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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: To evaluate the effectiveness of using a no-touch technology through aerosolization of hydrogen peroxide and silver ions, in the internal hygiene of public transport buses in the city of São Paulo. **Methodology:** A total of 294 samples were collected from 4 public buses. The transports were cleaned manually and subsequently disinfected with the aid of equipment, which promotes high-level disinfection of the environment, without the need for human intervention. The samples were sent to the Instituto Adolfo Lutz laboratory for analysis. **Results:** Collections on bus surfaces after manual cleaning with Sodium hypochlorite solution showed growth of fourteen microbial genera and after automated disinfection there was a reduction of 72% in bacterial genera and 100% in fungal genera. **Conclusions:** the complementary disinfection carried out with the aid of no-touch technology showed greater effectiveness in eliminating bacteria and fungi at concentrations of 3ml/m³ and 4ml/m³, respectively. Disinfection carried out at these concentrations showed a greater reduction in pathogenic microorganisms, which could contribute to a lower risk of contagion and illness for 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 year3. 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 (3,4)

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, potentially causing 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 infections9 and other fungal genera have also been linked to causing superficial, systemic or subcutaneous mycoses (9)

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. (10). 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 (11).

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 exposed wounds. (12)

Numerous conventional sanitizing processes can be used to decontaminate and sanitize various locations, such as hospitals, but these protocols may not be completely efficient. 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. (13)

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. (4,14)

Considering the importance of this topic, this study aimed to evaluate the effectiveness of using no-touch technology through aerosolization of hydrogen peroxide and silver ions in the internal disinfection of public transport buses 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 laboratory at the Instituto Adolfo Lutz (IAL).

SAMPLING

The sampling was divided into two categories: a) monitoring the effectiveness of hygiene using the manual method and, b) evaluating the effectiveness of disinfection using no-touch 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.

ABOUT NO-TOUCH TECHNOLOGY

It is a disinfectant, which acts synergistically for the high-level disinfection of internal environments, applied through automated equipment.

The aerosolization of the disinfectant promoted by the device allows the hydrogen peroxide and ionized silver nanoparticles to assume the physical-chemical behavior of gas, thus facilitating the uniform distribution of the disinfectant in a dry and uniform manner throughout the area.

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 that varied between 12 years, 6 years and 2 years.

The chosen vehicles followed the same route, circulating through the city's commercial center (between the Brás and Pinheiros regions), located in the metropolitan region and were evaluated when out of service and parked in the garage of the affiliated company.

BUS CLEANING

The buses were first cleaned by the garage's cleaning team, internally with a Sodium hypochlorite solution (the concentration of the product was not informed) in accordance with the affiliated company's protocol.

SAMPLE COLLECTIONS

In each bus, six microbiological samples were collected from the internal air and ten samples from high-contact surfaces. Air and surface collections were carried out after manual hygiene and after no-touch disinfection. The samples were collected from the surfaces using a swab, which was striated within the boundaries of a 10x2 cm template.

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

Before disinfecting transport with the technology, it was necessary to measure the cubic footage of the buses, to include this information in the equipment and thus be able to validate the disinfection protocols at concentrations of 1ml/m³, 2ml/m³, 3ml/m³ and 4ml/m³ of the disinfectant.

To validate the uniform reach of the aerosolized disinfectant solution inside each bus and correlate it with the reduction of microorganisms associated with the use of protocols at different concentrations of the product, we spread around 10 colorimetric chemical indicators throughout the interior of the bus, considering locations close to the analyzed surfaces, as well as in places considered difficult to reach, in relation to the positioning of the equipment, such as under the seat benches. This test was validated as positive when the color of the chemical indicator, white, was changed to orange, thus indicating contact with hydrogen peroxide.

AIR COLLECTIONS

Air samples for isolation of fungi and bacteria were collected using the Merck® MAS 100 impactor. To collect fungi, Petri dishes were used with modified Dicloran Rose Bengal culture medium and to collect bacteria, tryptone soy agar (TSB) and MacConkey (15,16) were used.

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 samples were placed in a thermal bag and sent to the Instituto Adolfo Lutz (IAL) laboratory, where they were processed in less than 24 hours, counting from the collection

time. The plates with samples for filamentous fungi were incubated in a bacteriological oven adjusted to 30º ± 2ºC for up to seven days for isolation and gender identification.

