Short communication

Transfer of *Salmonella* Enteritidis to four types of surfaces after cleaning procedures and cross-contamination to tomatoes

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ABSTRACT

The objectives of the present study were to evaluate the spread of *Salmonella* Enteritidis to different cutting boards (wood, triclosan-treated plastic, glass, and stainless steel) from contaminated poultry skin (5 log CFU/g) and then to tomatoes and to analyze the effect of different protocols used to clean these surfaces to control contamination. The following procedures were simulated: (1) no cleaning after handling contaminated poultry skin; (2) rinsing in running water; (3) cleaning with dish soap and mechanical scrubbing; and (4) cleaning with dish soap and mechanical scrubbing, followed by disinfection with hypochlorite. The pathogen was recovered from all surfaces following procedure 1, with counts ranging from 1.90 to 2.80 log, as well as from the tomatoes handled on it. Reduced numbers of *S.* Enteritidis were recovered using the other procedures, both from the surfaces and from the tomatoes. Counts were undetectable after procedure 4. From all surfaces evaluated, wood was the most difficult to clean, and stainless steel was the easiest. The use of hypochlorite as a disinfecting agent helped to reduce cross-contamination.

1. Introduction

Salmonellosis is considered one of the most widespread foodborne diseases in the world (Bollaerts et al., 2008), and Enteritidis is the main serotype responsible for human infections (Oliveira et al., 2006; Moore et al., 2007; Pang et al., 2007). Up to 87% of the sites where outbreaks occur are associated with foodstuffs prepared or consumed in households (van Asselt et al., 2008).

In this environment, it is estimated that about 40–60% of the cases of foodborne disease are caused by inadequate handling practices (de Jong et al., 2008), such as cross-contamination from cutting boards where raw poultry meat is handled along with other foodstuffs (Kusumaningrum et al., 2004; Parry et al., 2005; Luber, 2009; van Asselt et al., 2009). Several studies have evaluated the contamination of cutting boards, as well as issues related to the material used in the production of these utensils, and the ease of cleaning cutting boards. In a study conducted by Ravishankar et al. (2010), the rate of transfer of *Salmonella enterica* from poultry to lettuce handled with knives and on plastic cutting boards was studied under different scenarios. When utensils were not cleaned after they were used, the transfer rate was 1.25% from poultry to plastic and 45.62% from plastic and knives to lettuce.

Ak et al. (1994) assessed possible differences in the decontamination of cutting surfaces and observed that more bacteria were recovered from plastic than from wooden cutting boards. These authors recommended the use of wooden cutting boards in households. However, the results of a study carried out by Gough and Dodd (1998), with similar objectives and using *S.* Typhimurium, showed that wood presented a greater risk for cross-contamination than plastic. Other surfaces have also been studied. Moore et al. (2007) studied the recovery of *S.* Typhimurium from Formica, stainless steel, polypropylene and wood and observed greater recovery from Formica and stainless steel than from polypropylene and wood. The cleaning procedure, however, was not analyzed in this study.

From the methods used in cleaning of surfaces, studies have shown that water and soap alone are not enough to produce decontamination (Scott and Bloomfield, 1990, 1993; Cogan et al., 1999; Cogan et al., 2002; Barker et al., 2003). On the other hand, although disinfectants may be more effective in reducing *Salmonella* populations in household kitchens (Barker et al., 2003), there are few studies on their efficiency on different types of cutting surfaces and on the prevention of cross-contamination in households.

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DeVere and Purchase (2007) studied the efficacy of four domestic antibacterial products: two brands of cleaning wipes and two cleaning sprays. All of them possessed surfactant properties and were used to decontaminate wooden, plastic, triclosan-treated plastic and glass surfaces that were contaminated with *Staphylococcus aureus* and *Escherichia coli*. Except for one of the wipes, all of the other products were considered effective. The plastic surfaces were more difficult to sanitize than the wooden surfaces, which were easy to decontaminate.

Because the inadequate use of cutting surfaces, as well as the cleaning methods applied to them, may lead to the cross-contamination of ready-to-eat foods with *Salmonella*, the objectives of this study were to evaluate the spread of *S.* Enteritidis from contaminated chicken skin to different cutting surfaces and then to tomatoes handled on them under household conditions. Moreover, different protocols used to clean these surfaces were analyzed to propose control measures that may be easily adopted in households to prevent the contamination of foodstuffs.

