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Evaluation of Microbiological Cloth and Sponge Disinfection Methods in a Hospital's Food and Nutrition Unit

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HIGHLIGHTS

- Cloths and sponges are common contaminating utensils in a Food and Nutrition Unit
- Disinfection methods such as boiling and hypochlorite are efficient
- Pathogenic microorganisms adhere to the surface of cleaning cloths and sponges

Abstract: Food safety, within the context of the hospital's Food and Nutrition Units (FNU), is one of the key factors in ensuring the quality of the service. Thus, the aim of this study was to analyze the microbiological contamination of sponges and multipurpose cloths used in a hospital's FNU, as well as the effectiveness of different disinfection methods. The materials were submitted to viable cell count for total and thermotolerant coliforms, coagulase-positive *Staphylococcus*, and mesophiles; in addition, efficiency tests of the disinfection methods carried out by the FNU were done by scanning electron microscopy, antimicrobial susceptibility testing, biofilm formation capacity, and virulence factors. The analysis results confirmed the contamination of these utensils, and the disinfection methods used were efficient. Electromicroscopic analysis identified high bacterial adhesion after use, and the isolates were resistant to the antimicrobial penicillin and classified as poor biofilm producers. Sponge isolates showed greater virulence than other materials. Therefore, good practices in food services must follow strict hygiene and disinfection protocols to ensure the safety of the food produced.

Keywords: Sanitation; Disinfection; Food safety; Foodborne Diseases.

INTRODUCTION

The World Health Organization (WHO) estimates that 600 million people – almost one in ten individuals in the world – fall ill after eating contaminated food, and 420,000 people die each year, mainly due to inadequate food handling, poor hygiene of the physical structure, equipment, and utensils [1]. The search for food safety is a priority in many countries around the world, based on deaths and economic losses related to food poisoning [2].

This global data reflects in Brazil, where in 2018 there were 503 outbreaks, and 6,803 people became ill due to Foodborne Diseases (FBD), with 9 people dying as a result of it [3]. Offering safe food in hospital environments is essential, since the objective of such institutions is the recovery of health and comprehensive care to the patients, encompassing a set of care that includes food and nutrition, and the success of the treatment is possibly affected by factors such as the safety of the food offered [2,4].

In Food and Nutrition Units (FNU), hygienic-sanitary control must follow a strict protocol of techniques and methods as established by the Good Manufacturing Practices (GMP). However, when these measures are not strictly followed, pathogenic microorganisms can contaminate utensils and types of equipments, and transmit diseases [4]. In FNU, it is common to use cloths and sponges to sanitize equipment that cannot come into direct contact with running water, in addition to its prolonged use. This fact is justified by the difficulty in the sanitation processes since the structure of some establishments does not provide water points in all sectors. The undesirable prolonged use of these materials can lead to the multiplication of microorganisms, causing health risks for patients [5].

Kitchen sponges not only act as reservoirs for microorganisms but also as spreaders over surfaces, which can lead to cross-contamination of people's hands and of the food, which is considered the main cause of FBD outbreaks. Thus, cleaning cloths and sponges gain prominence, since they can transfer microorganisms in concentrations above the tolerated limit to surfaces and utensils used in food preparations [6]. These utensils can retain leftover food and serve as a reservoir for disease-causing microorganisms. Bacteria can remain on the surface of a given material for days after direct contact, which can increase cross-contamination [5,7]. For this type of material (cleaning cloths and sponges), it is recommended daily disposal after use, or propper sanitization, according to the current legislation (State Ordinance No. 78) [8]; for this reason, in the case of FNU, it is very important the commitment to establishing Standardized Operating Procedures (SOP), in order to provide satisfactory hygienic-sanitary conditions.

Therefore, the aim of this study was to analyze the microbiological contamination of sponges and of multipurpose dishcloths used in the hospital's FNU, as well as the effectiveness of different methods of disinfection.

MATERIAL AND METHODS

Characterization of samples and collection procedures

Dishcloths (100% cotton), multipurpose disposable cloths (Perfex®), and cleaning sponges (polyurethane) were collected from a hospital's FNU located in the city of Pelotas-RS, Brazil. The study was previously approved by its administration and then a checklist, which was developed for this study based on the State Ordinance No. 78, was applied with modifications [8]. After being collected, the materials were transported to the Laboratory of Microbiology of the College of Nutrition – Federal University of Pelotas (UFPEL) under controlled temperature conditions in a sterile plastic bag, and it was immediately analyzed.

Microbial quantification

For microbial quantification analysis of the samples, two collections were performed at the hospital's FNU: the first collection was performed after cleaning and sanitization, according to the SOP of the FNU, and the second collection was done after the end of the period of use, and prior to its sanitization.

For the microbiological analysis of the disposable multipurpose cloths, dish towels, and cleaning sponges that were divided into employees and patients, they were collected after the end of the shift, prior to its sanitization, and divided into three equal parts (17.5x35 cm), being that each part was intended for microbiological analysis of standard plate count, coagulase-positive *Staphylococcus* and total and thermotolerant coliforms. The samples were also sent to two laboratory sanitization processes, sodium hypochlorite (200 ppm) and boiling (15 min in a microwave oven), which were carried out in accordance with State Ordinance no. 78 [8].

For standard plate count, coagulase-positive *Staphylococcus*, total and thermotolerant coliform analyses, each sample was added to 225 mL of peptone water (0.1%) and shaken in a stomacher for 1 min.

