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Microbiological Air Quality in an Optical Clinic

Calidad microbiológica del aire en una clínica óptica

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ABSTRACT

Introduction: Air as a fluid is present in different spaces. It is likely to contain different pollutants, including pathogenic microorganisms, which, due to environmental factors, are dispersed in the hospital environment, where they are responsible for causing diseases in vulnerable populations. In addition to being considered a risk to health, nosocomial diseases generate very high treatment costs, ranging from 13 to 15 million pesos per illness during treatment. *Objective:* To determine the microbiological quality of the air in bathrooms, corridors, reception and storage of one optical clinic in Colombia. *Methods:* An initial visit to the clinic was carried out for a microbiological sampling of air with the MASS 100 equipment, based on a sampling route designed according to the ISO 14644 technical standard, in addition to the relative humidity and velocity percentage measurements of the wind for two weeks. Bacterial characterization was verified using a Vitek automated system. *Results:* It was identified that 64% of the sampling sections are above the limit established by the ISO 14644 standard of colony forming units (CFU) for clean areas. This study identified 14 bacterial genera, such as *Staphylococcus* (26.3%), *Pantoea* (10.5%), and *Sphingomonas* (5.3%). *Conclusions:* Although there is a high number of CFU in the clinic's air in the present study, most of these genera are saprophytic and opportunistic bacterial agents.

Keywords: Nosocomial bacterial, intramural air quality, optical clinic, environmental health.

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RESUMEN
Introducción: el aire como fluido está presente en diferentes espacios. Es probable que contenga diferentes contaminantes, incluyendo microorganismos patógenos, que, debido a factores ambientales, se encuentran dispersos en el entorno hospitalario, donde son responsables de causar enfermedades en poblaciones vulnerables. Además de ser consideradas como un riesgo para la salud, las enfermedades nosocomiales generan costos de tratamiento muy altos, que varían de 13 a 15 millones de pesos por enfermedad durante el tratamiento. *Objetivo:* determinar la calidad microbiológica del aire en los baños, los pasillos, la recepción y algunos espacios de almacenamiento de una clínica óptica en Colombia. *Método:* se realizó una visita inicial a la clínica para el muestreo microbiológico del aire con el equipo MASS 100, siguiendo una ruta de muestreo diseñada de acuerdo con la norma técnica ISO 14644, además de mediciones de porcentaje de humedad relativa y velocidad del viento durante dos semanas. La caracterización bacteriana se verificó utilizando un sistema automatizado Vitek. *Resultados:* se identificó que el 64% de las secciones de muestreo están por encima del límite establecido por la norma ISO 14644 de unidades formadoras de colonias (UFC) para áreas limpias. Este estudio identificó 14 géneros bacterianos, como *Staphylococcus* (26,3%), *Pantoea* (10,5%) y *Sphingomonas* (5,3%). *Conclusiones:* aunque el presente estudio evidenció un alto número de UFC en el aire de la clínica, la mayoría de estos géneros son agentes bacterianos saprófitos y oportunistas.

Palabras clave: infecciones bacterianas nosocomiales, calidad del aire intramural, clinica óptica, salud ambiental.

INTRODUCTION

The atmosphere does not have an autochthonous microbiota; however, it is a means for the dispersion of many types of microorganisms (bacteria, viruses, and fungi) from other environments, since some have created specialized adaptations that favor their survival and permanence (1). Therefore, it is important to identify these microorganisms found in bioaerosols that reach the indoors of different buildings such as homes, offices, hospitals, manufacturing industries, pharmaceutical industry, laboratories, classrooms, documentary archives, museums, libraries, and bookstores (2). It becomes even more important because, when analyzing the impact on the health of the people who circulate those areas, it is possible to mitigate the presence of pathogenic bacteria that are suspended and circulate in dust particles, fragments of dry leaves, skin, fibers of clothes, drops of water or drops of saliva eliminated when coughing, sneezing or talking (1); at the same time, the particles are recirculated through inadequate ventilation systems, which allows them to remain in buildings for long periods of time, influenced by environmental factors such as temperature, humidity, and wind (2). When they remain in buildings, the particles are introduced into the human body through the respiratory system, which generates an alteration of the state of health (3); this is the case of diseases such as influenza, severe acute respiratory syndrome (SARS), tuberculosis, pneumonia, and meningitis.

