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Original Article

QuEChER method for air microbiological monitoring in hospital environments

Iván Tavera Busso[1], Florencia Herrera[2], María F Tames[1], Ignacio González Gasquez[2], Lilia N Camisassa[2], Hebe A Carreras[1]

[1] *Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET and Departamento de Química, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina*
[2] *Hospital Provincial Domingo Funes, Ministerio de Salud. Dean Funes, Santa María de Punilla, Córdoba, Argentina*

Abstract
Introduction: Nosocomial pathogens have become a priority issue for public health, since they are responsible for increased morbidity and mortality in hospitalized patients and the development of multi-resistant microorganisms, as well. Recent studies found strong evidence that airborne transmission plays a key role in many nosocomial infections. Thus, we aim to develop a QuEChER methodology for the characterization of airborne microbial levels, analyzing potential variables that modify the air microbiological load.
Methodology: Particulate matter levels and suspended and settled bioaerosols were determined simultaneously employing optical sensors, Harvard impactors and settle plates, respectively. Environmental variables were also measured at different sites during different working shifts and seasons.
Results: We found a straightforward relationship between airborne particles, air exchange rates, and people influx. Levels of suspended microorganisms were related to fine particulate matter concentration, CO_2 and ambient temperature. A positive linear relationship ($R^2 = 0.9356$) was also found between fine particulate matter and CO_2 levels and air microbial load.
Conclusion: The QuEChER methodology is an effective methodology that could be used to improve the surveillance of nosocomial pathogens in developing countries hospitals where air quality is scarcely controlled.

Key words: hospital aerobiology; QuEChER; $PM_{2.5}$; CO_2; microbial load.

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Introduction

Hospital-acquired infections have become a priority health issue due to the high percentage of people affected, not only patients but also permanent staff working in these institutions. In fact, it has been demonstrated that 10% of infections acquired by hospitalized patients are nosocomial infections which can increase their morbidity and mortality. Even more, environmental microorganisms that are largely innocuous to healthy individuals represent severe risks for immunocompromised patients, those undergoing surgery or with burn wounds [1].

Recent studies found strong evidence that airborne transmission plays a key role in many nosocomial infections [2], suggesting hospital air quality could be a significant risk factor for patients. Season, weather conditions, indoor ventilation system, the intrusion of moisture, outdoor microbial load and number of occupants, visitors and human activities are factors that might modify indoor air quality, improving conditions for microbial growth and dissemination [3].

Airborne microorganisms or bioaerosols are transmitted through the air according to their size [4]. Thus, bioaerosols larger than 5 µm can only affect people located at a close distance while smaller particles (1 µm to 5 µm) can be aero-transported and affect people located at larger distances. Despite growing evidence demonstrating microorganisms [1] or even virulence factors such as antibiotic resistance genes [5] can be effectively transmitted through air, the contribution of airborne transmission in hospital infections has received less attention.

Previous studies about the prevention of nosocomial infections have focused on direct contact transmission. In contrast, current studies focused on air transmission since air quality within a hospital can vary throughout the building[3]. However, these studies have mainly centered on intensive care units and operating theatres or studied specific microbial loads [6,7]. Few

of them investigated biological and non-biological pollutants at the same time, their distributions at different working spaces or analyzed the variables that contribute to their spread [3,8]. Furthermore, novel methodologies of air monitoring are quite expensive, which represents a disadvantage for developing countries.

Nowadays, QuEChER methods (quick, easy, cheap, effective and rugged) are widely employed for a large variety of measurements, however, there is no such method for air microbiological monitoring, except for the settle plates that despite being rather old, it is the only one that could be considered QuEChER. Nevertheless, it does not accurately represent the suspended microorganisms responsible for diseases spreading [1]. Therefore, the aim of the present study was to develop a QuEChER methodology for the characterization of airborne microbial levels, estimating also the influence of potential variables that could modify the air microbiological load.

Methodology

Sampling site and study design

The study was conducted at the Domingo Funes Regional Hospital (DFH), a public institution located 60 km northeast from Córdoba city, in the middle of a sub-humid mountain forest. The hospital was originally built as a tuberculosis treatment center due to the climatic conditions of the location area. Consequently, the structure has wide corridors with large windows that ensure a high air exchange rate. Nowadays, this medium-complexity hospital is a regional reference center that covers a 250,000 inhabitants' area. Several services, such as intensive care unit, pediatrics, and adult outpatient medical specialties, neonatology care unit, general surgery, haemotherapy, laboratory and diagnostic imaging aid 200-300 inpatients per day, in two shifts, with higher demand during morning hours.

