Rapid Procedure for Biochemical Characterization and Serological Confirmation of Suspect Salmonella Isolates

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ABSTRACT

Fifty-two freshly processed broiler carcasses were examined for the presence of Salmonella by using a rinse method. Three selective plating media (bismuth sulfitc, brilliant green sulfa and Hektoen enteric) were compared. After 24 h of incubation, typical colonies were picked from each selective plate. An 8-h procedure to biochemically characterize (Micro-ID) and serologically (poly O and poly H) confirm Salmonella was then compared with a conventional procedure. Suspect Salmonella isolates were correctly classified from 63% of the carcasses with both the 8-h and conventional procedures. Of the 244 isolates confirmed to be Salmonella by conventional testing, 236 (97%) were also confirmed by the 8-h procedure. Brilliant green sulfa and Hektoen enteric agar were superior to bismuth sulfitc agar for Salmonella recovery. The 8-h procedure required less incubation time (8 h vs. 48 h) after colony formation, less incubation space, and less media preparation and cleanup than the conventional procedure.

Conventional procedures for examining foods and feeds for salmonellae involve preenrichment with nonselective media when the organisms in the sample are stressed, enrichment in selective media, selective plating to obtain isolated colonies, biochemical screening with triple sugar iron (TSI) and lysine iron (LI) agar slants, further biochemical testing and serological confirmation with somatic poly O and flagellar poly H antisera. These are very time-consuming procedures, i.e., 72 h are required for colonies to form (48 h if preenrichment is not used) and an additional 48 to 96 h for biochemical characterization and serological confirmation. Cox and Mercuri (1) proposed an alternative procedure requiring only 24 h from colony formation by concurrently doing biochemical tests and serology with the Minitek system (BBL).

With increased public awareness of food poisoning bacteria and possible increased scrutiny by regulatory agencies, it is particularly important to develop methods for rapid and accurate Salmonella detection. An enrichment serology (ES) procedure to detect Salmonella in dried foods and feeds using only broth cultures and serological reactions was proposed by Sperber and Deibel (10). Mohr et al. (8) used a 6-h ES procedure to screen for Salmonella from a variety of food products, and Surdy and Haas (11) used a 6-h modified ES procedure to detect Salmonella in dried soy products. While these procedures are very rapid, they alone, without biochemical characterization to complement serology, could result in false-negatives which are impossible to detect. The results of ES are highly correlated (>95%) with those of conventional procedures for recovering Salmonella, but they have not become widely accepted because of the reluctance of many microbiologists to forego biochemical characterization.

Silliker et al. (9) described a procedure for serologically confirming Salmonella from isolated colonies on selective plating media. This method is rapid and accurate, but since a battery of biochemical tests are not run, they recommend simultaneously inoculating TSI/LJ slants, which requires an additional 24 h of incubation. A 4-h system (Micro-ID) was found to be accurate for the biochemical characterization of Salmonella isolates (2, 3). Therefore, the objective of this study was to evaluate rapid biochemical (Micro-ID) and concurrent serological tests to confirm suspect-Salmonella colonies in 8 h, and to compare the Micro-ID serology procedure to the time-consuming conventional procedure.

MATERIALS AND METHODS

A total of 52 freshly processed broiler carcasses were rinse-sampled according to the procedure of Cox et al. (4). The rinse samples were incubated for 16 h at 37°C and then brilliant green sulfa (BGS) (1), Hektoen enteric (HE) and bismuth sulftc (BIS) agar plates were streaked for isolation. After 24 h of incubation at 37°C, up to three suspect Salmonella colonies per plate were picked and inoculated onto triple sugar iron (TSI) and lysine iron (LI) agar slants for the conventional confirmation procedure, and into tubes containing 0.7 ml of M broth (10) to run the modified 8-h confirmation procedure (Table 1).
TABLE 1. Confirmation procedure (8-h) for suspect-Salmonella isolates.

