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MONITORING THE ACTION OF AEROSOLIZED H_2O_2 AND THE IMPACT ON FUNGI AND BACTERIA IN THE INDOOR AIR OF SÃO PAULO ENVIRONMENTS

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Abstract: The microbiological dynamics of ambient air need to be monitored to ensure indoor air quality and minimize health risks, since that excessive exposure to bioaerosols may be associated with the development of various respiratory and/or systemic diseases. Regarding about decontamination and sanitation of several places, conventional sterilizing processes are being used, and the most frequent is the use of chemical products. The use of hydrogen peroxide (H_2O_2) as a decontaminant in indoor environments is already widely used as an antiseptic and is an effective antimicrobial agent because it has recognized biocidal action. Objective: This study aimed to characterize and evaluate the efficacy of hygienization by aerolized hydrogen peroxide against the presence of fungi and bacteria found in the indoor air of different environments with a large circulation of people from the city of São Paulo Methods: Five sample collection points were defined in the city of São Paulo; the air samples were collected before and after cleaning with aerolized hydrogen peroxide, using the Merck® MAS 100 NT air collector. One of the environments was analysed for 60 days to verify the effectiveness of maintaining the sanitation protocol with H_2O_2. The collected material was taken to the laboratory at the Adolfo Lutz Institute. Results: there were no large variations in the physical parameters during the application of H_2O_2, not even changes in the concentration of CO_2 and formaldehyde; aerolized H_2O_2 showed biocidal action for both microorganisms in the first assessment after application in the studied environment (3 hours). That there was a significant reduction in the amount of CFU/m^3 of microorganisms in the environment analysed for 60 days, with the greatest reduction in the first month, which was 61.5% (N=416) for fungi and 86.6% (N=1228) for bacteria. Conclusions: Hydrogen peroxide is a high-level disinfectant. Its already recognized biocidal action is related to the fact that this agent is an oxidizer. The maintenance of sanitization with H_2O_2 aerolized aeration was shown to be necessary given the gradual reducing potential in the CFU/m^3 of microorganisms that this method indicated in the analysed environment for 60 days.
Keywords: Air Pollution, Indoor; Fungi; Bacteria; Hydrogen Peroxide.

INTRODUCTION

The microbiological dynamics of ambient air need to be monitored to ensure indoor air quality and minimize health risks. Studies have been conducted on the frequency of microorganisms in the air, their implications on the human activity impact (HAI) indices and the measures that should be taken to prevent infections (CABO VERDE et al., 2015).

The World Health Organization (WHO) has determined that chemical and biological pollutants are the cause of sick building syndrome (SED). In these locations, the unbalanced concentration of chemical and biological pollutants between the outside air and the indoor air can contribute to the appearance of symptoms related to allergies, mental fatigue, headaches, and various forms of hypersensitivity. Monitoring the air circulation and the hygiene of the environment are ways to provide acceptable indoor air conditions. Within this strategy, the types of cleanings used to remove potential pathogenic bioaerosols from the air should be included to reduce the spread of infections; this includes appropriate cleaning strategies, the use of standardized hygiene protocols and the implementation of ventilation systems that help reduce the spread of microorganisms (ANA TÉRCIA BARIJAN, 2011; DEGOBBI et al., 2011; MARKLEIN et al., 2009).

In Brazil, the fundamental parameters

suggested for evaluating the quality of indoor environments are relative humidity, air temperature and speed, amount of fungi and bacteria present in the air, particulate matter and carbon dioxide. The resolutions and technical regulations are from the National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária - Anvisa), in accordance with the technical standards of the Brazilian Association of Technical Standards (ABNT) (ANA TÉRCIA BARIJAN, 2011).

Chemical and physical parameters characterize the internal and/or external air, but when monitoring biological parameters, such as the presence of microorganisms related to the internal circulation of people (activities performed inside buildings), a differentiated pattern is established, revealing variations in the concentrations of some species of microorganisms (GUO et al., 2021; SOUZA et al., 2019).

The composition of bioaerosols is variable, dynamic and relative to the origins of their components. Their structure and microbial cellular organization define their resistance to environmental conditions commonly used in sterilization, disinfection or asepsis processes, temperature and humidity; factors such as ventilation and air-conditioning systems can cause important changes in their characteristics (GONÇALVES; E BORGES, 2019; SOWIAK et al., 2018; VANETTI et al., 2016). Studies have shown that excessive exposure to bioaerosols may be associated with the development of various respiratory and/or systemic diseases, such as aspergillosis, allergic rhinitis, allergic fungal sinusitis, and toxic reactions that may also be associated with the environment (VAN LEUKEN et al., 2016).

