

Research Note

Transfer of *Salmonella montevideo* onto the Interior Surfaces of Tomatoes by Cutting†

CHIA-MIN LIN and CHENG-I WEI*

*Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida 32611-0370, USA*

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**ABSTRACT**

*Salmonella* contamination of precut watermelon, tomatoes, and cantaloupes was responsible for several outbreaks of salmonellosis. To better understand the relationship between bacterial doses and their transfer onto cut surfaces by using a knife, rifampicin-resistant *Salmonella montevideo* at 7, 70, 700, or 7,000 CFU in Butterfield’s buffer (BPB) or tryptic soy broth (TSB) was added to the stem scars of tomatoes. Tomatoes were cut from the stem scar to blossom end using a sterilized knife. After stem scars were aseptically removed, cut surfaces were placed on tryptic soy agar–rifampicin (TSA-RIF) plates or processed by a broth enrichment method to determine if *S. montevideo* had been transferred to the cut surface. *S. montevideo* was recovered in a dose-related fashion using both methods. A higher recovery rate was obtained with bacterial inocula in TSB than in BPB, and also with broth enrichment rather than the direct plating method. The distribution of the transferred *S. montevideo* on the cut surface of contaminated and noncontaminated tomatoes with a knife was related to the inoculum dose added to the stem scars. *S. montevideo* colonies were found to cluster at the stem scar region with the lower inoculum dose. However, when a higher inoculum dose was used, the colonies spread from the stem scar region to the center and bottom of cut tomatoes, or they were transferred to another uninoculated tomato by the contaminated knife. Therefore, the safety operation criteria recommended by FDA to wash fruits before cutting, to use clean and sanitized utensils and surfaces when preparing cut fruits, and to store the cut fruits below 7°C should be followed in preparing tomato slices to minimize salmonellosis outbreaks caused by this product.

Key words: *Salmonella montevideo*, tomatoes

*Author for correspondence. Tel: (352) 392-1991 Ext. 251; Fax: (352) 392-9467; E-mail: cwei@gnv.ifas.ufl.edu*
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the cut surface of a watermelon after its suspension was artificially swabbed on the rind and the watermelon cut with a clean knife. However, no quantitative data was given in this study. We still do not understand the relationship between the bacterial numbers on fruit surfaces and their introduction and/or transfer into the interior by means of a cutting knife, nor do we know the number of fruits that can be contaminated by a cutting knife that has previously been used to slice a contaminated fruit and then left unwashed before cutting another fruit. Therefore, the objectives of this study are to use tomatoes artificially contaminated with *S. montevideo* as the model system for obtaining such qualitative data to better understand the behavior of foodborne pathogens transferred from the surface to the interior of fresh fruits.

**MATERIALS AND METHODS**

**Tomatoes**

Mature green tomatoes (*Lycopersicum esculentum* cv. Agriset and cv. Solimar) were obtained from the Southwest Florida Research and Education Center at Immokalee, University of Florida. The tomatoes were kept at room temperature for ripening. Before each experiment, fully ripe tomatoes were washed under running tap water and wiped with paper towels.

**Bacterial culture**

A rifampicin-resistant strain of *S. montevideo* was obtained from Dr. Mark L. Tamplin, University of Florida at Gainesville. This bacterial culture was prepared from the Centers for Disease Control and Prevention isolate G4639, obtained from a patient in Illinois in July 1993. The culture was maintained in our laboratory on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) containing 0.1 g of rifampicin (Sigma Chemical Co., St. Louis, MO) per liter (TSA-RIF). On the day of the experiment, a bacterial colony was transferred to 15 ml of tryptic soy broth (TSB) (Difco) and incubated at 37°C for 7 h with constant shaking at 100 rpm. Bacterial density was determined by measuring the absorbance of the suspension at 540 nm using a Beckman (Fullerton, CA) DU-40 spectrophotometer. The suspension with an absorbance reading of 0.8 to 1.2 had a bacterial concentration of approximately $1 \times 10^9$ to $3 \times 10^9$ CFU/ml. The bacterial population was determined by surface plating in triplicate on TSA-RIF plates, using 0.1 ml of the serial dilutions of the bacterial suspension in Butterfield's phosphate buffer (BPB, pH 7.2) and a sterile glass rod. Bacterial colonies on the plates were counted after incubation at 35°C for 24 h.