Three transmission modes are relevant when studying indoor airborne pathogens in hospitals: aerosol clouds, droplet spray, and fomites [1]. To integrate these models, suspended and sedimented microorganisms were sampled in 7 different isolated sampling areas within the hospital: Adults Outpatient Offices (AOO), Bathrooms (B), Intensive Care Unit (ICU), Laboratory (L), Pre/Post Surgery Room (P/PSR), Paediatric Outpatient Offices (POO) and Recovery Room (RR) [9]. Except for the ICU, all locations have large windows that remain open during all daylight hours. In addition, all areas have vents in the ceilings connected to the central air conditioning system.

At each sampling area, active and passive monitors were located during the morning and afternoon shifts for a 20 minutes' period. In order to assess seasonal variations, two sampling campaigns were performed during warm (from February to April 2017) and cold (from July to September 2017) periods.

Environmental parameters

$PM_{2.5}$ (µg m^{-3}), temperature (ºC), relative humidity (%) and CO_2 (ppm) were measured at each sampling area employing the optical sensor Air Node (Air Visual, Goldach, Switzerland). CO_2 was determined as an indicator of the number of occupants in each area [10].

Microbiological air sampling

Passive monitoring

The concentration of settled microorganisms, typing and viability were assessed employing settle plates with different growth media. Aerobic mesophilic bacterial count was performed employing chocolate agar (PolyVitex, Biomerieux, Saint Louis, USA). Plates were incubated at 35 ± 1ºC for 7 days. The fungal load was assessed using malt extract agar (Oxoid, Hampshire, United Kingdom) supplemented with chloramphenicol (0.05%). Plates were incubated at 25 ± 1ºC for 7 days. Triplicate samples for each culture medium were collected to ensure sampling accuracy.

Bacterial phenotyping was based on morphology, Gram-staining, endospore formation, catalase activity and oxidase production [11]. Chromogenic agar (CPS ID3, Biomerieux, Saint Louis, USA) was also employed for phenotyping. Bacteria were grouped into morphological groups as Gram-positive cocci, Gram-negative cocci, Gram-positive rods, and Gram-negative rods according to their microscopic morphology. Some commonly found bacteria were identified using a miniaturized biochemical test (RapID, Remel, Thermo Fisher Scientific, Waltham, USA). Identification of filamentous fungi was carried out on material mounted in lactophenol blue and achieved through morphological characteristics listed in the illustrated literature [11]. The number of colony-forming units (CFUs) from each petri dish was corrected using the positive hole correction table MAS-100 provided by the supplier. The air bioburden values were expressed in CFUs m^{-2} h^{-1} and the limit of quantification was 10 CFU m^{-2} h^{-1} [12].

Active monitoring

The concentration of total suspended microorganisms (TSM) (bacteria and fungi), typing and viability were assessed employing a Harvard Impactor

at 12.5 L min^{-1} with an expected cut point a little bit over 2.5 µm. At each sampling site, 3 air samples were collected in sterile 47-mm polytetrafluoroethylene filters with a 1.0 µm pore (*Millipore*). Filters were then cultured in Petri dishes as described in Frankel *et al.* [12], employing the culture mediums previously mentioned.

Data analysis

Environmental parameters and microorganism's concentration were expressed as mean ± standard error. Statistical analyses were performed employing IBM SPSS 19.0 (IBM Corp., Armonk, USA). Values obtained at different locations, shifts and periods were compared using the one-way analysis of variance test with Tukey post hoc comparisons or Kruskal-Wallis non-parametric test, as appropriate. Differences with a p-value < 0.05 were considered statistically significant. Pearson coefficients were also calculated to evaluate associations between environmental parameters as well as principal component analysis (PCA).