1. Pick typical colony to 0.7 ml M broth.
2. Incubate M broth at 37°C for 6 h.
3. After 6 h, remove 2 loopfuls of M broth and run slide poly O agglutination.
4. After 4 h, if poly O (+), remove 0.2 ml M broth, add to 3.2 ml sterile saline and run Micro-ID.
5. After 6 h of incubation, run tube poly H agglutination on remaining 0.5 ml M broth and incubate for 2 h in a 50°C water bath.
6. After 8 h, read Micro-ID and poly H agglutination results.

In the conventional confirmation procedure, TSI/LI slants were incubated at 37°C for 24 h. Brain heart infusion (BHI) plates were streaked from TSI/LI agar slants exhibiting a typical Salmonella reaction to determine purity and to produce isolated colonies for agglutination tests. Standard poly O slide and poly H tube agglutination tests (6) were then made.

In the 8-h confirmation procedure (Table 1), biochemical tests and serology (both O and H) were performed concurrently. Biochemical confirmation was determined by using the Micro-ID system (General Diagnostics, Division of Warner-Lambert Company, Morris Plains, New Jersey).

TABLE 2. Comparison of an 8-h procedure with a conventional procedure for the detection of Salmonella on broiler carcasses.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Carcasses</th>
<th>Isolates</th>
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<tbody>
<tr>
<td>Conventional</td>
<td>33/52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>244/307&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>8-h</td>
<td>33/52</td>
<td>236/307</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of Salmonella-positive carcasses/number of carcasses sampled.

<sup>b</sup>Number of isolates confirmed to be Salmonella/number of typical isolates selected.

RESULTS AND DISCUSSION

The conventional and the 8-h confirmation procedures both showed positive recoveries of Salmonella from 33 of 52 (63%) of the carcasses sampled (Table 2). Of 244 isolates that were confirmed to be Salmonella by the conventional procedure, 236 (97%) were also confirmed by the 8-h procedure. Of the eight cultures identified by the conventional procedure but not by the 8-h system, seven were incorrectly identified because of an initially false-negative poly H agglutination test. However, because the isolates were biochemically confirmed as Salmonella by the Micro ID system, the poly H test was rerun and the cultures were correctly identified the second time. Only one culture was not properly identified as Salmonella by the Micro ID system.

The effectiveness of three commonly used selective plating media was compared with isolates from the conventional testing procedure. BGS and HE recovered Salmonella from 33 (63%) and 32 (62%) carcasses, respectively, whereas BiS only recovered Salmonella from 24 (46%) carcasses. Although BiS was not as efficient for recovery of Salmonella as the other two media in this study, its use is recommended because BiS is the only Salmonella plating medium not based on the fermentation of lactose and, therefore, is the only medium that would detect the 1% of salmonellae which are lactose-positive (7).

The percentage of confirmations, using conventional procedures, of typical, Salmonella-like colonies selected from the various plating media are shown in Table 3. BGS was the most efficient; 95% of the colonies tested were confirmed as Salmonella. Colonies of Proteus and a few other H<sub>2</sub>S-positive Enterobacteriaceae appeared similar to those of Salmonella on BiS and HE (5).

The advantages of the 8-h confirmation procedure over conventional procedures were: (a) 24 to 48 h less time between sampling and confirmation and (b) less time required for media preparation, inoculation and cleanup. The advantage of the 8-h procedure over the ES procedure was that both serological confirmation and biochemical characterization were obtained in 8 h. In the 8-h system, however, more isolates must be tested than in the conventional procedure because the 8-h procedure has no TSI/LI screening step, and 24 h longer were required than with ES.

Many factors must be considered when determining which procedure is best for detection of the presence of Salmonella in a food product. If rapid results are not required, then conventional procedures might be preferred; however, if release of a food product must be delayed until freedom from Salmonella contamination is assured, the 8-h procedure would be desirable.

In conclusion, the 8-h confirmation procedure agreed with the conventional procedure for 97% of the isolates tested. Also, all carcasses that were found to have Salmonella by conventional procedures also showed Salmonella by the 8-h procedure.

The time necessary for determining if a colony is Salmonella or not has been reduced by running the biochemical, poly O and poly H confirmation steps concurrently, rather than consecutively. Further study is underway to reduce the time from sampling of the food product to final confirmation of Salmonella to less than 24 h without sacrificing accuracy.

ACKNOWLEDGMENTS

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