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 (16)

After this period, the samples were sown using the exhaustion technique on plates containing the culture media TSB agar, mannitol salt agar, DRBC agar and MacConkey agar and incubated in bacteriological study at 35 ± 2ºC for up to 48 hours (16).

IDENTIFICATION OF THE SAMPLES

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), (17–19)

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 microbial genera.

After applying the protocol, there was a reduction of 72% in bacterial genera and 100% in fungal genera.

Chemical indicators placed inside the buses during the aerosolization of hydrogen peroxide with silver ions confirmed the uniform distribution of the product throughout the interior of the cars.

There was a gradual reduction in the presence of microorganisms from a concentration of 1mL/m3, the total reduction of bacteria occurred from 3mL/m3 and a total reduction of fungi from 4mL/m3.

All air samples after cleaning with the Sodium hypochlorite solution, except in the bus with an air conditioning system, showed concentrations outside the fungal standards recommended by ANVISA. 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 environment (garage).

A difference in the average concentration of UFC/m3 of fungi and bacteria isolated in the atmospheric air of the bus was observed according to the driving time (Table 3).

Sanitization at a concentration of 4mL/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 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 the hygiene protocols from 2mL/m3 and 4mL/m3, respectively; The bacterial genera Bacillus, Klebsiella, Micrococcus, Pantoea, Pseudomonas and Staphylococcus showed a significant reduction with the first concentrations of the aerosolized product and total elimination occurred with 3mL/m3 of the product; the other genera presented random characteristics (Table 4).

DISCUSSION

The presence of microorganisms inside public transport vehicles is considered widespread considering the number of people who use them and the air changes that occur on their routes, making transmission to humans possible (21); 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, not it has an impact on the reduction of bioaerosols, when monitored (22).

Some species of bacterial genera isolated in the air or on the surface of the buses studied, such as 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 (23).

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 (20,24)

The sanitization technology proved to be effective in distributing the sanitizing product throughout the bus environment, which was already expected, given the capacity of the system and the chemical compliance of the product (25,26).

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 (27)

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 (28).

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 (14,23,29).

No-touch technology 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 ($1mL/m^3$), showing high sensitivity to hydrogen peroxide and silver ions, a chemical compound already described in several research (30).

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 buses analyzed 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 (31).

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

group (23).

The genera Fusarium and Penicillium remained viable, but at low concentrations, after disinfection with 3mL/m3 of the aerosolized 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. (23).

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 automated sanitization. This fungus can cause meningitis in immunocompromised individuals and is basically spread through the air (32).

CONCLUSION

It can be stated that the disinfection carried out using the no-touch 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.

INTEREST CONFLICTS

The authors declare that there are no conflicts of interest.

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ANNEXES

Number of sample	ID. Bus	Running Time	Type of Hygiene	Disinfectant	Concentration
1A	32179	12 years	Manual	Hypochlorite	Not informed
1B	32179	12 years	No-touch technology	H_2O_2 6,6%	1ml/m³
2A	32403	6 years	Manual	Hypochlorite	Not informed
2B	32403	6 years	No-touch technology	H_2O_2 6,6%	1ml/m³
3A	32179	12 years	Manual	Hypochlorite	Not informed
3B	32179	12 years	No-touch technology	H_2O_2 6,6%	2 ml/m³
4A	32403	6 years	Manual	Hypochlorite	Not informed
4B	32403	6 years	No-touch technology	H_2O_2 6,6%	2 ml/m³
5A	32179	12 years	Manual	Hypochlorite	Not informed
5B	32179	12 years	No-touch technology	H_2O_2 6,6%	3ml/m³
6A	32403	6 years	Manual	Hypochlorite	Not informed
6B	32403	6 years	No-touch technology	H_2O_2 6,6%	3ml/m³
7A	32179	12 years	Manual	Hypochlorite	Not informed
7B	32179	12 years	No-touch technology	H_2O_2 6,6%	4ml/m³
8A*	32441	2 years	Manual	Hypochlorite	Not informed
8B*	32441	2 years	No-touch technology	H_2O_2 6,6%	4ml/m³
9A	31559	2 years	Manual	Hypochlorite	Not informed
9B	31559	2 years	No-touch technology	H_2O_2 6,6%	3ml/m³
10A	32179	12 years	Manual	Hypochlorite	Not informed
10B	32179	12 years	No-touch technology	H_2O_2 6,6%	4ml/m³

Subtitle: H2O2 6.6%: Hydrogen Peroxide 6.6%; Hypochlorite: Diluted Sodium hypochlorite; HDM®: No-touch technology

* presence of air conditioning system inside the bus.

