Research Paper

Evaluation of two recommended disinfection methods for cleaning cloths used in food services of southern Brazil

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Abstract

In the State of Rio Grande do Sul (RS), Southern Brazil, a good manufacturing practices regulation was published recommending two disinfection methods for cleaning cloths used in food services. The aim of the present study was to evaluate the efficacy of those methods. Cleaning cloths were sampled without prior notice at food services, on common working days. For the analyses, the cloths were divided in two sub-samples, being one of them microbiologically analyzed. The second sub-sample was further divided in two pieces and submitted to hand washing for two minutes. After that, one piece was boiled in water for 15 min and the other one was soaked in a 200 ppm sodium hypochlorite solution for 15 min. Both pieces of cloth were submitted to microbiological analyses. Cleaning cloths presented total aerobic mean counts of 6.9 ± 6.7 log/cm^2. All cleaning cloths presented coliform contamination, and 40% demonstrated mean counts of 6.2 ± 5.6 log/cm^2. Presumptive $S.\ aureus$ mean counts of 5.5 ± 4.9 log/cm^2 were found. No statistic correlation was observed among the number of meals served daily in the food services and the microbiological contamination levels. After washing and disinfection, microbiological counts were significantly ($p < 0.05$) reduced by both methods, achieving an approximately 5 log reduction. The reductions achieved by the sodium hypochlorite soaking method and the boiling method were not significantly different. Thus, it was possible to conclude that both recommended methods were suitable to disinfect cleaning cloths used in food services.

Key words: cleaning cloths, disinfection, The State of Rio Grande do Sul, Brazilian food services.

Introduction

The incidence of reported foodborne diseases has been increased worldwide (Norrung and Buncic, 2008; Sofos, 2008; Greig and Ravel, 2009;), including in Brazil (Oliveira *et al.*, 2007). The magnitude of this problem is bigger than reported by official surveillance bodies because the majority of the foodborne outbreaks are not officially reported. As previously described (Arbuthnott,1990)*,* less than 2% of the cases of foodborne acute gastroenteritis are detected by current surveillance/reporting process, and food services as bars, restaurants and hotels are among the most involved places where outbreaks have occurred. Many authors have indicated improper food preparation, inadequate storage or cooking and cross-contamination as the main cause of foodborne illnesses (Bean *et al.*, 1996; Scott, 1997; Simone *et al.*, 1997; Olsen *et al.*, 2000; Walker, 2003a). Cleaning cloths have been identified as an important potential cause of cross-contamination, representing a significant source of biological hazards. When contaminated cleaning cloths are rubbed on surfaces, organisms are invariably transferred to the surfaces of equipment and hands of food handlers in sufficient numbers to cause food contamination (Scott and Bloomfield. 1990b). Furthermore, the presence of food leftovers and the high humidity that cleaning cloths usually present could easily promote microbial multiplication under room temperature. Confirming the microbiological risk that cleaning cloths may represent, different species and counts of microorgan-

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isms have been found in cleaning cloths used in households and food services (De Wit *et al.*, 1979; De Boer and Hahne, 1990; Cogan *et al.*, 1999). Among the main factors that contribute to the frequent use of cleaning cloths in households and food services are the impossibility of moving heavy equipment for cleaning procedures, the presence of electric components which make it impossible to rinsing under running tap water, and also the lower cost of cotton cleaning cloths when compared to disposable cloths.

In order to reduce the microbiological contamination of cleaning cloths, a good manufacturing practices regulation (RIO GRANDE DO SUL, 2009) was published in 2009 by the Health Authority of the State of Rio Grande do Sul, Brazil. This regulation recommended two methods for disinfection of cleaning cloths in food services. It was the first Brazilian regulation to recommend the boiling of cloths in water for 15 min or their immersion in 200 ppm of sodium hypochlorite solution for 15 min, in order to disinfect the cleaning cloths. Even though both methodologies are well known and are assumed to be able to reduce microbiological counts of cleaning cloths, no scientific evaluation has been published so far or has been included in the aforementioned regulation.

Considering these aspects, the present study aimed to evaluate the efficacy of the two methods recommended by Portaria 78/2009 for disinfection of cleaning cloths used in food services.

