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COMPARATIVE STUDY OF PHYSICAL AND CHEMICAL METHODS FOR CONTROLLING BACTERIAL LOAD INDUCED ON DIFFERENT SURFACE PROTOTYPES

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Abstract: According to Patient Safety in Health Services, cleaning and disinfecting surfaces is the extraction of infectious agents, through the use of chemical or physical agents. The objective of this study was to verify the efficiency between chemical disinfection using Peroxide and the physical disinfection of high-frequency electric plasma with its bactericidal action on marble and Formica prototypes contaminated with the standard strain, ATCC (25922) *Escherichia coli*. The 20X20 surfaces of the prototypes, both marble and Formica, were contaminated with *Escherichia coli* strains (ATCC 25922) standardized by the McFarland scale, to compare the disinfection efficiency of using Peroxide MSCD® and plasma for the duration of 60s. RODAC® plates with PCA (*Plate Count Agar*) cultivation medium were used, thus it was possible to obtain a quantitative analysis of the contamination induced by the standard strain inoculum. The reduction percentage for the marble prototype with high-frequency electric plasma was 69.14% and for the Peroxide MSCD® it was 100%, and for the Formica prototype, 80% and 100% were obtained with the use of plasma disinfection. high frequency electrical and Peroxide MSCD®, respectively. Therefore, it was possible to prove the efficiency of these disinfection agents, based on standardized methods, despite the particularities of the surfaces and the microorganism studied, thus demonstrating the ability to control bacteria, strain of E. *coli* on prototype Formica and marble surfaces analyzed.

Keywords: Contamination. Surfaces. Disinfection. Bacterial load. Plasma Gases.

INTRODUCTION

According to Resolution No. 216, of 2004, environments such as establishments must follow the Manual of Good Practices, this document establishes hygienic-sanitary conditions, the maintenance and hygiene of facilities, equipment and utensils, such as confirming the quality of the prepared food. Through these actions, it is possible to avoid Foodborne Diseases (FADs). Around 88% of contaminations occur in restaurants, one of the most predominant among the different types of agents being *Escherichia coli* (E. coli), a bacterium that belongs to the Enterobacteriaceae family (MERENGUE *et al*., 2023).

Escherichia coli is one of the main bacteria that causes infections in children and adults. In recent decades, studies on this pathogen indicate that it is increasingly present in the lives of different populations, interacting and adapting to the environment (MERENGUE *et al.,* 2023).

According to Patient Safety in Health Services - Anvisa, 2010: cleaning and disinfection of surfaces, is the extraction of infectious agents, through the use of chemical or physical agents, from surfaces in the food area, industrial kitchens, restaurants, factories, inert. These agents are found in vegetative form, which can be viruses or bacteria.

Studies show that adequate cleaning and disinfection of surfaces can reduce around 99% of these infectious agents, however, when these procedures are not carried out, they can present the presence of microorganisms, which can be exposed on surfaces in hospitals, such as beds and equipment (BRAZIL, 2010). Furthermore, inadequate cleaning and disinfection can accelerate the wear of countertop surfaces, as well as contribute to the resistance of microorganisms, providing shelter and nutritional support for them (DISINFECTION, 2001).

Through chemical and physical control methods, it is possible to carry out sterilization, destroying all forms of microbial life, such as fungi, viruses and bacteria in vegetative and spore forms. There are classic physical methods such as the autoclave, which is a sterilization process using saturated steam under pressure, and the oven, which promotes dry heat with low penetration power (TIPPLE, 2011).

Chemical agents such as Peroxide MSCD®, the use of ethylene oxide, 2% glutaraldehyde, formaldehyde (alcoholic solution) and formaldehyde (10% aqueous solution), alcohols (70% and 80% solutions) are also well studied., heavy metals (mercury, chromium mercury, merthiolate; silver: silver nitrate; copper: copper sulfate) are considered chemical controls (CESAD, 2023). Disinfectants derived from oxidizing agents, such as hydrogen peroxide and peroxide compounds (sodium, zinc and benzyl), have germicidal action arising from the production of nascent oxygen (MORIYA; MÓDENA, 2008).

Peroxide MSCD® is a versatile disinfectant, used to sanitize surfaces, offering broad-spectrum action against microorganisms and can be applied to floors, door handles, handrails, etc. This product, based on Hydrogen Peroxide, offers three functions: cleaning, disinfection and eliminates the need for rinsing, eliminating 99.999% of microorganisms in a single application. Another important point about the Peroxide MSCD® product is that specific personal protective equipment is required for its application. This prevents accidents and ensures the safety of the individual during hygiene (ECOLAB, 2019).