Among the bacteria found in the air, it is very common to find Gram positive pleomorphic bacilli (*Corynebacterium*), Gram positive cocci (*Micrococcus* and *Staphylococcus*), and Gram negative bacilli (*Flavobacterium*, *Alcaligenes*) in lesser proportion (1). Of the fungi, *Cladosporium* is the one that predominates in the air, land, and sea, although it is also common to find other molds, such as *Aspergillus*, *Penicillium*, *Alternaria* and *Mucor*, and the yeast *Rhodotorula*. Viruses can also be found in the air and they are transported by it. Numerous human viruses (e.g., *Ortho* and *Paramyxovirus*, *Poxvirus*, *Picornavirus*) are transmitted by respiratory route, mainly in closed environments (4). The number of these microorganisms in the atmosphere changes according to height, the highest being close to the ground (especially at two meters, which is the microclimate of humans), decreasing at 200 meters and then becoming scarcer at 5000 meters. Their presence

is rare near the troposphere and non-existent in the stratosphere. The number of microorganisms in the air in populated areas depends on the industrial or agricultural activity in that area, as well as living beings and the amount of dust (5).

The time that microorganisms remain in the air depends on the shape, size and weight of the microorganism and the existence and potency of the air currents that sustain and elevate them. Adverse factors are the obstacles, which, when opposed to the winds, decrease their speed and drag power, and precipitations, which drag the suspended particles to the ground. In addition to the above, factors such as temperature, humidity, air currents and exposure to light condition the adaptation of bacteria to different spaces, which sometimes favors or discourages their reproduction and presence in the air of different microorganisms (1).

In general, low temperatures inhibit the growth of many microorganisms; however, some of them (e.g., molds and yeasts) develop well in cold environments. Other microbial species (e.g, *Aspergillus sp*, *Legionella pneumophila* or *Thermoactinomyces vulgaris*) reach their optimum development at elevated temperatures (5).

Very humid environments favor the development of fungi, bacteria and house dust mites. The movement of air contributes to the transport, maintenance and passage to the air of biological contaminants coming from the outside or contained in an interior reservoir. The degree and type of light can also favor or inhibit the development of microorganisms. For example, ultraviolet light inhibits such growth, and the absence of light prevents the formation of spores of some fungi (6).

Despite all this, we should not be alarmed by the content of bacteria in the air or in the human body, since humans have microorganisms called "normal flora" that coexist with the host in a normal state without causing disease (7). Likewise, the normal human flora from different points of view represents an important host defense mechanism. It contributes to the development of the immunological response, as has been demonstrated in animal models that are born and raised in sterile conditions (axenic individuals). These animals reveal a poor development of the various components of the immune system. The flora also helps to prevent the colonization of the skin or mucous membranes by bacteria that can be pathogenic. The germs to initiate the infection must, in general, begin by colonizing the epithelia, where they must compete with the members of the flora for factors such as cellular receptors and nutrients (7).

Despite living with bacteria in our bodies and in the air from an early age, we must pay close attention to the bacteria transported in the bioaerosols, which can become a transient flora—that is, a flora that varies from one human being to another and which is composed of germs that intermittently colonize a certain sector. This transient flora may include bacteria that are potentially pathogenic to the individual or other people who come in contact with it (8) or, even worse, in the case of hospitals, nosocomial-type bacteria that cause infections in admitted patients in a health care establishment in which the infection had not manifested or was not in the incubation period when they were admitted. It includes the infections contracted in the hospital but that manifest after hospital discharge and also the occupational infections of the establishment's staff (9).