Public and private places have been targets of concern regarding decontamination and sanitation; numerous conventional sterilizing processes are being used, and the most frequent is the use of chemical products. Chemicals have a greater effect on vegetative cells and show variations in their action, according to concentration and contact time. Microbial cell structure and organization define resistance to environmental conditions and to physical and chemical agents commonly used in sterilization, disinfection or asepsis processes. More technological processes are highlighted in the cleaning of internal environments, such as decontamination by aerolized chemical agents. The use of hydrogen peroxide (H_2O_2) as a decontaminant in indoor environments is already widely used in hospitals as an antiseptic and is an effective antimicrobial agent because it has recognized biocidal action. Applications in the form of steam plus chemical agents are also used frequently in hospital environments, which ensures standardization but only in these environments, since there are no sanitation models with this technology for other types of spaces (ANA TÉRCIA BARIJAN, 2011; DAVIES et al., 2011; R; A, 2016).

Aiming to understand and characterize hygienization with aerolized hydrogen peroxide in different environments, in addition to hospital environments, this study aims to characterize and evaluate the efficacy of hygienization by aerolized hydrogen peroxide against the presence of fungi and bacteria found in the indoor air of different environments with a large circulation of people from the city of São Paulo.

METHODOLOGY

CHARACTERIZATION OF COLLECTION POINTS AND HYGIENIZING PRODUCTS

Five sample collection points were defined in the city of São Paulo: fitness centre, post office, restaurant, school and office. The air samples were collected before and after cleaning with aerolized hydrogen peroxide at a concentration of 7.5%, plus 0.85% phosphoric

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acid (to maintain a low pH). The areas received a volume of 1 ml/m^3 of the product during the air cleaning process. After cleaning, the sites were closed for 30 minutes for the next test of product action time (WEBER; KANAMORI; RUTALA, 2016).

In the first stage of this study, the application of hydrogen peroxide was monitored for 24 hours, verifying the changes provided by the addition of the chemical in the air of the studied environment, observing when the chemical product act as a biocidae, biostatic or selective for fungi and bacteria distributed in the air. The air quality detector used was the Elitech Mod. Temtop M2000. This equipment is already used for the analysis of air patterns (LINLEY et al., 2012; SANTIAGO DURÁN CARO, 2021).

One of the environments (the school) was analysed for 60 days to verify the effectiveness of maintaining the sanitation protocol with H_2O_2 .

AIR COLLECTIONS

The air samples for the isolation of fungi and bacteria were collected using the impactor MAS 100 from Merck®. For the collection of fungi, modified Dicloran Rosa Bengala Culture medium ($DRBC_m$) was used, and for the collections of bacteria, soybean tryptone soybean broth (TSB), MacConkey and general chromogenic agar from Probac do Brasil® were used (CASTRO E SILVA et al., 2015; LACAZ, C. DA S.; PORTO, E. & MARTINS, 1991; ŁUKASZUK et al., 2017).

The volume of air collected per sample was 250 L, which allowed the analysis of the CFU/m^3 concentration and the isolation of the colony-forming units when multiplied four times (1000 L/m^3).

The collected material was taken to the laboratory at the Adolfo Lutz Institute where the samples were processed. The $DRBC_m$ plates were incubated at 30 ± 2 °C for up to seven days for isolation and identification of the genus (LACAZ et al., 1998; THOMAS J. WALSH, RANDALL T. HAYDEN, 2018).

The TSB, McConkey and chromogenic agar plates were incubated at 35 ± 2 °C for 48 hours. The bacterial isolates were subcultured and identified according to morphological characteristics and confirmed by the MALDI-TOF system (TRABULSI, LUIZ RACHID / ALTERTHUM, 2015).

PHENOTYPIC CHARACTERIZATION

MICROSCOPIC ANALYSIS

The analyses of the colonies of bacteria grown in the culture medium plates used in the air collections were performed by differentiating the morphology by Gram staining, and for the fungal colonies grown in DRBCm, the morphology was analysed by microscopy in lactophenol staining with cotton blue, observing pigmentation (hyaline or dematiaceous) and reproductive structures, for characterization of the genus (CHOWDHARY et al., 2014; G.S.HOOG, C., J. GUARRO, G. GENÉ, 2014; "Manual de Limpeza e Desinfecção de Superfícies. pdf — Português (Brasil)", [s.d.]; THOMAS J. WALSH, RANDALL T. HAYDEN, 2018).

IDENTIFICATION BY THE MALDI-TOF SYSTEM

The identification of the samples was performed by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) using MALDI Biotyper equipment (Bruker Daltonics, USA) (REEVE; BACHMANN, 2019).