2. Materials and methods

2.1. Preparation of the bacterial culture

An *S.* Enteritidis strain of avian origin resistant to nalidixic acid (NAL[+]) was used in this study. The strain, which was kept under refrigeration (<4 °C) in preservation agar (0.5 g meat extract, 1 g peptone, 0.5 g NaCl, 1.5 g agar, and 100 mL distilled water), was cultured in bismuth sulfide agar (BS − Oxoid) supplemented with 100 μg of nalidixic acid (Wintomylon) per mL of medium. Plates were incubated at 35 °C/24 h. After incubation, one colony isolated from the medium was transferred to a test tube containing 10 mL of brain heart infusion broth (BHI − Difco) and incubated at 35 °C/24 h. The inoculum was diluted tenfold to 10^{-10} in saline solution (SS − Vetec) 0.9%. *S.* Enteritidis NAL[+] was then quantified in duplicate on spread plates with BS supplemented with 100 μg of nalidixic acid per mL of medium. Plates were incubated at 35 °C/24 h. The objective of this step was to assess which dilution showed a concentration closest to 5 log CFU/mL of *S.* Enteritidis NAL[+], which was the contamination level used in the study.

Skin from different parts of the chicken (breast, drumsticks, and thighs) was collected from poultry slaughtered in an abattoir. The skin was cut into 5-g pieces, which were weighed (200 g) in sterile plastic bags, and inoculated with 50 mL of SS 0.9% containing enough *S.* Enteritidis to achieve a contamination level equal to 5 log CFU/g of skin. The mixture was homogenized for 3 min and kept under refrigeration for 5 min to improve the adherence of the microbial cells. After contamination, the skin was sampled to assess the initial microbial population.

2.2. Transfer and cross-contamination using cutting surfaces after different cleaning procedures

This study evaluated four types of materials used as cutting surfaces for food handling: pine wood, triclosan-treated plastic, tempered glass, and stainless steel.

For the assays on *S.* Enteritidis recovery and cross-contamination, an area of 100 cm² (10 cm × 10 cm) was determined on each cutting board. Cutting boards were sterilized in autoclave at 121 °C for 15 min before being used. They were then contaminated with a 5-g portion of the skin described above. The skin was placed on the cutting boards and gently rubbed for 1 min with circular movements. The cutting boards were then kept at room temperature for 3 min to improve the adherence of the microbial cells.

In procedure 1, one surface of each cutting board was sampled soon after, and the other was used in the cross-contamination assay. In this assay, tomatoes were cleaned manually with a sponge and neutral dish soap and then disinfected for 15 min in chlorine solution (percent active chlorine: 2.0%−2.5% w/w). Following these steps, the tomatoes were cut into small pieces. For procedure 2, surfaces were rinsed in cold water for 10 s and left to drain for 5 min. In procedure 3, besides rinsing, the surfaces were manually and vigorously scrubbed with a moist sponge and 1 mL of neutral liquid dish soap (sodium linear alkylbenzene sulfonate). In procedure 4, besides cleaning as described in procedure 3, the surfaces were sanitized with 250 mL NaClO (percent active chlorine: 2.0%−2.5% w/w) in a concentration equal to 5000 ppm for 1 min (Barker et al., 2003).

2.3. Enumeration of S. Enteritidis NAL[+]

Salmonella was recovered from cutting boards by means of alginate swabs sterilized by gamma radiation and moistened in buffered peptone water (BPW − Difco). After sampling, swabs were placed in test tubes containing 10 mL of BPW. Care was taken to make sure that the entire surface of the swab made contact with the entire 100-cm² area to be analyzed. The tubes containing the swabs were vortexed for 1 min.

Analytical units with 25 g of chicken skin and tomatoes were weighed in sterile plastic bags, diluted with 225 mL of BPW, and homogenized for 1 min in a stomacher. Decimal dilutions were carried out with BPW for all samples (swabs, tomatoes and skin) and plated on BS agar supplemented with 100 μg of nalidixic acid per mL of medium. Plates were incubated at 35 °C/24 h.

