Tolerance of *Salmonella* Enteritidis and *Staphylococcus aureus* to Surface Cleaning and Household Bleach


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ABSTRACT

Effective cleaning and sanitizing of food preparation sites is important because pathogens are readily spread to food contact surfaces after preparation of contaminated raw products. Tolerance of *Salmonella* Enteritidis and *Staphylococcus aureus* to surface cleaning by wiping with regular, microfiber, and antibacterial-treated cloths was investigated. Wiping with cleaning cloths resulted in a considerable reduction of microorganisms from surfaces, despite the greater difficulty in removing *S. aureus* than *Salmonella* Enteritidis. Depending on the cloth type, *S. aureus* were reduced on surfaces from initial numbers of approximately $10^5$ CFU/100 cm² to numbers from less than 4 CFU/100 cm² (below the detection limit) to 100 CFU/100 cm². Directly after the cloths were used to clean the contaminated surfaces, they contained high numbers of bacteria ($10^4$ to $10^5$ CFU/100 cm²), except for the disposable antibacterial-treated cloths, in which no bacteria could be detected. The tolerance of these pathogens to sodium hypochlorite was studied in the suspension test and in cloths. *S. aureus* showed a better tolerance for sodium hypochlorite than *Salmonella* Enteritidis. Inactivation of microorganisms in cloths required a higher concentration of sodium hypochlorite than was needed in the suspension test. Repeated exposure to sodium hypochlorite, however, resulted in an increase in susceptibility to this compound. This study provides essential information about the transfer of bacteria when wiping surfaces and highlights the need for a hygiene procedure with cleaning cloths that sufficiently avoids cross-contamination in the household environment.

Cross-contamination via contaminated equipment and poor personal hygiene during food preparation contributes to the transmission of infectious disease (20). Pathogens are readily spread to food contact surfaces and cleaning tools after preparation of contaminated raw products (8, 10, 11). Proper cleaning and sanitizing of kitchen sites is suggested to prevent the spread of microorganisms and cross-contamination to ready-to-eat food. This notion has led to the promotion of several commercial household sanitizers, disinfectants, and cleaning cloths. A variety of active chemical agents are included in these products, many of which have been used for years in antiseptics, disinfectants, or preservation products (18).

Under normal conditions, cleaning is adequate for household situations, but in some circumstances, such as a diseased family member or the handling of potentially contaminated food, disinfection is indicated (14). Sodium hypochlorite (household bleach) is widely recommended, particularly in the United States, for sanitizing kitchen sites because of its biocidal activity at very low levels and its availability. In the Netherlands, approximately 10% of households used sodium hypochlorite to decontaminate kitchen work surfaces and dishcloths (2). The biocidal activity of sodium hypochlorite is dependent on such factors as chlorine concentration, pH, temperature, and presence of organic material. Disinfection must be applied correctly to achieve an optimal effect on the reduction of microorganisms. Laboratory studies have shown that adaptation to disinfectants can occur when microorganisms are exposed to sublethal concentrations (16). However, little is known about the effect of repeated challenge of sodium hypochlorite to pathogens in cloths.

In this study, the performance of antibacterial-treated cloths was investigated and compared with that of regular and microfiber cloths with respect to reduction or elimination of *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) and *Staphylococcus aureus* from artificially contaminated surfaces. *Salmonella* Enteritidis, a gram-negative bacteria, has been the most important cause of *Salmonella* infections in Europe (4) and the United States (20) in the last 5 years. Being among the longest recognized of gram-positive pathogenic bacteria, *S. aureus* is frequently found in domestic kitchen areas, particularly on dry surfaces. The effects of household sodium hypochlorite on these bacteria were determined in suspension tests and in artificially contaminated cloths. Repeated exposure to sodium hypochlorite was studied in cloths. In addition, the efficacy of this product to reduce normal flora of household cloths was also determined.