During the execution of the identification protocol, the bacteria in pure colonies were plated on plates containing the same agar of origin and incubated for up to 24 hours in an oven at 35 ± 2 °C. After growth, a small amount of the colony was removed with

the aid of a loop, deposited on the plate of the apparatus and covered with 1 µL of the α-cyano-4-hydroxycinnamic acid (CHCA) matrix for reading. In turn, the fungal inocula were removed with a barbeque stick from a subculture in potato dextrose agar and incubated for 5 days at 30 °C. The fungus was then inoculated in 300 mL of Milli-Q water. The solution was homogenized until it dissolved, and then 900 µL of 99% ethanol was added. After the addition, the solution was centrifuged at 18,000 rpm for 2 minutes, and the supernatant was discarded.

The tube was left open for five minutes for total ethanol evaporation. Next, 50 µL of 70% formic acid was added and vortexed for 1 minute. Then, 50 µL of acetonitrile was added, and the mixture was vortexed again for another minute. At the end of this process, the tube was centrifuged at 18,000 rpm for another 2 minutes. For the transfer in the apparatus, 1 µL of the supernatant with 1 µL of CHCA matrix was used for reading (REEVE; BACHMANN, 2019).

RESULTS AND DISCUSSION

Analysing Table 1, there were no large variations in the physical parameters during the application of H_2O_2 during the study period, not even changes in the concentration of CO_2 and formaldehyde (HCHO). The lack of variability noted in these parameters may be explained by the internal environment, where variations of these chemicals were controlled (VANETTI et al., 2016).

Aerolized hydrogen peroxide showed biocidal action for both microorganisms in the first assessment after application in the studied environment (3 hours), and data was similar to the application of the product in other environments, such as hospitals and clinics (MARRA; SCHWEIZER; EDMOND, 2018; PASSARETTI et al., 2013; VANETTI et al., 2016).

Regarding the biocidal action of H_2O_2 on the isolated bacteria, we observed that the highest peak of action occurred in the first 6 hours after application of the sanitizer, with variations until the end of 18 hours. Studies show that there is greater sensitivity of gram-positive cocci to hydrogen peroxide. The variations recorded in the first 18 hours of the study are due to the concomitant presence of a greater number of bacilli than cocci in the analysed samples, considering that hydrogen peroxide has a total biocidal effect on bacilli at concentrations starting at 10% (TAOUFIQ, 2021).

The biocidal effect of hydrogen peroxide on the fungi was recorded three hours after aeration, leaving the environment with low CFU/m^3 rates. Only one fungal genus with hyaline characteristics remained in the air samples, which in turn did not present difficulties in increasing its CFU/m^3 numbers during the analysis. The emergence of another fungal genus, Dematiaceous, only occurred 6 hours after application when the product in the air had dissipated, according to the concentration of particles (PM2.5 and PM10); these data corroborate the information that melanized fungi are more resistant to hygienization by hydrogen peroxide than hyaline particles (IVANOVA et al., 2005).

The inhalable particles, sizes PM2.5 and PM10, showed an increase in their rate after application of H_2O_2 , with a recorded decrease of 96.9% of PM2.5 and 97.5% of PM10, which were probably carrying microorganisms present in the air in addition to dust particles (SOUZA et al., 2010).

Table 2 shows that there was a significant reduction in the amount of CFU/m^3 of microorganisms in the environment analysed for 60 days, with the greatest reduction in the first month, which was 61.5% for fungi and 86.6% for bacteria.

Regarding the fungal genera present, we

	BACTERIA			FUNGI			CHEMICAL STANDARDS				PHYSICAL STANDARDS		ACTION
EXPOSURE TIME	UFC/ m^3	Different isolates		UFC/ m^3	Different isolates		PM 2.5 (µg/ m^3)	PM 10 (µg/ m^3)	CO_2 (ppm)	HCHO (mg/ m^3)	TEMPERATURE (°C)	MOISTURE (%)	
		B	C		D	H							
0	>250	4	5	>250	4	1	015.4	022.3	406	0.001	21.8	86.3	-
3	0	0	0	20	0	1	380.7	676.3	425	0.002	24.6	86.3	BIOCIDAE
6	12	1	0	>250	0	1	011.5	016.5	387	0.001	25.4	73.8	SELECTIVE
9	76	2	5	>250	0	1	007.1	010.0	398	0.002	25.9	75.4	SELECTIVE
12	25	1	2	108	0	2	005.9	007.6	391	0.001	26.5	70.9	BIOESTATICS
15	48	2	2	213	2	1	005.7	007.8	579	0.001	26.3	73.4	BIOESTATICS
18	91	3	2	55	2	1	004.7	006.4	514	0.001	25.5	69.9	BIOESTATICS
21	>250	7	2	10	1	3	005.3	007.2	533	0.001	25.2	69.9	SELECTIVE
24	66	3	2	24	0	2	007.2	009.5	596	0.002	24.8	70.9	BIOESTATICS

