Total Rinse Method for Microbiological Sampling of the Internal Cavity of Eviscerated Broiler Carcasses

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A method is described for spray-rinse sampling the entire visceral cavity of broiler chicken carcasses for microbiological analyses. The method is designed to provide a more comprehensive sample than swabbing or excision of small areas.

During a microbiological study of the internal cavity of eviscerated broiler chicken carcasses it became desirable to develop a new method for sampling the total cavity surface. Sampling the internal cavity is inconvenient because of limited accessibility due to the size and location of the eviscerating cut. Also, irregularity of the cavity surface in the backbone area causes difficulty in obtaining representative samples with common methods.

Patterson (6) and Baldock (1) have reviewed numerous methods for sampling internal and external poultry carcass surfaces. These include swabbing areas of various sizes and locations with moistened cotton or alginate swabs, exciting small quantities of surface tissue which are subsequently blended or shaken, spray rinsing several square-centimeter areas, and rinsing parts or whole carcasses. These methods, with the exception of the total bird rinse, may not provide a representative microbiological assessment because of the limited areas sampled and the known variations of contamination of different parts of a carcass (4). We have devised a spray-rinse method for sampling the total internal cavity of broiler carcasses in order to achieve a more representative analysis of its microbiological condition.

MATERIALS AND METHODS

Rinse sampling procedure. Most sampling was performed in a commercial poultry processing plant on freshly eviscerated carcasses taken directly from the line immediately after the final wash. A carcass was placed on its back, and excess neck skin was removed by using sterile forceps and scissors. Next, a polystyrene disposable funnel was introduced, stem first, through the opening cut in the abdominal wall, passed through the visceral cavity with the aid of a sterile disposable pipette, and forced into the neck opening so that the stem protruded beyond the opening (Fig. 1). A tight seal was formed between the funnel and neck area muscles so that the funnel served as a rinse collection device when the bird was hung by the hocks on a shackle. The visceral cavity was then spray rinsed with two 50-ml quantities of sterile 1% (wt/vol) sodium citrate which drained through the funnel and was collected in a sterile milk dilution bottle. Spraying was done with a sterile disposable 50-ml syringe equipped with a 14-gauge stainless-steel cannula that was curved to about a 45-degree angle and cramped at the end to give a fan-type spray pattern. During rinsing the spray was directed into all parts of the cavity with as much pressure as could be applied by hand. A fresh syringe and funnel were used for each carcass, and the cannula was dipped in 95% ethanol and flame before each use.

Swab sampling procedure. Carcasses were sliced longitudinally through the breast with an alcohol-flamed knife and spread open to avoid contact with visceral cavity surfaces. The entire visceral cavity surface of each carcass was swabbed with three sterile alginate swabs moistened with sterile 1% sodium citrate. Each swab was vigorously rubbed over an area of about 150 cm² according to the “total objective swab (TOS)” method described by Mossel and Bichl (5). All three swabs were deposited in 30 ml of sterile 1% sodium citrate and shaken to dissolve the alginate.

Artificial contamination procedure. Recovery efficiency of sampling methods was determined using a nalidixic acid-resistant Salmonella typhimurium strain to artificially contaminate carcasses. On milli-liter of a saline suspension containing 10⁷ cells/ml prepared from an 18-h culture was randomly pipetted over the surfaces of each visceral cavity. A 5-minute contact time was allowed before sampling. All experiments using the S. typhimurium marker organism were performed in the laboratory.

Cultural methods. Rinse samples were stored in ice for transport to the laboratory. The delay between sampling and plating did not exceed 3 h. Total aerobic plate counts were determined by making appropriate dilutions in sterile 1% (wt/vol) sodium citrate followed by plating in Standard Methods agar (BBL). Plates were incubated 72 h at 20 C.

The S. typhimurium artificial contaminant was grown on brain heart infusion agar (Difco) incubated at 35 C. It was detected by surface plating dilutions of swab and rinse samples on MacConkey agar (Difco) containing 100 μg of nalidixic acid per ml.
Funnels. The size of the large end of the funnel cone was important relative to achieving the desired location and fit in the carcass neck opening. We found empirically that a cone diameter of 36 mm was suitable for the carcass size range normally encountered in this study. Since disposable funnels of this size were not commercially available, we cut larger funnels to size on a band saw. The funnels were then washed, heated for 5 min in 85°C water, and packaged in sterile plastic bags.