Taking into consideration, the composition of the Peroxide MSCD® product, hydrogen peroxide is also considered a sterilizing chemical agent, both in its liquid, gaseous and plasma form, inactivating bacteria, viruses, tuberculosis bacilli, fungi and some spores. It acts by producing free hydroxyl radicals that attack the lipid membrane of DNA and other elements of the microbial cell (MORIYA and MÓDENA, 2008).

Hydrogen peroxide is one of the most versatile oxidants that exists, superior to chlorine, chlorine dioxide and potassium permanganate; Through catalysis, H2O2 can be converted into hydroxyl radical, with reactivity inferior only to fluorine (MATOS et al., 2002).

Within the context of sterilization, we have high-frequency electrical plasma, which is defined as a partially or fully ionized gas, in electrical neutrality, composed of ions, electrons, photons, ultraviolet radiation, free radicals and chemically reactive neutral particles (MORFILL *et al.*, 2009; GOREE *et al.*, 2006).

Recent studies show that plasma has a broad spectrum of action against microorganisms and does not allow the development of resistance. It is used in industrial applications, such as surface modification, and is being employed in the medical and dental fields for disinfection and sterilization of materials. Plasma is divided into two types: high temperature, which requires high temperatures (around 3000ºC) to be produced and is less common, and low temperature, also called non-thermal, which can be generated from micro -waves and radio frequency, with gases under atmospheric pressure or low pressure, including vacuum environments. It is believed that the inactivation of microorganisms occurs due to damage to DNA caused by ultraviolet (UV) radiation or the cytotoxic activity of reactive species generated by plasma. Furthermore, protein modulations and induction of apoptosis may occur (RODRIGUES et al., 2019).

The high-frequency device consists of a generator, an electrode holder and several glass electrodes, which are generally hollow glass tubes with rarefied air or gas such as neon inside. The passage of current causes ionization of the gas molecules which, under strong energetic impact, become fluorescent. Its use has been carried out for years for antiseptic, bactericidal, fungicidal and germicidal purposes (OLIVEIRA, 2021).

Therefore, research in the area aims to employ techniques for viable alternatives in microbial control, such as the use of electric plasma technology, which gained prominence due to the various limitations of other methods, being an important means of controlling infections (SILVA *et al*., [s.d.]).

The objective of the present study was to compare physical disinfection using electric plasma with chemical disinfection using Peroxide and its bacteriostatic and/or bactericidal action on prototypes contaminated with the ATCC (25922) *Escherichia coli strain.*

METHODOLOGY

In the present study, the methodology adapted from David et al. was used. (2022). For the experiment, physical (high frequency plasma) and chemical (Peroxide) disinfection protocols were developed and adapted, testing a standard strain of *Escherichia coli* ATCC (25922). Furthermore, all biosafety standards were followed, as recommended by the ``Centro de Diagnósticos Laboratoriais`` – CDLAB laboratory, where all in vitro tests were carried out. To begin with, four prototypes were defined, two made of Formica and two made of marble, both with an area of 20x20cm², presenting different porosity and texture. Within the document, three groups of experiments were standardized: Control group (CG), Chemical disinfection group using Peroxide MSCD® (Pe) and Group using high frequency plasma (PL).

The bacteria chosen for the induced contamination of surfaces was the ATCC strain of Escherichia coli (25922). For the experiment it was necessary to use materials such as: platinum loop, test tube containing sterile saline, sterile swab and laminar flow. Finally, to check the bacterial load, RODAC® contact plates containing PCA medium – *Plate Count Agar* and an incubation oven were used to quantify microbial contamination of surfaces.

To begin the procedure, the bacterial inoculum was prepared in properly disinfected laminar flow, E. coli ATCC (25922) bacterial colonies were collected from the culture medium with the aid of a platinum loop, then diluted in sterile saline. Finally, the turbidity was evaluated on the McFarland scale with grading 2 (presenting a bacterial number of 6x108). All three groups: Control Group, Peroxide MSCD® Group (Pe) and High Frequency Plasma Group (PL) were exposed to contamination by the bacteria E. coli ATCC (25922), through a sterile swab soaked in the bacterial inoculum and seeded on the prototypes by exhaustion method.