Afterward, 1 mL of the homogenate was added to 9 mL of 0.1% peptone water, and serial dilutions were done up to 10^{-5}. The experiment was carried out in duplicate and the results were expressed in Colony Forming Units (CFU)/cm^2 of cloth and/or sponge.

Enumeration of mesophilic aerobic microorganisms

For the isolation of aerobic mesophiles, 1 mL of each dilution (10^{-1} to 10^{-5}) was added to plates containing Plate Count Agar (PCA), using the pour-plate technique. After solidification, the plates were incubated inverted at 35 ± 2 °C for 48 h. The number of samples CFU per cm^2 was obtained by multiplying the number of colonies by the dilution factor, and its product was divided by the area corresponding to the surface of the cloth and sponge, according to the methodology described by Silva and coauthors [9].

Enumeration of Staphylococcus aureus

One hundred microliters (=0.1 mL) of the dilutions (10^{-1} to 10^{-5}) were inoculated onto the surface of Baird-Parker Agar (BP). After complete incorporation, the plates were incubated inverted at 35 ± 2 °C for 48 h. The number of CFU/cm^2 of the sample was obtained by multiplying the number of colonies by the dilution factor, and its product was divided by the area corresponding to the surface, according to the methodology described by Silva and coauthors [9]. Confirmation of the isolates as *Staphylococcus aureus* was performed by the coagulase test.

Enumeration of total and thermotolerant Coliforms

To count the total coliforms, the method proposed by the United State Food and Drug Administration (USFDA) [10] was used with some modifications. One milliliter (1 mL) of aliquots of the above-mentioned dilutions were placed on plates containing Violet Red Bile Lactose agar (VRBA – Merck – Germany) dissolved and cooled to 45 °C with subsequent homogenization. After solidification, a 5 mL overlay of the same medium was added. Plates were incubated at 37 °C for 48 h. After the incubation period, the colonies count and total coliforms characteristics were performed (purple-red with 0.5 mm or more in diameter, surrounded by a reddish halo of bile salt precipitation). To confirm total coliforms, three to five colonies were removed from each plate and inoculated in tubes containing Brilliant Green Broth (Bile 2%) (Biobrás – Brasil). The tubes remained for 24 h in a water bath at 37 °C. After this period, Durham tubes that showed the presence of gas and turbidity were considered positive. The total coliform count was determined by multiplying the number of typical colonies by the inverse of the dilution, with the result being expressed in Log CFU/cm^2 of cloth and sponge.

The counting of thermotolerant coliforms was performed by seeding five characteristic colonies (intense pink with or without the presence of bile salt precipitate), from VRBA agar plates, in tubes containing 10 mL of *E. coli* (EC) broth (Difco – USA). Durham tubes that showed turbidity and the presence of gas after 48 h in a water bath at 45 °C were considered positive. The count was determined by the number of typical colonies counted by the inverse of the inoculated dilution of the positive tubes, the result being expressed in Log CFU/cm^2 of cloth and sponge.

Efficiency of the disinfection methods used by the FNU

To evaluate the cloths disinfection methods, sanitization with sodium hypochlorite (200 ppm) and boiling (15 min in a microwave oven) in the laboratory were applied as parameters. The clothes were hand-washed for 10 min, by the same person, using commercial neutral detergent purchased from a local store. After washing, the clothes were rinsed in running drinking water, then, one of the pieces was boiled in water for 15 min (microwave) and the other piece was placed in a solution of sodium hypochlorite (200 ppm) for 15 min, followed by rinsing in running potable water [8].

After cleaning, the clothes were placed in 225 mL of 0.1% peptone water and vigorously shaken. A volume of 0.1 mL of the homogenate was inoculated directly into plates containing PCA, BP, and 1 mL of the homogenate in VRBA, and incubated in tubes containing VB and EC. The plates and/or tubes were incubated at the ideal temperature for each of the isolated microorganisms, for 48 h. The experiment was performed in duplicate and the results were expressed in Log CFU/cm^2 of cloth.

Scanning electron microscopy (SEM)

The SEM was performed to qualitatively assess bacterial adhesion in the samples. The fixation and dehydration method used was the critical point drying, with a protocol provided by the Electronic Microscopy Center of the Federal University of Rio Grande do Sul (UFRGS). The samples were first washed with 10 mL of 1x phosphate-buffered saline (PBS) solution and immersed in a fixative solution containing glutaraldehyde (25%). After a minimum period of seven days of fixation, the samples were washed three times with a washing solution containing 0.2 M phosphate buffer and distilled water at a ratio of 1:1 v/v. In each wash, the samples were immersed in wash solution for 30 min [11]. Then, dehydration was performed with increasing concentrations of acetone (30 to 100%). After reaching 100% acetone concentration, the samples were placed in a critical point dryer (Critical Point Dryer - Balzers® CPD030), to replace the acetone inside the cells with CO_2. Once the dehydration process was completed, the samples were metalized with gold (Sputter Coater - Balzers® SCD050) to allow visualization under the microscope. The images were obtained using SEM (JSM 5800 – JEOL®) and performed at the Laboratory of Nanotechnology of the Materials Engineering Course – Federal University of Pelotas (UFPel).

Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed according to the standard disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI) [12], and the tested isolates were classified as sensitive or resistant. Isolates from cleaning cloths and sponges were previously inoculated on plates containing Mueller-Hinton Agar (MH) and incubated at 35 °C for 24 h. After the incubation period, the microorganism colonies were resuspended in sterile saline solution (0.9%) and measured spectrophotometrically at 0.150 ± 0.02 (Optical Density - OD_{600nm}), which corresponds to the turbidity pattern of 0.5 on the Scale of McFarland. Then, with the aid of swabs, plates containing MH agar were inoculated with the standardized solution containing six antimicrobials commonly used in hospitals: clindamycin - 2 µg (CLI), chloramphenicol - 30 µg (CLO), vancomycin - 30 µg (VAN) erythromycin - 50 µg (ERI), meropenem - 20 µg (MER) and penicillin - 10 µg (PEN), all of the Laborclin brand. After incubation, the diameter of the inhibition zones of the different tested antimicrobials was measured. The experiment was carried out in triplicate in three independent experiments, and data were expressed in mm.

Assessment of the biofilm formation capacity of the isolates

For the analysis of the capacity of biofilm formation and virulence and resistance factors, eight isolates were selected in total, being two isolates of *E. coli* (sponge and multipurpose dishcloth for patients), two of *Salmonella* (patient dishcloth and kitchen dishwashing sponge), two of *Staphylococcus* and two of mesophilic isolates (sponge and multipurpose kitchen dishcloth).

The biofilm formation capacity of the cleaning cloths and sponge isolates was performed according to the methodology described by Stepanovic and coauthors [13]. Isolates were previously inoculated onto plates containing BHI and incubated at 35 °C for 24 h. Microtiter plates were aliquoted with 180 mL of sterile Brain Heart Infusion (BHI). After overnight incubation, the microorganism colonies were resuspended in saline solution and spectrophotometrically adjusted to 0.150 ± 0.02 (OD_{600nm}). Then, 20 µL of the solution was inoculated into each one. *Staphylococcus aureus* ATCC 25923 was used as a positive control and, as a negative control, BHI broth. Plates were covered and incubated at 35 °C for 24 h. After growth, cultures were aspirated with a multichannel pipette and the microplates were washed three times with saline solution. The microplate was inverted onto absorbent paper to dry, and the samples were further fixed with 150 mL of methanol (CH_3OH) for 20 min.

After this period, methanol was discarded and the plates were kept inverted overnight. Plates were stained with 150 µL crystal violet (5 g L^{-1}) for 15 min. Then, they were inverted and the excess was removed under running water. After a short drying period, 150 µL of ethanol (95% v/v) was added. The plates were kept at room temperature for 30 min and the absorbance was measured in a microplate reader (Anthos 2010, Type 4894 17550) at 450 nm. Based on the optical density (OD) produced by biofilms, strains were classified into the following categories: no biofilm production (0), weak (+), moderate (++), or strong biofilm producer (+++), as previously described by Stepanovic and coauthors [13]. The OD cutoff point (ODC) was defined as three standard deviations above the mean OD of the negative control. Strains were classified as follows: OD ≤ Odc = no biofilm production, Odc<OD ≤ (2 x ODC) = poor producer, (2 x ODC) <OD ≤ (4 x ODC) = moderate producer (4 x Odc) <OD = strong producer. The experiment was carried out in triplicate in three independent experiments.

Analysis of virulence and resistance factors

Gelatinase

The detection of gelatinase production was performed according to Marra and coauthors [14]. The microorganisms were inoculated into tubes containing 4 mL of BHI broth and 12% gelatin and incubated for 48 h at 37°C. After incubation, the tubes were placed in an ice bath for 30 min, without shaking. *Staphylococcus aureus* ATCC 25923 was used as a positive control. The result was interpreted as negative (solid medium) or positive (liquid medium). The experiment was carried out in duplicate in two independent experiments.

DNase Activity

DNase activity was tested as described by Bannerman [15], with adaptations, using DNase Agar (Oxoid, São Paulo, Brazil). The isolates were striated directly on the plate with DNase Agar and incubated for 24 h at 37 °C. After the incubation time, the plate was covered with 1N hydrochloric acid for 3 min. The formation of a clear halo around the colonies was considered indicative of a positive result. *Staphylococcus aureus* ATCC 25923 was used as a positive control. The experiment was performed in duplicate independent experiments.

Hemolytic Activity

The eight isolates were tested for hemolytic activity according to Foulquié-Moreno and coauthors [16], using Blood Agar (7% v/v horse blood) and incubation at 37 °C for 48 h. The interpretation of the results was carried out as follows: α-hemolysis - strains that produced green zones around the colonies; γ-hemolysis - had no effect on Blood Agar plates (will be considered non-hemolytic). Strains that have blood lysis zones around the colonies were classified as hemolytic (β-hemolysis). The experiment was carried out in duplicate independent experiments.

RESULTS

Microbial quantification and efficiency of disinfection methods by FNU

A checklist was applied to verify the hygienic-sanitary conditions of the hospital's FNU, through which it was observed that the Good Practices Manual (MBP) and SOP are executed at the FNU under the supervision of the nutritionist responsible for production. In addition, it was reported that the sanitation and disinfection procedures were carried out in accordance with the current legislation [8].

Cleaning cloths

According to State Ordinance no. 78 [8], when cleaning cloths are used on surfaces that come into contact with food, they must be replaced every 2 h, not exceeding 3 h, and may only be used again after cleaning. Disposable (multipurpose) cleaning cloths, on the other hand, follow the same rules, with the exception that they cannot be reused. For this reason, in the present study, the multipurpose disposable cloths (Perfex®) were not analyzed for their cleaning efficiency.