Results

To assess microbiological indoor air quality at different sampling sites and different sampling periods, we first performed a qualitative analysis of the microbial species recovered (Table 1). In both sampling periods, the microorganisms isolated from airborne samples were also isolated in settle plates, the other way around was not always true. This fact was particularly evident during the cold period: 3 to 5 out of 10 suspended species were also isolated in settle plates. On the contrary, during the warm period, the proportion was 7 to 10 out of 10 isolated species. This result can be related to ambient temperature, since during the cold season doors and windows remain closed most of the time, thus preventing the natural ventilation. Even more, during this period there is a high number of patients with respiratory affections, which increase the microorganism's biodiversity [1]. On the other hand, differences between sedimented and suspended microorganisms can be also related to particles aerodynamic diameter, since bioaerosols larger than about 0.3 µm, which contains most of the bacterial and

Table 1. Species isolated at different sampling sites during the cold and warm period within the Domingo Funes Hospital.

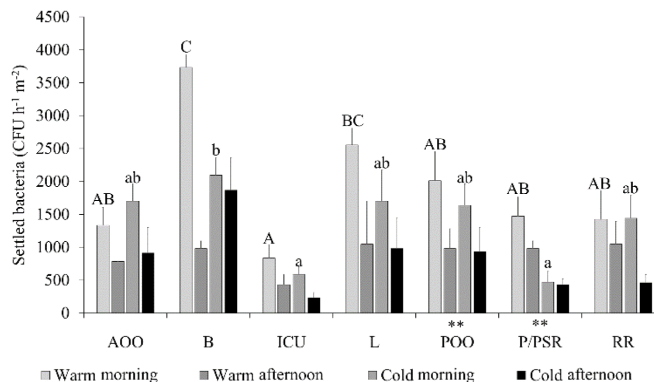
Location	Warm		Cold		
	Sedimented	**Suspended**	**Sedimented**		**Suspended**
AOO	*Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Bacillus spp.* *Enterobacter aeroges* *Klebsiella pneumoniae* *Nocardia spp.* *Pseudomonas stutzeri*	*Methicillin-resistant* *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus lugdunensis* *Streptococcus agalactiae*	*Bacillus spp.* *Pseudomonas aeruginosa* *Methicillin-resistant* *Staphylococcus aureus*
B	*Acinetobacter baumanii* *Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Actinomyces spp.* *Aspergillus spp.* *Bacillus spp.* *Corynebacterium spp.* *Nocardia spp.* *Proteus penneri*	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus hominis* *Streptococcus agalactiae*	*Aspergillus spp.* *Bacillus spp.* *Proteus penneri* *Pseudomonas aeruginosa* *Staphylococcus aureus*
ICU	*Actinomyces spp.* *Aspergillus spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Actinomyces spp* *Corynebacterium spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Acinetobacter baumanii* *Aspergillus spp.* *Bacillus spp.* *Nocardia spp.* *Pseudomonas aeruginosa*	*Pseudomonas stutzeri* *Staphylococcus aureus* *Staphylococcus epidermidis* *Streptococcus agalactiae* *β-hemolytic Streptococcus*	*Acinetobacter baumanii* *Pseudomonas aeruginosa* *Staphylococcus aureus* *β-hemolytic Streptococcus*
L	*Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Aspergillus spp.* *Bacillus spp.* *Cellulomonas spp.* *Corynebacterium spp.* *Enterobacter aerogenes*	*Nocardia spp.* *Pseudomonas stutzeri* *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus hominis* *Streptococcus agalactiae*	*Aspergillus spp.* *Bacillus spp.* *Corynebacterium spp.* *Nocardia spp.* *Staphylococcus aureus*
POO	*Aspergillus spp.* *Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Aspergillus spp.* *Bacillus spp.* *Corynebacterium spp.* *Enterobacter aerogenes* *Nocardia spp.* *Proteus penneri*	*Pseudomonas aeruginosa* *Pseudomonas stutzeri* *Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus hominis* *Streptococcus agalactiae*	*Aspergillus spp.* *Bacillus spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*
P/PSR	*Actinomyces spp.* *Corynebacterium spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Aspergillus spp.* *Bacillus spp.* *Corynebacterium spp.* *Klebsiella pneumoniae*	*Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus* *Staphylococcus epidermidis*	*Aspergillus spp.* *Staphylococcus aureus* *Staphylococcus epidermidis*
RR	*Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Actinomyces spp.* *Corynebacterium spp.* *Nocardia spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*	*Actinomyces spp.* *Aspergillus spp.* *Bacillus spp.* *Cellulomonas spp.* *Nocardia spp.* *Pseudomonas aeruginosa*	*Staphylococcus aureus* *Staphylococcus epidermidis* *Staphylococcus hominis* *Staphylococcus simulans* *Streptococcus agalactiae*	*Aspergillus spp.* *Bacillus spp.* *Pseudomonas aeruginosa* *Staphylococcus aureus*

fungal spores, tend to settle out [13]. Regardless of the sampling period, the areas with the lowest microbiological load (ICU, P/PSR, and RR) also showed the lowest number of species.

