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ANALYSIS AND CONTROL OF THE BACTERIAL LOAD OF TWO DIFFERENT PROTOTYPES AFTER CLEANING AND DISINFECTION METHODS

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Abstract: The concept of health can be properly defined as "[...] the result of the conditions of food, housing, education, income, environment, work, transportation, employment, leisure, freedom, access and possession of land and access to services of health". Among these factors, food stands out, having a direct influence on the quality of life and health of individuals, providing maintenance, prevention and recovery of well-being, essentially because it is a basic human need. Episodes of DTAs (Foodborne Diseases) related to household incidence are of great proportion, demonstrating the need for greater attention to the use of the ten golden rules of the World Health Organization (WHO) for the safe preparation of food. The use of microbial control measures can minimize the occurrence of possible contamination in places where food is prepared, utensils and equipment, as well as the furniture in the place, in order to reduce the percentages of DTAs. The study aimed to evaluate the antimicrobial action of two cleaning and disinfection methods on different materials: marble and formica. The analysis was standardized in order to quantify the microbial load after contamination induced with the *Escherichia coli strain* (ATCC 25922) on the surfaces of the prototypes, comparing two different methods for their decontamination, using neutral detergent and 70% alcohol and the disinfectant Peroxide MSCD®, a control group was also established. The analysis and quantification of microorganisms present was performed using RODAC® plates with PCA culture medium in contact with the surfaces of the prototypes. The reduction/elimination, by quantitative analysis, of CFU/cm² was observed in the prototypes, after cleaning/disinfection on both surfaces of the materials. It is concluded that the cleaning/disinfection protocol was efficient in microbial control, using neutral detergent plus 70% alcohol and MSCD® Peroxide, with complete elimination (100%) of the bacterial load from both surfaces of the prototypes.
Keywords: Contamination. Surfaces. Disinfection. Bacterial load.

INTRODUCTION

The concept of health can be properly defined according to the National Health Conference (1986) as "[...] the result of the conditions of food, housing, education, income, environment, work, transportation, employment, leisure, freedom, access and tenure to land and access to health services". Among these factors, food stands out, having a direct influence on the quality of life and health of individuals, providing maintenance, prevention and recovery of well-being, essentially because it is a basic human need.

The preparation of food, as well as the relationship with the conditions involved during the process, is extremely important, since pathologies linked to its contamination can be triggered by improper handling of food. Among the classes of contaminants there are physical, chemical or biological agents. Foodborne diseases (DTA) are caused by biological contaminants - microorganisms in sufficient quantity to affect the health of the population, being associated with hygienic-sanitary conditions during food preparation, and may manifest as poisoning, infections or toxinfections, " which they may present in a chronic or acute form, with characteristics of an outbreak or isolated cases, with localized or disseminated distribution and with different clinical forms" (MINISTRY OF HEALTH; SECRETARY OF HEALTH SURVEILLANCE; DEPARTMENT OF EPIDEMIOLOGICAL SURVEILLANCE, 2010) being of importance for public health at national and global level.

To guarantee food safety, the Good Manufacturing Practices (GMP) determine hygienic-sanitary conditions for food preparation, the cleaning of the installation, utensils and equipment, as well as the furniture that are in place, in order to guarantee the quality end of the product and avoid intercurrences, such as DTA's. Such manufacturing standards show necessary practices and conduct both in production and commerce establishments, as well as in the residential environment. The cleaning process proved to be effective in eliminating microorganisms on intentionally contaminated surfaces (GRAZIANO et al., 2013), including cleaning and disinfection procedures.

Cleaning consists of removing unwanted mineral and/or organic substances, such as soil, dust, grease and other dirt. Disinfection is capable of reducing, by physical method and/or chemical agent, the number of microorganisms to a level that does not compromise the hygienic-sanitary quality of the food (BRASIL, 2004).

Physical and chemical methods can be used to carry out cleaning and disinfection processes, whether on fabrics, instruments or surfaces. Being able to carry out the use of chemical methods through alcohol, detergents and disinfectants. Some examples of antiseptic actives with germicidal applicability are compounds of iodine, iodophors, chlorhexidine, alcohol, soap and detergents, chlorine and chlorinated derivatives, silver compounds, oxidizing disinfectants, phenolic derivatives, aldehydes and furan derivatives (MORIYA; MÓDENA, 2008).

Ethyl alcohol has a bactericidal action, capable of coagulating the protein of bacteria, in addition to being fungicidal and partially virucidal, being used as a component of antiseptics. Detergent agents remove dirt, debris and impurities from both skin and surfaces. Disinfectants derived from oxidizing agents such as hydrogen peroxide and peroxide compounds (sodium, zinc and benzyl) have a germicidal action arising from the production of nascent oxygen (MORIYA; MÓDENA, 2008).