**Determination of the rate of bacterial detection following cutting of inoculated tomatoes**

Twenty-five μl of bacterial suspension at $2.8 \times 10^2$, $2.8 \times 10^3$, $2.8 \times 10^4$ or $2.8 \times 10^5$ CFU/ml in BPB or TSB was placed on the stem scars of 10 tomatoes to yield a final population of 7, 70, 700, or 7,000 CFU. Two tomatoes inoculated with 25 μl of BPB or TSB served as the negative control. The positive control was 4 tomato halves inoculated with 7 CFU of *S. montevideo* in 25 μl of BPB or TSB on the cut surface. Bacterial suspension on the stem scar or cut surface of tomato halves was allowed to dry at room temperature with circulating air.

After the bacterial suspension in BPB or TSB on the stem scar had dried, each tomato was cut vertically from the stem scar to the blossom end with a knife previously covered and wiped with 95% ethanol and then flame. The stem scars of the tomato halves were removed using another alcohol-sterilized knife and the tomato halves were individually placed with the cut side down on a TSA-RIF plate, moved back and forth on the whole agar surface and then discarded. The positive control was processed similarly after removal of the stem scar. Colonies found on TSA-RIF plates following incubation at 35°C for 24 h were streaked on xylose lysine deoxycholate (XLD) and bismuth sulfite (BS) agar plates. The colonies showing typical characteristics of *Salmonella* spp. on both agar plates were then confirmed with triple sugar iron (TSI) and lysine iron agar (LIA).

The comparison with bacterial suspension in BPB or TSB was conducted simultaneously. The experiments were repeated four times and the rate of bacterial detection at each inoculation dose in 80 tomato halves was determined.

Four tomatoes each with an inoculum dose of 7, 70, 700, or 7,000 CFU in 25 μl of TSB on the stem scar were used for determination of bacterial recovery by applying the broth enrichment method of Lin et al. (10). After the tomatoes were cut into halves and the stem scars removed as previously described, each tomato half was cut again in a parallel direction with the first cut from stem scar to blossom end to obtain two portions. The 25-g slice between the first and second cut was then homogenized in a sterile blender jar with nine volumes (wt/vol) of half-strength TSB (½ TSB) for 30 s at low speed followed by another 30 s at high speed, while the remaining tomato was discarded. After the homogenate was transferred into a sterile flask and incubated at 35°C for 6 h with constant shaking at 100 rpm, a 2-ml aliquot was transferred into 18 ml of selenite cystine broth (SC) or tetrathionate broth containing brilliant green and iodine-KI. Following incubation of the mixture for 15 h at 35°C with constant shaking at 100 rpm, a loopful of each selective broth was streaked onto XLD and BS plates. Colonies with typical characteristics of *Salmonella* spp. on these plates following incubation at 35°C for 24 h were confirmed by culture on TSA-RIF, TSI, and LIA. The experiment was repeated once.

Studies were also conducted to determine if prolonged incubation for 4 h of cut tomatoes at room temperature would enhance bacterial recovery on the cut surface. Two sets of 5 tomatoes each were inoculated at the stem scars with $6.6 \times 10^3$ CFU in 25 μl of TSB. After the bacterial suspension had dried, the tomatoes were cut into halves through the stem scar to blossom end. One set of tomato halves (10) was placed on TSA-RIF plates immediately after the stem scars were removed as previously described, while the other half was covered with aluminum foil and left at room temperature for 4 h before being placed on TSA-RIF plates. The procedures for recovering and confirming *S. montevideo* were the same as previously described. The experiment was repeated once.

