Recovery of Salmonellae from Broiler Carcasses
by Direct Enrichment

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ABSTRACT

Each of four serotypes of Salmonella (S. anatum, S. montevideo, S. saint-paul, S. typhimurium), inoculated at low levels on broiler carcasses (ca. 20 cells/carcass) was detected by direct enrichment of the whole carcass rinse fluid with either Selenite Cystine Broth (SC) or Selenite Brilliant Green Broth (SGB). Neither Selenite Brilliant Green Sulfur Broth (SBGS) nor TT Broth was effective in detecting the serotypes unless the entire broiler carcass with the rinse fluid was incubated with either of these enrichment broths. SBG and SC were effective as direct enrichment broths for recovering pure cultures of the four serotypes subjected to sublethal heat treatment (53°C for 1 min) approximating that to which broiler carcasses are subjected during the commercial scalding process.

A number of different procedures have been used for detection of Salmonellae in processed poultry carcasses; but as yet, none has been accepted as standard for this food product. There is disagreement as to the sampling method, choice of enrichment media, selective plating media, confirmation methods and whether the initial step in the procedure should involve preenrichment of the sample in a nonselective broth or direct enrichment in a selective broth.

Edel and Kampelmacher (4) have recommended preenrichment for some foods because sublethal injury to salmonellae could occur during processing. Many investigators have used or recommended lactose broth as the preenrichment medium in the analysis of poultry carcasses for Salmonella (2,3,9,11,12,23). Other investigators working with raw food samples or poultry used the preenrichment technique but with nutrient broth (13), mannitol broth (16) or buffered peptone (25) instead of lactose. Still others have used or recommended direct enrichment techniques for this type product (10,24). The International Commission on Microbiological Specifications for Foods (15) recommends a modified procedure of Surkiewicz et al. (23) for isolating salmonellae from poultry. The modification involves shaking the carcass in a plastic bag with 300 ml of lactose broth, followed by the addition of 300 ml of double-strength liquid-enrichment broth.

Many different enrichment broths are available, so if the decision is made to use the direct enrichment method for detecting salmonellae on poultry carcasses, the question will arise as to which one(s) to use. It has been suggested that the optimum procedures and methodology for Salmonella should be determined for every different food product (8,14). The purpose of our study was to compare the efficiency of four commonly used enrichment media for direct enrichment of processed broiler carcasses.

MATERIALS AND METHODS

Experiment 1

Eviscerated broiler carcasses were obtained from a local processing plant. A strain of Salmonella typhimurium, resistant to 100 ppm of nalidixic acid, was used as the marker organism in this study. Cells of this culture were grown on Brain Heart Infusion (BHI) Agar (Difco) slants for 18 h and then washed from the slant with sterile physiological saline solution. The suspension was then diluted with sterile saline solution to an optical density (OD) of 0.2 at 540 nm. measured with a Bausch and Lomb Spectronic 20 spectrophotometer. Then 0.1 ml of the diluted suspension which contained 20 or fewer cells of the marker organism, was rubbed into skin on the breast, the thigh and under the wing of each of 40 carcasses with a sterile bent glass rod. The number of cells inoculated was determined from 0.1 ml aliquots spread on 10 plates of BHI agar. The inoculum for this experiment ranged from 6 to 20 cells per carcass.

After inoculation, each carcass was individually placed in a plastic bag, 300 ml of Selenite Brilliant Green (SGB) Broth (Difco) were added and the contents vigorously shaken for 1 min. The bird and the broth were then incubated together in the bag for 24 h at 35-37°C. After incubation, three loopsful of broth from each bag were streaked onto plates of MacConkey Agar (Difco) containing 100 µg of nalidixic acid per ml. After 24 h of incubation at 35-37°C, isolated colonies were picked from the plates and tested serologically to confirm the presence of the marker organism.

The above procedure was repeated with three other enrichment broths, Selenite Brilliant Green Sulfur (SBGS) Broth (Difco), Selenite Cystine (SC) Broth (Difco) and TT Broth (Difco).

Experiment 2

The procedure for this experiment was identical to that described for Experiment 1 with the following exceptions. Each inoculated carcass was placed in a polyethylene bag with 270 ml of sterile water and...
vigorously shaken for 1 min. The carcasses, after draining into the bag for 30 sec was removed and 30 ml of a concentrated solution of one of the four enrichment broths (SBG, SBGS, SC, TT) which yielded a single-strength final concentration was added to the rinse fluid. The rinse sampling bag was placed in an 800-ml beaker and incubated for 24 h at 35.37 °C.