Chlorine as an antibacterial active is the most potent of germicides, but it can be annulled by organic matter together with an alkaline pH. It is also not recommended for instrument disinfection, due to associated corrosiveness. Chlorinated solutions at a concentration of 5% are potent germicides indicated for the disinfection of instruments and utensils, causing irritation to tissues and must not be used as antiseptics (MORIYA; MÓDENA, 2008).

As a disinfectant agent, with a focus on action against bacteria such as *Escherichia coli* and *Staphylococcus aureus*, the use of alcohols is evident. Despite being common agents in the human microbiota, they can cause serious and potentially lethal medical events such as bacteremia and septic shock (TORTORA et al., 2017).

Episodes of FBDs related to the incidence at home are of great proportion, demonstrating the need for greater attention to the use of the WHO's ten Golden Rules for safe food preparation (WORLD HEALTH ORGANIZATION, 2002). Quality parameters indicate a direct link between biological contamination and food health, and it is important to use control measures to prevent, reduce to an adequate level or eliminate a physical, chemical or biological agent that compromises the hygienic and sanitary quality of the food (BRASIL, 2004).

In view of this, the analysis was standardized in order to quantify the bacterial load after contamination induced on surfaces of marble and formica prototypes, comparing two different methods for their decontamination,

using neutral detergent and 70% alcohol or peroxide MSCD® disinfectant as methods of cleaning and disinfection.

METHODOLOGY

In this study, two different surfaces, formica and marble, were evaluated. Three experimental groups were divided according to the surface of the piece, being a Control Group, a Cleaning and Disinfection Group using neutral detergent and 70% alcohol components, and a Peroxide MSCD® Disinfection Group.

In order to provide the necessary means for contamination, decontamination and subsequent analysis of the surfaces, two prototypes of formica with an area of 20x20cm, two prototypes of marble with an area of 20x20cm, strains of Escherichia coli (ATCC 25922), platinum loop, *tube* of assay containing sterile saline, sterile *swab,* laminar flow, neutral detergent, 70% alcohol, Peroxide MSCD®, disposable towels, RODAC® contact plates containing PCA – *Plate Count Agar medium* and incubation oven.

Initially, in laminar flow, a bacterial inoculum was prepared from the standard strain of *E. coli with standardized turbidity at graduation 2 on the* McFarland scale (approximate number of bacteria $6x10^{8}$), in which bacterial colonies were collected from the medium of culture with the aid of a platinum loop and then diluted in sterile saline contained in the test tube. All groups were exposed to induced contamination, using a sterile *swab* soaked in the bacterial inoculum and seeding using the depletion method.

Each piece was submitted to the collection of only one control sample, using RODAC® contact plates, previously identified and containing the PCA medium, used for the total count of microorganisms. The culture medium was placed in direct contact with the surface, performing a movement similar to that of a stamp, and repeating it throughout the entire area of the piece.

After collecting samples from the Control Group, the Cleaning and Disinfection Group, composed of prototype 1 Formica and prototype 1 Marble, was subjected to the cleaning and disinfection process using neutral detergent and 70% alcohol. With the aid of a damp disposable towel containing neutral detergent, the surface was cleaned using one-way movements and then the surface was dried. Then, using a disposable towel soaked in 70% alcohol, disinfection was performed using one-way movements. After complete evaporation, samples were collected using the same methodology applied to the Control Group, using RODAC® plates in direct contact with the entire surface of the parts.

Next, the Peroxide MSCD® Disinfection Group, composed of prototype 2 Formica and prototype 2 Marble, was exposed to the Peroxide MSCD® disinfectant. The solution was deposited over the entire area of the parts, with the aid of a spray bottle, and the product was allowed to evaporate for about 3 to 4 minutes. Samples were collected using the same methodology previously described, using RODAC® plates in direct contact with the entire surface of the parts.

Samples from all groups were incubated in an oven at 37ºC for 24 hours. After incubation, the Colony Forming Units (CFU/surface) were counted and the microbial loads found on each surface before and after the application of the control methods were compared. The percentage reduction was calculated from the formula $\%R = \frac{Ci-Cf}{Ci} \times 100$. The laboratory tests were carried out at the Center for Laboratory Diagnostics - CDLAB, in accordance with all biosafety protocols.