Materials and Methods

Sampling procedures

Thirty five used cotton cleaning cloths were collected from the kitchen of 13 food services located in Porto Alegre city, Rio Grande do Sul. Among the food services, 10 were restaurants and three were industrial kitchens. The selection of the food services included in the study was based on the following criteria: to prepare at least 250 daily meals; to serve the food in buffet. In all the commercial food services at least the basic Good Manufacturing Practices (GMP) procedures were implemented, while in six of them GMP manual or GMP certification were provided. At the time of sampling, all the industrial kitchens had implemented GMP manual and Sanitarian Standard Operation Procedures (SSOP), and had a nutritionist engaged for the process control. One of the industrial kitchens prepared approximately 7,000 daily meals, while the other two prepared 750 and 320 daily meals, respectively. Prior to the sampling, the authorization of the food service's manager was requested. The samplings were conducted on different weekdays, without previous information of the food handlers. After being used for at least two hours in the kitchen, two cleaning cloths were collected in each commercial restaurant (n = 26), and three in each industrial kitchen (n = 9), totalizing 35 cleaning cloths samples. The collected samples were placed in sterile plastic bags and transported un-

der refrigeration to the Food Microbiology Laboratory of Instituto de Ciência e Tecnologia de Alimentos - ICTA/UFRGS.

Preparation of cleaning cloths for analyses

The cleaning cloths were measured and the size was standardized (35 cm x 70 cm) using a disinfected scissor and gloved hands. After that, the cloths where divided into two sub-samples (35 x 17.5 cm). On sub-sample was used to determine the initial number of bacteria in the cleaning cloth. The other sub-sample was further divided in two pieces of equal size (17.5 x 17.5 cm), which were submitted to the two disinfection methods.

Cloth contamination analyses

The first sub-sample of each 35 cleaning cloths was suspended in 500 mL of 0.1% peptone water (Merck, Darmstadt, Germany), and agitated by hand, for 10 min. An 1 mL- aliquot of the homogenate was added to 9 mL of 0.1% peptone water and successive decimal dilutions were carried out. Aliquots (20 µL) of each dilution were plated, in duplicate, on Plate Count Agar (PCA, Merck, Darmstadt, Germany), Baird Parker egg yolk-tellurite Agar (BP, Merck, Darmstadt, Germany) and Violet Red Brilliant Agar (VRBA, Merck, Darmstadt, Germany) for enumeration of Total Aerobic Counts (TAC), presumptive *Staphylococcus aureus* (grey/black shiny colonies with or without lipase activity) and presumptive coliforms (red, surrounded by reddish precipitation zones with 1 to 2 mm colonies), respectively. Plates were incubated at 30 °C, for 24-48 h and the colonies were counted. Presumptive *S. aureus* were confirmed by the coagulase test (FDA, 1992). Results were expressed as log $\pm$ SD cfu/cm^2 of cloth.

Evaluation of disinfection methods

The second sub-sample was divided in two equal pieces (70 units of 17.5 x 17.5cm) and submitted to the two disinfection methods recommended by Portaria 78/2009 (RIO GRANDE DO SUL, 2009*)*. All cloth samples (n = 70) were hand washed for approximately two minutes, by a same person, using potable water (0.2% residual chlorine) and 2 mL of a commercial neutral detergent (Trademark Minuano, Brazil) purchased from a retail supermarket. Microbiological counts were measured after hand washing in three cleaning cloths in order to check if this step contributes significantly with the microbiological reductions. This procedure was carried outin only three cloths because the aim of the present study was to evaluate the efficacy of the disinfection procedures, assuming that hand washing must be done as a previous step of disinfection. After the washing, half of the cloths (n = 35) were rinsed with potable water and one piece was boiled in 500 mL of water for 15 min. The time counting was started immediately after the water started to boil. The other half of the cloths (n = 35) were soaked in a 200ppm sodium hypochlorite solution (10 mL

of Q-Boa, 2,5% active chlorine, diluted in 1 liter of potable water) for 15 min, followed by rinsing with potable water. The concentration of free chlorine of the solutions used in the experiments was determined with a chlorine test kit (CHEMetrics, Inc., Calverton, VA). After disinfection, the cloths were placed into 250 mL of 0.1% peptone water and agitated vigorously. A volume of 20 μL of the homogenate was directly inoculated on Petri dishes containing PCA, BP and VRBA for the enumeration of TAC, presumptive *S. aureus* and coliforms, respectively. All the counts were performed in duplicates, after incubation at 30 °C, for 24 - 48 h. Results were expressed as log ± SD cfu/cm^2 of cloth.