MATERIAL AND METHODS

Bacterial strains and growth conditions. Two foodborne pathogens, *Salmonella* Enteritidis (phage type 4, chicken product isolate) and *S. aureus* (196E toxin producer, human isolate), were...
TABLE 1. Types of cloths used in the study and their antibacterial effect by the diffusion test (*n* = 3)

<table>
<thead>
<tr>
<th>Cloth Code</th>
<th>Type</th>
<th>Antibacterial agent</th>
<th>Dimension (cm²)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Reusable microfiber&lt;sup&gt;a&lt;/sup&gt;</td>
<td>——</td>
<td>20 by 20</td>
<td>Salmonella Enteritidis: 0, S. aureus: 0</td>
</tr>
<tr>
<td>B</td>
<td>Reusable viscose</td>
<td>——</td>
<td>38 by 40</td>
<td>Salmonella Enteritidis: 0, S. aureus: 0</td>
</tr>
<tr>
<td>C</td>
<td>Reusable sponge</td>
<td>——</td>
<td>18 by 20</td>
<td>Salmonella Enteritidis: 0 ± 0.2, S. aureus: 0</td>
</tr>
<tr>
<td>D</td>
<td>Reusable sponge</td>
<td>Unspecified</td>
<td>18 by 20</td>
<td>Salmonella Enteritidis: 7 ± 1, S. aureus: 22 ± 7</td>
</tr>
</tbody>
</table>
| E          | Disposable wetted napkin | Anionic surfactants | 18 by 18 | S. aureus: 4 ± 1, n.a.

<sup>a</sup> Microfibers are fibers with a weight/length ratio of <1 decitex (1 decitex = 1 g/10,000 m). They adsorb dirt by static electricity when used dry or by capillary force when damp (19).

<sup>b</sup> Used one time and washed. n.a., not applicable.

obtained from the National Institute of Public Health and the Environment, The Netherlands. The stock cultures were maintained at −80°C in cryo vials (Greiner Bio-one GmbH, Frickenhausen, Germany) containing a stationary-phase culture in brain heart infusion (Difco, Becton Dickinson, Sparks, Md.) broth with 25% (vol/vol) glycerol (Fluka-chemica, Buchs, Switzerland) and glass beads (~2 mm, Emergo, Landsmeer, The Netherlands). Strains were cultured by transferring one glass bead to 10 ml of brain heart infusion broth, followed by incubation for 18 to 20 h (overnight) at 37°C. Serial dilutions were prepared in peptone saline solution (PSS: NaCl 8.5 g/liter) and neutralized bacteriological peptone (1 g/liter, Oxoid, Basingstoke, UK). Tryptone soy agar (TSA; Oxoid) was used to enumerate the test bacteria. In some cases, selective media were used: mannitol lysine crystal violet brilliant green agar (Oxoid) for *Salmonella* Enteritidis and Baird Parker egg yolk–tellurite agar (Oxoid) for *S. aureus*. All plates were incubated for 24 h at 37°C.

**Performance of regular and sanitizer-treated cloths.** Five different cloths from local supermarkets—one disposable and four reusable, listed in Table 1—were used. The antibacterial effects of the cloths were initially studied using the agar diffusion test. The cloths were cut in approximately 1-cm-diameter pieces and placed on freshly prepared TSA surfaces that were inoculated with appropriate dilutions of overnight cultures. For the reusable cloths, both new and used cloths were examined. The used cloths had been used once in a previous experiment, washed with hot water with anionic-active detergent, rinsed with water, and allowed to dry at room temperature. The inhibition zones were measured after incubation for 24 h at 37°C.

The ability of the cloths to clean and decontaminate surfaces was investigated on stainless steel (AISI type 304 standard, ODS, Barendrecht, The Netherlands) surfaces placed in a laminar hood without airflow at room temperature (22 to 25°C, 40 to 45% relative humidity). The surface areas were 50 by 80 cm² and were disinfected with 800 ppm of sodium hypochlorite solution (obtained from a local supermarket) for 15 min, washed in hot water with anionic-active detergent, and rinsed with water, and allowed to dry at room temperature. The inhibition zones were measured after incubation for 24 h at 37°C.