According to the results obtained, it can be observed that in the samples of dishcloths sanitized by the FNU itself, total and thermotolerant coliform counts were found in the sample from the patients' dishes (Table 1). Likewise, when analyzing the multipurpose disposable dishcloths and cloths collected after use in the shift, the presence of total and thermotolerant coliforms was observed (Table 1).

Table 1. Counting total and thermotolerant coliforms in disposable and multipurpose dishcloths used in a Hospital's Food and Nutrition Units (FNU) in the city of Pelotas-RS. Data were expressed as mean Log CFU/cm2 ± standard deviation.

Sample	Cloths sanitized and sanitized by FNU		Cloths after use in work shift		Boiled cloths		Sanitized cloths in accordance with legislation	
	TC**	FC	TC	FC	TC	FC	TC	FC
	----- CFU/cm^2 -----							
ET*	0.00±0.0	0.00±0.0	5.56x10^2±0.0	5.73X10^2±0.00	0.0±0.0	0.0±0.0	00.0±0.0	0.0±0.0
PD	3.78x10^2±0.05	5.14x10^2±0.00	5.97x10^2±0.0	5.79X10^2±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PMDF	NC****	NC	5.98x10^2±0.0	NC	NA****	NA	NA	NA
PMDP	NC	NC	5.98x10^2±0.0	NC	NA	NA	NA	NA

*ET: employee dish towel; PP: patient dishcloth; EDMC: Employee Disposable Multi-Purpose Cloth and PDMC: Patient Disposable Multi-Purpose Cloth
**TC: Total Coliforms; FC: Thermotolerant Coliforms
***NC: not collected as it is a disposable item in FNU
****NA: not analyzed

The cleaning and sanitization of the dishcloths were also carried out by the current legislation [8]: cleaning for 10 min and after immersion in a chlorinated solution for 15 min (200 ppm) or boil in a microwave oven (15 min). Through the results obtained, it was observed that there was no growth of any of the microorganisms analyzed (Table 1).

Table 2 presents the results from the materials sanitized by the FNU itself. Through the obtained data, it can be observed that there was no presence of mesophiles and coagulase-positive S*taphylococcus*. However, after use in the work shift, it was observed mesophiles and coagulase-positive S*taphylococcus* in both analyzed cloths (Table 2).

When analyzed in this study, no count of any of the evaluated microorganisms was observed in the sanitized wipes, in accordance with the current legislation [8].

Table 2. Coagulase-positive *Staphylococcus* and mesophilic count in disposable and multipurpose dishcloths used in a hospital's Food and Nutrition Units (FNU) in the city of Pelotas-RS. Data were expressed as mean Log CFU/cm^2 ± standard deviation.

Sample	**Sanitized cloths by FNU**				**Cloths after use in work shift**			
	ET*	PD	EDMC	PDMC	ET	PD	EDMC	PDMC
	---- CFU/cm^2 ----							
Mesophiles	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	4.02x10^1±0.01	4.33x10^1±0.23	6.33x10^3±0.04	5.42x10^2±0.07
Coagulase-positive *Staphylococcus*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.70x10^1±0.00	2.70x10^1±0.00	3.40x10^1±0.28	3.92x10^1±0.25

**ET: employee dish towel; PP: patient dishcloth; EDMC: Employee Disposable Multi-Purpose Cloth and PDMC: Patient Disposable Multi-Purpose Cloth

Sponges

After the analyses, total coliform count in sponges was observed after cleaning and sanitization by the FNU itself, with no detection of thermotolerant coliforms (Table 3).

However, when analyzing both sponges (sanitization of dishes from patients and employees) after use during the shift, a significant number of total coliforms was observed for patients and employees (5.98x10^2 and 5.98x10^2 Log CFU/cm^2, respectively), with the same being seen for thermotolerants coliforms (5.67x10^2 and 5.73x10^2 Log CFU/cm^2, respectively), as shown in Table 3.

According to the data described in Table 4, the presence of mesophilic microorganisms and coagulase-positive *Staphylococcus* were not observed when the sponges sanitized by the FNU were analyzed. In contrast, after use during the shift, the presence of mesophilic aerobic microorganisms was observed both in

sponges from the cleaning of dishes used by patients and employees. When analyzing the presence of coagulase-positive *Staphylococcus*, a low cell count ($4.10x10^1$) was observed in both sponges.

Table 3. Total coliform (TC) and thermotolerant coliform (FC) counts in cleaning sponges used in a hospital's Food and Nutrition Units (FNU) in the city of Pelotas-RS. Data were expressed as mean Log CFU/cm² ± standard deviation.

Sample	Sponges sanitized by FNU		Sponges after use in work shift	
	TC**	FC	TC	FC
	----- CFU/cm² -----			
SP*	$3.85x10^2$±0.00	0.00±0.0	$5.98x10^2$±0.00	$5.67X10^2$±0.00
ES	$3.81x10^2$±0,05	0.00±0.0	5.98 x 10^2±0.00	$5.73X10^2$±0.00

*SP: cleaning sponge for patient dishes; ES: employee dishwashing sponge
**TC: Total Coliforms; FC: Thermotolerant Coliforms

Table 4. Counting of mesophiles and *Staphylococcus aureus* in cleaning sponges used in a hospital's Food and Nutrition Units (FNU) in the city of Pelotas-RS. Data were expressed as mean Log CFU/cm² ± standard deviation.

