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EVALUATION OF THE EFFECTIVENESS OF AUTOMATED DISINFECTION MEDIATED BY HYDROGEN PEROXIDE AND SILVER ION AEROSOLIZING EQUIPMENT IN PUBLIC BUSES IN THE CITY OF SÃO PAULO, BRAZIL

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Abstract: Goal: evaluate the effectiveness of using no-touch technology through aerosolization of hydrogen peroxide and silver ions, known as HyperDryMist® (HDM), in the internal hygiene of public transport buses in the city of São Paulo. **Methodology:** A total of 294 samples were collected on buses, divided into two categories. The buses were sanitized using a manual cleaning protocol and HDM® technology. The samples were sent to the Mycology Center of the Adolfo Lutz Institute for analysis. **Results:** Collections on bus surfaces after manual cleaning with sodium hypochlorite solution showed growth of fourteen genera of microorganisms and, after applying the protocol with HDM®, there was a reduction of 72% of bacterial genera and 100% of fungal genera, with 3mL /m^3 and 4mL/m^3 of product applied, respectively. Conclusions: disinfection carried out using HDM® technology was more effective in eliminating CFU/m^3 of bacteria and fungi at concentrations of 3ml/m^3 and 4ml/m^3, respectively. These concentrations substantially reduced the spread of microorganisms and the possibility of contamination of employees and users of public transport.

Keywords: Environmental monitoring; Sanitizers; Air; Bacterium; Fungi; Means of transport.

INTRODUCTION

The city of São Paulo is the most populous municipality in all of South America, with more than 20 million inhabitants; therefore, it has a problem common to all megacities: air pollution [1].

Air pollution generated by human activities, in addition to influencing the climate, has direct effects on the health of the population in general. In megacities, the main sources of air pollution are vehicle emissions, followed by gas emissions from industries and the burning of biomass. Indoor air quality also plays a prominent role when related to airborne diseases[2].

The public transport bus fleet in the city of São Paulo is made up of 15 thousand vehicles and it is estimated that almost three billion people use this means of transport per year [3]. Environments with a high flow of people, such as public transport vehicles, allow opportune occasions to modify the composition of indoor/outdoor air, in addition to the spread of microorganisms [4].

Air has chemical, physical and biological elements in its composition, and the slice that represents its biological part is called bioaerosol[5].

Bioaerosols constitute around 30% of suspended particles in both the atmospheric air of urban and rural regions, and the majority are composed of fungi and bacteria. These microorganisms use particulate material (pollen, insect fragments, human skin scales and hair) as a substrate for their maintenance and multiplication[6].

Some environmental factors, such as ventilation, temperature and humidity, modify atmospheric air, promoting the dispersion and concentration of bioaerosols; the same happens in indoor environments, where the mechanisms used for environmental comfort, such as air conditioning systems, can cause the same changes in these places[7].

Fungi are complex microorganisms widely used in various areas of science, but they are also considered opportunistic pathogens for humans and a threat to environments, which can cause diseases and property losses in certain situations, respectively[8]. When talking about opportunistic pathogens, fungi are related to skin and/or respiratory and systemic infections in patients with immunological compromise. The Aspergillus genus is the most directly involved in these cases of severe systemic and pulmonary infections. [9] and other fungal genera have also been linked to

causing superficial, systemic or subcutaneous mycoses [10].

Bacteria, on the other hand, gain great prominence because they are microorganisms that trigger countless benefits or harm to humans. They are present in several natural microbiota, such as the skin and intestine of humans and animals, maintaining the physiology of these organs and promoting the maintenance of the health of these individuals, also improving their immunity.[11]. However, around 3% of these microorganisms are pathogenic, causing infectious diseases, which occur when this pathogen colonizes the body or causes poisoning, which occurs when a toxin produced by these microorganisms is ingested; Infectious diseases are the main cause of deaths in the world, the majority of which occur in underdeveloped countries[12].

Man is subject to many of these diseases, since the human body can harbor several microorganisms due to exposure to the environment and other risk factors. There are several ways for microorganisms to enter human systems, such as orally, nasally, piercing, sexual intercourse and open wounds[13].