This is the reason why solutions are found through epidemiological research, studies of living conditions, and local diagnoses with emphasis on risk assessment. For example, in 2009, in order to avoid the problems caused by nosocomial microorganisms found in the intramural air of different hospital areas, the treatment of infected patients in 29 high-complexity institutions in Bogotá cost 727 trillion pesos that could have been invested in prevention of transmission of nosocomial diseases, without human loss (10).

The biggest issue with these diseases is the emergence of resistance to commonly used antibiotics.

This resistance is mainly due to the abusive, and sometimes inadequate, use of antibiotics. The presence of multi resistant microorganisms (MMR) has important repercussions for patients and the system (costs, epidemic outbreaks, and morbidity and mortality) (11).

Based on the costs and the impact of the microorganisms found in the bioaerosols, the objective of this research was to determine the microbiological quality of the air present in the restrooms, corridors, reception and storage of the one optical clinic in Colombia.

MATERIALS AND METHODS

Initial assessment visit

An initial assessment visit was made to the optical clinic, in which the sampled spaces were identified: the reception room and the restroom in the main hallway, access corridors to doctor's offices, as well as the men's and women's restrooms. A preliminary sampling was carried out that allowed us to adjust the conditions for using the equipment, the sampling times, and the analysis of the results.

Routes and sampling points

After compilingx the pertinent information, the mapping is done with the support of the infrastructure area to establish the air routes and the sampling points according to ISO 14644. In total, a total of 598 sampling points were calculated and evaluated for the entire clinic, which were carried out twice a day, in the morning (8:00 am – 12:00 pm) and in the afternoon (1:30–4:30 pm).

Air samples and microbiological identification

The Mass 100 equipment was used for microbiological analysis of the air, with an intake of a constant value of 100 liters/min for 5 min and including a 90 mm petri dish with nutrient agar that allowed the impregnation of the microbial sample. The samples were incubated at 37 °C for 24 hours, and then we proceeded to obtain the concentration of colony forming units (CFU) and transfer them with a sterile loop. The most representative colonies were transferred to trypticase soy agar for subsequent identification with the VITEK automated system (Biomerieux, Marcy l'Etoile, France), which uses Vitek 2-GP cards® (Reference 22218 for Gram-positive microorganisms) and Vitek® 2- GN cards (Reference 414163 for Gram negative microorganisms) (13).

Air speed and humidity

In this study, the PCE-A420 hand cup anemometer was used for measuring wind speed and humidity, which is equipped with Robinson cups or propellers attached to a central axis, whose rotation, proportional to the wind speed, is conveniently recorded; in the magnetic anemometers, this rotation activates a tiny electrical generator that facilitates an accurate measurement. It is also a device that has a measurement resolution of 0.1 m/s, and the bucket system responds to the minimum airflow speeds (13).

RESULTS

Population during sampling

The following table shows the number of people who passed through the clinic during the sampling days in the morning hours (See Table 1).

These data were categorized according to the type of population into adults (including administrative staff, students, and visitors) and children. In the two sampling moments, the number of people of the administrative staff and students is constant, while the visiting population varies according to the number of people attending the clinic during the morning and evening hours. It can also be observed that, in the first and second floors, there is a greater influx of people with up to 103 people than in the third and fourth floors, with 48 people.

Table 1. **Number of people in the clinic during sampling**

Sampling day	Floor	Population			Total population
		Adults		Children	
		Administrative and students	Visitors		
Day 1	1	9	17	10	103
	2	15	37	15	
Day 2	1	9	14	7	87
	2	15	32	12	
Day 3	1	9	19	9	92
	2	15	30	10	
Day 4	3	9	19	2	48
	4	1	17	-	
Day 5	3	9	15	2	47
	4	1	20	-	
TOTAL		92	220	67	347

Source: own work

From the colony count, a total value per day and per sampling floor is obtained, as shown in Table 2. These values are obtained by counting all the colonies observed in the petri dishes used during the sampling day.

