Original Article

Survival and transfer of microorganisms from kitchen sponges to surfaces of stainless steel and polyethylene

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Abstract

Introduction: Contaminated sponges might lead to cross-contamination in kitchens since they can transfer microorganisms to surfaces where microorganisms can survive for hours or days and contaminate food. The main objective of this study was to evaluate the transfer and the survival of bacteria from kitchen sponges to surfaces of AISI 316 stainless steel and polyethylene.

Methodology: Twenty-four sponges were collected from industrial kitchens in the state of Rio Grande do Sul and aseptically split into two equal parts. One part was subjected to enumeration of heterotrophic microorganisms, faecal coliforms, coagulase-positive *Staphylococcus* and search detection of *Salmonella enterica.* The other part was rubbed on surfaces of AISI 316 stainless steel (12 sponges) or polyethylene (12 sponges). The transfer and survival of microorganisms was quantified by swab collection and pour-plate method using plate count agar.

Results: All sponges were contaminated by heterotrophic microorganisms (average of 6.8 log CFU/sponge) and 83.3% with faecal coliforms (average of 5 log CFU/sponge). None of the sponges were contaminated by *S. enterica* and/or coagulase-positive *Staphylococcus*. The average transfer of microorganisms varied between 3.3 and 5.5 log CFU/cm^2 for stainless steel and from 3.5 to 5.6 log CFU/cm^2 for polyethylene. Although the survival rate decreased over time, more than 1 log CFU/cm^2 of heterotrophic microorganisms survived after 24 hours on both surfaces.

Conclusions: The sponges used in food services were significantly contaminated and could transfer large amounts of microorganisms to surfaces of AISI 316 stainless steel and polyethylene.

Key words: kitchen sponges; microbiological contamination; survival on stainless steel and polyethylene

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Introduction

Cross-contamination is one of the major factors responsible for food-borne disease outbreaks [1] and in various stages of food preparation [2].

According to Mattick *et al*. [3], cleaning and disinfecting surfaces and utensils may prevent cross-contamination in kitchens because they promote the physical removal of food residues and the chemical inactivation of microorganisms. According to these researchers, cross-contamination is often associated with contamination of dishes or surfaces with washing water, contaminated sponges, or contaminated items placed in contact with them.

Several studies have already revealed that cloths and sponges can be important disseminators of pathogens and can transfer bacteria to surfaces and utensils, leading to cross-contamination of food [4,5,3].

The common materials used in surfaces that come into contact with foods in industries and kitchens are stainless steel and polyethylene. However, the surfaces of these materials are irregular when observed microscopically, thus facilitating the deposition of organic matter and food residues, and contributing to microbial attachment and survival [6].

According to Kusumaningrum *et al*. [5], bacteria that were transferred from sponges to surfaces could survive for hours on stainless steel surfaces thus increasing the risk of cross-contamination. Therefore, due to the frequent use of sponges in kitchen cleaning processes, the main objective of this study was to assess the transfer and survival of microorganisms from sponges used in food services to surfaces of AISI 316 stainless steel and polyethylene.

Methodology

Sponge sampling

A total of 24 synthetic polyurethane sponges were collected from two industrial kitchens (*i.e.*, 12 sponges were collected from each kitchen) located in Rio Grande do Sul (RS) State, southern Brazil. The industrial kitchens were situated inside metallurgic industries and each one served approximately 1,200 daily meals, prepared by teams of approximately 12 food handlers. Both kitchens had implemented Good Manufacturing Practices (GMP), had a GMP manual describing all sanitizing procedures, and had Sanitarian Standard Operation Procedures (SSOP) implemented. All food handlers were trained in GMP procedures. Surfaces of equipment and utensils were routinely washed with neutral detergent and potable water and disinfected with 70% ethylic alcohol. The kitchens had a professional nutritionist controlling the food preparation and sanitizing procedures. The water quality of the kitchens was monthly controlled by microbiological analysis according to Brazilian regulations [7]. The water was absent of faecal coliforms in 100 ml tested and had less than 500 cfu/ml of heterotrophic microorganisms.

