sterilization (clean sector); and storage room and distribution of sterile materials (clean sector).^{3,7} The cleaning area is a dirty sector because it contains contaminated medical devices with the most diverse organic secretions from the attended patients and the microorganisms carried by them. Studies have shown that aerosols are generated during manual cleaning activities using devices under pressurized water and due to the operation of ultrasonic cleaners.^{8–11} However, the need to maintain a negative air pressure differential in the CSSD clean room is not supported by scientific evidence. The negative pressure system requires costs for its installation in the hospital CSSD, often requiring structural renovations, as well as maintenance costs. In addition, considering the CSSD cleaning area which is an area of constant transfer of medical devices both from care units for processing through counters and doors and outlets to the staging area, anterooms are necessary to maintain the programmed negative pressure. Some studies^{12–14} have shown a reduction in the effectiveness of particle containment in the isolation room with human trafficking with particle transfer to adjacent areas by the opening and closing of doors, even with a pressure differential of 2.5–20 Pa. Thus, the recommendations for areas intended for cleaning medical devices to have a negative pressure system is questionable.

There is also no consensus so far regarding the number of air changes per hour in the negative pressure system CSSD cleaning area. The American Society of Heating, Refrigerating and Air-Conditioning Engineers⁴ establishes a minimum of two air changes per hour and a total of six air changes per hour. The Association for the Advancement of Medical Instrumentation¹⁵ sets 10 air changes per hour as a minimum. The American Institute of Architects⁵ only states that the total air changes per hour should be six, while the Centers for Disease Control and Prevention, USA,⁶ recommends the same parameters proposed by the AIA.⁵

Considering the risk of a service which does not have negative pressure in the cleaning room, a relationship of this determination with the occupational safety risk of professionals working in this sector and in the areas adjacent to the CSSD cleaning room can be an important new information to this field. However, the risk of air contamination of already

cleaned medical devices in the preparation area is refuted considering that they will still be sterilized. There is also no justification for occupational chemical risk for professionals working in areas adjacent to cleaning, as the chemicals routinely used in the cleaning room are detergents consisting of low toxic potential products. Given the growing concern about the safety of health professionals in their work environment, which is legitimate, as well as implementing decision-making based on proven scientific evidence, this study aimed to assess the impact of negative pressure on the cleaning area of the CSSD by evaluating the microbiological air quality of this sector and the preparation area of medical devices.

2 | METHODS

2.1 | Sampling locations

Indoor air collections were performed in the cleaning and preparation rooms of two CSSDs of a 662-bed private tertiary general hospital. One of the CSSDs had a negative pressure system in the cleaning area and the other did not have negative pressure, and both had central air conditioning systems. The use of a high-pressure steam precleaning device (Steamer[®]) was established as inclusion criteria to select the CSSDs to participate in this study. This equipment has been gaining space in cleaning cannulated products, but it causes mist to form during its use; thus, constituting a challenging scenario for maintaining ambient air quality and right humidity.

The negative pressure CSSD had an anteroom before entering the cleanroom so that negative pressure was properly maintained. The cleaning room had an area of 58.00 m² and the preparation room had an area of 102.00 m². In the CSSD without negative pressure, the cleaning room had an area of 42.60 m² and the preparation room of 111.00 m². Both CSSD (with and without negative pressure) had a 99.9% filtration absolute filter in the air conditioning system. The negative pressure CSSD anteroom had a class F8 bag filter.

Air was also collected from the environment outside the hospital in order to use as a comparative control group, being conducted in an outdoor square around the hospital.

The collections were performed in quintuplicate both in the cleaning area and in the preparation area of each CSSD, while a daily air collection was performed for the outdoor air sampling. The total sampling was 624 plates, since four different culture medium types were used for each collection as described below.