Both the Formica (1 and 2) and Marble (1' and 2') pieces were subjected to negative and positive control, using previously identified RODAC® contact plates, with PCA medium to perform the total count of the microorganism., which were placed directly in contact with the surfaces, making contact movements with the surface (stamp). The negative control RODAC® plates were used as prototypes without contamination with E. coli ATCC (25922), thus being used as a safety method, ensuring that the surfaces did not present other contamination. Positive control RODAC® plates were used after propagation of the E. coli ATCC (25922) strains in the prototypes.

After collecting the control groups, the Peroxide MSCD® Group (Pe), composed of the Formica (1 and 2) and Marble (1 and

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2) prototypes, was subjected to chemical disinfection, by spraying Peroxide MSCD® on the surfaces of the materials, and allowed the spontaneous evaporation of the product for a period of 3 to 4 minutes. Then, the stamp movement was carried out in direct contact throughout the entire area, on each piece with the identified RODAC® plates.

Continuing, with the High Frequency Plasma Group (PL), composed of the Formica prototype (1 and 2) and the Marble prototype (1´ and 2´), a specific support was used and adapted for the application of electric plasma at a frequency 1500 Hz, with a current of approximately 100mA, on both surfaces with a distance of 0.5 cm between the device and the prototypes, for 60s for each space in the area, accounting for a total of 20 minutes for the entire prototype area. Soon after, on each piece, two RODAC® plates were placed in direct contact, simulating stamp movement, across the entire area.

All groups were incubated in an oven at 37ºC for 24 hours. After this period, the Colony Forming Units were counted (CFU/surface), and thus the microbial loads found on each surface (formic and marble) were compared before and after the application of the control methods. The reduction percentage was calculated using the formula:

%R = Ci-Cf x 100/ Ci.

RESULTS

Based on experiments carried out on marble and Formica surfaces, results were obtained from RODAC® plates which, due to their counting characteristics, identified a reduction in CFU/cm² on the tested surfaces after the application of the agents (plasma and peroxide). The values were represented as a percentage, being an average of the reduction of the tests carried out according to

%R = Ci-Cf x 100/ Ci.

In the tests carried out with the prototypes and the use of RODAC® plates to evaluate contamination on different surfaces with different porosities, data were obtained in CFU per cm² and the average of the triplicate of the experiment which, based on these results, was calculated as a percentage of reduction through averaging.

In the marble prototype group, using high frequency plasma, the results were as follows for the control group (CPPL) in the first experiment, the colony count was 85 CFU/cm², in the second experiment, this count was 4 CFU/cm² and the third repetition, the count was 194 CFU/cm² and therefore presented an average of 94 CFU/cm². In the test group, which used high-frequency plasma (TPL), the colony count was reduced to 23 CFU/cm² for the first experiment, 0 CFU/cm² for the second repetition of the experiment and the third repetition, a reduction was obtained of 64 CFU/cm², with an average of 29 CFU/cm², which represents an average reduction of 69.14%. In the same prototype used, the Peroxide MSCD® in the control group (CPPe), presented results for the first experiment, a count of 20 UFC/cm², in the second experiment, this count was 169 UFC/cm² and finally, the third count resulted at 166 CFU/cm², with an average value of 118 CFU/cm². In the test group, which used Peroxide MSCD® (TPe), the colony count was reduced to 0 CFU/cm² for both the first, second and third repetitions of the experiment, with an average of 0 CFU/cm², which represents an average 100% reduction.

In the Formica prototype group using high frequency plasma, the results were as follows for the control group (CPPL), in the first experiment, the colony count was 24 CFU/cm², in the second experiment, this count was 46 CFU /cm² and the third repetition, the count was 104 CFU/cm², thus presenting an average of 58 CFU/cm². In the test group, which used high-frequency plasma (TPL), the