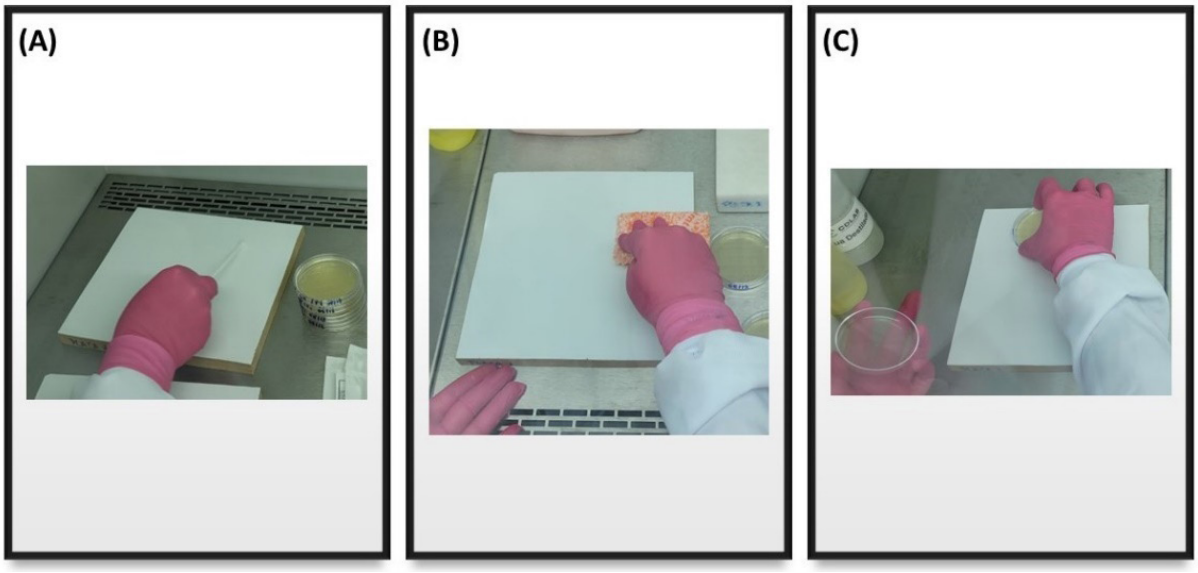

Figure 1 – Methodological procedure performed on the formica prototype: contamination induced by means of inoculum of *E. coli* with *swab* (A); cleaning and disinfection with neutral detergent and 70% alcohol (B); sample collection by means of a stamp with a RODAC® plate (C).

Source: Authors (2022).

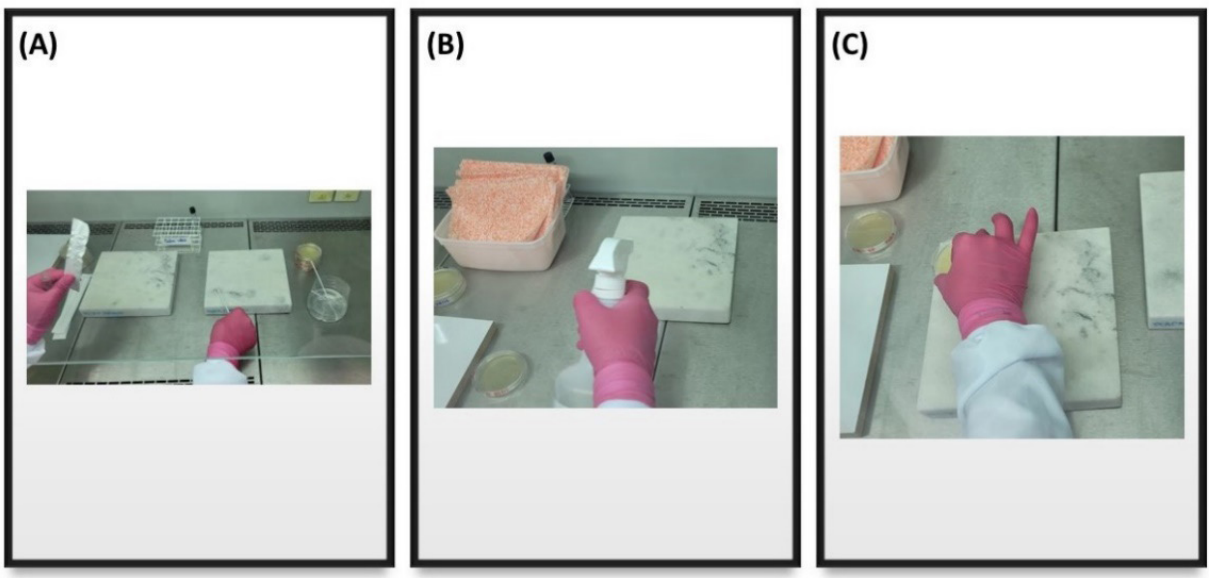

Figure 2 – Methodological procedure carried out on the marble prototype: contamination induced by means of *E.coli inoculum* with *swab* (A); disinfection with Peroxide MSCD® (B); sample collection by means of a stamp with a RODAC® plate (C).