The effect of sodium hypochlorite on artificially contaminated cloths and repeated exposure experiment. The efficacy of sodium hypochlorite to decontaminate artificially contaminated cloths was investigated with viscose cloths (19 by 20 cm²; cloth B). Each cloth was placed in a stomacher bag with 10 ml of an appropriate dilution of an overnight culture to obtain initial counts of approximately 10⁶ CFU/100 cm², followed by the addition of 10 ml of sodium hypochlorite solution at final concentrations of 500 and 800 ppm. The log reductions were investigated at 10, 30, and 60 min of exposure after adding hypochlorite.
the cloths were sampled for the second time by the same procedure as described before.

The effect of repeated exposure to sodium hypochlorite was investigated in cloths contaminated with pathogens and treated with hypochlorite at final concentration of 650 ppm for 10, 30, and 60 min. The experiments were prepared and sampled as described previously. After the cloths were kept at room temperature in a stomacher bag for 24 h, they were challenged with hypochlorite for the second time at the same final concentration for 10, 30, and 60 min, followed by sampling in which each treated cloth was homogenized with 50 ml PSS in a Stomacher at 260 rpm for 60 s, as described previously. The procedure was repeated with 500 ppm of hypochlorite solution.

Effect of sodium hypochlorite on naturally contaminated household cloths. Household cotton cloths were collected after approximately 1 week of use. Each cloth was cut into four pieces, and one piece of each cloth was sampled immediately as described above. One liter of hypochlorite solution in tap water at a final concentration of 2.400 ppm, as recommended by the manufacturer, was used to wash the other pieces of the cloths for approximately 30 to 60 s. Each piece of cloth was then removed from the solution, kept in a stomacher bag at room temperature, and sampled after 15 and 60 min and after 24 h. Plate count agar (Oxoid) was used to enumerate the total aerobic counts, and violet red bile glucose agar (Oxoid) was used for total Enterobacteriaceae incubated for 24 to 48 h at 30°C and for 24 h at 37°C, respectively.

Scanning electron microscopy. To visualize the arrangement of the cells on cloths, scanning electron micrographs were prepared. Naturally and artificially contaminated sponge cloths were cut into pieces (0.5 by 0.5 cm²) and fixed with 3.5% glutaraldehyde (Sigma-Aldrich) in 0.1 M cacodylate buffer (Sigma-Aldrich), pH 7.2. The cells were dehydrated once in a 10, 30, 50, 70, and 96% ethanol series; dehydrated twice in 100% ethanol; and critical point dried. Finally, cells were sputter coated with 10-nm platinum and viewed with a model JSM-6300F scanning electron microscope (JEOL, Peabody, Mass.).

Statistical analyses. Each experiment was carried out three or more times on different days, and no less than two replicates were used in each experiment, except for the scanning electron microscopy. Data analyses were performed on SPSS for Windows 95/98/NT/2000, release 10.1. P < 0.05 was considered statistically significant.

RESULTS

Performance of regular and sanitizer-treated cloths. The reusable cloths (A and B) did not show inhibition of Salmonella Enteritidis and S. aureus with the diffusion agar test (Table 1). Cloth C showed a small zone of inhibition on S. aureus plates when new (1 ± 0.2 mm), most likely because of chemical residues from the production process. Only cloth D, which was wetted with antibacterial solution, demonstrated a considerable zone of inhibition on Salmonella Enteritidis plates (7 ± 1 mm), but the effect disappeared when the cloths were used once and washed. Cloth D showed the largest inhibition zone with S. aureus (22 ± 7 mm), and the effect was still apparent at a lower level (15 ± 6 mm) when the cloths were used once. The disposable cloth that was available impregnated in chemical solution (cloth E), showed a slight antibacterial effect on S. aureus (4 ± 1 mm) but not on Salmonella Enteritidis plates.