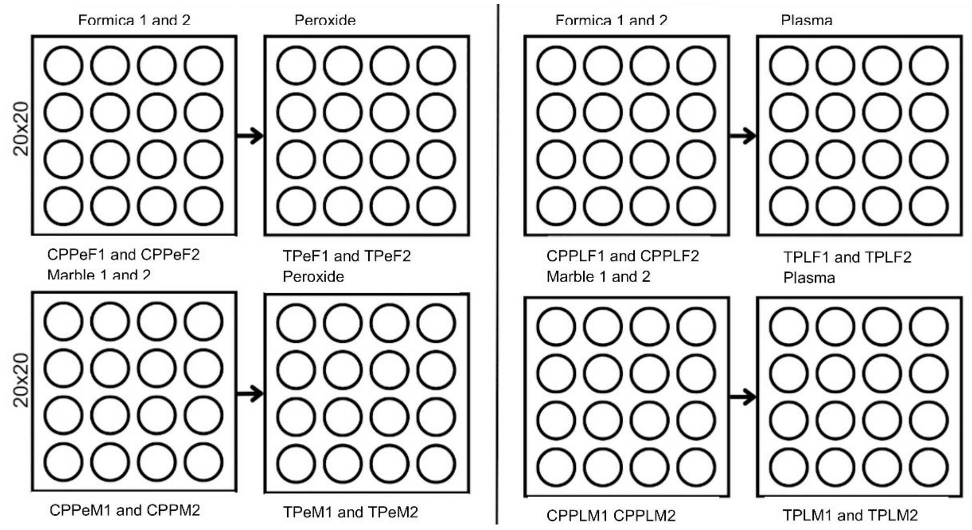

Figure 1 - Table of experiments of marble and Formica prototypes (20cm²) induced contamination of ATCC (25922) E. *coli physical* and chemical disinfection

Formica 1 and 2: peroxide control formica 1 (CPPeF1) and control 2 (CPPeF2), Peroxide test formica 1e 2 (TPeF1) and (TPeF2), Marble 1 and 2: control of marble peroxide 1 (CPPeM1) and control 2 (CPPeM2), Peroxide marble test 1 and 2 (TPeM1) and (TPeM2). Formica 1 and 2: plasma control formica 1 (PL FC1), test plasma formica 1 and 2 (TPLF1) and (TPLF2). Marble 1 and 2: plasma control marble 1 (CPPLM1) and control 2 (CPPLM2), test plasma marble 1 and 2 (TPLM1) and (TPLM2).

Source: Authors (2023)

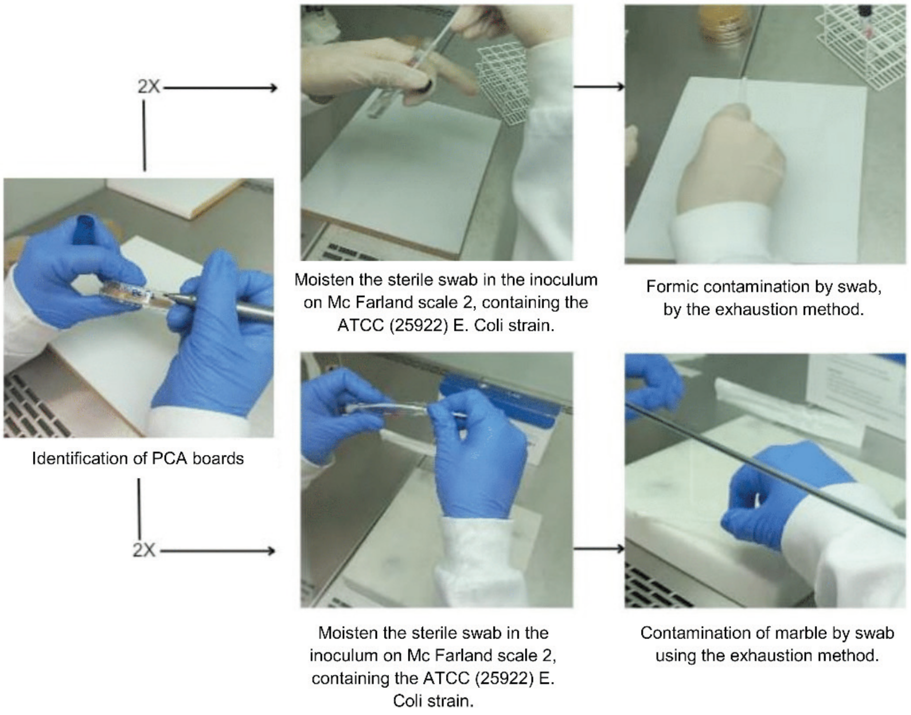

Figure 2 - Schematic of the first step of the experiment: identification of the RODAC® plates and the contamination induced by the marble and Formica prototypes by the exhaustion method

Source: Authors (2023)