Although there are no guidelines for indoor $PM_{2.5}$ concentration, the values measured were always below the WHO outdoor daily guideline concentration (25 µg m^{-3}). Despite shifts, the cold period showed significantly higher particle concentrations than the warm period at all sampling sites, except for the L (Supplementary Figure 1). This fact may be related to the low ventilation during winter that increases indoor particles' concentration [14]. On the other hand, $PM_{2.5}$ levels in the L may be due to the high density of patients and staff in this area, regardless of the season. Considering shifts, a higher particles' concentration was observed during the morning than afternoon shifts although mean values were not statistically different. Also, no differences were observed among sampling sites in the afternoon shifts neither during the warm nor the cold period, which can be related to the less influx of people during this shift.

Two environmental parameters also showed differences between periods and shifts (Table 2). Levels of CO_2 increased during morning shifts when the hospital is fully operational, and during the cold periods due to the scarce air exchange rate. This agrees with temperature values, since more ventilated sampling sites, such as B, L, AOO, and POO, showed indoor

Figure 1. Concentration of settled bacteria at different sampling sites within the Domingo Funes hospital.



Bars with the same letters do not have significant statistical differences for the same period and shift. Asterisks indicate statistical differences between periods (no shift distinction); "**"; $p < 0.01$.

temperature values similar to the outdoor ones. On the other hand, the most critical services (ICU, P/PSR, and RR) presented more stable temperature values, as expected. No differences were found regarding relative humidity.

The microbiological analysis showed statistical differences between shifts and periods only in settled bacteria (Figure 1) and fungi (Figure 2). Regarding periods, both parameters tend to be higher during the warm season, but only the P/PSR sampling site showed statistical differences. This effect may be attributable to

Table 2. Environmental parameters measured at different sampling sites within the Domingo Funes Hospital.

Parameter	Sampling Site	Warm		Cold		p-values	
		Morning	Afternoon	Morning	Afternoon	Period	Shift
Temperature (ºC)	AOO	21.8 ± 0.4	21.7 ± 0.6	19.6 ± 0.6	19.7 ± 0.5	**	
	B	22.2 ± 0.3	22.3 ± 0.5	19.9 ± 0.9	20.2 ± 0.4	***	
	ICU	22.8 ± 0.3	22.9 ± 0.2	20.8 ± 1.8	21.9 ± 0.2		
	L	22.1 ± 0.6	22.9 ± 0.5	19.5 ± 1.1	20.6 ± 0.5	**	
	POO	21.8 ± 0.6	22.0 ± 0.6	19.0 ± 0.8	19.6 ± 0.5		
	P/PSR	23.1 ± 0.8	23.9 ± 0.5	21.9 ± 1.3	22.3 ± 0.8	***	
	RR	23.0 ± 0.4	23.1 ± 0.3	22.3 ± 0.4	22.6 ± 0.2		
Relative Humidity (%)	AOO	63.8 ± 7.8	66.1 ± 5.7	60.9 ± 6.4	63.4 ± 5.1		
	B	62.6 ± 7.8	68.0 ± 4.1	60.0 ± 5.9	63.2 ± 6.4		
	ICU	60.3 ± 7.0	64.8 ± 5.1	63.5 ± 4.3	61.4 ± 5.0		
	L	60.2 ± 6.4	64.8 ± 4.8	62.2 ± 4.2	64.7 ± 5.8		
	POO	63.7 ± 7.0	65.7 ± 5.1	61.6 ± 6.1	63.3 ± 5.2		
	P/PSR	59.3 ± 7.9	68.4 ± 7.7	61.3 ± 8.5	58.8 ± 5.4		
	RR	58.8 ± 8.1	63.4 ± 6.9	61.7 ± 6.5	58.8 ± 4.6		
CO_2 (ppm)	AOO	670 ± 191	415 ± 138	688 ± 69	598 ± 47		
	B	663 ± 167	451 ± 158	716 ± 99	502 ± 74		
	ICU	683 ± 185	493 ± 192	644 ± 290	704 ± 152		
	L	927 ± 232	374 ± 130	799 ± 81	763 ± 208		
	POO	712 ± 184	349 ± 111	770 ± 137	567 ± 40		*
	P/PSR	535 ± 147	336 ± 157	502 ± 56	544 ± 107		
	RR	489 ± 121	344 ± 112	583 ± 164	521 ± 59		