The ability of the cloths to remove or reduce bacteria from artificially contaminated stainless steel surfaces is shown in Table 2. In general, the results indicate that Salmonella Enteritidis and S. aureus were still found on surfaces at 0.6 to 2.0 log units (CFU/100 cm²) when the surfaces had just been cleaned, with the exception of the surfaces cleaned with cloth E, in which the bacterial counts were below the detection limit (log N < 0.6 CFU/100 cm²). For Salmonella Enteritidis, the type and condition (new or used) of the cloth did not influence the performance (P = 0.16 and 0.09, respectively). In addition, 15 min of air drying after wiping resulted in a significant reduction of Salmonella Enteritidis bacterial counts on surfaces (P = 0.00).

In contrast, cloth type did affect the removal or reduction of S. aureus (P = 0.00), whereas 15 min of air drying did not significantly reduce the counts of this bacterium on surfaces (P = 0.28). The condition of the cloths also did not influence their ability to reduce numbers of S. aureus (P = 0.64).

Directly after using the cloths to clean the contaminated surfaces, they contained high numbers of bacteria (10² to 10³ CFU/100 cm²), except cloth E, in which the bacteria could not be detected (below the detection limit, log N < 2.0 CFU/100 cm²). The bacterial loads in cloths varied among the cloth types (P = 0.00), both for Salmonella Enteritidis and S. aureus, but were not significantly affected by the condition of the cloths (P = 0.65 and 0.28 for Salmonella Enteritidis and S. aureus, respectively). These results indicate that during cleaning, microorganisms were transferred from the surfaces to the cloths, and depending on the type of the cloth (with or without antibacterial component), the numbers of microorganisms were stable or reduced.

Effect of sodium hypochlorite in suspension test. The results of the suspension tests, with the addition of 3 g/liter bovine serum albumin as the interfering substances to simulate dirty conditions, are shown in Figure 1. In general, S. aureus demonstrated a better tolerance to sodium hypochlorite than Salmonella Enteritidis. At concentrations of 200 ppm, after 60 min of exposure, S. aureus were reduced 2 log units, whereas Salmonella Enteritidis decreased approximately 4 log units. At 300 ppm, higher reductions were found for Salmonella Enteritidis as well as S. aureus, and at 400 ppm, 5-log reductions were obtained for Salmonella Enteritidis and S. aureus after 30 and 60 min of exposure, respectively.

Effect of sodium hypochlorite on artificially contaminated cloths and the repeated exposure experiment. The effect of commercial sodium hypochlorite solutions on the reduction of Salmonella Enteritidis and S. aureus in cloths is shown in Figure 2. Although the experiments were carried out in the absence of food residues or interfering substances, decontamination of microorganisms in cloths required higher concentration of hypochlorite than in suspension tests. At 500 ppm hypochlorite, numbers of Salmonella Enteritidis and S. aureus were slightly reduced at the exposure time of 60 min. When the cloths were left at room temperature for 24 h after wringing the solution out,
TABLE 2. Counts of Salmonella Enteritidis and Staphylococcus aureus on wiped surfaces and in cloths

<table>
<thead>
<tr>
<th>Cloth</th>
<th>Condition</th>
<th>n</th>
<th>Surface counts (log CFU/100 cm²)</th>
<th>Cloth counts (log CFU/100 cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Directly after wiping</td>
<td>15 min after wiping</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>Salmonella Enteritidis</strong></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>New</td>
<td>4</td>
<td>0.8 ± 0.3</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Used</td>
<td>4</td>
<td>0.6 ± 0.0</td>
<td>&lt;0.6 ± 0.0</td>
</tr>
<tr>
<td>B</td>
<td>New</td>
<td>6</td>
<td>1.1 ± 0.4</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Used</td>
<td>6</td>
<td>1.1 ± 0.3</td>
<td>&lt;0.6 ± 0.0</td>
</tr>
<tr>
<td>C</td>
<td>New</td>
<td>4</td>
<td>1.2 ± 0.4</td>
<td>&lt;0.6 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Used</td>
<td>4</td>
<td>0.9 ± 0.5</td>
<td>&lt;0.6 ± 0.0</td>
</tr>
<tr>
<td>D</td>
<td>New</td>
<td>4</td>
<td>1.0 ± 0.4</td>
<td>&lt;0.6 ± 0.0</td>
</tr>
<tr>
<td></td>
<td>Used</td>
<td>4</td>
<td>0.8 ± 0.3</td>
<td>&lt;0.6 ± 0.0</td>
</tr>
<tr>
<td>E</td>
<td>New</td>
<td>8</td>
<td>&lt;0.6 ± 0.0</td>
<td>&lt;0.6 ± 0.0</td>
</tr>
</tbody>
</table>