B = Bacilli; C = Cocci; D = Dematiaceous; H = Hyaline

Table 1 - Monitoring of physical, chemical and biological patterns during the application of hydrogen peroxide for 24 hours in an environment

		Fungi		Bacteria			
Number in days	moment	DRBC CFU/ m^3	Genera	SOYBEAN UFC/m^3	CHROMO UFC/m^3	MACCONKEY UFC/m^3	Genera
1	Before hygienization	416	*Aspergillus Penicillium Neurospora*	1228	12	12	*Exiguobacterium Glutamicibacter Micrococcus Serratia Staphylococcus*
	After hygienization	336	*Aspergillus Neurospora*	176	0	0	*Bacillus Exiguobacterium Micrococcus Staphylococcus*
30	Before hygienization	160	*Neurospora*	164	12	0	*Bacillus Micrococcus Paenibacillus*
	After hygienization	52	*Bipolaris Neurospora*	32	12	8	*Serratia*
60	Before hygienization	140	*Aspergillus Penicillium Rhizopus*	172	380	32	*Bacillus Klebsiella Micrococcus Staphylococcus Stenotrophomonas*
	After hygienization	0	- -	0	20	32	*Acinetobacter*

Table 2 - Microbiological monitoring of hygiene performed at school for 60 days

can mention that they were renewed during the analysed period, and the diversity was maintained, except for the gradual reduction of CFU/m^3 after immediate hygiene with H_2O_2. In the presentation of bacterial genera, we can say that there was a similarity in the frequency of the genera, except for the genera *Staphylococcus* and *Serratia*, where we observed an oscillation. This fact is explained by the action of the product and the renewal of air, since students and teachers maintained their daily routines in the analysed environment, and the partial elimination of microorganisms kills vegetative forms but not necessarily the spores of microorganisms in the Air (FERREIRA, 2017; UDUMAN et al., 2002).

Fungi belonging to the genera *Fusarium* and *Rhizopus* showed no growth after hygienization with H_2O_2 and fungi belonging to the genera *Aspergillus, Cladosporium, Curvularia, Paecilomyces*, *Penicillium* and *Neurospora* were present after cleaning. The genus *Bipolaris* appeared once in this study after school cleaning. These data show that the action of sanitization with H_2O_2 is not fungicidal for most fungal genera present in the air but biostatic (WAWRZYK et al., 2020) (Table 3).

The genera *Bacillus* spp., *Micrococcus* spp., *Paenibacillus* spp. and *Pantoea* spp., isolated before cleaning was performed in the studied environments, are not relevant to human pathogens (BRAGOSZEWSKA; BIEDROŃ; HRYB, 2019; CABO VERDE et al., 2015; GRAÇA et al., 2013). Table 4 indicates that only the genus *Bacillus* remained dispersed after cleaning in the restaurant (KEMPF et al., 2005; MELLY; COWAN; SETLOW, 2002).

The other bacterial genera found and identified in the air of the analysed environments (*Acinetobacter* spp., *Enterobacter* spp., *Enterococcus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Serratia* spp., *Staphylococcus* spp. and *Stenotrophomonas* spp.) have already been described as pathogens (BIER et al., 2017; BLAZEJEWSKI et al., 2015; BROOKE, 2012; CAMPOS et al., 2021; CHANG et al., 2013; HSUEH et al., 2002; IOSIFIDIS et al., 2013; RODRÍGUEZ-MEDINA et al., 2019; UDUMAN et al., 2002).

The diversity of the genus *Staphylococcus* allowed us to show its growth after cleaning with H_2O_2 in the samples collected in the office, considering that some species are more resistant to this chemical agent (OTTER; YEZLI; FRENCH, 2012).

Air cleaning in public environments against bacterial composition was effective when used at a concentration of 7.5%, reducing or even eliminating the presence of bacterial genera in samples collected after the use of H_2O_2 in decontamination (MISHRA; IMLAY, 2012).

Hydrogen peroxide acts by producing destructive hydroxyl free radicals that can attack membrane lipids, DNA and other essential components of the cell. Catalase, produced by facultative aerobic and anaerobic organisms that have cytochrome systems, can protect cells from metabolically produced hydrogen peroxide, degrading hydrogen peroxide into water and oxygen. This defence is overcome by the concentrations used for disinfection.