Numerous conventional sterilizing processes can be used to decontaminate and sanitize various locations, such as hospitals, but these protocols may not be completely efficient. [14]. Nowadays, more technological processes are gaining prominence, such as gaseous decontamination with chemical agents; gaseous decontamination occurs when a chemical disinfectant agent is dispersed in the form of a gas to decontaminate a specific location, such as hospital rooms, and becomes advantageous, since areas that are difficult to sanitize are reached by the disinfectant agent and its biocidal efficacy[15].

Urban buses present difficulties in their hygiene due to their internally irregular surfaces such as safety bars, seats, turnstiles, among others, which can allow the presence and colonization of microorganisms on their surfaces, causing these vehicles to become fomites that carry diseases, leading to passenger contamination when coming into contact with these surfaces and even with the circulating air[16].

Considering the importance of this topic, this study aimed to evaluate the effectiveness of using no-touch technology (HDM®) through the aerosolization of hydrogen peroxide and silver ions in the internal disinfection of public transport bus environments in the city of São Paulo.

METHODOLOGY

A total of 294 samples from the interior of four buses were collected in a garage of a public transport company in the city of São Paulo, over a period of five weeks.

Among the samples collected, 144 samples of the vehicles' internal air were taken, 6 external air control samples and 144 samples of high-touch surfaces, namely: steering wheel, seat and vertical and horizontal safety bars.

The samples were analyzed by the Adolfo Lutz Institute (IAL).

SAMPLING

Sampling was divided into two categories: a) monitoring the effectiveness of cleaning using the manual method with sodium hypochlorite and b) evaluating the effectiveness of cleaning using HDM® technology.

In order to understand possible deviations related to microbial behavior and growth, temperature and humidity control was carried out inside each bus during sample collection and external concentration of microorganisms[17].

TECHNOLOGY: NO-TOUCH HYPERDRYMIST®

The no-touch HDM® technology, manufactured by the Swiss company 99 Technologies®, consists of a micro-aerosolizing modulator model 99MB and a disinfectant, whose active ingredient is hydrogen peroxide at a concentration of 6.6% associated with silver salts and coformulants (99S®).

The 99MB is a robot intended for high-level disinfection of non-critical environments and surfaces. Commonly used in healthcare establishments, this robot's technology can be applied in any type of indoor environment, including land, air and sea transport.

This disinfection process promotes the aerosolization of hydrogen peroxide and silver ions into the environment. The action of coformulants added to the disinfectant allows droplets with a size of less than 0.5 microns to assume the physical-chemical behavior of gas, expanding dryly and uniformly throughout the environment, providing greater effectiveness in eliminating forms infectious, without harming or generating incompatibility with materials and electronic components usually present in internal transport environments.

BUS SELECTION

In this study conducted over five weeks, four buses from a public transport company in the city of São Paulo were evaluated, randomly, two vehicles per week, with running times of 12 years, 6 years and 2 years.

The chosen vehicles had a common route that circulates in the city's commercial center (between the Brás and Pinheiros regions), in the metropolitan region and were analyzed when out of activity and parked in the garage of the affiliated company.

BUS CLEANING

The buses were internally sanitized with sodium Hypochlorite solution (the concentration and proportion of dilution carried out for cleaning were not informed) in accordance with the protocol of the affiliated cleaning company. Afterwards, they were cleaned using disinfection protocols with HDM® technology.

SAMPLE COLLECTIONS

In each bus, two external air samples were collected for control, six microbiological samples from the internal air and ten samples from high-contact surfaces, three air samples and five surface samples, after manual hygiene and after the use of technology HDM®, respectively.

The five weeks of collection were subdivided into five hygiene protocols: four testing protocols, with gradual concentrations of 99S® and a confirmation protocol, using from the first concentration that, in the testing protocols, was able to eliminate the presence of microorganisms (Table 1).

Before disinfecting transport with HDM® technology, it was necessary to measure the cubic footage of the buses, to include this information in the modulator, so that the amount of disinfectant dispersed was proportional to the protocol used per mL/m^3.