Source: Authors (2022).

RESULTS

The control group showed growth of CFU in both materials as recommended, and in group 1 (formic prototype) a smaller growth of colonies was observed, being 27 CFU/ cm² in prototype 1 and 5 CFU/cm² in prototype 2, while in group 2 (marble prototype) a greater amount of colony growth was identified, being found in both prototype 1 and prototype 2 a value greater than 100 thousand CFU/ cm². The cleaning and disinfection group with neutral detergent and 70% alcohol (L&D group), as well as the disinfection group using Peroxide MSCD® (DP group) made it possible to observe the efficiency of cleaning and disinfection, with the total absence of growths of colonies, carrying 0 CFU/ cm². In this way, a 100% reduction of microorganism was achieved on both surfaces and plates of group 1 (formica prototype) and group 2 (marble prototype).

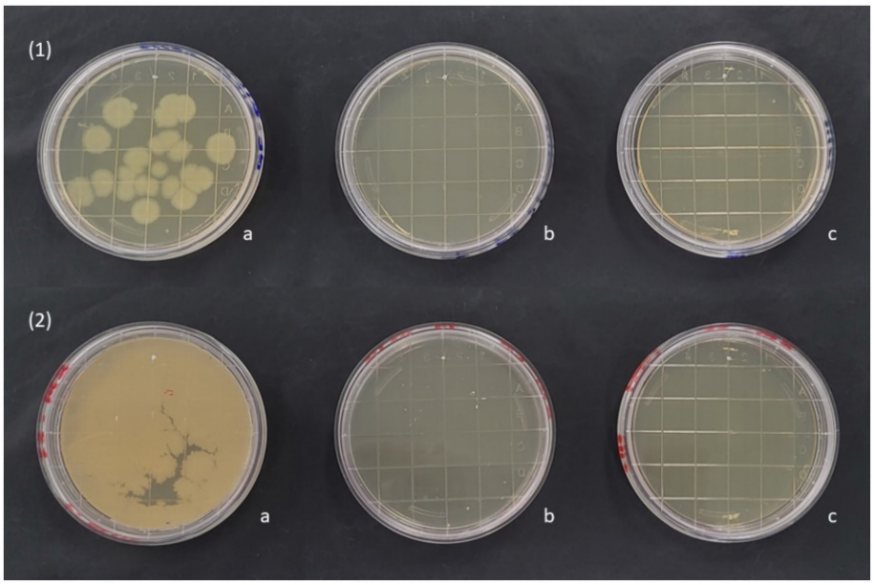

Figure 3 – Determination of microbial load 1: test performed on the surface of the Formica 1 prototype in the control group (a); cleaning and disinfection group (b); disinfection group with Peroxide MSCD® (c); 2: test carried out on the surface of marble prototype 1 of the control group (a); cleaning and disinfection group (b); disinfection group with Peroxide MSCD® (c).

Source: Authors (2022).

Surfaces Formica 1	**Group control** 27 UFC/cm²	**L&D Group** 0 UFC/cm²	DP Group 0 UFC/cm²	**% reduction** 100%
Formica 2	5 UFC/cm²	0 UFC/cm²	0 UFC/cm²	100%
Marble 1	Greater than 100,000 CFU/cm2	0 UFC/cm²	0 UFC/cm²	100%
Marble 2	Greater than 100,000 CFU/cm2	0 UFC /cm²	0 UFC/cm²	100%

Table 1 – Quantification of CFU/cm² obtained in each experimental group and their percentage of reduction.

Source: Authors (2022).

DISCUSSION

Based on the results of the surface analysis of the different prototypes, after cleaning and disinfection with neutral detergent and 70% alcohol or Peroxide MSCD® with an efficiency of 100% obtained in both material compositions of the prototypes and applied cleaning and disinfection methods, it can be verified their effectiveness, functionality and bactericidal action in a standard strain of *E. coli*.

Cleaning with a detergent solution has an action to reduce surface tension, provided by the surfactant in its composition, which "modifies the properties of water, reducing surface tension, facilitating its penetration on surfaces, dispersing and emulsifying dirt. The detergent has the function of removing both water-soluble and non-water-soluble dirt" (BRASIL, 2010).