Sample	Sponges sanitized by FNU		Sponges after use on the work shift	
	SP*	ES	SP	ES
			----- CFU/cm² -----	
Mesophiles	0.00±0.0	0.00±0.0	$6.10x10^3$±0.00	$6.40X10^3$±0.06
Coagulase-positive *Staphylococcus*	0.00±0.0	0.00±0.0	$4.10x10^1$±0.00	$4.10X10^1$±0.16

*SP: cleaning sponge for patient dishes; ES: employee dishwashing sponge

Scanning Electron Microscopy (SEM)

According to the results obtained through the electromicroscopic analysis, high bacterial adhesion was verified after the use of cleaning materials (dishcloths and sponges) during one shift at the FNU. The Figures show images of materials used as controls, which were from the pantry dishes, used by the patients and sanitized, represented by the letter A (Figures 1, 2, and 3).

Figure 1 shows the images referring to the analyzes carried out on samples of cleaning sponges (polyurethane). As analyzed in Figure 1A (control), microorganism adhesion to the polyurethane surface was not observed. However, in Figures 1B, 1C, and 1D, from the cleaning of kitchenware in the pantry, and 1E, 1F, and 1G, from the cleaning of the patients' dishes after a shift, the adhesion of microorganisms was observed, with characteristics of biofilm formation.

Figure 2 presents images referring to the analyzes carried out on samples of dish towels (100% cotton). As shown in Figure 2A (control), adhesion and biofilm formation by microorganisms on the surface of the cloths was not observed. However, in Figures 2B, 2C, and 2D, from the cleaning of kitchenware in the pantry, and 2E, 2F, and 2G, from the cleaning of the patients' dishes after the shift, the adhesion of microorganisms was observed.

Similar results were found in Figure 3, which shows the images referring to the analyzes performed on samples of multipurpose disposable cloths (Perfex®). As shown in Figure 3A (control), microbial adhesion was not observed on the surface of the material analyzed, which is mostly cotton. However, in Figures 3B, 3C, and 3D, from the cleaning of FNU dishes, adhesion of microorganisms was observed, with characteristics of biofilm formation.

In samples from dishcloths, disposable multipurpose cloths (Perfex®), and sponges, from the cleaning of patient dishes and pantry, there was a thick biofilm layer covering the surface of the material. At higher magnifications, it was observed that the biofilm is composed of numerous bacteria, probably exuded by these microorganisms. Under a 5,000-fold magnification, the bacteria presented different shapes and arrangements, bacillary and cocci, arranged in chains.

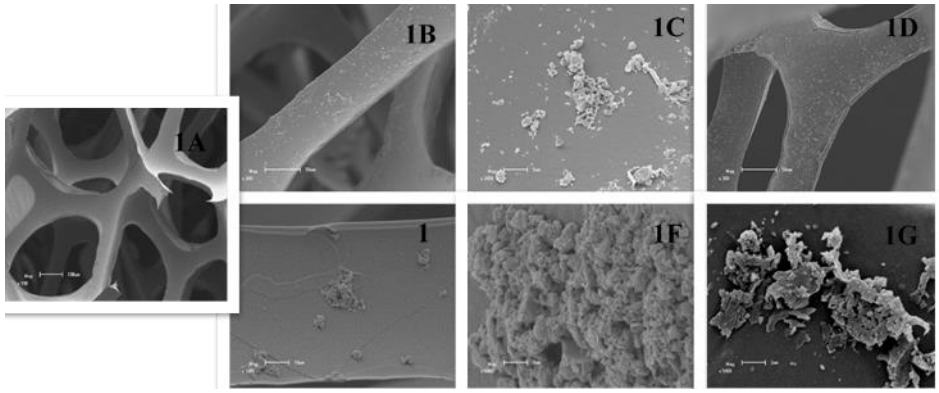

Figure 1. Scanning Electron Microscopy analysis of biological sponge samples used in food production in the hospital' area. (A): control sponge; (B, C, and D): sponge from the pantry dishes; (E, F, and G): sponge from the patients' dishes.

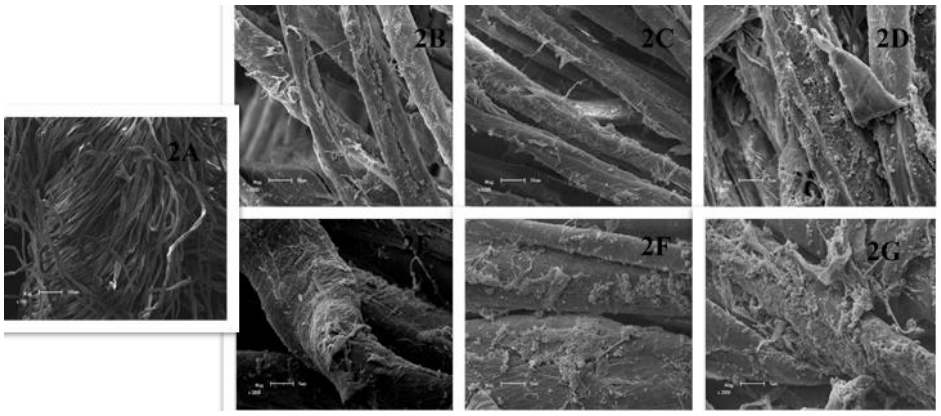

Figure 2. Scanning Electron Microscopy analysis of biological samples of cloth used in food production in the hospital area. (A): control cloth; (B, C, and D): cloth from the drying of crockery in the pantry; (E, F, and G): cloth from the drying of the patients' dishes.

