Transfer of Firmly Attached $^{32}$P-Salmonella typhimurium from Raw Poultry Skin to Other Surfaces

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ABSTRACT

Salmonellae adhere firmly to poultry skin during processing. Loosely attached bacteria cross-contaminate work surfaces. This study was undertaken to determine if firmly attached bacteria present a health hazard through transfer to work surfaces. Attached $^{32}$P-labeled S. typhimurium cells were serially rinsed with 2 to 4 L of Salmonella-free potable tap water or with sterile 0.85% NaCl. Rinsing removed 61 to 89% of attached labeled cells. However, after rinsing, 11 to 39% of cells remained attached, and of these, 3 to 10% were able to detach and transfer from skin to stainless steel surfaces. It was concluded that large rinse volumes may not remove all attached salmonellae from poultry skin surfaces and the potential for cross-contamination does exist.

Contamination of raw poultry with human enteric pathogens, such as Salmonella, has been, and continues to be of concern to public health authorities and to the poultry industry. Poultry is frequently implicated as a source of bacterial food poisoning, often in domestic environments or food service establishments where cross-contamination of foods can occur (1). Furthermore, the cost of product recall in the food industry due to foodborne disease is very high (8).

Bacteria attach or adhere firmly to poultry carcasses during processing (2,3,4,5,6,7). However, the mechanism(s) by which they attach and factors influencing the degree of attachment have not been fully elucidated. Some investigators believe that while it may be impossible to eliminate pathogens from poultry carcasses, those that are firmly attached are potentially less hazardous than those cells that are loosely attached because loosely attached bacterial cells may detach easily and contaminate work surfaces, kitchen utensils and other foods (2). This cross-contamination of the work environment would then pose a potential health hazard; whereas, cells which are firmly attached would probably remain attached to the carcass, be destroyed during cooking, and pose no health risk.

Therefore, this study was undertaken to evaluate the potential for transfer of firmly attached $^{32}$P-labeled Salmonella typhimurium cells from raw poultry skin to other surfaces.

MATERIALS AND METHODS

Use of radioactively labeled bacterial cells was assumed to be more sensitive and more rapid than conventional microbiological procedures. Selection of $^{32}$P (as orthophosphate) was based on short half-life and ease of counting. Active Salmonella typhimurium (ATCC 14028) was allowed to grow in nutrient broth (Difco) for 24 h at 35°C. Stationary phase cells were harvested by centrifugation (1600 x g, 9°C for 10 min) and washed using sterile 0.85% saline solution. Washed cells were resuspended in 0.06 M sodium phosphate buffer (pH 7) containing 0.1% glucose and 200-400 μCi of $^{32}$P (as orthophosphate) to yield approximately a 10⁹ viable cells/ml of buffer. These conditions had been predetermined to yield optimal $^{32}$P uptake (mean count of 1.2 x 10⁵ cpm/ml of cell suspension). Following 3 h of incubation at 35°C in a water bath shaker (Aqua Therm Water Bath Shaker, New Brunswick Scientific, New Brunswick, N.J.), the cells were harvested, washed and resuspended in 0.06 M sodium phosphate buffer, and used.

Two pieces of excised breast skin (12 cm²) from the same plucked but unviscerated broiler carcass which was obtained from a commercial processing plant were inoculated with 20 μl buffer containing 2 x 10⁷ $^{32}$P-cells. A different broiler was used in each experiment. Cells were spread on the entire piece of skin using a sterile hockey stick. Inoculated skin pieces were covered with foil (without skin contact) to prevent drying and held for 1 min. One skin piece was used as a control to determine recovery, the other was rinsed and then subjected to the following treatments.

(a) The inoculated skin pieces (one per treatment) were rinsed 10 times with 200 ml of sterile 0.85% saline solution (A) or with tap water (B) for a total rinse of 2000 ml and each experiment was repeated twice.

(b) The inoculated skin pieces were rinsed 30 times with 100
ml of sterile 0.85% saline solution (C) to yield a total rinse volume of 3000 ml; this experiment was repeated three times.

(c) The inoculated skin pieces were rinsed 15 times with 200 ml of tap water (D) for a total volume of 3000 ml; this experiment was repeated twice.

(d) The inoculated skin pieces were rinsed 20 times with 200 ml of tap water (E) for a total of 4000 ml; this experiment was repeated three times. Cells which did not rinse off were considered firmly attached.

Each rinse volume was collected separately and filtered through a 0.45 μm Metricel Membrane filter (25 mm, Gelman Sciences, Inc., Ann Arbor, MI). The filter was then placed in a scintillation vial containing 10 ml of ScintiVerse II (Fisher Scientific Co., Fair Lawn, N.J.) and counted in a liquid scintillation counter (Beckman LS5801: Beckman Instruments, Inc., Fullerton, CA). The instrument was programmed to count all samples to ±2% error or for 10 min, whichever occurred first. All samples were corrected for background. Viability of cells was confirmed by plating on BG Sulfa Agar (Difco) and incubating for 35°C for 24 h.

After the final rinse, the inoculated skin side was brought in contact with a stainless steel surface and held in place for 1 min. To avoid losses which may result from maceration, the whole skin piece, which was relatively small, was then placed in a scintillation vial and counted as previously described. The stainless steel surface was swabbed with filter paper which was placed in a scintillation vial and counted to determine the transferability of any cells. Inoculated control skin pieces were placed in a scintillation vial without rinsing and counted as above. All data were computed to percentages based on total radioactive counting of the 32P-cells recovered from rinses, skin and the stainless steel surface. Results from counting were corrected for the half-life of 32P.