RESULTS AND DISCUSSION

Recovery of rinse solution. Success of the method depended mainly upon recovery of a high percentage of rinse solution and efficient removal of microbial contaminants. A total of six replications with 30 samples each was performed over a period of several months. Recoveries from a 10-sample segment of a typical experiment are shown in Table 1. The means, standard deviations, and coefficients of variability for all replicates are given in Table 2. The overall mean for 180 samples was 90.09 ml recovered (standard deviation, 9.16; coefficient of variability, 10.16). Analysis of variance among replication means indicated no significant difference \( F = 1.57 < F_{0.05(V_1, V_2, V_3, V_4, V_5, V_6, V_7, V_8, V_9, V_{10}, V_{11}, V_{12}, V_{13}, V_{14}, V_{15}, V_{16}, V_{17}, V_{18}, V_{19}, V_{20}, V_{21}, V_{22}, V_{23}, V_{24}, V_{25}, V_{26}, V_{27}, V_{28}, V_{29}, V_{30})} = 2.21 \) and a standard error of 1.65. Failure to recover all the rinse solution may have been caused by leakage around funnels, absorption by carcass tissues, or drainage missing the collection bottle.

Microbial counts. Total aerobic counts from a typical trial are given in Table 1. We selected the unit, microorganisms per carcass visceral cavity, (i) because it was adequate and convenient for our experiments and (ii) because of the difficulty of determining visceral cavity surface area. Comparison of our counts with those obtained by other visceral cavity sampling methods using gravimetric (2) or surface area (8) units is not possible. Although no data are available in the literature relating carcass weight to visceral cavity surface area, the methods described by Goresline and Haugh (3) and Simonsen (7) could be used to obtain this information and thereby permit determination of counts per square centimeter.

A comparison of total aerobic plate counts and recovery of an artificially applied \( S. typhimurium \) contaminant was made on a count per visceral cavity basis using the mean of 10 samples for both rinse and swab methods. The mean total aerobic plate counts \((1.1 \times 10^9/cavity)\) for the rinse method was 4.2 times greater than that obtained by the swab method \((2.6 \times 10^7/cavity)\). Recovery efficiency of \( S. typhimurium \) was 68% by rinsing and only 9% by swabbing. The differences between the mean \( \log_{10} \) total aerobic plate counts per cavity obtained by the two methods, as well as the mean \( \log_{10} S. typhimurium \) recovered per cavity, were statistically significant at the 5% level of confidence (see Table 3). Thus, the rinse method gives higher total counts and better recovery efficiency of artificial contaminants.

Practical experiences. Mention should be made of some experiences encountered using the total internal rinse method. Occasionally a
TABLE 3. Comparison of internal rinse and swab sampling methods applied to broiler visceral cavities for total aerobic plate count (TPC) and recovery of artificially inoculated S. typhimurium*

<table>
<thead>
<tr>
<th>Sampling method</th>
<th>Mean log$_{10}$ TPC per visceral cavity*</th>
<th>Mean log$_{10}$ S. typhimurium recovered per visceral cavity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swab</td>
<td>6.93</td>
<td>5.87</td>
</tr>
<tr>
<td>Rinse</td>
<td>7.81</td>
<td>6.83</td>
</tr>
<tr>
<td>Mean difference*</td>
<td>0.88</td>
<td>0.96</td>
</tr>
</tbody>
</table>

*Average weight per carcass, 3.5 pounds (ca. 1.6 kg).
* Mean of 10 samples.
* Artificial inoculum, $10^7$ S. typhimurium cells per carcass.
* Differences statistically significant at the 5% level of confidence.

could be sampled per hour. Also a small area of the neck region was not included in the rinse because of the position of the funnel, but this area was very small relative to the total visceral cavity surface area.

We think that our total internal rinse method provides more representative microbiological samples of the highly irregular visceral cavity surface of broiler carcasses than other cavity sampling methods.

LITERATURE CITED