In disinfections carried out with HDM® technology, some colorimetric chemical indicators, hydrogen peroxide reagents, were added inside the buses to validate the uniform dispersion of the product inside the vehicle; The chemical indicators before disinfection are white in color and, after reacting with hydrogen peroxide, they turn orange.

AIR COLLECTIONS

Air samples for isolation of fungi and bacteria were collected using the Merck® MAS 100 impactor. To collect fungi, modified Dicloran Rose Bengal culture medium was used and to collect bacteria, tryptone soy agar (TSB) and MacConkey agar were used[18].

The volume of air collected per sample was 250L, which allowed analyzing the concentration of colony-forming units (CFU/m3) impacted in the culture medium.

The collected material was taken to the mycology laboratory at the Instituto Adolfo Lutz (IAL), where the samples were processed. The plates with the samples for bacteria and fungi were incubated in a bacteriological oven adjusted to 37ºC ± 2ºC and 30º ± 2ºC for up to 48 hours and seven days for isolation and gender identification, respectively.

SURFACE COLLECTIONS

Surface samples were collected using a swab soaked in sterile saline in an area of 20cm2. The swabs were placed in tubes containing BHI (Brain Heart Infusion) broth and taken to the IAL, where they were screened and incubated at 37ºC for up to 24h.[19].

After this period, the samples were sown using the depletion technique on plates containing the culture media TSB agar, mannitol salt agar, DRBC agar and MacConkey agar and incubated in a bacteriological oven at 35 ± 2ºC for up to 48h.[19].

SAMPLE IDENTIFICATION

The bacterial and fungal isolates were phenotypically characterized by polyblastic taxonomy and the genus identification was confirmed by mass spectrometry (Matrix-Assisted Laser Desorption Ionization- Time of Flight Mass Spectrometry or MALDI-TOF MS) using the MALDI Biotyper equipment (Bruker Daltonics, USA)[20].

RESULTS

Air samples in an external environment, outside the bus, were used to count CFU/m3 (colony forming unit per cubic meter), as recommended by RDC nº 9 of 01/16/2003, published by the National Health Surveillance Agency (ANVISA); isolates from external samples were not identified.

Collections on bus surfaces after manual cleaning with sodium hypochlorite solution showed growth of fourteen types of microorganisms.

After applying protocols with HDM® technology, there was a 72% reduction in bacterial genera and 100% in fungal genera (Table 2).

The chemical indicators placed inside the buses during the application of the sanitizer revealed that the hydrogen peroxide was efficiently distributed throughout the interior of the cars, promoting a gradual reduction in the presence of microorganisms from a concentration of 1mL/m3. The total reduction of bacteria occurred from 3mL/m3 and the total reduction of fungi from 4mL/m3.

All air samples after cleaning with sodium hypochlorite solution, except in the bus with an air conditioning system, showed concentrations outside the fungal standards recommended by ANVISA (Table 3). The maximum value for microbiological contamination must be <750 CFU/m3 of fungi, for the internal/external ratio (I/E) it is 1.5, where I was the amount of fungi inside the bus and E, the amount of fungi in the outdoor environment17 (garage).

A difference in the average concentration of CFU/m3 of fungi and bacteria isolated in the atmospheric air of the bus was observed according to the driving time (Table 4).

Using HDM® technology, the application of hydrogen peroxide with silver ions at a concentration of 3mL/m3 reduced 100% of microorganisms present in the air (Figure 1).

The presence of the Byssochlamis and Mucor genera was detected only after cleaning with HDM® technology at 1mL/m3; the genera Cladosporium and Fusarium presented a random frequency throughout the study and the other fungal genera suffered a gradual or absolute reduction after cleaning protocols with HDM® technology from 2mL/m3 and 4mL/m3, respectively (Table 5).

The bacterial genera Bacillus, Klebsiella, Micrococcus, Pantoea, Pseudomonas and Staphylococcus showed a significant reduction with the first concentrations of 99S®, being completely eliminated with 3mL/m3 of the product; the other genera presented random characteristics (Table 6).