The sampled sponges were in use for at least one day in the kitchens and they were collected after previous contact with and verbal consent from the technicians working at the establishments. The sponges were collected using aseptic latex gloves, placed inside sterile plastic bags, and transported at temperatures < 5ºC to the Microbiological Research and Diagnostic Laboratory of the University of Western Santa Catarina Sao Miguel do Oeste, SC, Brazil, to be analysed.

Microbiological analyses

In the laboratory, the sponges were aseptically split into two equal parts. Next 100 ml of 0.1% peptone water (AES Chemunex, Bruz Cedex, France) with 0.1 ml of 10% sodium thiosulphate was added to one part and then mixed in a Stomacher (ITR, Esteio, RS, Brazil) for 60 seconds. Later, this part of the sponge was subjected to quantification of heterotrophic microorganisms (HM), faecal coliforms (CF), coagulase-positive *Staphylococcus* (SA), and investigation of *S. enterica* (SAM). The methods used for analyses were those described by the Regulation number 62 of 26 August 2003, published by the Brazilian Ministry of Agriculture and Food Supply (MAPA) [8] that follows methods recommended by the Compendium of Methods for the Microbiological Examination of Foods - APHA [9].

The HM counts were performed by the pour-plate method using plate count agar (PCA) (Merck, Darmstadt, Germany), while the quantification of CF was carried out using the overlay technique with violet red bile agar (VRBA) (Merck). Characteristic colonies of faecal coliforms were confirmed in EC broth (Merck). The SA counts were performed using the spread-plate technique on Baird-Parker agar (Difco, Basingstoke, United Kingdom). The characteristic colonies (black with halos) were subjected to Gram staining and biochemical tests for confirmation (catalase, coagulase and thermonuclease) [8].

All counts were performed in triplicate and on plates containing 25 to 250 colonies. The results were expressed in log CFU/sponge.

For the *S. enterica* investigation, 25 ml of 0.1% peptone water were used in which the sponge was hydrated after its sampling. This aliquot was added to 225 ml of 1% buffered peptone water (Merck) and incubated at 36°C for 20 hours. After that, 1 ml of this sample was inoculated in tubes containing selenite-cystine broth (Merck) and tetrathionate broth (Merck). These were incubated at 41°C ± 0.5°C for 24 hours. The samples were then striated in brilliant green agar and xylose lysine deoxycholate agar (Merck) and incubated at 36°C ± 1°C for 18 to 24 hours. Characteristic colonies were confirmed by biochemical and serological tests according to MAPA [8] and APHA [9]. The results were expressed as presence or absence of *Salmonella*.

Transfer and survival of heterotrophic microorganisms on surfaces of AISI 316 stainless steel and polyethylene

The other part of the 24 sponges was rubbed separately on surfaces of AISI 316 stainless steel or polyethylene. A total of 12 sponges were used for each surface.

Each sponge was hydrated with 30 ml of 0.1% peptone water (AES, Bruz Cedex, France) containing 30 µl of 10% sodium thiosulphate and then rubbed five times on the AISI 316 stainless steel surface (10 x 10cm) and on the polyethylene surface. Afterward, the contaminated surfaces were sampled using a sterile swab previously immersed in 10 ml of 0.1% peptone water, rubbed on the surface (10x10cm) of the materials in three different directions, and subsequently put into test tubes containing 0.1% peptone water. The swab was agitated for 30 seconds in a magnetic stirrer and the bacterial suspension was subjected to decimal dilutions in 0.1% peptone water. Next the total count of HM was performed by seeding

using the pour-plate technique in plate count agar and incubated at 36°C ± 1°C for 48 hours. During the evaluation period, the surfaces were kept at room temperature of about 20°C using air conditioning. Counts were performed at 0, 1, 2, 3, 4 and 24 hours after contamination of the surfaces with sponges.

All counts were performed in triplicate on plates containing 25 to 250 colonies. The results were expressed in log CFU/cm^2.

Statistical analysis

The differences of the microbial survival on surfaces were evaluated using the Student T-test. The T-Student analysis was used to compare the average counts of two different groups to verify statistically significant differences between them. Data analyses were performed on the SPSS (Statistical Package for Social Sciences, IBM, Chicago, USA) release 12.1. A p-value of < 0.05 was considered statistically significant.