Aerolized hydrogen peroxide showed bactericidal and fungicidal activity

According to the results shown in Figure 1, the action of hydrogen peroxide as an environmental sanitizer was effective, as previously reported in other studies (AHMED; MULDER, 2021; IVANOVA et al., 2005; TAOUFIQ, 2021).

In environments where the flow of people is always high, such as in schools and restaurants, the action of H_2O_2 was more efficient in reducing the CFU/m^3 concentration of bacteria.

Genera	Gym		Post office		Restaurant		Office		School	
	A	B	A	B	A	B	A	B	A	B
Aspergillus	X	X	X	X	X	X			X	X
Bipolaris										X
Cladosporium	X	X	X	X			X	X		
Curvularia			X	X						
Fusarium	X		X				X			
Neurospora									X	X
Paecilomyces	X	X	X	X			X	X		
Penicillium	X		X	X			X	X	X	X
Rhizopus			X						X	

Table 3 - Fungal genera present in internal air samples before and after cleaning with hydrogen peroxide (H_2O_2) in different environments of the city of São Paulo

Genera	Gym		Post office		Restaurant		Office		School	
	A	B	A	B	A	B	A	B	A	B
Acinetobacter					X				X	X
Bacillus			X		X	X	X		X	
Enterobacter	X									
Micrococcus	X		X				X		X	
Paenibacillus			X							
Pantoea			X							
Pseudomonas					X					
Serratia									X	
Staphylococcus			X				X	X	X	
Stenotrophomonas									X	
Enterococcus									X	
Klebsiela variicola									X	

Table 4 - Bacterial genera present in internal air samples before and after cleaning with hydrogen peroxide (H_2O_2) in different environments of the city of São Paulo

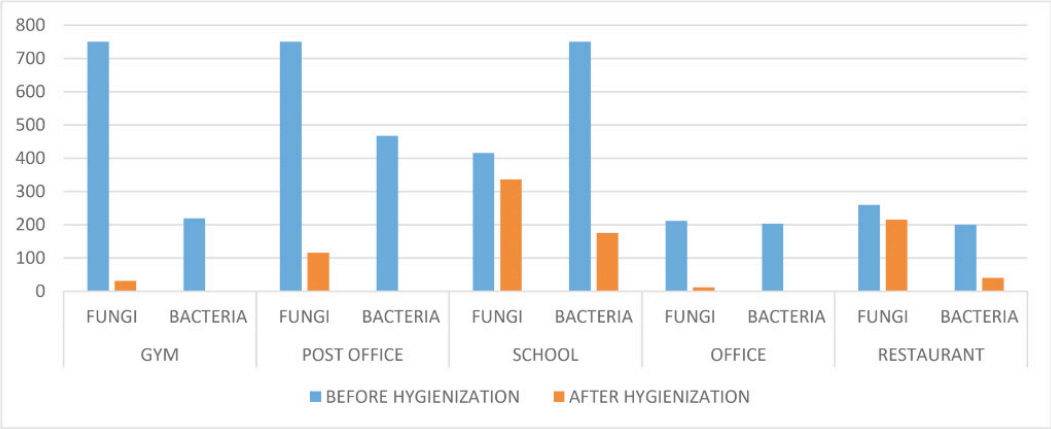

Figure 1: Distribution of CFU/m³ of fungi and bacteria before and after cleaning with hydrogen peroxide

CONCLUSION

The 24-hour monitoring aided in the decision-making power on the best way to collect and analyse the microbiological samples of the study, providing knowledge about the dispersion of peroxide in the environment that received this chemical treatment.

Hydrogen peroxide is a high-level disinfectant. Its already recognized biocidal action is related to the fact that this agent is an oxidizer. This implies protein denaturation, rupture of cell membrane permeability, and inactivation of microorganisms depending on the time, temperature and concentration of the product applied.

The maintenance of sanitation with H_2O_2 aerolized aeration was shown to be necessary given the gradual reducing potential in the CFU/m^3 of microorganisms that this method indicated in the analysed environment for 60 days.

Hydrogen peroxide is a high-level disinfectant. Its already recognized biocidal action is related to the fact that this agent is an oxidizer. The maintenance of sanitation with H_2O_2 aerolized aeration was shown to be necessary given the gradual reducing potential in the CFU/m^3 of microorganisms that this method indicated in the analysed environment for 60 days.

The results of the study allow analyzing the expansion of the use of aerolyzed hydrogen peroxide in other environments outside hospital environments, since positive results were shown in the decontamination of internal air from varied environments due to the safety and properly application of this chemical.

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