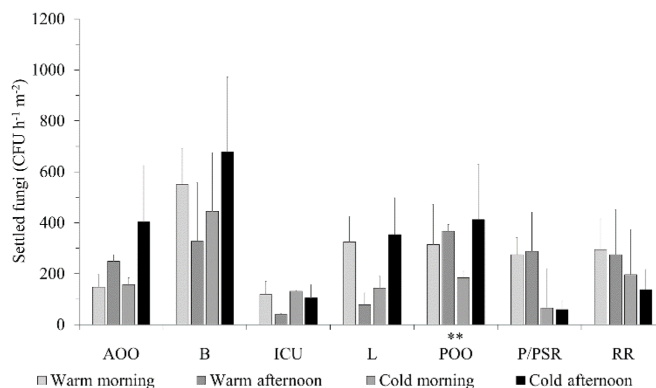
Ref.: " * ", $p < 0.05$; " ** ", $p < 0.01$; " *** ", $p < 0.001$.

the air-cooling system that moves the air mass from the roof to the floor, pulling down microorganisms and increasing settling times, thus reducing their concentration in the air [1]. In contrast, settled bacteria concentration showed statistical differences between sampling sites, with highest values during the morning shifts. Despite a similar trend, no differences were found for fungi concentrations, which may be due to the high data variability. The cleanest areas at the hospital (ICU, P/PSR, and RR) had the lowest bacteria concentration during cold afternoons, while the most crowded ones, such as L and B, had the highest values during warm mornings. This fact seems to be related to the influx of people since a cleaning shift is performed at the end of the morning hours and there are only a few people attending the hospital during the afternoon. No reference values were found for the bathroom area. It is noticeable that despite the fact settle plates are becoming obsolete, microbial concentrations at each sampling site were below the limit values of the 1978 Fisher index of microbial air contamination [15]. However, in order to compare with air microbiological quality guideline values, overall means of TSM were calculated for each sampling site (Figure 3). The laboratory presented the highest levels of TSM, compared to the other sampling sites, which may be related to the high influx of people at this service. However, no significant differences were found between sampling areas or shifts.

Associations between environmental variables and sampling sites were assessed with a PCA (Supplementary Figure 2, Supplementary Table 1). Two principal components were obtained, which explain 81.1 % of the data variability. The first component was driven by temperature and the second component by $PM_{2.5}$, CO_2, and TSM. Levels of $PM_{2.5}$, CO_2, and TSM were mainly associated with the L, while relative humidity and settled microorganisms where associated with the B, AOO, and POO. In contrast, the temperature was associated with ICU, P/PSR, and RR, which are the lowest ventilated areas.

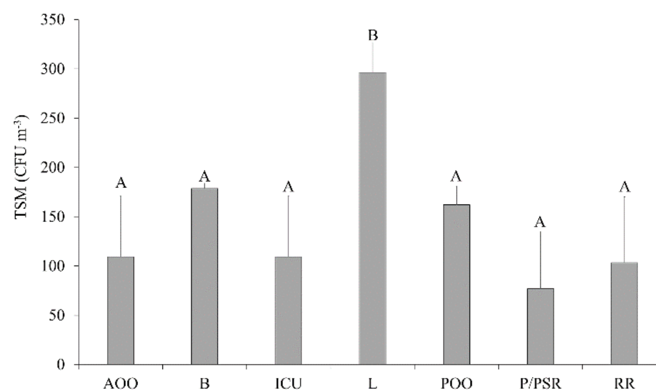
Finally, in order to predict the TSM concentrations and to estimate the influence of different TSM predictors, we performed a multiple linear regression analysis employing those environmental variables that were significantly associated with TSM (data not shown). Several models were assessed and compared employing the adjusted R^2 value. We finally selected a model that retained CO_2, $PM_{2.5}$ and settled bacteria as significant predictors (Equation 1) through a stepwise regression method ($p < 0.05$) (Adjusted $R^2 = 0.9356$).