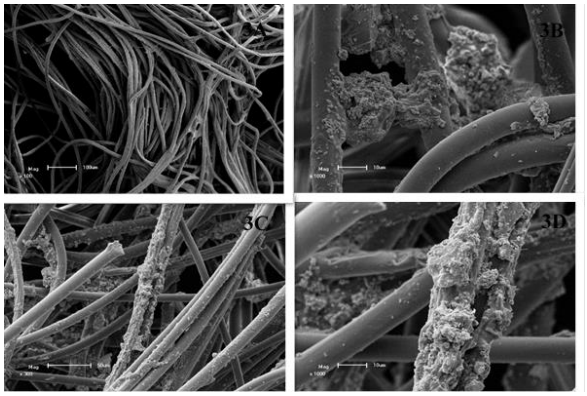

Figure 3. Scanning Electron Microscopy analysis in biological samples of disposable multipurpose cloth used in food production in the hospital area. (A): Disposable multipurpose cloth control; (B, C, and D): disposable multipurpose cloth used in Food and Nutrition Units (FNU).

Antimicrobial susceptibility testing

A total of 30 isolates were submitted to antimicrobial susceptibility analysis. According to the results obtained and to the classification of CLSI [12], it was possible to observe that all isolates were resistant to the antimicrobial penicillin; the same can be seen when using the antimicrobial clindamycin, except for the isolate referring to the kitchen dishcloth, which was classified as an intermediate. It is noteworthy that the mesophilic microorganism isolated from the disposable multipurpose kitchenware cloth showed resistance to all antimicrobials tested, except for chloramphenicol, which was classified as an intermediate. Likewise, the *Staphylococcus* dishcloth isolate from the patients was resistant to all the antimicrobials tested, with the exception of the antimicrobial meropenem, which was sensitive. Resistance to vancomycin, erythromycin, and clindamycin was also observed in the mesophilic dishcloth isolated from employees' dishes, and the kitchen dish sponge' microorganisms were resistant to vancomycin, chloramphenicol, and clindamycin.

Assessment of biofilm formation capacity

For this analysis and for those related to virulence factors, two isolates of *E. coli* were selected (dish sponge and multipurpose disposable tablecloth for patients), *Salmonella* (dishcloth for patients' dishes and sponge for employees' dishes), *Staphylococcus* (dishwasher sponge and multipurpose disposable kitchenware cloth) and mesophiles (dishwasher sponge and multipurpose disposable kitchenware cloth). Based on the results obtained, only one of the mesophile isolates (disposable multipurpose kitchenware cloth) was classified as a non-biofilm producer, and the others were classified as poor biofilm producers (Table 5).

Table 5. Classification of isolates according to biofilm production.

ISOLATE	SAMPLE	CLASSIFICATION
Escherichia coli	SP*	Weak biofilm producer
	MTP	Weak biofilm producer
Coagulase-positive *Staphylococcus*	ES	Weak biofilm producer
	MTP	Weak biofilm producer
Salmonella spp.	DPD	Weak biofilm producer
	ES	Weak biofilm producer
Mesophiles	ES	Weak biofilm producer
	MCE	Non-biofilm producer

*SP: sponge from the patients' dishes; MTP: multipurpose disposable tableware for patients; ES: employee dish sponge; DPD: dishcloth from the patients' dishes; MCE: disposable multipurpose cloth for employee dishes

Phenotypic analysis

Gelatinase

The isolates were submitted to the test for detection of gelatinase production, according to the previously established methodology. Only four isolates were positive (staff dish sponge – *Salmonella*; patient dish sponge - *E. coli*; employee dish sponge and dishcloth - *Staphylococcus*). It is noteworthy that all isolates classified as gelatinase positive were collected after the shift use period.

DNase activity

DNase activity was tested according to the methodology previously described. After an incubation period, by covering the plates with hydrochloric acid, it was possible to identify the formation of halos around the colonies of five of the analyzed samples (kitchenware sponge and patients' dishcloth - *Salmonella*; sponge and multipurpose cloth tableware from patients - *E. coli*; kitchen dish sponge - *Staphylococcus*).

E. coli isolates from both materials, sponge and multipurpose cloth, from the patients' dishes, were classified as positive; while about *Staphylococcus* isolates, only the sponge from the employees' crockery showed a positive result. When analyzing the *Salmonella* isolates, the sponge from the employees' dishes and the patients' dishcloth was positive in the DNase test. It should be noted that all samples with a positive result were collected after the shift use period.

Hemolytic Activity

The eight selected isolates were tested for hemolytic activity after an incubation period of 48 h, as provided for in the methodology used. As a result, it can be inferred that five samples were classified as β-hemolysis (patient dishcloth - *Salmonella*; sponge and multipurpose disposable dishcloth from patients - *E. coli*; sponge and multipurpose disposable dishcloth from the patient kitchen - *Staphylococcus*), being these coming from the patients' dishcloth (*Salmonella* isolated), sponge and multipurpose cloth, used on patients' dishes, for *E. coli,* and sponge and multipurpose cloth, used for cleaning the employees' dishes, for *Staphylococcus*. The materials were collected after the period of use in the shift. Thus, the isolates mentioned above-presented lysis with rupture of the plasma membrane. The isolates analyzed for mesophilic

microorganisms and *Salmonella* isolates from the sponge used in cleaning the patients' dishes were classified as y-hemolysis, in other words, absence of hemolysis, since no lysis zones were identified in the agar culture medium blood.