Besides the quantification of the agent, the presence of *Salmonella* was also determined in the BPW homogenate containing the swab and/or 25 g of tomatoes. These mixtures were incubated at 35 °C/24 h. After pre-enrichment, the pathogen was isolated in BS agar supplemented with 100 μg of nalidixic acid per mL of medium. Plates were incubated at 35 °C/24 h.

2.4. Statistical analysis

Each trial was carried out 10 times, and microbiological analyses were performed in duplicate. A statistical analysis of the data was carried out by non-parametric statistics based on Kruskall−Wallis test to compare the surfaces submitted to each cleaning procedure, and Friedman's test was used to compare the different cleaning procedures with respect to each type of surface. Results were analyzed using 5% as the significance level (Conover, 1971).

3. Results and discussion

The mean count of *S.* Enteritidis NAL[+] in samples of skin used in the contamination procedures was 5.11 log CFU/g. Table 1 shows the median, maximum and minimum counts of *S.* Enteritidis NAL[+] recovered from cutting boards made of wood, triclosan-treated plastic, glass, and stainless steel, after simulating the four different cleaning procedures, and the cross-contamination to tomatoes handled on the boards.

In procedure 1, fewer cells were recovered from wood than from the other surfaces ($p < 0.01$). Abrishami et al. (1994) reported that 88% of *E. coli* inoculum was not recovered from wood 10 min after inoculation due to the penetration of the bacteria caused by the capillarity of the material. Thus, we can consider the hypothesis that soon after handling the contaminated skin on wooden surfaces, part of the inoculum as "absorbed" and became unavailable to the swabs used to recover it.

Moore et al. (2007), who studied the recovery of *S.* Typhimurium cells from different surfaces, showed that more cells were

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Table 1
S. Enteritidis NAL$^+$ transferred to surfaces after four cleaning procedures and cross-contamination to tomatoes.

	1	2	3	4
Transfer to surfaces (log CFU/cm^2)				
Wood	1.90 (2.80–1.20)[b1A2]	1.63 (2.04–0.85)[aA]	0.30 (0.85–<0*)[aB]	<0 (<0–<0)
Plastic	2.73 (2.83–2.37)[aA]	<0 (0.70–<0)[bB]	<0 (<0–<0)[bB]	<0 (<0–<0)
Glass	2.62 (2.94–2.04)[aA]	0 (0.78–<0)[bB]	<0 (<0–<0)[bB]	<0 (<0–<0)
Stainless steel	2.80 (2.95–2.51)[aA]	<0 (0–<0)[bB]	<0 (<0–<0)[bB]	<0 (<0–<0)
Cross-contamination to tomatoes (log CFU/g)				
Wood	2.51 (3.10–1.81)[aA]	1.80 (2.65–<1**)[aAB]	<1 (1.70–<1)[aB]	<1 (<1–<1)
Plastic	2.71 (3.46–1.88)[aA]	<1 (2.00–<1)[bB]	<1 (<1–<1)[aB]	<1 (<1–<1)
Glass	2.31 (3.54–1.90)[aA]	<1 (2.00–<1)[bB]	<1 (<1–<1)[aB]	<1 (<1–<1)
Stainless steel	2.45 (3.34–1.60)[aA]	<1 (<1–<1)[bB]	<1 (<1–<1)[aB]	<1 (<1–<1)

[1]Different letters indicate differences between surfaces for the same procedure ($p < 0.05$).
[2]Different letters indicate differences between procedures for the same surface ($p < 0.05$).
*Undetectable counts ($<1.0 \times 10^0$ CFU/cm^2).
**Undetectable counts ($<1.0 \times 10^1$ CFU/g).

recovered from stainless steel than from wood. The objective of treating plastic with triclosan is to produce a cutting board with antibacterial activity on its surface (Kalyon and Olgun, 2001). However, this effect was not observed in the present study (Table 1) when triclosan-treated cutting boards were compared with glass and stainless steel ($p > 0.01$).

It should be emphasized that the type of surface did not influence Salmonella counts in tomatoes handled on surfaces that were not cleaned (Table 1). Differently, Moore et al. (2007) observed that counts of S. Typhimurium transferred to contaminated cucumbers were greater when cucumbers were handled on stainless steel and Formica cutting boards compared with those recovered when cucumbers were handled wood and plastic.