DISCUSSION

The presence of microorganisms inside public transport vehicles is considered to be quite widespread, taking into account the number of people who use them and the air changes that occur on their routes; the isolation of microorganisms on the surface and in the air after cleaning with sodium hypochlorite solution, reveals that the product does not act completely effectively on the surface, taking into account that the cleaning process with this product is mechanical/manual and, does not impact the reduction of bioaerosols when monitored[21].

Some species of bacterial genera isolated in the air or on the surface of the buses studied include: *Acinetobacter*, *Bacillus*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Pseudomonas*, *Serratia* and *Staphylococcus* and, the fungal genera: *Rhodotorula* and *Cryptococcus* are human pathogens, described in several serious cases of hospital infections or by ingestion [22].

During collections, the legislation in force in Brazil for closed environments was used as a reference for monitoring microorganisms. It recommends collection with air impactors and analysis of the frequency of human pathogens present in the air, however, there is no official parameter from regulatory bodies that define these parameters for transport vehicles.[17,23].

The HDM® technology proved to be effective in distributing the sanitizing product throughout the bus environment, which was already expected, given the system's capacity and the product's chemical compliance[14,24].

Hydrogen peroxide already has a well-established biocidal effect and, associated with silver ions, promoted the total reduction of bacteria after using a concentration of 3mL/m3, which corroborates the results found in other studies using similar technologies[25].

The variation in the average UFC/m3 of microorganisms in buses due to the running time and the presence of a seal for the air conditioning system found in a vehicle, allowed us to analyze that the structural characteristics of transport favor the concentration and maintenance of microorganisms within closed environment, even if it is adequately sanitized[26].

The presence of bacteria in the air considered pathogenic to humans after cleaning buses with sodium hypochlorite raises important possibilities of respiratory or contact contamination. During their travels, people come into contact with indoor air, inhaling microbiological particles and often touch their hands to mucous membranes, opening the door to contagion for microorganisms dispersed on surfaces.[16,22,27].

The HDM® was able to completely reduce all bacterial genera that are pathogens to humans, with relevance to genera such as *Bacillus and Staphylococcus*; These bacteria were present in high concentrations after cleaning with sodium hypochlorite solution and suffered a drastic reduction in the first concentration of product (1mL/m³), showing high sensitivity to hydrogen peroxide and silver ions, a chemical compound already described in several searches[28].

As well as bacteria, several anemophilic fungi were isolated in air samples inside the buses; basically, they are classic components of bioaerosols. This finding allowed us to verify that the composition of the internal air of the analyzed buses is similar in its diversity to the external air in the places where they pass, but not in the concentration found, allowing us to understand its composition[29].

The random frequency of some fungal genera is justified, as air collection by impaction collects a sample volume and their circulation reveals their main characteristic, which is that they belong to the anemophile group[22].

The genera Fusarium and Penicillium remained viable, but at low concentrations, after cleaning with 3mL/m3 of the 99S® product. It is known that these fungi are found in different places and can be considered a natural part of many environments that do not need to be sterile to be healthy. What is surprising in this study is that hydrogen peroxide associated with silver ions at this concentration showed growth of fungal species and not the fungicidal effect as already mentioned, which can be explained by residual recontamination by fungi from the environment[22].

Another record of interest was the finding of two positive samples for a capsulated yeast belonging to the Cryptococcus genus on two surfaces on different buses. The two surface samples were collected before cleaning with the HDM® protocol. This fungus can cause meningitis in immunocompromised individuals and is basically spread through the air[30].