Results

Microbiological analyses

The 24 analyzed sponges showed HM scores ranging from 4.1 to 10 log CFU/sponge, with an average of 6.8 log CFU/sponge. From these sponges, 83.3% had CF in quantities ranging from 3 to 9.7 log CFU/sponge, with an average of 5 log CFU/sponge. None of the evaluated sponges had SA or SAM.

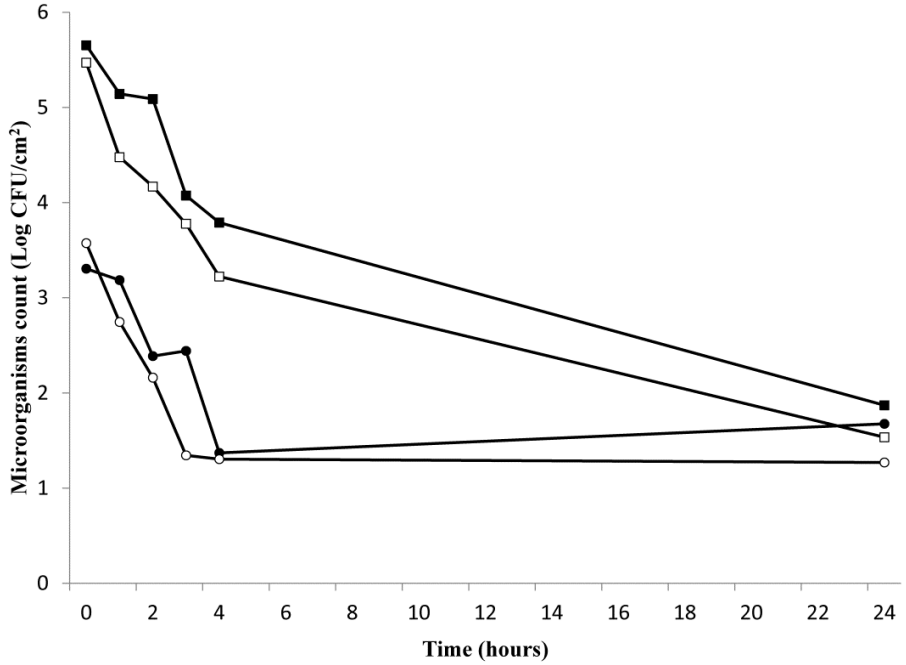
Transfer and survival of heterotrophic microorganisms on surfaces of AISI 316 stainless steel and polyethylene

Due to the large variation of contamination by HM, the sponges were divided into two groups for this experiment. The first group (group 1) was composed of sponges contaminated with 7 to 10 log CFU/sponge and the second group (group 2) was composed of sponges contaminated with 4 to 6.9 log CFU / sponge.

The transfer of microorganisms (time zero in Figure 1) was higher by sponges from group 1 than from group 2. The group 1 sponges transferred an average of 5.5 log CFU/cm^2 of the initial contamination of HM to AISI 316 stainless steel surfaces and 5.6 log CFU/cm^2 to polyethylene surfaces, whereas group 2 sponges transferred an average of 3.3 log CFU/cm^2 and 3.5 log CFU/cm^2 of de HM to the surfaces of AISI 316 stainless steel and polyethylene, respectively (Figure 1).

One hour later, there was a reduction of approximately 1 log CFU/cm^2 of HM from group 1 sponges present on the surfaces of AISI 316 stainless

Figure 1. Survival of HM transferred from cleaning sponges to surfaces of AISI 316 stainless steel and polyethylene in different levels of contamination



Group one: sponges with 7 to 10 log CFU/sponge; **Group two**: sponges contaminated with 4 to 6.9 log CFU/sponge

■ Group one in polyethylene
□ Group one in AISI 316 stainless steel
● Group two in AISI 316 stainless steel
○ Group two in polyethylene

steel and of 0.51 log CFU/cm^2 on polyethylene surfaces. For transfer of HM from group 2 sponges, there was a reduction of 0.2 log CFU/cm^2 and 0.8 log CFU/cm^2 for the stainless steel and polyethylene surfaces, respectively (Figure 1).