According to resolution N 216, of September 15, 2004, the removal of unwanted mineral and/or organic substances, provided by the cleaning method, is one of the first steps to carry out disinfection and its effectiveness. Disinfection using 70% alcohol is capable of denaturing proteins that make up the cell wall of microorganisms, providing bactericidal, virucidal, fungicidal and tuberculocidal action, not only having a sporicidal action. Being indicated for furniture in general, having immediate action and easy application (BRAZIL, 2010).

According to the Surface Cleaning and Disinfection Guidelines, disinfection is defined as a physical-chemical process capable of eliminating most pathogenic microorganisms. As a chemical agent with greater applicability, there are disinfectants, which have three levels: high, medium and low, with 70% alcohol at medium level, not being effective against bacterial spores (ARAÚJO et al., 2019).

In addition to the range of disinfectant substances, hydrogen peroxide stands out as one of the most versatile oxidants, with great applicability and medical importance, being widely used in this field. Hydrogen peroxide is a potent oxidant and acts through catalysis, that is, decomposition of the H_2O_2 molecule, converting it into a hydroxyl radical (OH), such a reaction is capable of inferring stress to cells exposed to the compound resulting in in their death (MATTOS et al., 2002).

According to Tadei et al. (2021), the commercial hydrogen peroxide, Peroxide MSCD®, demonstrated effectiveness when applied to MDF and marble surfaces, where the percentage of reduction obtained was 100%, validating the results obtained in this study.

Corroborating the results of the present study, as well as the techniques used, Graziano and his collaborators, in 2013, demonstrated the disinfectant effectiveness of 70% alcohol by two techniques: direct application on surfaces (Control Group) and another in the previous cleaning of the surface (with water and detergent) for subsequent application of 70% alcohol (Comparative Control Group). With an average of 99.9999% reduction of microorganisms in a total of 84 plates for each experimental group, with significant growth only in 15 plates of the Comparative Control Group.

Escherichia *coli* comprises a group with a high diversity of Gram-negative bacteria, being a common member of the intestinal microflora of humans and animals. It has pathogenic strains such as Shiga toxigenic *E. coli* (STEC), an important food-borne pathotype. As a subset, there is enterohemorrhagic *E. coli* (EHEC), which causes hemolytic uremic syndrome (HUS) in addition to other serious human diseases (NEWELL; LA REGIONE, 2018).

Due to its present mutability, variety of strains and pathogenicity, it is important to study the control mechanisms and preventive methods for contamination. Once incident microbial resistance related to these microorganisms (VILA et al., 2016).

In vitro studies using *E. coli* strains to test the 70% alcohol disinfection method demonstrate the effectiveness of its action on microorganisms (DUTRA et al., 2022). According to the in vitro study, presented by Dutra and his collaborators in 2022, the applicability of the disinfectant (70% alcohol) is effective if applied to the strains on the material surfaces of the present study.

According to Librelotto and Ferrari (2016), melamine formaldehyde, popularly known as formica, is the material used for covering furniture in general, its purpose is to attribute aesthetics and protection to the wood used as a base. Its surface is usually uniform and without considerable porosity, not being favorable to the adhesion of microorganisms and suggesting a greater possibility of success in relation to cleaning and disinfection methods.

A study carried out by Centro Universitário Autónomo do Brasil, Curitiba - PR, induced contamination of formica and granite surfaces by microorganisms of clinical importance and analyzed the reduction of their microbial load after being subjected to disinfection with 70% alcohol. The research presented satisfactory results when comparing the analyzes of formica and granite surfaces, where alcohol applied to formica showed better performance (BERNARDI; COSTA, 2017).

According to Bernardi and Costa in 2017, granite is considered a material with imperfections, since the frequent use of chemical agents can cause abrasion and increase the porosity of the material, making them unsuitable for use in the long term, thus relate the results obtained in the control group with greater adhesion to the material after induced contamination.

The results found in the literature, referring to the cleaning and disinfection of surfaces, with 70% alcohol and hydrogen peroxide, together with the list of materials submitted to this process, are in agreement with the results obtained in this experiment, where it was possible to evaluate the effectiveness of these chemical agents used both in domestic environments and in public and health environments.

CONCLUSION

In accordance with the previously established objectives, it can be concluded that the cleaning and disinfection methods using neutral detergent and 70% alcohol and the disinfectant Peroxide MSCD® were equally effective and applicable, since both methods used presented 100% of percentage of reduction of the bacterial load, both on the formica surface and on the marble surface, although the latter has greater porosity.

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