CONCLUSION

It can be stated that the disinfection carried out using the no-touch HDM® technology tested during this study was more effective in eliminating CFU/m³ of bacteria and fungi at concentrations of 3mL/m³ and 4mL/m³, respectively. These concentrations substantially reduced the spread of microorganisms and the possibility of contamination of employees and users of public transport.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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ANNEXES

Number of sample	ID. Bus	Mileage time	Type of hygiene	Disinfectant	Concentration
1A	32179	12 years	Manual	Hypochlorite	Not informed
1B	32179	12 years	HDM®	H_2O_2 6,6%	1ml/m³
2A	32403	6 years	Manual	Hypochlorite	Not informed
2B	32403	6 years	HDM®	H_2O_2 6,6%	1ml/m³
3A	32179	12 years	Manual	Hypochlorite	Not informed
3B	32179	12 years	HDM®	H_2O_2 6,6%	2 ml/m³
4A	32403	6 years	Manual	Hypochlorite	Not informed
4B	32403	6 years	HDM®	H_2O_2 6,6%	2 ml/m³
5A	32179	12 years	Manual	Hypochlorite	Not informed
5B	32179	12 years	HDM®	H_2O_2 6,6%	3ml/m³
6A	32403	6 years	Manual	Hypochlorite	Not informed
6B	32403	6 years	HDM®	H_2O_2 6,6%	3ml/m³
7A	32179	12 years	Manual	Hypochlorite	Not informed
7B	32179	12 years	HDM®	H_2O_2 6,6%	4ml/m³
8A*	32441	2 years	Manual	Hypochlorite	Not informed
8B*	32441	2 years	HDM®	H_2O_2 6,6%	4ml/m³
9A	31559	2 years	Manual	Hypochlorite	Not informed
9B	31559	2 years	HDM®	H_2O_2 6,6%	3ml/m³
10A	32179	12 years	Manual	Hypochlorite	Not informed
10B	32179	12 years	HDM®	H_2O_2 6,6%	4ml/m³

Table 1 – Characterization of buses and hygiene protocols applied during the study on public transport buses in the city of São Paulo, Brazil.

Subtitle: H2O2 6,6%: Hydrogen Peroxide 6.6%; Hypochlorite: Diluted sodium hypochlorite; HDM®: HyperDryMist® no-touch technology. *presence of air conditioning system inside the bus.

Microrganism	Number of isolated colonies		Reduction rate between protocols (%)
	Sodium hypochlorite	HDM*	
Acinetobacter	2	0	100
Bacillus	12	4	75
*Cryptococcus**	2	0	100
Cupriavidus	1	0	100
Enterobacter	2	0	100
Enterococcus	1	0	100
Escherichia	1	0	100
Kocuria	3	2	66
Lactobacillus	1	0	100
Microbacterium	1	0	100
Pantoea	3	1	66
Pseudomonas	2	1	50
*Rhodotorula**	3	0	100
Staphylococcus	22	6	64
* Fungal genera			

Table 2 – Frequency of microorganisms isolated on high-contact surfaces inside buses after manual and no-touch cleaning protocols carried out in the city of São Paulo, Brazil.

Sample	Internal concentration (CFU/m3)	External concentration (CFU/m3)	I/E coefficient	COMPLIANCE
01A 02A	1589 1509	128	12,4 11,8	NC NC
03A 04A	1136 1307	236	4,8 5,5	NC NC
05A 06A	**countless*** **countless***	520	* *	NC NC
07A 08A	1659 53**	604	2,7 0,1	NC C
09A 10A	**countless*** 1493	292	* 5,1	NC NC

Table 3 – The I/E ratio coefficient of UFC/m3 concentration in buses after cleaning with sodium hypochlorite solution in the study carried out in the city of São Paulo, Brazil

* I/E coefficient incalculable due to exacerbated I value;

** bus with internal air conditioning system.

NC: non-compliant; I/E coefficient >1.5

C: conformity; I/E coefficient <1.5

Running time/sanitation protocol	Average CFU/m³	
	BAC	FUNG
2 YEARS		
Sodium hypochlorite	117	260
HDM®	2	12
6 YEARS		
Sodium hypochlorite	62	284
HDM®	6	128
12 YEARS		
Sodium hypochlorite	290	463
HDM®	11	119

Table 4 – Average concentration of CFU/m³ of fungi and bacteria in the internal air of vehicles after the application of hygiene protocols on buses stratified by running time in the study carried out in the city of São Paulo, Brazil