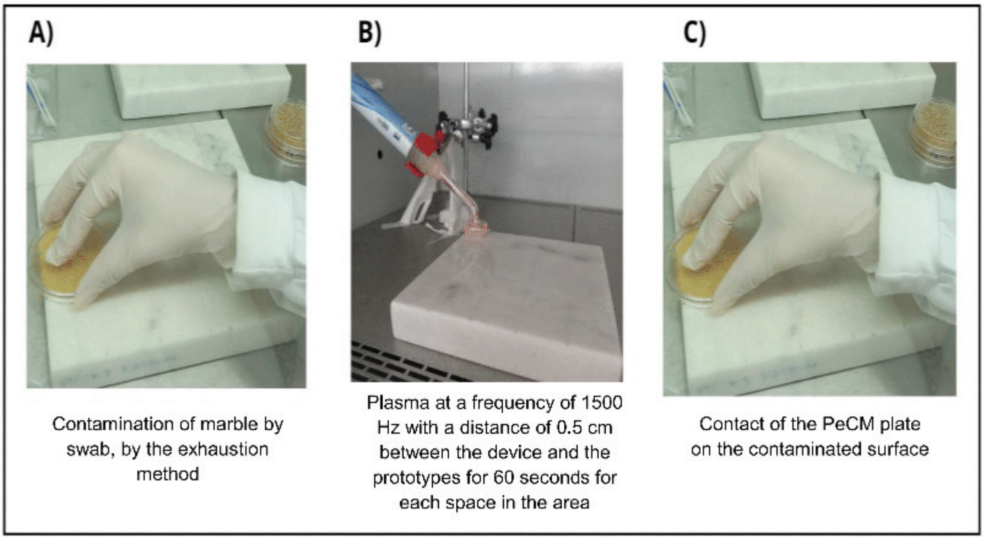

Figure 3 - Methodology of sequential steps (A, B and C) using high frequency plasma in the marble prototype

Source: Authors (2023)

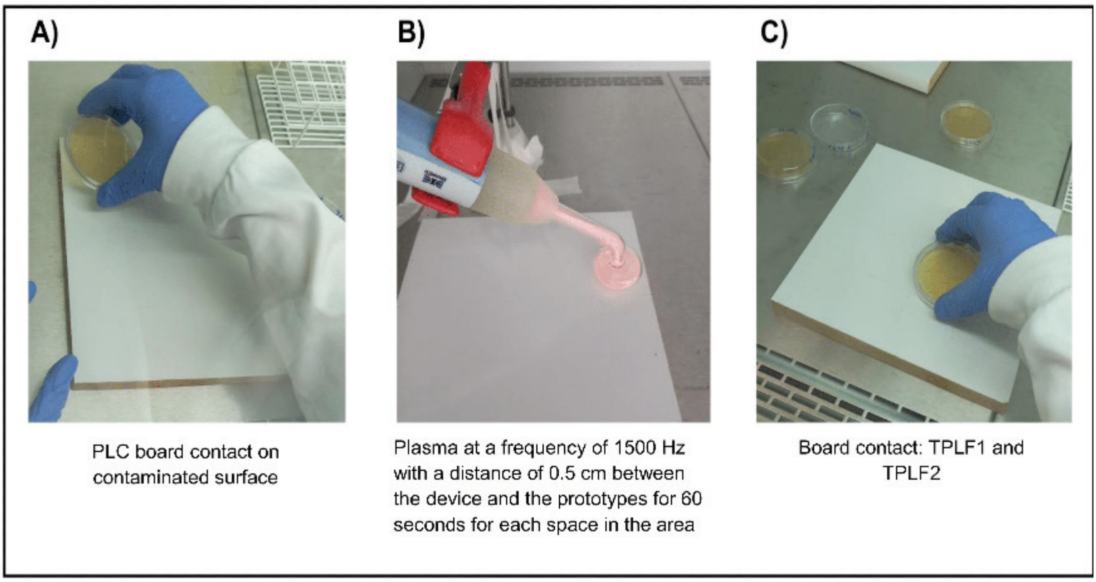

Figure 3 - Methodology of sequential steps (A, B and C) using high frequency plasma in the Formica prototype

Source: Authors (2023)