2.2 | Sample collection

Six-stage Andersen solid impaction sampler equipment (Universal Electric Co., model 81KE14F, USA) was used for

TABLE 1 Distribution of central tendency measures of the colony forming units (CFU) recovered according to the temperature, relative humidity and culture medium, of the studied clean room and preparation CSSDs with and without negative pressure, and the outside environment

	T ^a	RH ^b	Negative pressure	Culture medium	N	Mean	SD	Median	Minimum	Maximum	Microorganism
Cleaning	18.6	60.8	No	Sabouraud Agar	5	154.6	45.1	167	76	188	<i>Rhodotorula</i> spp.
	21.1	62.8	Yes		5	66.2	26.3	54	43	104	<i>Penicillium</i> spp. <i>Aspergillus niger</i>
	18.6	60.8	No	Blood Agar	5	87.4	54.5	109	25	139	<i>Bacillus subtilis</i> <i>Micrococcus</i> spp.
	21.1	62.8	Yes		5	81.8	61.2	53	29	181	<i>Penicillium</i> spp. <i>Bacillus subtilis</i> <i>Micrococcus</i> spp. <i>Rhodotorula</i> spp. <i>Aspergillus niger</i>
Preparation	20.9	51.8	No	Sabouraud Agar	5	117.0	36.6	113	77	175	<i>Aspergillus niger</i>
	23.1	53.8	Yes		5	74.2	15.2	77	51	90	<i>Penicillium</i> spp. <i>Aspergillus niger</i>
	20.9	51.8	No	Blood Agar	5	122.8	151.6	63	37	393	<i>Rhodotorula</i> spp. <i>Bacillus subtilis</i>
	23.1	53.8	Yes		5	49.0	28.0	44	25	93	<i>Penicillium</i> spp. <i>Bacillus subtilis</i> <i>Micrococcus</i> spp. <i>Rhodotorula</i> spp. <i>Aspergillus niger</i>
Outside	25.6	62	No	Sabouraud Agar	3	192.0	52.8	221	131	224	<i>Aspergillus niger</i>
	26.1	61.6	Yes		3	128.7	35.9	138	89	159	<i>Penicillium</i> spp. <i>Aspergillus niger</i>
	25.6	62	No	Blood Agar	3	165.3	64.3	183	94	219	<i>Bacillus subtilis</i> <i>Rhodotorula</i> spp.
	26.1	61.6	Yes		3	76.3	36.5	61	50	118	<i>Penicillium</i> spp. <i>Bacillus subtilis</i> <i>Micrococcus</i> spp. <i>Aspergillus niger</i>

^aAverage temperature in degrees Celsius (°C).

^bAverage relative humidity (%).

ambient air collection. This was laid out on a table with a height of 1.0 m. A single type of culture medium was placed at all stages of the Andersen for each collection. The collection order was standardized considering the disposition of the culture media in the Andersen stages as follows: the first collection was performed placing the Sabouraud glucose agar culture medium in all stages of the Andersen, which is selective for fungi; the second collection was conducted with blood agar culture medium, which is a rich and nonselective medium indicated for recovering Gram-positive and -negative bacteria, as well as fungi; the third collection was performed with legionella selective agar medium, which is a selective culture medium for *Legionella* spp.; finally, the fourth collection was conducted with Lowenstein-Jensen agar culture medium, which is selective for mycobacteria. The air flow rate in the equipment was 28.3 L/min and the air sampling time for each collection was 20 minutes, which corresponded to a volume of 566 L of air passage for each collection.

Air sampling outside the hospital was performed prior to starting the air collection in the CSSD cleaning and preparation room to enable a comparison of the outdoor and indoor air contamination using the same four culture media as described above.

Air was collected in the cleaning room and then in the preparation room after collecting the outside air. The ambient air samples from both the cleaning room and the preparation room were performed during times of intense workload. Hot water showers, pressure guns, high-pressure steam equipment, an ultrasonic washer, and washers' disinfectors were in use during the clean room collections. The scenario was characterized as the moment of maximum release of droplets and aerosols in the environment due to the cleaning activity of medical devices being performed.

Room temperature and relative humidity were monitored during the experiments using a calibrated digital thermohygrometer (Incoterm, 7664, China).