Table 2. **Total number of colonies**

Sampling day	Floor	Number of colonies	Total colonies
Day 1	1	1436	2674
	2	1238	
Day 2	1	928	1936
	2	1008	
Day 3	1	1486	2944
	2	1458	
Day 4	3	914	1777
	4	863	
Day 5	3	458	990
	4	532	
Total			10321

Source: own work

This table shows that day 3 of the sampling had the highest number of colonies with respect to floors 1 and 2, with a value of 2944, while day 4 had the highest value for floors 3 and 4, with 1777 colonies.

The colors on Table 4 are based on the amount of CFU given in the sampling for each of the petri dishes used, so that the values are distributed in a range of pollution, taking into account the maximum valued by ISO 14644 (i.e., 100 CFU/ m^3). Table 4 also differentiates each section of the clinic with its pollution range and, similarly, Table 3 indicates which part of the clinic each section refers to.

Table 3. **Clinic sampling areas**

Floor	Section	Comments
1	1	Reception's southern area
	2	Reception's central area
	3	Reception's northern area
	4	Men's restroom (administrative staff)
	5	Women's restroom (administrative staff)
2	1	Western area's corridor
	2	Central area's corridor, next to the stairs
	3	Eastern area's corridor
	4	Men's restroom
	5	Orthoptic corridor
	6	Pediatric waiting room 2
	7	Pediatric waiting room 1
3	1	Western area's corridor
	2	Central area's corridor, next to the stairs
	3	Eastern area's corridor
	4	Women's restroom
	5	Adult exam area's corridor
	6	General exam area's corridor
4	1	Western area's corridor
	2	Central area's corridor, next to the stairs
	3	Eastern area's corridor
	4	Men's restroom
	5	Warehouse (equipment)
	6	Warehouse (archive)

Source: own work

Table 4. **Bacterial load ranges in CFU values**

Minimum	Maximum	Color	Pollution status	Areas
150	200		Very high	1st floor (section 1 and 2)
100	150		High	1st floor (section 2 and 4)
50	100		Medium	1st floor (section 5) 2nd floor (section 1, 2, 3, 4, 5, 6, and 7) 3rd floor (section 1, 2, 3, 5, and 6) 4th floor (section 1, 2, 3, and 4)
0	50		Low	3rd floor (section 3) 4th floor (section 5 and 6)

Source: own work

Microbiologic identification

Table 5 shows the results obtained in the sampling, revealing that a total of 14 different bacterial genera were found, in which the *Staphylococcus* genus is the most common one, with a presence of 26.3%. Most of the bacteria found are opportunistic and can colonize the skin and mucous membranes, including *Staphylococcus haemolyticus* and *Sphingomonas paucimobilis*, which have occasionally been reported to cause pathologies such as endopftalmitis and conjunctivitis (15, 23). Only one pathogenic species was found, namely *Brucella melitensis*, which has been known to cause diseases in animals and in humans affected by systemic brucellosis (24).

Environmental factors

The following graphs show the values obtained with the anemometer, including the wind speed and relative humidity per sampling section evaluated every day during the morning and afternoon hours.

Wind

The wind speed in the first floor did not have considerable variations in the different days, only in the restrooms, since the ventilation grids that these spaces have do not work optimally. This is because, being non-mechanical ventilation ducts, there is no constant air flow, which causes changes in wind volumes; as such, the reception and main entrance did not exceed 2 m/s.

In the second floor there is a higher air flow, in which the values obtained were above 2 m/s, particularly in the pediatrics wing, where a wind current is formed through the corridor, entering through the windows of the north and south wings of the clinic, which makes this an area with high wind flow compared to other areas of the clinic.

On the third floor there is constant wind flow throughout the day, allowing microorganisms to enter and keeping them suspended, thus increasing the amount of CFU during the day.

On the fourth floor, the wind speed was quite low during the sampling, especially in the warehouse area, which has no windows or mechanical ventilation, encouraging the appearance of moisture and dust particles, which would be important to investigate through a sampling of surfaces.