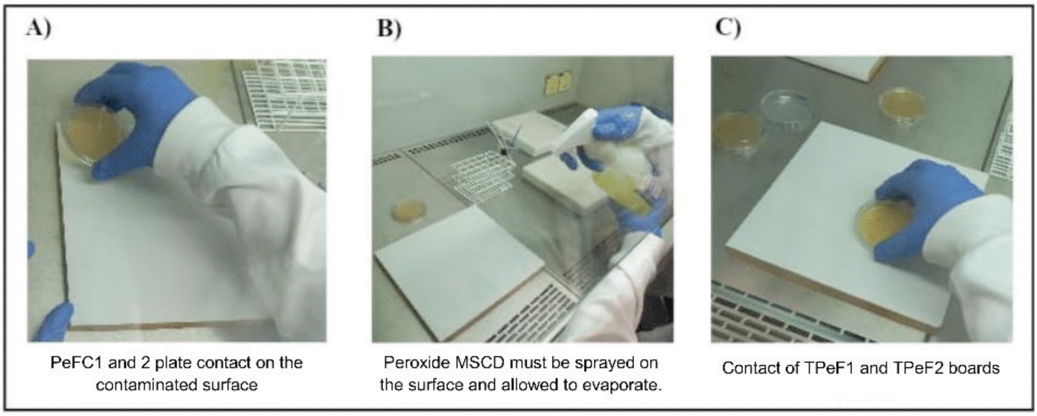

Figure 4 - Methodology of sequential steps (A, B and C) using Peroxide MSCD® on the Formica prototype

Source: Authors (2023)

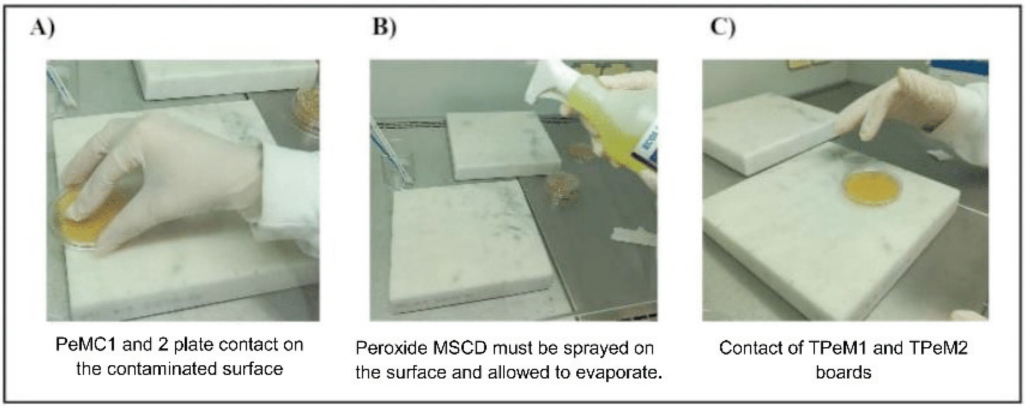

Figure 5 - Methodology of sequential steps (A, B and C) using Peroxide MSCD® on the marble prototype

Source: Authors (2023)

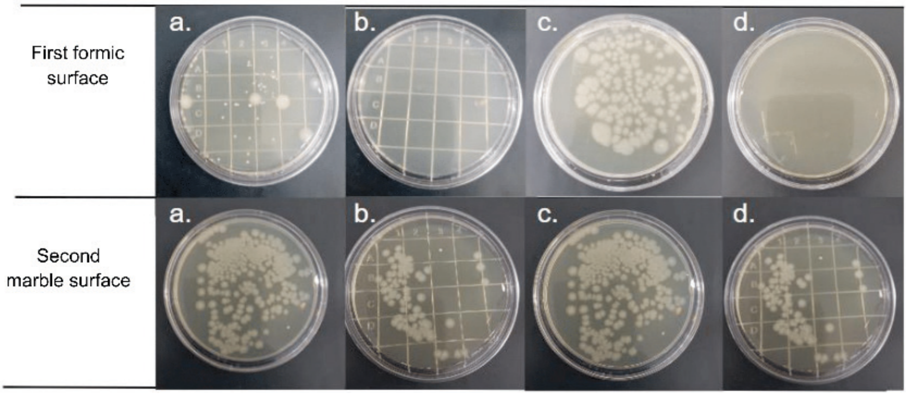

Figure 6 - Determination of microbial load in the first row: test carried out on the surface of the Formica prototype (a) control group; (b) high-frequency plasma disinfection group; (c) disinfection group with Peroxide MSCD®; second row: test carried out on the surface of the marble prototype in the control group (a); high-frequency plasma disinfection group (b); disinfection group with Peroxide MSCD®

Caption: a: High frequency plasma control (CPPL); B; High frequency plasma (TPl) test; c Positive control Peroxide MSCD® (CPPe); d: Peroxide MSCD® Test (TPe)