**Bacterial transfer by using a cutting knife from inoculated to uninoculated tomatoes**

Four groups of 5 tomatoes each were inoculated on the stem scars with a bacterial population of $4.4 \times 10^3$, $5.4 \times 10^4$, $6.6 \times 10^4$, or $5.0 \times 10^5$ CFU in 25 μl of TSB. After the suspension had dried, a knife previously covered and wiped with 95% ethanol and then flame was used to cut the inoculated tomato into two halves from the stem scar to blossom end. This contaminated knife without further sterilization was then used to cut, in sequence, 2 uninoculated tomatoes (4 halves). The stem scars were removed from the 2 inoculated tomato halves but not the 4 uninoculated halves. The inoculated and uninoculated halves were each placed on a TSB-RIF plate, with the cut side facing the agar surface, to recover *S. montevideo* as previously described. Bacterial colonies were confirmed by culture on XLD and BS plates, and then TSI and LIA.
slants. The occurrence of S. montevideo on each set of tomato halves (the inoculated and uninoculated) was then determined. The experiment was repeated once or twice.

Bacterial distribution on the cut surface of tomato halves

Four tomatoes were each inoculated with $6.25 \times 10^3$, $6.25 \times 10^4$, or $9.5 \times 10^5$ CFU of S. montevideo in 25 µl of TSB at the stem scar. After the suspension had dried, the tomatoes were cut through the stem scar to the blossom end into 2 halves. Without removing the stem scar, each half tomato was placed with the cut side down on a TSA-RIF plate for 1 min. After the tomato halves were discarded, the TSA-RIF plates were marked for stem scar position and then incubated at 35°C for 24 h. The distribution of bacterial colonies on the TSA-RIF plates was photographed.

RESULTS AND DISCUSSION

Outbreaks of food-borne illness from consumption of fresh produce are, in most cases, caused by cross-contamination from other food items or by handlers. However, some outbreaks are caused by the contaminated foods themselves rather than by mishandling (Beuchat, 1996). S. montevideo added to the stem scars can be introduced into the interior of tomatoes by the physical action of cutting. Both the bacterial population and recovery method affected the detection of S. montevideo on contaminated tomato halves. Except for those tomatoes inoculated with 7 CFU, the use of direct plating method recovered S. montevideo in a dose-related fashion from all inoculation levels (Table 1). A higher recovery rate was obtained from tomatoes inoculated with S. montevideo in TSB than in BPB. The broth enrichment method offered better recoveries than the direct plating method (Table 1). It also allowed detection of bacterial contaminants on the cut surface of tomatoes previously inoculated with 7 CFU of S. montevideo onto the stem scars. This is because S. montevideo introduced and/or transferred into tomatoes by using a knife could recover and then proliferate in the enrichment broth during incubation. However, the broth enrichment method was more complicated than direct plating. It also took 1 more day to complete the assay.

The results also showed that the introduction and/or transfer of bacterial contaminants by using a cutting knife could occur at a bacterial population as low as $<10$ CFU at the stem scar (Table 1). The failure to detect S. montevideo on the cut surface of tomatoes inoculated with 7 CFU of the microorganism by the direct plating method does not mean that the bacteria were not introduced and/or transferred by the cutting knife onto the interior of the fruits. TSB has been demonstrated to be a better support for S. montevideo survival on tomato surfaces as well as a better protector against the bactericidal effect of aqueous chlorine (11). These attributes could contribute to the higher recovery efficiency achieved with the bacterial suspension in TSB compared to recovery in Butterfield’s buffer. Under natural conditions, the contaminated bacteria are usually delivered by surface organic substances which could provide a better microenvironment for bacterial survival on fresh produce.

<table>
<thead>
<tr>
<th>Inoculum dose on stem scar (CFU of S. montevideo)</th>
<th>Direct plating</th>
<th>Broth enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>None: BPB or TSB</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CFU on cut surface:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 (direct plate)</td>
<td>100 (80/80)</td>
<td>100 (48/48)</td>
</tr>
<tr>
<td>7 (enriched)</td>
<td>100 (80/80)</td>
<td>100 (80/80)</td>
</tr>
<tr>
<td>70</td>
<td>3.8 (3/80)</td>
<td>6.3 (5/80)</td>
</tr>
<tr>
<td>700</td>
<td>16 (13/80)</td>
<td>25 (20/80)</td>
</tr>
<tr>
<td>7,000</td>
<td>28 (22/80)</td>
<td>36 (29/80)</td>
</tr>
</tbody>
</table>

Therefore, the removal of organic substances from the surface of fresh produce by washing will serve as a control point for surface sterilization.