2.3 | Cultivation and isolation

After the samples were collected, they were incubated in a microbiological incubator (LabIncubator, model 2503, Fanem[®], Brazil) at a temperature of $35 \pm 2^\circ\text{C}$. The plates with the fungal selective media (Sabouraud agar) and *Legionella* spp. (selective legionella agar) remained in the incubator for up to 15 days, blood agar for 5 days, and Lowenstein-Jensen mycobacterial selective agar for up to 60 days of incubation. Sample readings were taken daily with colony counts on an electronic colony counter (Quimis[®], model 02958, Brazil).

2.4 | Microbiological identification

The fungi identification was carried out through macro- and microscopic morphological characteristics and preliminary identification of the bacteria through the Gram method, but no identification methods were employed as there was no recovery of *Legionella* spp. or mycobacteria.

2.5 | Statistical analysis

Descriptive analyses were performed and three-factor ANOVA, Wilcoxon-Mann-Whitney test and linear regression were applied.

3 | RESULTS

Most of the isolated microorganisms in both CSSDs were fungi. The identified bioaerosols were as follows: *Penicillium* spp., *Aspergillus niger*, *Rhodotorula* spp., *Micrococcus* spp. and *Bacillus subtilis*. The *Penicillium* spp. genus was not isolated in the CSSD without negative pressure. Neither *Legionella* spp. nor mycobacteria were isolated in the collected air samples. Regarding sedimentation of microorganisms in the different Andersen air sampler stages, only *Penicillium* spp. was not isolated at Stage 6.

The negative pressure CSSD presented a smaller count of CFU in both the cleaning room and the preparation room when compared to the CSSD without negative pressure, according to the descriptive analysis of the data presented in Table 1.

The average concentration of bioaerosols in the CSSD cleaning and preparation rooms without negative pressure was 273.15 and 206.71 CFU/m³ of fungi, respectively, while the average concentration of bioaerosols in the environment outside the hospital was 339.22 CFU/m³ of fungi. The average concentration of bioaerosols in the CSSD with negative pressure was 116.96 CFU/m³ of fungi in the cleaning room, 131.10 CFU/m³ in the preparation room, and 227.32 CFU/m³ of fungi outside the hospital. The following findings regarding the inside/outside ratio were obtained: it was 0.5 in the cleaning room of the CSSD with negative pressure and 0.58 in the preparation room; while the ratios in the cleaning and preparation rooms for the CSSD without negative pressure were 0.8 and 0.6, respectively.

The load of isolated bioaerosols in the negative pressure CSSD was statistically lower ($P = .01541$) than the CSSD without negative pressure. In making this comparison considering the cleaning and preparation rooms of both CSSD with and without negative pressure, it is possible to verify that the number of colonies simultaneously falls in the cleaning and

TABLE 2 Estimation distribution, standard error, p-value, and confidence intervals of isolated bioaerosols in CSSD cleaning and preparation rooms with and without negative pressure, according to temperature and relative humidity

Term	Estimate	Std. error	Statistic	P-value	Conf. low	Conf. high
(Intercept)	-567.68	364.89	-1.56	0.1268	-1302.60	167.24
Temperature	20.79	9.82	2.12	0.0399	1.00	40.57
Humidity	3.77	2.26	1.67	0.1021	-0.78	8.33
Cleaning room	80.66	62.31	1.29	0.2021	-44.84	206.15
Preparation room	63.81	59.27	1.08	0.2873	-55.55	183.18

preparation room when the CSSD has the negative pressure system in the cleaning room.

Regarding the relationship of temperature and relative humidity compared to the count of colonies, in Table 2 it can be seen that the number of colonies increased by an average of 20.79 CFU at each temperature degree, and there is an average increase of 3.77 in the number of colonies compared to the relative humidity.

4 | DISCUSSION

The present investigation showed that the negative pressure system in the CSSD cleaning room contributed to a quantitative reduction in the ambient air contamination level by bioaerosols. By making this comparison considering the cleaning and preparation rooms of both CSSDs with and without negative pressure, it is possible to verify that the number of colonies simultaneously falls in the cleaning and preparation room when the CSSD has the negative pressure system in the cleaning room; this might be expected as there is forced exhaustion of air out of the building.