The maximum value obtained is 0.35 m/s. The wind speed values obtained during the sampling can be observed in Figure 1.

Relative humidity

Next, the relative humidity obtained during the sampling for the different floors and for the different sections of the clinic in which the sampling was carried out is shown (Figure 2).

According to the UNE 100713: 2005 standard, the minimum and maximum relative humidity should be 45% and 55%, respectively (14). According to

TABLE 5. **Results obtained in the sampling**

FLOOR/SECTION	GENDER	SPECIES	NOSOCOMIAL
2 - Section 3 – Orthoptics	*Roseomonas*	*gilardii*	NO
2 - Section 4,3 – Pediatrics	*Sphingomonas*	*paucimobilis*	YES (16)
1 - Section 2 – Reception			
1 - Section 3 – Reception			
3 – Section 1,3 – Hall			
2 - Section 1,2 – Hall			
2 - Section 1,2 – Hall	*Brucella*	*melitensis*	NO
2 – Restroom			
2 - Section 1,2 – Hall	*Rhizobium*	*radiobacter*	NO
1 – Men's restroom	*Micrococcus*	*luteus/lylae*	NO
1 – Women's restroom	*Staphylococcus*	*epidermidis*	YES (16) (17)
1 - Section 1 – Reception	*Aerococcus*	*viridans*	YES (18)
4 - Section 3,2 – Repository	*Pantoea*	*spp*	YES (19)
2 - Section 1,3 – Hall	*Staphylococcus*	*haemolyticus*	YES(20)
2 - Section 4,3 – Pediatrics			
3 – Restroom			
4 - Section 1,3 – Hall			
2 – Restroom	*Rothia*	*mucilaginosa*	NO
2 - Section 1,1 – Hall	*Staphylococcus*	*saprophyticus*	NO
1 - Women's Bathroom	*Vibrio*	*fluvialis*	NO
1 - Section 2 – Reception	*Kocuria*	*rosea*	NO
2 - Section 1,2 – Hall	*Alloiococcus*	*otitis*	NO
2 - Section 3 – Orthoptics	*Staphylococcus cohnii*	*ssp cohnii*	YES (21)
2 - Section 4,2 - Pediatrics			
3 - Section 3 - Functional optometry	*Comamonas*	*testosterone*	NO
3 - Section 1,1 - Hall	*Pantoea*	*agglomerans*	YES (19)
3 - Section 4 - Functional optometry	*Staphylococcus*	*aureus*	YES (22)
2 - Section 4,1 - Pediatrics	*Gardnerella*	*vaginalis*	NO

Source: own work

the results, the relative humidity is maintained in a range between 45% and 62%, thus surpassing the values established by the norm. The zones with the highest values of relative humidity obtained (all above 60%) are in the orthoptic wing on the second floor, the corridor on the third floor, and the corridor and men's restroom in the fourth floor, *aureus*, *Gardnerella vaginalis*, *Roseomonas gilardii*, *Sphingomonas paucimobilis*, *Brucella melitensis*, *Rhizobium radiobacter*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Aerococcus viridans*, *Pantoea Spp*, *Staphylococcus haemolyticus*, which are associated with 28 different points of the clinic, as seen in the results in Table 5.

DISCUSSION

The results of the Vitek, allowed the identification of the following bacterial morphotypes: *Rothia mucilaginosa*, *Staphylococcus saprophyticus*, *Vibrio fluvialis*, *Kocuria rosea*, *Alloiococcus otitis*, *Staphylococcus cohnii*, *Ssp cohnii*, *Comamonas testosteroni*, *Pantoea agglomerans*, *Staphylococcus*

The most identified genus was *Staphylococcus*, with five different species, namely *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, *S. cohnii cohnii*, and *S. aureus*; this is due to the ubiquitous capacity of these microorganisms and their ability to generate exotoxins or enzymes, which is directly related to their presence in the skin and mucosa of healthy people (16).

