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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 cm^2 (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-cm^2 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