The bioaerosol concentration in the CSSD without negative pressure system in the cleaning room was only 273.15 CFU/m³ of fungi and 206.71 CFU/m³ of fungi in the even smaller preparation room. Review studies^{16,17} which evaluated the standards and guidelines for indoor air quality by bioaerosols established by government agencies in various countries showed that there is no consensus on bioaerosol concentration, and fungi are mostly used as indoor air quality markers, ranging from less than 100 CFU/m³ to more than 1000 CFU/m³. There is no clarity on the reasons why fungi were elected as markers of ambient air contamination, perhaps due to the possibility of sensitization to respiratory allergies. Many of these guidelines were proposed by expert consensus considering absolute or relative numbers (indoor/outdoor comparison) and did not evaluate the effect of fungal concentration on the health of individuals. These parameters arbitrated by regulations are controversial, as there is no evidence linking fungal spore coefficients to a safe level of worker exposure.

The World Health Organization¹⁸ states that health-relevant indoor microbial pollutants are largely heterogeneous. As people are often exposed to multiple biological and chemical agents simultaneously, it is complex to accurately estimate individual species of microorganisms and other biological agents that are responsible for health effects, and it is almost impossible to identify them. Although it is difficult to conclusively determine which agents cause adverse health effects, the WHO states that an excessive level of any of these agents in the environment becomes a potential health risk; however, it does not determine the load it considers excessive or which microorganisms would characterize health threats.¹⁸

The Centers for Disease Control and Prevention⁶ recognizes the limitations on microbiological air sampling, ie, a lack of standards linking fungal spore levels to infection rates to a safe level of exposure. Added to this is the difficulty of tracking the precise source of a fungus and distinguishing between infections acquired in the healthcare setting or in the community.

Some studies^{19,20} state that the health risks associated with airborne fungi are mainly linked to the spread in wards containing immunocompromised patients, in which species such as *Aspergillus flavus*, *Aspergillus fumigatus*, *Blastomyces*, *Coccidioides* and *Cryptococcus* are potentially threatening to the lives of these patients. In healthy people working indoors, exposure to fungi and their spores can trigger sensitization and induction of a clinically symptomatic allergy such as rhinitis, asthma and sinusitis.^{20,21} Fungi associated with such reactions and which may be commonly isolated in the interior environment include species of *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria*.^{19,20} Although these studies have associated some fungal genera with respiratory allergies, the concentration required to trigger such reactions is not determined.

Systematic reviews considering both epidemiological studies in humans²² as well as experimental studies in animals²³ were intended to derive bioaerosol exposure limits based on health effects. The reviews concluded that none of the studies reviewed provided adequate dose-response ratios to establish such exposure limits. The limitations identified in the studies analyzed by Walser et al (2015) were as follows: heterogeneity of health effects; lack of studies with valid data for dose-response assessment; insufficient exposure

assessment; and a diversity of methods employed to measure microorganisms in bioaerosols. Given the impossibility of determining exposure limits from the review of human studies, the same group performed another review evaluating animal studies.

In a systematic review,²³ the four included studies exposed guinea pigs and mice to different fungi genera with an exposure dose concentration ranging from 105 to 109 spores/m³ for a period of 4–12 weeks. An increase in the number of macrophages, neutrophils, eosinophils, and lymphocytes was reported in all included studies following exposure to spores or fungal conidia, with significant inflammation following exposure to *Aspergillus fumigatus*. Despite the suggestion of a relationship between dose-response and time dependence, the results of the studies could not be directly compared given the wide variability and poor description of exposure conditions. Thus, the authors concluded that further experimental studies need to be conducted to highlight and establish bioaerosol concentration limits capable of causing health effects.