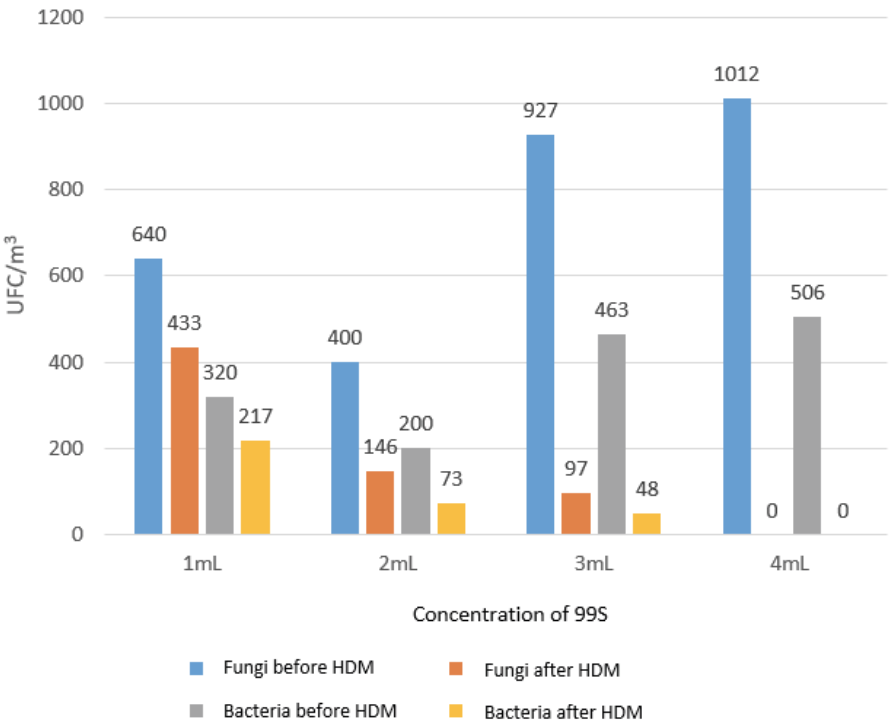

Figure 1 – Comparison of CFU/m3 of fungi and bacteria in the internal air of buses before and after cleaning with HDM® at different concentrations in the study carried out in the city of São Paulo, Brazil

Gender	Positive samples per hygiene protocol (N)				
	Hypochlorite	HDM® 1 mL/m³	HDM® 2 mL/m³	HDM® 3 mL/m³	HDM® 4 mL/m³
Aspergillus	4	0	0	0	0
Byssochlamis	0	2	0	0	0
Cladosporium	3	0	1	0	0
Epicoccum	2	0	0	0	0
Fusarium	3	0	0	1	0
Mucor	0	1	0	0	0
Penicillium	8	2	1	1	0
Scedosporium	1	0	0	0	0
Syncephalastrum	1	0	0	0	0
Trichoderma	2	1	0	0	0
Rhodotorula	3	0	0	0	0

Table 5: Distribution of positive samples for fungal genera isolated in the internal air of buses after application of weekly hygiene protocols in the study carried out in the city of São Paulo, Brazil

Gender	Positive samples per hygiene protocol (N)				
	Hypochlorite	HDM® 1 mL/m³	HDM® 2 mL/m³	HDM® 3 mL/m³	HDM® 4 mL/m³
Acinetobacter	0	0	1	0	0
Bacillus	16	5	1	0	0
Klebsiella	2	0	0	0	0
Kocuria	0	0	1	0	0
Kurthia	0	0	1	0	0
Lecleria	0	1	0	0	0
Lysinibacillus	1	0	0	0	0
Micrococcus	8	0	1	0	0
Paenibacillus	1	0	0	0	0
Pantoea	7	0	1	0	0
Pseudomonas	7	1	0	0	0
Serratia	0	1	0	0	0
Staphylococcus	14	2	0	0	0
Streptomyces	1	1	0	0	0

Table 6 – Distribution of positive samples for bacterial genera isolated in the internal air of buses after application of weekly hygiene protocols in the study carried out in the city of São Paulo, Brazil