Experiment 3
A low level inoculum (approximately 12 cells) of an 18-24 h culture of nalidixic-acid resistant S. typhimurium was pipetted directly into each of 10 milk dilution bottles, containing 100 ml of SBG, and incubated 24 h at 35.37 °C. A loopful of broth from each bottle was then streaked onto plates of MacConkey Agar containing 100 ppm nalidixic acid. After a 24 h incubation of these plates at 35.37 °C, colonies were tested serologically to confirm them as our marker organism. The experiment was repeated with SBGS, SC and TT as the enrichment broth.

Experiment 4
Each of 20 freshly processed raw broiler carcasses was dermally inoculated with a mixed suspension containing approximately 20 cells each of S. anatum, S. montevideo and S. saint-paul. The sites inoculated were breast, thigh and under the wing. The number of cells of each serotype in 0.1 ml of the suspension was determined by the procedure described for the inoculum used in Experiment 1. Each carcass was placed in a plastic bag, with 300 ml of SBG broth, shaken vigorously for 1 min and incubated in the bag with the broth for 24 h at 35.37 °C. Next, three loopful of the broth were streaked onto each of two BG Sulfa Agar (Diffco) plates. After 24 h of incubation at 35.37 °C, 10 typical colonies were picked (five from each plate) for each of the 20 samples, inoculated onto slants of Lysine Iron (LD) Agar (Difco), and incubated 24 h at 35.37 °C. L1 agar slants exhibiting a typical salmonella reaction were subcultured onto BH agar plates for determination of purity; then these cultures were serologically confirmed to be one of the three serotypes inoculated.

This experiment was repeated with another 20 carcasses and SC instead of SBG broth.

Experiment 5
Individual suspensions of S. anatum, S. montevideo, S. saint-paul and S. typhimurium each containing about 20 cells, were inoculated into 10 tubes each containing 3 ml of sterile physiological saline tempered to 53.3 °C. The tubes were put in a 53.3 °C water bath and held for 1 min. Then, the contents of each tube were transferred to 97 ml of SBG broth. After 24 h of incubation at 35.37 °C, three loopful of the broth were streaked onto each of two BG Sulfa Agar plates and the procedures previously described in Experiment 4 were used to recover the inoculated organism. Experiment 5 was replicated 10 times, five times each with SBG broth and SC broth.

RESULTS AND DISCUSSION
In experiments 1 and 2 (Table 1) direct enrichment into SBGS, SC and TT successfully recovered an inoculum of very few cells of S. typhimurium 100% (40/40) of the time when the carcasses were incubated in the bag with the enrichment broth. SBG yielded recovery in 75% (30/40) of the samples. However, when the carcass was not incubated with broth, results were significantly different. In this experiment SBG and SC performed at a 100% efficiency (40 out of 40 positive recoveries); however, the efficiencies of SBGS and TT in recovering the marker organism were drastically reduced. SBGS yielded only 10% positive recoveries of the inoculated organism, while TT was unable to produce a positive in any of the 40 samples. In Experiment 3 (Table 2), direct introduction of the marker organism into the various enrichment broths produced results nearly identical to those obtained in Experiment 2. The findings presented in Tables 1 and 2 suggest that SBGS and TT may exert a lethal or toxic effect on our marker organism. TT broth contains tetrathionate and thiosulfate and Palumbo and Alford (18) observed a lethal effect on bacteria by this tetrathionate-thiosulfate combination. The actual mechanism of this lethality is not known. However, since both tetrathionate and thiosulfate react with free sulfhydryl groups of enzymes and cause their inactivation (19,20), tetrathionate broth may interfere with the synthesis and/or activity of sulfur-containing enzymes or cell wall and membrane components. Palumbo and Alford (18) also reported that the tetrathionate-thiosulfate combination initially reduced the numbers of many organisms, but that regrowth followed. Since the inoculum level in our studies was so low, such an initial effect could have caused elimination of the marker organism. The toxic effect of TT broth was not observed in Experiment 1 (Table 1) when the carcass was incubated in the rinsing fluid. A possible explanation could be that in Experiment 1 some of the inoculated cells were protected against these toxic effects by the skin and tissues of the carcass. The TT broth plus the broiler carcass is a complex mixture of continuously changing compounds due to the variety of microorganisms present, so that protection in the very early stages of incubation could be critical.