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

Microorganism	Number of isolated colonies		Reduction rate between protocols (%)
	Sodium hypochlorite	No-touch technology	
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.

Running time/sanitation protocol	Average of UFC/m³	
	BAC	FUNG
2 YEARS		
Sodium hypochlorite	117	260
No-touch technology	2	12
6 YEARS		
Sodium hypochlorite	62	284
No-touch technology	6	128
12 YEARS		
Sodium hypochlorite	290	463
No-touch technology	11	119

Table 3: 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

Gender	Positive samples per hygiene protocol (N)				
	Hypochlorite	HDM® 1 mL/m³	HDM® 2 mL/m³	HDM® 3 mL/m³	HDM® 4 mL/m³
Bacteria					
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
Fungi					
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
Aspergillus	4	0	0	0	0
Byssochlamis	0	2	0	0	0
Cladosporium	3	0	1	0	0
Epicoccum	2	0	0	0	0

Table 4: 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

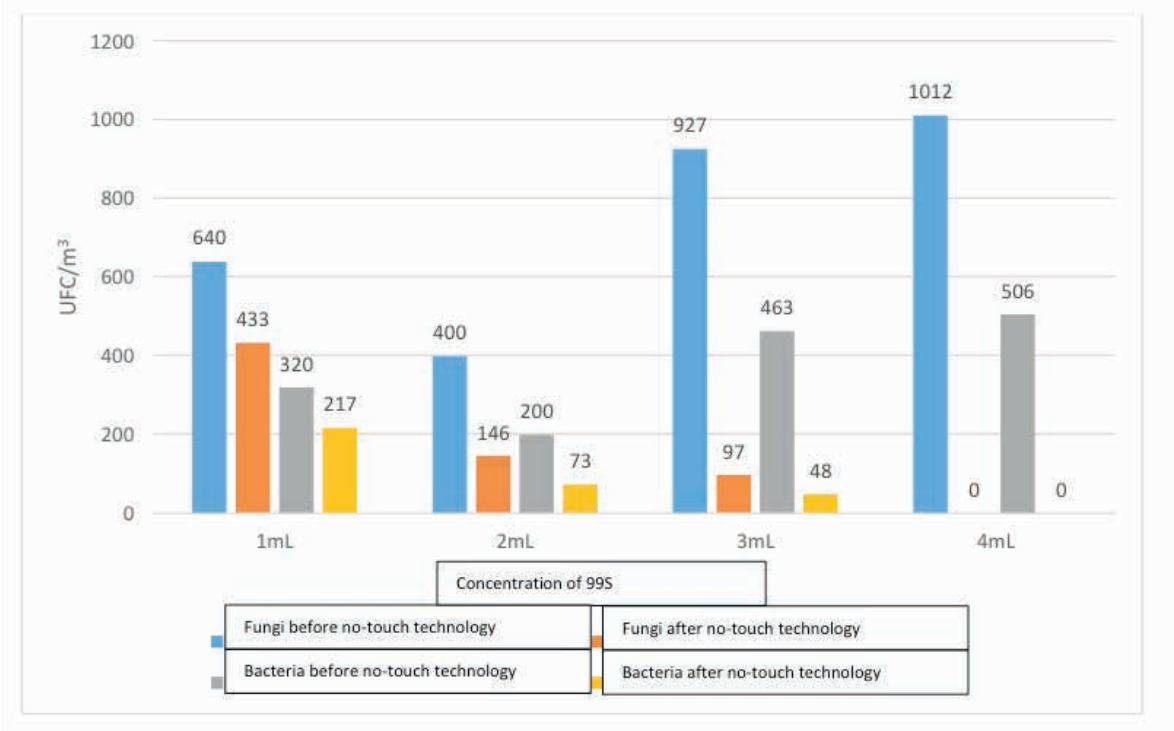

Figure 1: Comparison of CFU/m3 of fungi and bacteria in the internal air of buses before and after cleaning with no-touch technology at different concentrations in the study carried out in the city of São Paulo, Brazil