Statistical analysis

Counts were performed in duplicates and submitted to variance analysis (ANOVA) in Microsoft Excel (Microsoft Corp. Redmond, WA). Differences were considered significant when $p < 0.05$.

Results

The number of bacteria in cleaning cloths was highly variable, however an expressive contamination was observed in all samples. The number of TAC varied from 4.3 log cfu/cm^2 to 8.0 log cfu/cm^2, with a mean of 7.03 ± 6.73 log cfu/cm^2. The majority, *i.e.*, 46% of the cleaning cloths presented counts of 6.0 log cfu/cm^2, followed by samples with 5.0 log cfu/cm^2 (28%) and 7.0 log cfu/cm^2 (20%). As expected, all samples showed expressive microbiological contamination and it was not possible to correlate the bacterial counts with the number of daily meals prepared by the food services. Figure 1A demonstrates the frequencies of TAC on the cleaning cloths sampled.

The presumptive coliform number on the cleaning cloths varied from 2.6 to 7.2 log cfu/cm^2 per cloth, and 40% of the samples presented counts around 6.0 log cfu/cm^2, 31% presents 5.0 log cfu/cm^2, and 9% demonstrated 4.0 log cfu/cm^2 (Figure 1B) The mean contamination was 6.21 ± 5.69 log cfu/cm^2 and in 14% of the cloths presumptive coliforms were not detected.

Contaminations with presumptive *S. aureus* ranged from 4.0 log cfu/cm^2 up to 6.4 log cfu/cm^2, with mean numbers of 5.55 ± 4.96 log cfu/cm^2. Among the cleaning cloths tested, 23% were contaminated with approximately 4.0 log cfu/cm^2 of presumptive *S. aureus*. The same percentage (23%) of cloths demonstrated counts of 5.0 log cfu/cm^2. Two cloths (6%) presented counts of 6.0 log cfu/cm^2. Presumptive *S. aureus* was not detected in 48% of the cloths tested. Frequencies of presumptive *S. aureus* found in the cleaning cloths are demonstrated in Figure 1C.

In general, it was not possible to identify a clear correlation among TAC, presumptive coliforms and presumptive *S. aureus* counts. Some cloths presenting high counts
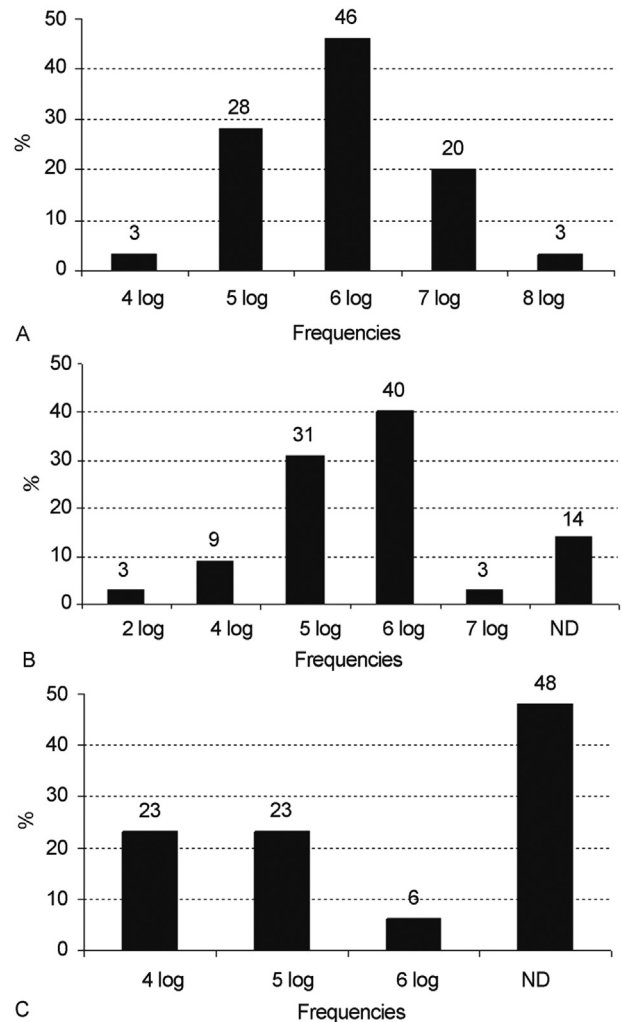
Figure 1 - Frequencies of Total Aerobic Counts (A), presumptive coliform counts (B) and presumptive *Staphylococcus aureus* counts (C) in cleaning cloths collected in food services of Southern Brazil.

of TAC did not demonstrated presumptive coliforms and/or presumptive *S. aureus*.