Figure 2. Concentration of settled fungi at different sampling sites within the Domingo Funes hospital.



Asterisks indicate statistical differences between periods (no shift distinction); "**"; p < 0.01.

Figure 3. Total suspended microorganisms (TSM) concentration at different sampling sites within the Domingo Funes hospital.



Bars with the same letters do not have significant statistical differences.

$$TSM = CO_2 \times 0.3730 + PM_{2.5} \times 9.590 + Settled\ bacteria \times 0.02523 - 194.5 \qquad (1)$$

Discussion

In the present study, we assessed the air microbiological quality at different services within a medium-complexity hospital. We showed that some microbes related to nosocomial infections, such as *Acinetobacter baumanii, Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus,* and β-hemolytic *Streptococcus*, are effectively disseminated through the air. Many isolated microorganisms were also reported by other authors as constituents of the normal air flora in hospitals [1,3]. Still, some of them are responsible for several hospital-acquired infections [16]. Indeed, according to the DFH laboratory

microbiological service report, bacteria such as *Acinetobacter baumanii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the most frequent etiological agents of nosocomial infections. Furthermore, some other species isolated in the present study, such as methicillin-resistant *Staphylococcus aureus* (AOO) and β-hemolytic *Streptococcus* (ICU), are concerning since they may cause infections in hospitalized patients that are hard to treat [11,17].

Regarding particles, overall $PM_{2.5}$ concentrations were similar or lower than those reported by different authors for similar locations [9,18,19]. The low $PM_{2.5}$ values measured in the present study may be a consequence of the hospital location since indoor concentrations are usually related to outdoor levels. These results suggest a straightforward relationship between airborne particles, air exchange rates, and people influx within the hospital, which is supported by the high CO_2 concentrations measured in the most crowded areas [20]. Despite the lack of information from other hospitals in Argentina, our results are in accordance with data found for hospitals in other countries. Fransson, *et al*. informed CO_2, temperature and relative humidity values in an orthopedic ward in Sweden comparable to those measured in the P/PSR and RR sampling sites [18]. On the other hand, Baurès *et al*. who analyzed environmental variables in two French hospitals, reported similar temperature values and low humidity and CO_2 concentration than the values measured at the L, P/PSR, and RR sampling sites [8]. These differences may be attributable to building characteristics, the presence of air-conditioned and specific activities performed at each service.

Nowadays, there are no updated indoor guidelines for microbial load in hospitals. The existing one is nearly 30 years old. Furthermore, several authors argued about the establishment of quantitative guidelines due to the lack of connection to human dose/response data and the absence of standardized protocols [16]. In the present study, the TSM measured at all sampling sites, except the P/PSR, exceeds the maximum acceptable microbiological limits of 150 CFU m^{-3} (bacteria plus fungi) suggested by the WHO for hospitals environments [21]. Our results demonstrate that indoor air quality in this hospital does not comply with the WHO standard, despite the fact this reference value may be outdated. Furthermore, available regulations worldwide are established exclusively for operating theatres, without considering other indoor environments [1]. However, our mean TSM concentrations were below other non-hospital guideline values, such as the American Conference of Governmental Industrial Hygienist (500 CFU m^{-3}) [22]. Despite methodological differences, our results are comparable to those reported by several other previous studies. Verde *et al*. informed microbiological air counts a bit higher in the AOO/POO and P/PSR of a medium/high-complexity hospital in Portugal [3]. In addition, Ortiz *et al*. found similar CFU m^{-3} values in the RR and ICU of a Spanish hospital [23].

Microbiological air quality monitoring in vulnerable environments such as hospitals is a major issue that requires in-depth analysis. Indeed, some estimations reported in the literature could not reflect the real microbiological burden if environmental factors such as the number of people in a room are not considered. Modern monitoring methods, although more accurate, may be expensive to afford, therefore the assessment of the air microbial burden at different places simultaneously within a hospital is difficult to accomplish. On the other hand, settle plates, although cheap and reliable for the assessment of suspended microorganisms, may have some failures since microbes lower than 5 µm size may not be efficiently sampled [1]. In fact, when predicting TSM concentrations only with settled bacteria and settled fungi we obtained the lowest regression coefficients (Adjusted R^2 0.0207 for bacteria and 0.0010 for fungi), which suggest these parameters do not correctly reflect the air microbiological burden. Therefore, we controlled other environmental variables and introduce in a regression model in order to improve its predictive ability.