The average counts of HM decreased significantly at subsequent exposure periods for both groups and surfaces. For example, the average bacteria scores for group 1 sponges after four hours suffered a reduction of around 2.27 log CFU/cm^2 on stainless steel and 1.86 log CFU/cm^2 on polyethylene, while for group 2 the reductions were 1.94 log CFU/cm^2 and 2.24 log CFU/cm^2 on stainless steel and polyethylene surfaces, respectively, after four hours (Figure 1).

After 24 hours, there was an average decrease of about 2.9 log CFU/cm^2 in bacterial counts of group 1, while bacterial counts of sponges of group 2 remained at almost equal levels on the surfaces. After this exposure period, the number of viable microorganisms from groups 1 and 2 was nearly equal (between 1.26 and 1.87 log CFU/cm^2).

Regarding the survival of microorganisms on surfaces of stainless steel and polyethylene, the statistical analysis (p values <0.05%) demonstrates no significant differences between the surfaces tested (Table 1).

Discussion

The results of the present study demonstrate that the sponges used in kitchens may be contaminated by microorganisms, which corroborates with several previous studies [4,10,11,12]. For example, the counts of HM and CF in our study were similar to the results presented by Kusumaningrum *et al*. [5] and Josephson and colleagues [4] who showed that used sponges had counts of HM and CF between 6-7 log UFC/sponge in the Netherlands and the United States, respectively.

The presence of CF in kitchen sponges is a reason for concern because these microorganisms are used as indicators of faecal contamination, and may indicate the presence of pathogenic bacteria. According to Keeratipibul and colleagues [13], the presence of coliforms is worrying because it reflects inadequate sanitary conditions. Such contamination in sponges may come from raw or cooked contaminated food, inadequate hygienic practices during food preparation, absence of disinfection procedures, cross-contamination due to contaminated surfaces, and storage in places where there is humidity and high temperatures after contamination, making possible bacterial multiplication.

In the present study we did not analyse the transfer and survival of faecal coliforms; this omission may be a limitation of our study, because it was not the objective. We have chosen to analyse the transfer and survival of heterotrophic microorganisms instead of faecal coliforms because this former bacterial group may encompasses coliforms, pathogenic and potentially pathogenic microorganisms, and spoilage microorganisms.

According to the study of Mattick *et al*. [3], kitchen sponges can be contaminated during the washing of dishes contaminated with microorganisms that can, in turn, be transferred to surfaces. This observation is important because, according to Kusumaningrum *et al*. [12], some pathogens such as *E. coli* can survive for days in sponges, which can increase the risk of food cross-contamination.

Table 1. P values (< 0.05%) of survival and transfer of microorganisms from kitchen sponges to surfaces of stainless steel and polyethylene

	Surfaces	**Time (hours)**					
		0	1	2	3	4	24
Group 1 (Sponges contaminated with 7 to 10 log CFU / sponge)	AISI 316 stainless steel	0,633	0,449	0,300	0,666	0,472	0,659
	Polyethylene	0,633	0,454	0,304	0,667	0,477	0,659
Group 2 (Sponges contaminated with 4 to 6.9 log CFU / sponge)	AISI 316 stainless steel	0,646	0,442	0,798	0,218	0,944	0,482
	Polyethylene	0,650	0,443	0,798	0,218	0,944	0,492

T-Student analysis performed on the SPSS (Statistical Package for Social Sciences) release 12.1

In our study, an absence of both SA and SAM was observed, in contrast to the results of Josephson and colleagues [4], who analysed 100 sponges and reported that 66 sponges presented an average of 3 log CFU/sponge of SA and one sponge had SAM. It should be emphasized that the low frequency of SA and SAM and high counts of HM and CF observed in our investigation have also been found in other studies [4,10], and although SA and SAM are less frequent in sponges, their presence represents a high risk in kitchens since they are the main pathogens that cause food-borne illnesses in different countries [1].