DISCUSSION

Cleaning utensils, such as sponges and multipurpose cloths, are potential sources of cross-contamination and potential spreading agents of pathogenic microorganisms [17]. It is common to use cloths and sponges in the procedures and techniques used to clean benches, utensils, and equipment in most food services. The materials used in the composition of cloths and sponges facilitate the accumulation of food residues, which favors contamination by microorganisms [18]. For this reason, it is recommended to properly sanitize them in accordance with state legislation [8].

With regard to microbiological standards for surfaces and equipment, there is no specific Brazilian legislation, for this reason, the values found were compared with the current legislation, RDC No. 12 [19], which presents the allowed reference standards for ready-to-eat foods, where the coliforms are reference value is $5x10^2$. In the present study, values of $1x10^2$ were obtained in all analyzed samples, proving to be adequate, according to the current legislation. Many studies demonstrate high counts of microorganisms in materials similar to those analyzed in this study [5,17,18]. Several factors linked to the incorrect handling of cloths and sponges by employees during food preparation, combined with the absence or inefficiency of adequate sanitation procedures, and conservation of materials under humidity at room temperature, can be an elucidation for the counting of microorganisms found in this paper [20].

In the present study, a count of microorganisms was found in dishcloths sanitized by the FNU, demonstrating the inefficiency in the process of cleaning and sanitizing the cloths. The presence of coliforms in the analyzed cloth samples deserves emphasis, mainly due to the fact that the contamination was found in the dishcloth samples from the patients' dishes (PD), sanitized by the FNU. This fact must be taken seriously, since the presence of microorganisms is not expected in properly sanitized cleaning cloths and, as these samples come from the hospital environment, they can bring numerous risks to the health of hospitalized patients, especially the immunocompromised [21]. In addition, it was observed the presence of total coliforms in sponges after cleaning by the FNU itself, but no count for thermotolerant coliforms. When analyzing both sponges (sanitization of dishes from patients and employees) after the shift, the presence of total and thermotolerant coliforms was observed.

A study carried out in a Vietnamese hospital found high counts of viable bacteria, mainly coliforms, in kitchen utensils, especially cutting boards and knives, which showed viable bacteria above 100 CFU/10 cm^2, and positive for coliforms / *E. coli*. In addition to these, the tables destined for preparations were also a source of *E. coli* [2]. Total and thermotolerant or fecal coliforms are indicative of poor hands and/or utensils hygiene [22], posing a risk to employees' and patients' health in the hospital environment [23].

When analyzing the presence of mesophilic microorganisms, the samples of dishcloths and sponges sanitized by the FNU did not show microbial count, thus, indicating efficiency in the cleaning and sanitization performed. In contrast, microorganism count was observed in the multipurpose disposable cloths and sponges analyzed after use in the shift. For these materials, there are no microbiological standard reference values. Silva [22] presents a reference value classified as satisfactory for counting mesophiles on benches and surfaces of 50 CFU/cm^2 ($5x10^1$ CFU/cm^2). According to the American Public Health Association (APHA) [24], equipment is considered clean when it has a standard plate count ≤ 2 CFU/cm^2 ($0.2x10^1$ CFU/cm^2).

The dishcloths used for the staff and patients' dishes had values between $4.02x10^1$ and $4.33x10^1$ CFU/cm^2 of mesophiles after the period of use in the shift. By comparing these values with the references cited above, it can be seen that although they are acceptable according to Silva [23], for APHA [24] these findings are above the recommended values. On the other hand, when analyzing the multipurpose cloths and sponges, after use in the shift, all samples from the dishes of patients and employees had microorganisms count considered above the value allowed by both references. The use of indicator microorganisms, such as mesophilic bacteria and Enterobacteriaceae, such as *E. coli* and total coliforms, are important tools for quality systems, as they indicate, in their presence, failures in the cleaning process that can easily be carried out effectively with detergent, running water and sanitization with 70% alcohol [21]. A study carried out on contact surfaces in a Moroccan hospital found between 3.94 log CFU/cm^2 and 1.56 log CFU/cm^2 for mesophilic aerobic bacteria, with the highest average being 3.94 log CFU/cm^2 for raw meat countertops [26].

In the present study, values between $2.70x10^1$ to $3.92x10^1$ CFU/cm^2 were also found for coagulase-positive *Staphylococcus* in disposable dishcloths and multipurpose disposables, respectively. As for sponges, after use in the shift, $4.10x10^1$ CFU/cm^2 were found, with all samples following RDC No. 12, which

determines the allowed limit for ready-to-eat foods of up to 10^3 for coagulase-positive *Staphylococcus*/g [19]. As expected, sponges sanitized by FNU did not have coagulase-positive *Staphylococcus*.