<table>
<thead>
<tr>
<th>TABLE 1. Efficacy of four enrichment broths for recovery of Salmonella artificially inoculated on broiler carcasses.</th>
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<tbody>
<tr>
<td><strong>Media</strong></td>
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<tr>
<td><strong>No. of positives/No. of carcasses</strong></td>
</tr>
<tr>
<td>SBG</td>
</tr>
<tr>
<td>SBGS</td>
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<td>SC</td>
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<td>TT</td>
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aStrain of S. typhimurium resistant to nalidixic acid.
bMean inoculum range was 6-20 cells per carcass.
cMean inoculum range was 12-18 cells per carcass.

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<th>TABLE 2. Recovery of Salmonella typhimurium inoculated directly into various enrichment broths.</th>
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</tr>
<tr>
<td>SBG</td>
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<td>TT</td>
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aMean inoculum was 12 cells per carcass.

The inhibitory properties of SBG and SBGS arise from the combined activities of selenite brilliant green and sodium taurocholate (22). While the addition of sulfapyridine (17) has been shown to increase the selectivity of SBG and promote recovery of salmonellae amidst large numbers of extraneous organisms, it may be that the combined effects of sulfapyridine, selenite brilliant green and sodium taurocholate, are in certain instances, toxic (or at least cause an initial reduction in numbers) to certain salmonella serotypes. Such a possibility would explain the poor performance of SGBS
in our study (Table 1 and 2). Our results with this medium agree with those of Yamamoto et al. (26) who found SBGS to be too selective and less effective than SC or tetrathionate brilliant green for isolation of salmonellae from turkey tissues and fecal samples. In addition, Carlson and Snoeyenbos (1) found that SBGS produced major reduction of salmonellae between 24 and 48 h of incubation, especially at 43 C. They concluded that this medium was totally unsatisfactory for some salmonellae strains. In contrast, Fagerberg (5) and Fagerberg and Avens (6, 7) found SBGS to be superior to 11 other enrichment broths for recovering salmonellae from contaminated turkey carcasses.

Since recovery of the marker organism was best with SBG and SC in the first three experiments, in Experiment 4 we tested whether they would enable us to recover several salmonella serotypes amidst the extraneous organisms found on broiler carcasses. In every instance, both SBG and SC allowed us to recover low numbers of cells of S. anatum, S. montevideo and S. saint-paul (Table 3). According to Fagerberg and Avens (8) an ideal enrichment broth should not be too inhibitory to salmonellae yet should be selective enough to prevent overgrowth by competitive organisms. Our data suggest that SBG and SC met this requirement for isolating these three serotypes of Salmonella from broiler carcasses.

**TABLE 3. Comparison of SBG and SC for recovery of S. anatum, S. montevideo, and S. saint-paul from broiler carcasses.**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Serotype</th>
<th>No. of positives/No. of carcasses</th>
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<tbody>
<tr>
<td>SBG</td>
<td>S. anatum</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td>S. montevideo</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td>S. saint-paul</td>
<td>20/20</td>
</tr>
<tr>
<td>SC</td>
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<td>20/20</td>
</tr>
<tr>
<td></td>
<td>S. montevideo</td>
<td>20/20</td>
</tr>
<tr>
<td></td>
<td>S. saint-paul</td>
<td>20/20</td>
</tr>
</tbody>
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2 Mean inoculum for S. anatum was 15 cells per carcass.
3 Mean inoculum for S. montevideo was 23 cells per carcass.
4 Mean inoculum for S. saint-paul was 18 cells per carcass.

It has been suggested that sublethal injury can occur during processing of raw meats and poultry and that, therefore, the examination of such foods for salmonellae should include a preenrichment step (4). During processing, broilers are commonly scalded for 1 min in 53-C water and this process could conceivably result in injury to salmonellae. In Experiment 5 we found that pure cultures of S. anatum, S. montevideo, S. saint-paul or S. typhimurium at low levels (about 20 cells) subjected to these conditions could be detected by enrichment in either SBG and SC without a preenrichment step (Table 4). Bacteria on broiler carcasses during commercial scalding are probably exposed to even less heat than that in this experiment because of the insulating effects of feathers, skin and organic matter in the scald water; so sublethal injury to salmonellae present would not be likely. The results of this experiment indicate that salmonellae cells on carcasses receiving a 53-C scald during processing could be recovered by use of SBG or SC enrichment broths.

**REFERENCES**