Source: Authors (2023)

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colony count was reduced to 1 CFU/cm² for both the first and second repetitions of the experiment, while the third repetition was to 33 CFU/cm², taking as average a value of 12 CFU/cm², which represents an average reduction of 80%. Using the same prototype group, the control group (CPPe), presented a result for the first experiment of colonies 85 CFU/cm², in the second experiment, this count was 72 CFU/cm² and in the third repetition, the count was 104 CFU /cm² with an average of 90 CFU/cm². In the test group, which used Peroxide MSCD® (TPe), the colony count was reduced to 0 CFU/cm², both for the first, second and third repetition of the experiment, respectively with an average of 0 CFU/cm², which represents an average reduction of 100%.

Marble Group	UFC/cm²	Average	% Reduction from average
CPPL	1º 85 UFC/cm² 2º 4 UFC/cm² 3º 194 UFC/cm²	94 UFC/cm²	69,14 %
STPL	1º 23 UFC/cm² 2º 0 UFC/cm² 3º 64 UFC/cm²	29 UFC/cm²	
CPPe	1º 20 UFC/cm² 2º 169 UFC/cm² 3º 166 UFC/cm²	118 UFC/cm²	100%
TPe	1º 0 UFC/cm² 2º 0 UFC/cm² 3º 0 UFC/cm²	100 UFC/cm	

Table 1- Quantification of the triplicate of the experiment in CFU/cm² obtained in the marble group prototype by high frequency plasma (Pl) and peroxide (Pe), the average and its reduction potential

Subtitle: Plasma positive control (CPPL); plasma test (TPL); CPPe peroxide positive control; peroxide test (TPe)

Source: Authors (2023)

Formica Group	UFC/cm²	Average	% Reduction from average
CPPL	1º 24 UFC/cm² 2º 46 UFC/cm² 3º 104 UFC/cm²	58 UFC/cm²	80 %
TPL	1º 1 UFC/cm² 2º 0 UFC/cm² 3º 33 UFC/cm²	12 UFC/cm²	
CPPe	1º 85 UFC/cm² 2º 72 UFC/cm² 3º 104 UFC/cm²	90 UFC/cm²	100%
TPe	1º 0 UFC/cm² 2º 0 UFC/cm² 3º 0 UFC/cm²	0 UFC/cm	

Table 2- Quantification of the triplicate of the experiment in CFU/cm² obtained in the Formica group prototype by high frequency plasma (Pl) and peroxide (Pe), the average and its reduction potential

Subtitle: Plasma positive control (CPPL); plasma test (TPL); CPPe peroxide positive control; peroxide test (TPe)

Source: Authors (2023)

DISCUSSION

The average reduction in tables 1 and 2, based on physical disinfection by plasma in the marble group, showed a reduction of 69.14% compared to the Formica surface, which showed a reduction of 80%. Marble is considered a surface with greater porosity compared to Formica, this porosity can influence the germicidal action of the plasma. Since there is difficulty in disinfecting and cleaning surfaces, especially materials that present or may present porosities and fissures, which consequently become reservoirs of microorganisms (FERNANDES; FERNANDES; RIBEIRO FILHO, 2000a).

Formica, on the other hand, has fewer pores in its structure, which inhibits the proliferation of bacteria and retains less dirt (MADEIRANIT, 2023). Therefore, in relation to chemical disinfection, it was possible to observe faster drying in comparison and still observe two 100% reduction rates. It is then necessary to evaluate the cost-benefit of the materials, taking into consideration, that the two results with peroxide were exceptional. Therefore, when considering the choice between these materials, the analysis is not only of the reduction rates, but also of the time, cost and effectiveness of the disinfection method, which are fundamental for accurate decision making.

In relation to the porosity of the surfaces, mineral countertops, mechanical action during cleaning, the use of abrasive products such as steel wool, brushes, soaps and similar are not recommended for cleaning, this action can remove microscopic mineral particles, promoting erosion (DEPARTAMENTO, 1999) of certain surfaces. Therefore, the use of new disinfection agents, which promote less friction and damage, can be a good contribution to the reduction and proliferation of microorganisms.