After hand washing and disinfection by both methods, the mean counts of all the microorganisms evaluated decreased significantly ($p < 0.05$). Considering the mean numbers of TAC observed in the cloths before disinfection, the majority of the samples presented approximately 5.0 log cfu/cm^2 reduction. Hand washing carried out before disinfection contributed with approximately 1.0 log/cm^2 reduction.

The cloths boiled in water for 15 min demonstrated TAC counts varying from not detected (< 1 log cfu/cm^2) to 3.3 log cfu/cm^2. Among all boiled samples tested for TAC, 48% presented counts around 1.0 log cfu/cm^2, 29% presented counts of approximately 2.0 log cfu/cm^2 and in 20% of the samples microorganisms were not detected. Only two boiled cloths (6%) demonstrated presumptive coliforms (counts around 1.0 log cfu/cm^2) and in the major-

ity of the cloths (94%) these microorganisms were not detected. Counts of presumptive *S. aureus* varied from not detected to 1.8 log cfu/cm^2, and in 89% of the samples presumptive *S. aureus* were not detected.

Cloths washed and disinfected by 200 ppm sodium hypochlorite solution for 15 min demonstrated TAC counts varying from not detected to 3.0 log cfu/cm^2. Only two samples (6%) presented counts with approximately 3.0 log cfu/cm^2, eight samples (23%) presented counts around 2.0 log cfu/cm^2 and 21 samples (60%) demonstrated counts around 1.0 log cfu/cm^2. Microorganisms were not detected in five samples (14%).

After 200 ppm sodium hypochlorite disinfection, the remaining populations of presumptive coliforms varied from not detected up to 3.0 log cfu/cm^2. Two samples presented counts around 3.0 log cfu/cm^2, one sample (3%) presented counts of 2.0 log cfu/cm^2, four samples (11%) presented counts around 1.0 log cfu/cm^2 and in 27 samples (77%) presumptive coliforms were not detected. Concerning presumptive *S. aureus*, populations varied from not detected to 2.1 log cfu/cm^2, two samples (6%) demonstrated counts around 2.0 log cfu/cm^2, six samples (17%) presented counts around 1.0 log cfu/cm^2 and in 27 samples (77%) these microorganisms were not detected. Comparing the results of the two disinfection methods, boiling was able to eliminate viable microorganisms in a higher number of samples.

Discussion

Cleaning cloths are widely used in food services in many countries, including Brazil. Reusable cleaning cloths are frequently found in food services, being used to wipe surfaces, remove food and detergent residues and other cleaning procedures. It is known that during their use cleaning cloths can become heavily contaminated and therefore are considered an important potential source of cross-contamination (Mackintosh and Hoffman, 1984; Tebbut, 1986; Scott and Bloomfield, 1990b; Bloomfield and Scott, 1997; Scott, 1999).

Cleaning cloths presenting high levels of microbial contamination have been reported by several authors. Typically, the contamination levels reported varied from 2.0 to 6.0 log cfu/cm^2 *(Davis et al.*, 1968; Scott and Bloomfield, 1990a, 1990b)*. The results found in our study demonstrated TAC varying from 4 up to 8 log cfu/cm^2 of cloth. Considering the mean contamination (7.03 ± 6.73 log cfu/cm^2) and the cloth area (70 x 35 cm), a common cloth with a homogeneous distribution of bacteria could contain approximately 9.3 log cfu.

The wide range of counts demonstrated in our study could be explained by the different ways of use of cleaning cloths in food services. At sampling, it was not investigated how long the cloths were in use and if they were sanitized before or during the use. Additionally to the diversity of uses of cleaning cloths, high contamination numbers commonly found in cleaning cloths can be explained by their cotton fiber structure and the frequent presence of organic matter, which may promote microbial attachment and protection (Kusumaningrum *et al.*, 2003). However, it is important to point out that in the present study we considered that the distribution of bacteria in cleaning cloths was homogeneous and the piece of cloth investigated was representative of the entire cloth.