|        |               |   | **S. aureus**                   |                               |
|        |               |   |                                 |                               |
| A     | New           | 6 | 1.1 ± 0.7                       | 1.0 ± 0.7                     |
|       | Used          | 4 | 1.1 ± 0.5                       | 1.0 ± 0.5                     |
| B     | New           | 8 | 1.7 ± 0.9                       | 1.5 ± 0.9                     |
|       | Used          | 6 | 2.0 ± 1.0                       | 1.5 ± 0.7                     |
| C     | New           | 4 | 1.4 ± 0.2                       | 1.2 ± 0.3                     |
|       | Used          | 4 | 1.4 ± 0.3                       | 1.1 ± 0.4                     |
| D     | New           | 4 | 0.7 ± 0.2                       | 0.8 ± 0.2                     |
|       | Used          | 4 | 1.0 ± 0.5                       | 0.9 ± 0.5                     |
| E     | New           | 8 | <0.6 ± 0.0                      | <0.6 ± 0.0                    |

a Data represent enumeration on TSA. Values are mean ± SD. The log detection limit on surfaces was 0.6 CFU/100 cm² and in cloths was 2.0 CFU/100 cm². n, number of experiments.

b The log initial numbers on surfaces were 5.8 ± 0.6 and 5.2 ± 0.2 CFU/100 cm² for Salmonella Enteritidis and S. aureus, respectively.

c The initial numbers of target microorganisms in cloths, both new and used, were below the detection limit (log N < 2.0 CFU/100 cm²).

levels of microorganisms remained high (~10⁵ CFU/100 cm²).

At 800 ppm with 10 min exposure time, 2-log reductions were found for Salmonella Enteritidis, whereas S. aureus was reduced with approximately 1 log unit. After 24 h, bacteria were still found in these cloths at approximately 10⁴ CFU/100 cm². At 30 and 60 min exposure time, approximately 3-log reductions were observed for Salmonella Enteritidis and S. aureus, but the next day, the counts in cloths decreased below the detection limit (log N < 2.0 CFU/100 cm²). These results indicate that inactivation of pathogens in cloths requires high concentrations of hypochlorite and a long exposure time.

The effect of repeated exposure to sodium hypochlorite was investigated in cloths. The first challenge to sodium hypochlorite at a final concentration of 650 ppm for 10, 30, and 60 min resulted in reductions of Salmonella Enteritidis and S. aureus of between 1 and 1.5 log units. When these cloths were subsequently removed from the solution, kept at room temperature after wringing out, and treated with the same concentration on the next day, the numbers of microorganisms decreased below the detection limit. Experiments with a lower hypochlorite concentration (500 ppm) also resulted in an increase in susceptibility to this product. At this concentration, the first challenge resulted in less than a 1-log reduction of the count numbers of Salmonella Enteritidis and S. aureus after exposure times of 10, 30, and 60 min. The second challenge on the next day resulted in a 4-log reduction after 10 min of exposure and in a reduction below the detection limit after 30 and 60 min exposure.

**Effect of sodium hypochlorite on naturally contaminated household cloths.** Eighteen cloths (18 by 18 cm²) used daily in households contained total aerobic counts between 10⁵ and 10⁶ CFU per cloth and total Enterobacteriaceae between 10⁵ and 10⁶ CFU per cloth. After the cloths were washed with hypochlorite solution at concentration of 2,400 ppm and subsequently left at room temperature for 15 and 60 min, the total aerobic counts in cloths were reduced approximately 4 log units (Fig. 3). After 24 h, the levels of the microorganism in the cloths were approximately 10³ CFU per cloth, for both total aerobic and total Enterobacteriaceae counts. High variability was observed among the count numbers in the cloths, as indicated by a large standard deviation.
FIGURE 2. Effect of sodium hypochlorite at concentrations of (A) 500 and (B) 800 ppm on *Salmonella* Enteritidis and *Staphylococcus aureus* in artificially contaminated cloths (n = 3). Sampling was carried out at time 0, 10, 30, and 60 min, directly after exposure with hypochlorite and 24 h after the cloths were removed from the hypochlorite solution and subsequently kept at room temperature. Data represent enumeration on TSA. \( \log N < 2.0 \text{ CFU/100 cm}^2 \).