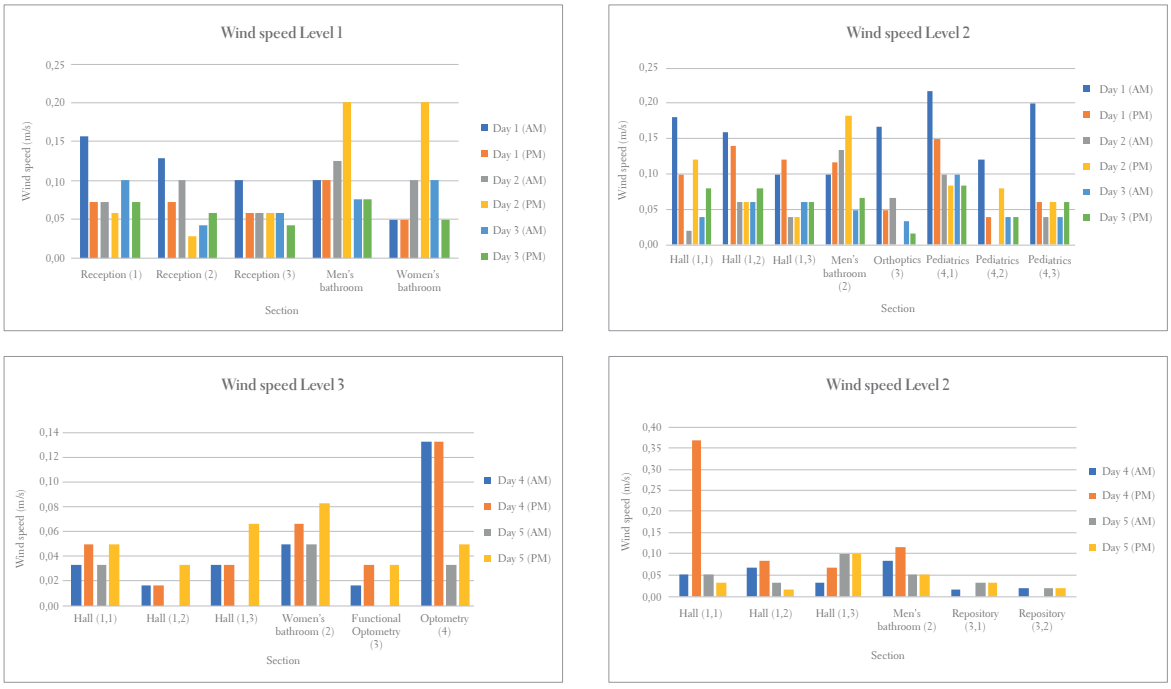

FIGURE 1. **Wind speed during sampling**

Source: own work

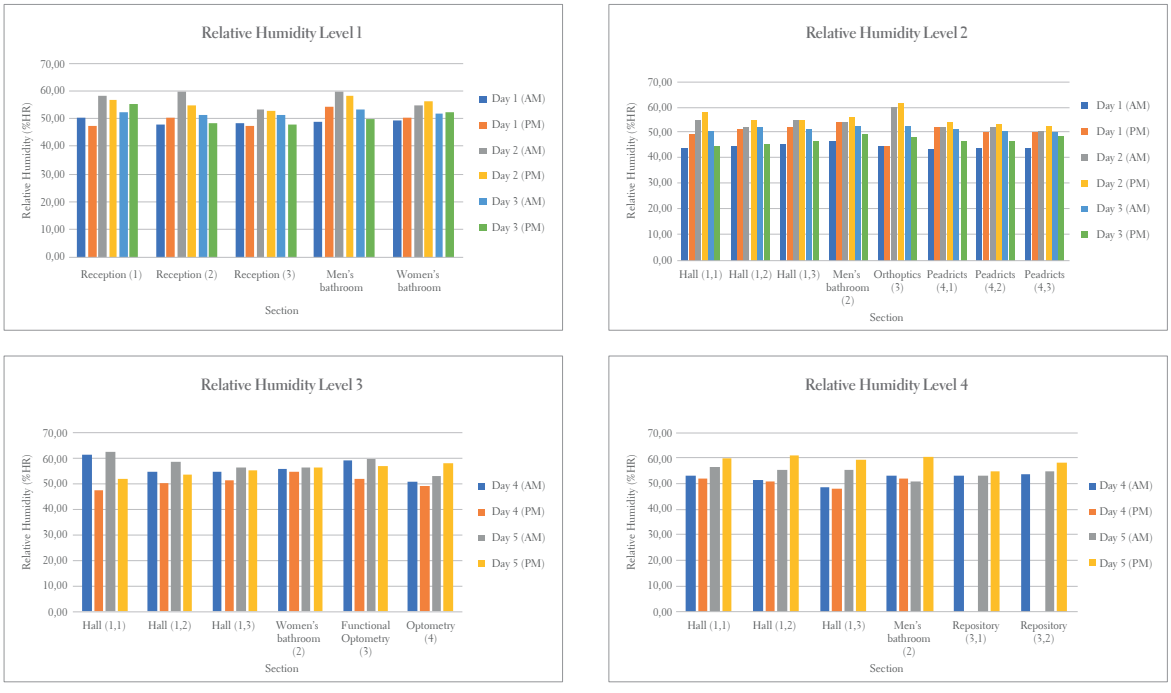

FIGURE 2. **Relative humidity during sampling**

Source: own work

Staphylococcus aureus is the bacteria with the highest pathogenicity in the *Staphylococcus* genus. In fact, twenty to forty percent (20–40%) of people who have their nostrils colonized by this microorganism (16) were isolated from the ophthalmology clinic where infectious pathologies are treated, knowing already that the *Staphylococcus* genus is directly related to conjunctivitis, as demonstrated by the study conducted by Hernández (17) in 2005. This study showed that emphasis should be made on some highlighted areas in the bacterial load map done by researches with the information provided with the CFU, which made it possible to identify that 64% of the sampling sections are above the limit established by the CFU's ISO 14644 for clean areas. This indicates that there are specific factors that increase or sustain the bacteria number and distribution, such as the variables of wind speed (ventilation) and relative humidity, which did not show considerable variations based on Procedural Technical Standard (NTP) 589. However, they could form an environment conducive to bacteria such as *Staphylococcus aureus*, *Brucella melitensis*, *Staphylococcus epidermidis*, and *Sphingomona paucimobilis*, which were identified as the microorganisms with the highest infectious potential. Finally, it is recommended to measure to air in future studies every hour in order to create a profile and compare it with the number of bacteria. This might considerably reduce and keep control of all the bacteria that are present in the optical clinic.

It is important to guarantee ventilation in hospitals so as to provide the adequate hygienic conditions to protect the patients and professionals who work there, and it is also important to perform the thermal treatment of the environment. From the outlook of the prevention of occupational hazards, the ventilation of workplaces is a measure of collective protection that allows eliminating or reducing the content of polluting agents that may be present in the environment (25). This aspect is not found in the clinic; there is no mechanical ventilation system that establishes a continuous air circulation, only natural ventilation, which is not enough, thus making it necessary to have extractors to allow the humid air of the clinic to circulate. As stipulated by the UNE 100713:2005, the minimum relative humidity should be 45% and the maximum should be 55% (25); however, according to the results, the minimum relative humidity was never below 45% and sometimes the maximum value exceeded the 55% mentioned in the NTP. This generates condensation due to the water content in the air, causing it to settle on floors, ceilings, walls and food, thus promoting the growth of bacteria. For this purpose, and in order to control health risks, it is proposed to keep the windows open to avoid increasing relative humidity and, in the unventilated places of the clinic, place humid air extractors and dry air fans in order to stabilize the air and avoid bacterial dispersion, thanks to the effect of general ventilation by dilution (26).

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