Coliforms have been adopted as food safety and hygiene indicator for many foods (Jay *et al.*, 2005). As previously described (Gerba *et al.*, 2001) coliforms and other microorganisms can be used as indicators of microbial hazard in quantitative microbial risk assessment. Our study recorded mean numbers of presumptive coliforms on cloths of 6.4 log cfu/cm^2, and only in five samples they were not detected. The presumptive coliform contamination of cleaning cloths may be attributed to several factors as: improper handling during food preparation, contamination by raw materials, absence of disinfection procedures, cross-contamination by food handlers, conservation under room temperature and high humidity.

The contamination level of presumptive *S. aureus* observed in this study was distributed across a relative wide range of values, since in several samples these microorganisms were not detected (48%), while in others the counts reached 6 log cfu/cm^2. As well known, *S. aureus* is a widespread opportunistic pathogen that can cause food-borne illnesses (Parnes, 1997) and is a common inhabitant of human nose, throat and skin (Arbuthnott, 1990). This pathogen can produce heat-stable toxins responsible for causing food poisoning (Hein *et al.*, 2005). Improper manipulation by personnel was described as being the principal factor for the presence of *S. aureus* in foods (Hatakka *et al.*, 2000). As previously reported (Kusumaningrum *et al.*, 2003), *S. aureus* can survive in surfaces over four days and can be easily transferred to food by cross-contamination. As a mesophilic microorganism, the room temperature found in food service kitchens can provide an adequate environment for the multiplication and toxin production. Our results reported mean counts of presumptive *S. aureus* of 5.6 log cfu/cm^2, which may indicate poor personal hygiene practices and a potential risk of cross-contamination by cleaning cloths. On the other hand, 48% of the cloths did not presented presumptive *S. aureus*, and this interesting result indicates that such microorganism may not be present in cleaning cloths, even though they are mostly in direct contact with food handlers hands.

In the present study, the number of bacteria was significantly (p < 0.05) reduced in contaminated cleaning cloths by both methods recommended by Portaria 78/2009, *i.e.* boiling in potable water for 15 min or soaking in 200ppm solution of sodium hypochlorite for 15 min. It is well known that cleaning process is one important step to reduce contamination from surfaces due the mechanical re-

moval of dirt, soil and microorganisms (Kusumaningrum *et al.*, 2003), however cleaning alone is not sufficient to prevent the presence of pathogenic microorganisms and cross-contamination. By hand washing, the microbial count reductions observed in our study were of approximately 1.0 log, demonstrating the need of disinfection methods. One limitation in the present study is that only three cleaning cloths were analyzed after hand washing as an illustrative information because the aim of the study was to compare the two recommended disinfection methods and both should be carried out after hand washing. Together, washing and disinfection procedures were able to reduce around 5 logs of microorganisms, demonstrating that these two easy to perform and not expensive methods are effective.

The disinfection of cleaning cloths appears to play an important role in decontamination process, since microorganisms can be removed from them resulting in microbial inactivation. As found by several authors, adhered bacteria appear to be less sensitive to cleaning and disinfection products than bacteria in suspension (Frank and Koffi, 1990; Briandet *et al.*, 1999; Stopforth *et al.*, 2002).

Sodium hypochlorite has an important biocidal activity and is widely recommended worldwide for the disinfection process, however its biocidal activity depends on several factors such as chlorine concentration, pH, temperature and the presence and quantity of organic matter (Kusumaningrum *et al.*, 2003*)*, besides the time of exposure to the solution. Vegetative bacteria are susceptible to chlorine concentrations of 2 to 500 ppm in environments with low organic matter (Bessems, 1998). In a study conducted in household environments (Rusin *et al.*, 1998), it was demonstrated that disinfection using hypochlorite products in combination with regular cleaning could significantly reduce bacterial contamination of cleaning cloths. However, the use of 4000 ppm hypochlorite solution was not sufficiently effective to reduce contamination of cleaning cloths when they were heavily contaminated (Scot and Bloomfield. 1990b).

In our study, the slightly better reduction levels demonstrated by the boiling method may be partially explained by the better capacity of removing organic matter of this procedure. The high temperature and agitation of the cloths during boiling could facilitate organic matter removal and consequently the microbial inactivation. Boiling in water was also described as an efficient procedure for reduce contamination in cleaning cloths elsewhere (Parnes, 1997; Ikawa and Rossen, 1999; Anonymous, 2000).

Based on the results of the present study, cleaning cloths used in food services of Southern Brazil were highly contaminated, however the disinfection procedures recommended by the regulation 78/2009 (RIO GRANDE DO SUL, 2009) were able to significantly reduce their microbial contamination.

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