FIGURE 3. Effect of sodium hypochlorite solution (2.400 ppm) on the total aerobic counts (TAC) and total *Enterobacteriaceae* (Entero’s) of naturally contaminated household cloths (n = 18).

FIGURE 4. Scanning electron micrograph of naturally contaminated household sponge cloths; (A) \( \times 2,000 \) and (B) \( \times 10,000 \).

The scanning electron micrographs indicated that the cloths had a complex structure (Fig. 4), which might offer a protective microenvironment for microorganisms where attachment could be facilitated. Furthermore, food residues were observed in household cloths, covering the bacteria to some extent. This arrangement possibly facilitated additional protection to microorganisms against direct exposure to hypochlorite.

**DISCUSSION**

Cleaning refers to the mechanical removal of dirt and soil from an object area. In addition, during cleaning, microorganisms are removed from surfaces, as demonstrated in this study. The different cloths used to wipe artificially contaminated surfaces demonstrated similar considerable reduction of *Salmonella* Enteritidis (about 4.5 to 5 log units), indicating that the performance of all cloths was comparable. Some variation might be associated with the amount of pressure applied to the cloths during surface wiping. The numbers of cells used in the experiments (\( \sim 2 \) to \( 6 \times 10^3 \) CFU/100 cm\(^2 \)) were similar to the *Salmonella* counts found on chopping boards after preparation of *Salmonella*-contaminated chicken carcasses (9).

The reduction of *S. aureus* by wiping was influenced by the cloth type. The disposable cloth (cloth E) performed better than the other cloths. The additional effect of this cloth on bacterial reduction was possibly a result of the anionic surfactant left on surfaces, resulting in inactivation of any remaining microorganisms. A statistical test between the performances of the reusable cloths, thus excluding cloth E, also demonstrated significant differences, indicating that some of these cloths performed better than others in the reduction of *S. aureus* from surfaces. Although some variation could also be associated with the wiping practice, in general, it was more difficult to remove *S. aureus* from surfaces than *Salmonella* Enteritidis using the cloths, most likely because of the characteristics of the microorganism. As found in our earlier study, *S. aureus* survived better on stainless steel surfaces (15) than *Salmonella* Enteritidis. Particularly at high initial levels, *S. aureus* was present in clumps that might provide some protection to the innermost cells against drying (23) and wiping. Possibly because of this clump structure, 15 min of air-drying did not significantly reduce *S. aureus* on surfaces, next to intrinsical tolerance against dry conditions.

An important observation is that, during cleaning, microorganisms were transferred from surfaces to the cloths, which can potentially cause cross-contamination. Our previous study demonstrated that microorganisms were readily spread from kitchen sponges to surfaces, with transfer rates of approximately 20 to 40% (15). Some kitchen cloths are specially treated with particular chemicals for disinfection purposes, such as cloths D (reusable) and E (disposable). This treatment resulted in differences in bacterial counts remaining in the cloths (i.e., in cloth E, the numbers were below the detection limit). Additionally, when the reusable cloths were used once and washed, their antibacterial effect was reduced or disappeared, as demonstrated with the agar diffusion test. However, a single use did not lower the disinfecting activity that could lead to statistical differences in the count numbers on cloths. The variations between the results of the agar diffusion test and the cloth exposure experiment were most likely a result of the ease or difficulty of diffusion of the antibacterial component into the agar medium. As described above, cloth E showed no effect on *Salmonella* Enteritidis and only a small inhibition zone on *S. aureus* by the agar diffusion test, but it demonstrated significant bacterial reduction in the cloths. Furthermore, the antibacterial component in cloth D probably diffused...
better to the agar medium, resulting in considerable inhibition of Salmonella Enteritidis and S. aureus on plates, although the disinfecting effect in cloths resulted in less than a 2-log reduction compared with the regular cloths.