The spread of infectious microorganisms in the hospital environment is common, especially for *Staphylococcus* that are present in the handlers' skin and nasopharynx [25]. In a study done by Guimarães, Ferreira and Soares [26], which evaluated the microbiological conditions of utensils used in the food production process in a commercial restaurant, the results showed 80% contamination of coagulase-positive *Staphylococcus* in the evaluated utensils, demonstrating the result of poor sanitation. As in the study by Touimi and coauthors [27], who observed *S. aureus* and coagulase-negative staphylococci with a frequency of 50 and 83.31% for the microorganisms, respectively, on vegetable cutting boards in a hospital food unit in Morocco.

Bacterial adhesion can be observed on the materials that make up both dishcloths (cotton) and disposable multipurpose cloths (Perfex®), and sponges (polyurethane). Under certain conditions, these attached microorganisms interact with surfaces and initiate the cell multiplication process [28]. It is observed from the microscopy images that the surface of the material (polyurethane) is porous, with cracks typical of its structure, which are suitable for microorganisms' shelter.

According to Berber, Bueno and Bonaldo [29], the polyurethane that constitutes the sponge has small cracks in its structure, similarly, the cotton cloth has cracks and pores. These characteristics facilitate the residual deposition of food, and serve as a facilitator for the agglomeration of microorganisms in their structures, together with environmental factors, such as adequate humidity and temperature, making them conducive to the development of microorganisms, with possible multiplication and biofilm formation [30]. Biofilm easily adheres to different types of materials and surfaces, in addition, when subjected to heat, it can form a deposit or a very adherent crust that protects new microorganisms and hinders the cleaning process [31].

The findings of the present study point to the ability of microorganisms to form biofilms, even though most of them have been classified as poor formers, taking into account the fact that the materials analyzed were collected from a hospital's FNU. Therefore, it is important to take into consideration the appropriate type of material to be used in this environment, as well as the most suitable chemical products for the correct sanitation [32]. The scanning electromicrographs obtained in this study showed the diversity of microorganisms found on the surface of the samples in the different materials analyzed, and also the formation of biofilms that accumulated on the extracellular matrix in multiple layers, that culminated in a bacterial community that could also spread over the surface of the material through the detachment of some microorganisms from its colony, and transport to an adjacent area [30,33]. The data presented regarding the count of microorganisms in the analyzed materials corroborate with those found in the SEM analyses, in which high microbial adhesion was observed in dishcloths, multipurpose disposable cloths, and cleaning sponges. It is also noteworthy that when the cleaning materials were subjected to the sanitization process by sodium hypochlorite (200 ppm) and/or by boiling, the microorganisms isolates showed susceptibility to antimicrobial, demonstrating a broad spectrum, being effective against Gram-negative and Gram-positive bacteria. Similar results were found in a study where 76% of isolates from the Enterobacteriaceae family were sensitive to the antimicrobial drug chloramphenicol. It usually has a bacteriostatic action, but it can also act as a bactericide in high concentrations or against some microorganisms with high sensitivity [34].

Several studies have analyzed antimicrobial resistance, as it imposes great costs on society and challenges the control of infectious diseases, jeopardizing progress in health outcomes through increased morbidity and mortality [35,36,37]. To ensure safety, it is important to determine the potential for virulence [38]. The results referring to virulence analyses revealed that only four isolates (*Salmonella* kitchen dish sponge, *E. coli* patients' dish sponge, and kitchen dishcloth and sponge for *Staphylococcus*) tested positive for gelatinase and, among them, three were from sponges collected after the shift use period. Bioactive peptides such as casein, gelatin, collagen, hemoglobin, and others are hydrolyzed by gelatinase [39]. A study analyzed eight samples containing isolates from Brazilian foods and found no gelatinase enzyme activity in any of them [40]. The same occurred in a study where 17 samples from fermented products containing *Lactobacillus* spp. with probiotic potential were negative for gelatinase activity [41].

Infection of the host by the DNase enzyme causes degradation of the nucleic acid (DNA), and this virulence factor is little observed when using samples from food and surfaces, being more common in clinical samples [42]. Almeida Júnior and coauthors [43] isolated samples from food and observed that none of the isolates had DNase enzyme activity. These results corroborate most of the isolates in the present study, differing only in five samples (patients' sponge and dishcloth for *Salmonella*, sponge and multipurpose patient dishcloth for *E. coli* and kitchenware sponge *Staphylococcus*) which showed DNA degradation activity. The detection of deoxyribonuclease (DNase) activity, an exoprotein that catalyzes the hydrolysis of

phosphodiester bonds in the DNA structure, it's important to distinguish pathogenic from non-pathogenic microorganisms in the microbiota, being as important as coagulase [44].

Regarding hemolytic activity, five of the samples were classified as β-hemolysis (*Salmonella* patient dishcloth, sponge, and multipurpose patient dishcloth for *E. coli* and sponge and multipurpose kitchen dishcloth for *E. coli* and S*taphylococcus*), collected after the shift period of use. Hemolysins are different according to their lytic action on red blood cells, and the beta and alpha types are the most important in the pathogenesis of infections [44].

CONCLUSION

Therefore, it is of great importance the implementation of effective utensils cleaning methods in hospital environments for the production of safe food for patients and staff. The findings of this study demonstrate that kitchen utensils are vehicles for the propagation of pathogenic microorganisms, when not properly sanitized or discarded, so, following the standard protocols for disinfection and disposal of cloths and sponges, as described in the legislation, becomes an essential procedure. It should be noted that the use of different cleaning techniques, such as microwaving and boiling, are effective methods of elimination of the evaluated microorganisms, being alternative procedures options to be implemented as good practices measures in food and nutrition services.

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