Viability of 32P-cells was determined in separate experiments as follows. Each rinse portion was divided into two equal parts and filtered separately. One filter was placed in a tube containing 9 ml of peptone (1%) (Difco), incubated at 35°C for 24 h then streaked on BG sulfa agar plates. The other filter was used for scintillation counting. After rinsing and contact with the stainless steel surface, inoculated skin pieces were stomached 1 min in 10 ml of saline (0.85%) solution, serially diluted and spread on BG sulfa agar (Difco) plates. After skin contact, the stainless steel was placed in a plastic bag containing 10 ml of saline (0.85%) solution and rinsed by shaking manually for 1 min. Serial dilutions of the rinse were spread on BG sulfa agar plates. All spread plates were incubated at 35°C for 24 h.

RESULTS AND DISCUSSION

Use of 32P-labeled cells was initially assumed to be a more sensitive and rapid technique than conventional microbiological procedures. The technique proved to be rapid but a degree of variability was observed in 32P recovery from the rinses, probably due to a dilution factor which reduced radioactive counts to background level.

From 81 to 100% radioactive counts were recovered from the control (unrinsed) skin which established the basic recovery rate. Theoretically, the total recovery during the experiments from skin pieces, rinses and the stainless steel surface should have been approximately the same as recovered from the control. In practice, recovery varied between 33 and 84% of counts from the controls (Table 1). The best total recovery (84%) was obtained when the 3000 ml saline solution rinse was used. It was not possible to account for these losses by any refinements or improvements in technique of rinsing, handling of glassware and samples or by taking time-lapses and 32P half-life into account. Mean radioactivity recovered from rinses was 91% and mean total recovery from the experiments (rinses, skin, and stainless steel surface) was 55% of that, leaving a mean loss of 36% radioactivity not accounted for. Much greater variation in recovery occurred in the combined counts from rinsed skin, rinses and stainless steel than from control skin, with most of the variation occurring in the counts from the rinse water or saline solution indicating that the lost radioactivity probably occurred during the rinsing procedure. This observation was verified by determining the presence or absence of viable cells in each rinse portion, on skin and stainless steel. By enrichment procedures, viable cells were recovered in one experiment from 15 of 20 rinses, whereas only 6 of these rinses had a radioactive count above background level. In another experiment, 19 of 20 rinses contained viable counts, whereas radioactivity above background level was detected in only 4 of these samples. Viable cells were recovered from the stomached skin pieces (105/ml) after rinsing and following contact with the stainless steel surface, and from the stainless steel surface (103/ml of rinse). These results explain the apparent "losses" in radioactivity which, due to the low level of 32P uptake by the cells, was diluted in the rinses to levels below detection by labeling procedures.

There was no particular pattern to the number of bacteria that were rinsed off in the 10 to 30 rinse portions that made up the total rinse volume. The largest number (cpm) that rinsed off sometimes occurred in the initial and other times in the later rinse portions. Statistical analysis of the data showed that there was no significant difference in the number of cells that were rinsed off by saline solution or water and no significant differences were found due to rinse volume used. However, in all experiments, rinsing removed a significantly larger number of cells than were left on the skin (P<.10).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean cpm in spread-inoculum</th>
<th>Control skin (unrinsed)</th>
<th>Skin, rinses, stainless steel (combined count)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3234</td>
<td>2801</td>
<td>87</td>
</tr>
<tr>
<td>B</td>
<td>3365</td>
<td>3122</td>
<td>93</td>
</tr>
<tr>
<td>C</td>
<td>2168</td>
<td>1996</td>
<td>92</td>
</tr>
<tr>
<td>D</td>
<td>1598</td>
<td>1601</td>
<td>100</td>
</tr>
<tr>
<td>E</td>
<td>1214</td>
<td>982</td>
<td>81</td>
</tr>
</tbody>
</table>

*0.02 ml of inoculum (2 X 107 cells).
*Based on cpm recovered from control skin which established level of expected recovery.
When broiler breast skin pieces were rinsed 10 times using a total volume of 2000 ml of tap water or saline solution, 62 to 63% of the radioactivity recovered was in the rinses, 33 to 34% remained on the skin pieces after contact with a stainless steel surface, and 3 to 5% was transferred to the stainless steel surface (Table 2). A similar pattern was observed for skin and 5 to 10% was transferred to the stainless steel surface. Approximately one-third of the counts remained attached even after extensive rinsing with up to 2000 to 3000 ml of saline solution or tap water, most of the bacteria which attach to poultry skin and which cannot be rinsed off are able to detach and contaminate other surfaces.

In conclusion, the technique of using labeled salmonellae in these studies could not be used with low levels of cells, as had been hoped, because of the low level of $^{32}$P uptake by cells and the dilution factors inherent in the experiments. Even though data presented do not represent absolute values because of dilution factors, none-the-less, the point was effectively made that some of the bacteria which attach to poultry skin and which cannot be rinsed off are able to detach when in contact with work surfaces and, consequently, pose a realistic health hazard because of their ability to cross-contaminate the food preparation environment.

**REFERENCES**


4. Lillard, H. S. 1986. Role of fimbriae and flagella in the attachment of $^{32}$P-Salmonella typhimurium to broiler breast skin. J. Food Sci. 51:54-56