In procedure 2, the recovery of S. Enteritidis NAL$^+$ from wooden cutting boards was significantly greater ($p < 0.01$) than from the other materials (Table 1). Abrishami et al. (1994) also reported that after cleaning with cold water, more bacteria remained adhered to wood than to plastic and that rinsing alone was not able to remove bacteria from wooden cutting boards as easily as from plastic cutting boards.

Although the results obtained for procedure 2 showed that water significantly removed ($p < 0.01$) the S. Enteritidis NAL$^+$ population from triclosan-treated plastic, glass and stainless-steel cutting boards, compared with procedure 1 (Table 1), these results should be analyzed with caution because there was a large number of samples of both surfaces and tomatoes that tested positive for Salmonella (Table 2). This finding indicates that rinsing only with cold water is not the best method to clean cutting boards after handling raw chicken.

After procedure 3, small amounts of the microorganism were recovered from wood and were undetectable on triclosan-treated plastic, glass, stainless steel, and tomatoes (Table 1). After handling poultry meat contaminated with S. Newport on plastic surfaces, Ravishankar et al. (2010) also observed low counts (<1 log CFU/g or cm^2) of the microorganism on the surfaces after washing with soap, warm water, and vigorously scrubbing and on lettuce later handled on these surfaces.

Cleaning procedure 3 showed significantly reduced ($p < 0.01$) S. Enteritidis NAL$^+$ populations on all surfaces compared with procedure 1 (Table 1). Consequently, S. Enteritidis cells were not detected in the tomato samples handled on triclosan-treated plastic, glass and stainless steel (Table 1). However, when the results of Salmonella detection are analyzed, positive results were observed for surfaces and tomatoes (Table 2), demonstrating that the procedure was able to reduce the number of, but not completely remove, pathogen cells. Thus, cross-contamination may occur. This result was also obtained by researchers who used soap and water to clean cutting boards (Scott and Bloomfield,

1990, 1993; Cogan et al., 1999; Cogan et al., 2002; Barker et al., 2003; de Jong et al., 2008).

When hypochlorite was used to clean the surfaces, Salmonella detection showed that although there was a reduction in the number of positive samples of all surfaces and of tomatoes handled on triclosan-treated plastic, glass and stainless steel when compared with the other procedures (Table 2) and that no statistical difference ($p > 0.01$) was observed between the surfaces and tomatoes with respect to Salmonella counts (Table 1), only stainless steel and tomatoes handled on it showed to be negative after procedure 4 (Table 2). These results demonstrate the superiority of stainless steel when compared with the other materials in terms of ease of cleaning and safety for food handling due to the elimination of the risk of cross-contamination.

Regarding the methods used to recover microorganisms from contaminated surfaces, the literature presents several alternatives. However, there is no consensus on a standard method (Moore and Griffith, 2002). One of the disadvantages of using swabs is that they are flexible and possibly inefficient because the pressure applied to the surface during sampling is too light (Moore et al., 2007). This fact may explain the low counts obtained during the recovery of the pathogen from the surfaces analyzed. However, some studies report the use of alginate swabs, which were used in our study, is the best and most sensitive method of surface sampling (Cliver, 2006).

It may be concluded that cutting boards are critical factors when handling raw foods contaminated with pathogenic microorganisms. Among the surfaces analyzed, wood was considered to be the most difficult to clean and stainless steel was the easiest. Cleaning with cold water and dish soap followed by vigorous scrubbing and rinsing, practices commonly followed in household kitchens to clean cutting boards, may reduce but not eliminate the risk of exposure to the pathogen. By comparing the various cleaning procedures and types of surfaces, stainless steel cleaned with water and dish soap and vigorously scrubbed, rinsed, and disinfected with

Table 2
Rates of positive samples for S. Enteritidis NAL$^+$ after transfer to surfaces and cross-contamination to tomatoes after four different cleaning procedures.

	1	2	3	4
Transfer to surfaces (in %)				
Wood	100	100	100	77
Plastic	100	100	46	8
Glass	100	100	77	31
Stainless steel	100	77	31	0
Cross-contamination to tomatoes (in %)				
Wood	100	100	100	100
Plastic	100	100	100	31
Glass	100	92	77	54
Stainless steel	100	77	15	0

hypochlorite showed to be the safest material for handling tomatoes because it achieved the lowest level of cross-contamination.

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