The plasma used demonstrated its versatility and can be applied in different fields, such as hospital, food and therapeutic. Studies conducted by Ziuzina in 2014 demonstrated the effectiveness of cold plasma in controlling Salmonella *typhimurium*, E. *coli* and L. *monocytogenes* isolated from tomatoes, cherry tomatoes, strawberries and lettuce. The results of these studies revealed remarkable inhibition of the growth of Salmonella *typhimurium* isolated from tomato after 10 seconds with plasma. Similarly, the growth of lettuce isolates was inhibited after 30 seconds, and the presence of E. coli was significantly reduced after 45 seconds of exposure to high-frequency plasma. Despite time variations, from 10 seconds to 300 seconds, the results indicated an effective inhibitory action (RODRIGUES, 2019). While on surfaces, the cold plasma polymerization of bioactive groups carried out by Pegalajar-Jurado et al. (2014), with 20W of power, the films presented moderately hydrophobic contact angle values (~80°) that provided bactericidal activity with the ability to reduce the adhesion of E. coli and S. *aureus* cells by 98% and 64%, respectively.

The same finding can be seen in the present study, where there was a significant reduction in the time of 60 seconds on surfaces. However, it is plausible to consider that prolonging the exposure time could result in an even more significant reduction in CFU/cm², possibly reaching 100%. These findings highlight the promising application of high-frequency plasma in the inhibition of microorganisms on various surfaces, pointing to possible advances in the effectiveness of the method with the optimization of the protocol.

It was noted in the work Liao et al. (2017), that the efficiency of the plasma to successfully inactivate the microorganism depends on some issues, such as the type of plasma used, the type of surface, as well as which microorganism is being subjected to physical disinfection. Likewise, factors that influence the action of the plasma must be taken into consideration, such as the characteristics of the microorganism, the cellular matrix, pH, exposure time and even the ambient temperature (RODRIGUES, 2019). High frequency plasma, according to data available on the BCMED website, which presents data about the device, demonstrates physiological effects, such as bacterial, germicidal, fungicidal and antiseptic action, it is also considered multifunctional and can act both in hair treatments, such as skin cleansing and other facial techniques.

A study was carried out with different types of hygiene for washing lettuce, using running water, fermented acetic alcohol, hydrogen peroxide and an acetic mixture of alcohol and hydrogen peroxide. The results obtained demonstrated that peroxide presented the second-best result with a 90% reduction in microorganisms, the most effective result was a 94.8% reduction by combining hydrogen peroxide with fermented acetic alcohol (QUINTÃO, 2023).

The rates of hospital infection by *Staphylococcus aureus* resistant to methicillin, *Enterococcus* resistant to vancomycin and *Clostridium difficile*, showed a reduction through the use of hydrogen peroxide-based disinfectant incorporated into a container of disposable tissues used once a day. Another study, in which the contamination of C. *difficile* spores was used in empty hospital rooms disinfected with eight products, one of which demonstrated positive results, which was hydrogen peroxide (OLIVEIRA, 2017). Through this research, it was possible to note the ability that hydrogen peroxide has to combat microorganisms, even in their spore forms.

For the present research, the E. coli ATCC (25922) strain was used, which is a facultative anaerobic organism, most common in the human intestine, belonging to the Enterobacteriaceae family. Several virulence factors are shared by most members of these species, such factors include the ability to produce a highly reactive lipopolysaccharide at the cell envelope, ability to produce type 1 mannose-binding fimbriae (hair-like appendages of adhesive surfaces) and (in many strains) the ability to produce an antiphagocytic capsule and sequester iron (MERENGUE et al., 2023). Due to all these factors, the diversity of infectious diseases caused by these species of E. *coli* is understandable, and the importance of creating mechanisms to combat proliferation, from food handling environments and even in hospital environments, is clear.

CONCLUSION

The present work demonstrated the capacity for physical disinfection with the use of electric plasma and chemistry using Peroxide MSCD®, both revealed to have a germicidal action to reduce microorganisms, specifically the E. coli ATCC (25922) strain. The chemical agent Peroxide, on both surfaces, demonstrated an efficiency of 100% in reducing the average bacterial load of CFU/cm^2. While high-frequency plasma showed a percentage reduction of 80% and 69.14% for Formica and marble surfaces respectively, however, the plasma application time and the porosity of each surface must be taken into consideration.

It was possible to identify through this study the capacity of both agents, despite the particularities of the surfaces and microorganisms. This study proved, after the standardization of several protocols, that it is possible to reduce CFU/cm^2 after using both control methods analyzed. These results not only improve our understanding of the efficiency of these agents, but also provide valuable insights for practical applications, driving advances in the control of microorganisms, the appropriate management of food and countertops in different environments across different segments.

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