The risk of cross-contamination by sponges during the cleaning of utensils and surfaces is high, as these objects can be a major source of bacteria [11]. Such microorganisms can survive for weeks in sponges [5] and can therefore be transferred to surfaces [5,3]. In this study, it was observed that naturally contaminated sponges can transfer a large number of microorganisms to surfaces that are rubbed with them. Kusumaningrum *et al*. [5] artificially contaminated kitchen sponges with *S. aureus*, *S.* enteritidis and *C. jejuni*. After rubbing the sponges on stainless steel, they could verify that they transferred between 21% and 43% of the initial inoculums placed on the sponges. According to these researchers, the physiological characteristics of bacteria are factors that influence the survival of the microorganisms because *S.* enteritidis and *S. aureus* remained viable on the surface for up to 96 hours while *C. jejuni* was not detected after four hours of exposure.

Other materials may also be contaminated by sponges, as shown by Mattick *et al*. [3] in their study conducted in England, where artificially contaminated sponges transferred *E. coli* and *Salmonella* sp. to laminate countertop surfaces. These researchers also reported that the number of microorganisms transferred to the surfaces depends on the initial contamination; that is, the greater the amount of bacteria in sponges, the greater the amount transferred. Similar results have been demonstrated in the present study, in which group 1 sponges (containing 7 to 10 log CFU/sponge) transferred an average of 5.5 log CFU/cm^2 from the initial contamination to AISI 316 stainless steel and polyethylene surfaces, while group 2 sponges (containing 4 to 6.9 log CFU/sponge) transferred approximately 1.9 log CFU/cm^2.

The results of this study also showed that the number of bacteria transferred to stainless steel and polyethylene decreased rapidly during the exposure of these surfaces to room temperature. Regardless of the amount transferred, the number of surviving bacteria was very low after 24 hours compared to the initial number of microorganisms.

In the present study, the rapid reduction found in the time interval between 0 and four hours for both groups and material surfaces can be attributed to the decrease of moisture on the materials. According to Tebbut *et al*. [14], bacterial suspensions on surfaces can form clumps, avoiding dehydration and functioning of microbial cells.

In the present study, significant differences in the number of surviving bacteria after 24 hours at room temperature were not observed; however, Kusumaningrum *et al*. [5] stated that the higher the concentration of bacteria on surfaces and residue of organic material in sponges, the higher the survival rate of microorganisms.

In must be emphasized that the sponges analysed in our study probably contained different amounts of organic matter (data not evaluated), which may have influenced the survival of microorganisms. The influence of organic matter in the bacterial transfer and survival was not evaluated by us and it is one of the limitations of our study.

The present study showed no significant differences in the transfer and survival of microorganisms on the surfaces of stainless steel and polyethylene. Similar results were demonstrated by Malheiros *et al*. [15], who found that *S. aureus* was transferred in similar amounts to stainless steel and polyethylene surfaces from cubes of chicken artificially contaminated. It is noteworthy that the surfaces of materials evaluated in this study were new, without grooves, which is not always observed during food service routines, where polyethylene-cutting boards and stainless steel tables are often scratched. Scratched utensils and surfaces may become highly contaminated by sponges, helping the rapid formation of biofilms. Many pathogens found in sponges, dish cloths, and many kitchen surfaces are able to form biofilms, which could increase the possibility of cross-contamination in these environments [16,17].

Based on the large numbers of microorganisms that can be transferred from a contaminated sponge, the control of hygiene in food services to reduce sources of contamination as well as the implementation of procedures for sponge disinfection are very important.

Conclusion

The results of this study showed that the sponges used in food services were significantly contaminated and could transfer large amounts of microorganisms to

surfaces of AISI 316 stainless steel and polyethylene. Although the amount of microorganisms transferred to the surfaces was high, there was a reduction in the number of microorganisms over time, and the reduction was greatest in the first four hours of exposure to room temperature. Even so, viable microorganisms were still found after 24 hours of exposure. Thus it is recommended that sponges are disinfected daily since they can transfer microorganisms to surfaces and increase the risk of cross-contamination in kitchens.

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