According to the “Medical diagnostics for indoor mold exposure” guideline developed by German and Austrian medical societies,²¹ most fungal species are classified in risk group 1 and only *Aspergillus fumigatus* and *A. flavus* species are in risk group 2. Risk 1 corresponds to biological agents which are unlikely to cause disease in humans, while biological agents that can cause disease in humans and may be a health hazard to workers are in risk group 2, but their spread to the general population is unlikely and there are effective preventative measures and treatment. Considering this risk classification in the present study, all identified biological agents are classified in risk group 1. Microorganisms isolated from the air were *Penicillium* spp., *Aspergillus niger*, *Rhodotorula* spp., *Bacillus subtilis*, and *Micrococcus* spp. It was observed that the negative pressure CSSD presented a larger variety of microorganisms in each collection compared to the CSSD without negative pressure. *Penicillium* spp. was not isolated in the CSSD without negative pressure. This fungus is not classified in the health risk group established by the National Institute of Safety and Hygiene in Spain.²⁴

In contrast to the microorganisms previously discussed of fungal origin, *Bacillus subtilis* is a Gram-positive bacterium with the ability to form spores and survive in the environment for years, been found in the air, soil, water, and vegetation. Occasionally, it can cause disease in immunocompromised individuals. Bioaerosols with *B. subtilis* and *Micrococcus* have been isolated in other studies of indoor air in hospital environments.^{25,26}

All microorganisms isolated in the present study are distributed in nature and have been isolated in the hospital environment in several studies.^{25–29} It should be noted that *Aspergillus niger* presented the lowest cytotoxicity among

the fungi evaluated in a study that collected air samples in several hospitals, especially in locations with immunocompromised patients.³⁰

In addition to an analysis of indoor air quality regarding the contamination level by fungi and bacteria in general in the present study, specific analyses for *Legionella* spp. and mycobacteria were also conducted, given the occupational health risks. As CSSDs contain hot water source whether through taps, ultrasonic washers, washers’ disinfectors and high-pressure steam equipment, research into the presence of *Legionella* spp. became important. Moreover, mycobacteria were also important to investigate, especially *M. tuberculosis*, considering that health products contaminated by this microorganism and aerosolized in the cleaning process could become a health risk to professionals working in this sector. However, there was no growth of these microorganisms in the samples.

5 | CONCLUSIONS

The findings of the present investigation showed that the negative pressure system in the CSSD cleaning room had impact in this environment and in the preparation room, contributing to a quantitative reduction in bioaerosols. On the other hand, the average bioaerosol concentration can be considered low when compared to the limits set by government agencies in many countries, even in the CSSD without this air treatment system. In addition, airborne pathogens known to be pathogenic such as *Legionella* spp. and *Mycobacterium tuberculosis* were not recovered in the present investigation. Furthermore, the number of microorganisms in all the investigated CSSD environments was lower than in the compared external environment.

It is noteworthy that the load and type of microorganisms present in any ambient air are circumstantial, unstable and mainly dependent on microbial disseminators present in the place, whether people or activities. In this sense, a CSSD is not conclusively condemned as an occupational risk because it does not have a negative pressure system in the cleaning room setting. Another point that endorses the question of whether this sector is required to contain a negative pressure system is the lack of referential standards capable of relating safe levels of bioaerosol exposure and health effects so that there is no evidence of occupational health risks. Given this, the authors of this study consider that the requirement for the CSSD cleaning room to have negative pressure should not be imposed in the regulations until the health risk relationship by bioaerosol concentration is scientifically proven.

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DISCLOSURE

No ethical approval and informed consent were required, as there were no humans involved in the samples. Neither clinical trial nor animal study were applicable. *Conflict of interest*: There is no conflict of interest to declare by any author.

AUTHOR CONTRIBUTIONS

Drs Almeida, Bruna, and Graziano had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Drs Almeida, Bruna, and Graziano. Acquisition, analysis, or interpretation of data: Drs Almeida, Bruna, and Graziano. Critical revision of the manuscript for important intellectual content: Drs Graziano and Gambale. Isolation, culture, and analyses of the microorganisms: Drs Mimica, Navarini, Sasagawa, and Moriya.

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