Other authors already observed significant correlations between suspended microorganisms and the variables employed as predictors. For instance, Frankel *et al*. informed a Pearson coefficient of 0.57 between suspended bacteria and inhalable particles at familiar homes [16]; Liu *et al*. reported a strong relationship between CO_2 levels and air bacterial count in two schools (r = 0.84 and 0.90) [24]. Regarding microbes, some studies have reported strong correlations between total settled and suspended microorganisms. These authors also suggest a linear regression function to estimate the air microbial burden. Napoli *et al*. reported significant correlation coefficients between 0.74 and 0.82 in operating rooms at different hospitals [7] and Haas *et al*. showed high correlation coefficients for *S. aureus* and *A. niger* when comparing impaction vs. sedimentation methods under controlled conditions [25].

Conclusions

Several studies have demonstrated that air quality in health centers is a major public health issue since microbes that cause hospital-acquired infections can be effectively transported by air. In the present work, we demonstrated that the use of settle plates underestimates the real burden of suspended organisms since settled microorganisms do no correlate with suspended ones. In addition, we were able to develop a model to predict the concentration of total suspended microorganisms by identifying environmental variables that enhance the microbiological burden, such as the number of people in a room, air exchange rate, hospital location, cleaning frequency and the presence of air conditioning systems. Furthermore, $PM_{2.5}$ levels, CO_2 concentration, and outdoor temperature were the main microbial load predictors.

To our knowledge, this is the first study of this kind in Latin America. We demonstrated that a quick, simple, cheap and reliable methodology is effective to assess, both qualitatively and quantitatively, the air microbiological burden in a health center environment. We acknowledge that a direct extrapolation to other conditions might not be completely reliable without standardization. However, our model identifies the variables that influence the air microbial load in a health center, thus their control would reduce the risk of infections transmission. These results may provide guidance for the study of indoor air quality in hospitals, employing simple and cost-effective techniques which are particularly important for developing countries where air quality is scarcely controlled.

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Corresponding author

Iván Tavera Busso, PhD
Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET and Departamento de Química, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina. Av. Vélez Sarsfield 1611. X5016GCA. Córdoba. Argentina.
Tel +54 351 5353800 ext 29771
Email: ivantaverabusso@unc.edu.ar

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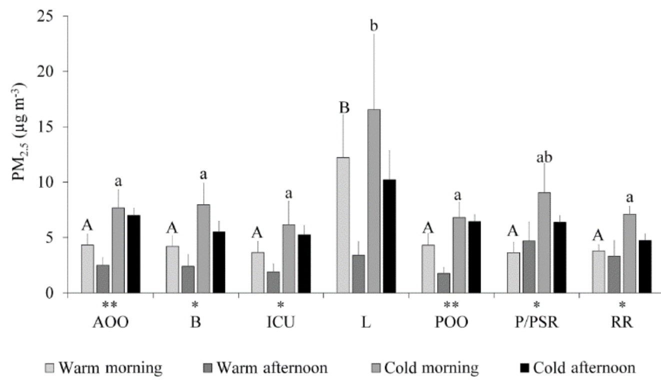
Annex – Supplementary Items

Supplementary Table 1. Eigenvectors obtained in the principal component analysis.

Variable	PC 1	PC 2
Bacteria - Settled	-0.42	-0.25
CO_2	-0.30	0.49
Fungi - Settled	-0.36	-0.45
$PM_{2.5}$	-0.30	0.50
Temperature	0.43	0.17
TSM	-0.41	0.38
Relative Humidity	-0.40	-0.27

Supplementary Figure 1. $PM_{2.5}$ concentration at different sampling sites within the Domingo Funes hospital.



Different letters indicate statistical differences between sites for the same period and shift. Asterisks indicates statistical differences between periods; "*"; $p < 0.05$; "**"; $p < 0.01$.

Supplementary Figure 2. Association between measured variables with different sampling sites within the Domingo Funes hospital.