Some studies have described alternative procedures to reduce bacterial contaminants in cloths (13, 21), such as boiling in water for 5 min. In addition, recommendations of suitable hygiene procedures for use in the domestic environment, including decontamination of cloths, have been published (3). When chemical disinfection is a choice for bacterial reduction in cloths, the correct use of these disinfectants (e.g., the correct concentration and the correct exposure time) should be applied. The biocidal activity of disinfectants such as sodium hypochlorite is dependent on external factors. For example, organic material from the environment interferes with the available chlorine, resulting in a decrease in the effectiveness of sodium hypochlorite, as demonstrated in suspension tests under dirty conditions compared with clean conditions. In another study, it was demonstrated that, in a suspension test, 9.2 times the concentration of hypochlorite solution was needed to achieve a 5-log reduction of S. aureus under dirty conditions compared with clean conditions (6).

The intrinsic susceptibility (tolerance) of microorganisms to chemical agents varies depending on the species and the antimicrobial agent (12). Furthermore, the test methodology used for the evaluation influences the tolerance of the microorganisms. Low concentrations of available chlorine (2 to 500 ppm) are active against vegetative bacteria in environments with low organic matter (6). However, at 500 ppm of hypochlorite, the levels of Salmonella Enteritidis and S. aureus were not reduced noticeably when the test was carried out on cloths, even though no additional organic matter interfered. The structure of the cloths might offer a protective microenvironment where microbial attachment and survival is facilitated. Furthermore, in naturally contaminated household cloths, 2,400 ppm of hypochlorite solution, as recommended by the manufacturer, did not result in a total reduction of the microflora of the cloths, although in some cases, a reduction of more than 4 log units was observed. The differences between cloths were possibly a result of the degree of bacterial contamination; the presence of organic materials, including food residues; or the attachment of bacteria on cloths. Organic matter could interfere with the hypochlorite or protect the microorganisms from direct contact with the disinfectants. As observed with scanning electron microscopy, bacteria were found in household cloths covered by organic matter from the environment.

Repeated exposure to hypochlorite, however, resulted in increased susceptibility of microorganisms to this product, most likely because of membrane damage. After the first exposure to hypochlorite, with concentrations of 650 ppm in brain heart infusion broth for 30 min, membrane damage was observed in more than 70% of the cell population, as determined by epifluorescence microscopy after cell staining using LIVE/DEAD BacLight bacterial viability kits. The second challenge caused further damage, resulting in nearly 100% dead cells (data not shown). The integrity of the cell membrane is important for the folding of proteins and the integrity of the DNA (7). Both hypochlorous acid and the hypochlorite ion are strong oxidizing agents that can attack cell walls and impair cellular membrane functionality, resulting in increased membrane permeability (17, 22).

This study has demonstrated that effective cleaning of food preparation sites is important. Wiping of surfaces with regular cloths resulted in a considerable reduction of pathogens such as Salmonella Enteritidis. Subsequent air drying resulted in a further reduction to a level at which the risk for cross-contamination is very low. When bacteria such as S. aureus or other opportunistic bacteria have to be eliminated from surfaces, disposable chemical-impregnated cloths can be indicated. Our study also demonstrated that, during cleaning, microorganisms were transferred from surfaces to the cloths, and inactivation of microorganisms in cloths required higher concentrations of sodium hypochlorite than in the suspension test, although the experiments were carried out in the absence of interfering substances. When organic matter from the environment is present in the cloths, which occurs regularly in practical situations, the effectiveness of a disinfectant will be less, requiring a higher concentration of the disinfectant. Instead, boiling the cloths for 5 min or washing at ≥60°C could be indicated (3, 13).

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