The storage of cut tomatoes for 4 h at room temperature did not enhance the recovery rate of about 50% for S. montevideo. The colony numbers on TSA-RIF plates with the tomato group placed immediately after cutting did not differ from that following 4 h of incubation (data not shown). However, Salmonella spp. were reported to grow rapidly in tomato slices stored at 22 or 30°C for 24 h (2).

The distribution of the introduced and/or transferred S. montevideo from stem scar to the center of the tomatoes by a cutting knife was related to the inoculum dose applied at the stem scar. At the lower inoculum dose of $6.25 \times 10^3$ CFU, S. montevideo colonies were found to cluster at the stem scar region on TSA-RIF plates. However, as the inoculum levels were increased, the colonies were found to spread from the stem scar region to the center and bottom of cut tomatoes along the cutting direction of the knife (Fig. 1). When the inoculum dose added to the stem scar was increased further, bacteria could contaminate and remain on the knife and then be transferred by the knife to the cut halves of the second tomato (Table 2). Again, such transfer and/or introduction of bacterial contaminants from contaminated to noncontaminated tomatoes by means of a cutting knife was also related to the bacterial loads present in the contaminated tomatoes. For example, S. montevideo was not detected from the cut surface of the uninoculated tomatoes using an unsterilized knife which had been used to first cut tomatoes inoculated at the stem scar with $4.4 \times 10^3$ or $5.4 \times 10^4$ CFU (Table 2). However, when the inoculum population was increased to $6.6 \times 10^3$ and $5.0 \times 10^6$ CFU, S. montevideo was recovered from the first set of uninoculated tomatoes (Table 2). No bacteria were recovered from the second set of uninoculated tomatoes due to losses of bacterial contaminants from the knife following cutting the first set of uninoculated tomatoes.

These findings confirm the results of previous studies by Gayler et al. (7) and the Center for Disease Control (5) that the inside of watermelons could be contaminated with Salmonella spp. during slicing either from the contaminated...
TRANSFER OF SALMONELLA MONTEVIDEO INTO TOMATOES WITH A KNIFE

FIGURE 1. Distribution of S. montevideo on the cut surface of representative tomato halves by using a knife to cut through the stem scars of tomatoes inoculated with $6.25 \times 10^3$, $6.25 \times 10^4$, or $9.5 \times 10^5$ CFU in 25 μl of tryptic soy broth. The arrow indicates the position of the tomato stem scar.

TABLE 2. The percent detection of Salmonella montevideo on different sets of tomatoes following transfer from the contaminated stem scar of the first set of tomatoes with a cutting knife

<table>
<thead>
<tr>
<th>Inoculum dose (CFU S. montevideo)</th>
<th>1st set</th>
<th>2nd set</th>
<th>3rd set</th>
</tr>
</thead>
<tbody>
<tr>
<td>$4.4 \times 10^3$</td>
<td>33 (10/30)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$5.4 \times 10^4$</td>
<td>60 (18/30)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$6.6 \times 10^5$</td>
<td>93 (28/30)</td>
<td>10 (3/30)</td>
<td>0</td>
</tr>
<tr>
<td>$5.0 \times 10^6$</td>
<td>95 (19/20)</td>
<td>45 (9/20)</td>
<td>0</td>
</tr>
</tbody>
</table>

Precautions should be taken in handling fresh produce and fruits to minimize such contamination from the surface to the interior of the foods or from a contaminated to a noncontaminated food by using a cutting knife. The U.S. Food and Drug Administration has recommended that retail establishments wash melons before cutting and use clean and sanitized utensils and surfaces when preparing cut melons. In addition, all cut melons need to be stored at below 45°F (7°C) and displayed or served within 4 h if they are not refrigerated (8). These same safety criteria for preparing cut melons should also be employed in handling tomato slices. Since S. montevideo can survive on the surface of tomatoes for days, especially at the stem scars and growth cracks (11, 12), it is especially important to wash the fruits and remove stem scars and growth cracks before preparing tomato slices to minimize salmonellosis outbreaks from consumption of this food.

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REFERENCES