EVALUATION OF *SALMONELLA* TEST METHOD OF SRI LANKA STANDARDS INSTITUTION

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ABSTRACT

This study was conducted to evaluate the microbiological test method of Sri Lanka Standards Institution to detect *Salmonella* in foods. The SLS method was compared with the method published by Food and Agriculture Organization for detection of *Salmonella* by applying it to several food items. Black pepper, cinnamon, cardamom, cloves, and milk powder were artificially contaminated with *Salmonella* serotype H culture and uninoculated eggs were used for the study. A control test was carried out without adding the inoculum. Method of preparation of the food samples for isolation of *Salmonella* and the pre-enrichment media used, are specific for each food to be tested in the FAO method. However, SLS method employs the same preparatory procedure and the pre-enrichment media for almost all foods. Both methods gave positive reactions for artificially inoculated black pepper, cardamom, and milk powder. In the tests carried out for cloves and cinnamon by SLS method no positive reactions were observed whereas FAO method was able to identify the serotype H in these two spices. The anti-microbial substances in cloves and cinnamon appear to inhibit the growth of *Salmonella* by SLS method. In the fresh egg sample that was tested by the SLS method colonies were observed only in XLD agar, but not in Brilliant Green agar. These discrepancies observed for the test results for *Salmonella* leave room to suspect the applicability of the SLS method to foods.

INTRODUCTION

Microbiological testing is important to detect pathogenic microbes in foods and to minimize health hazards due to pathogens. In Sri Lanka, foods are tested to detect pathogenic microorganisms (*Salmonella, Staphylococcus aureus*) according to the methods published by Sri Lanka Standards Institution. *Salmonella* is a common pathogen in many of the processed foods and an estimated 80 million cases of food borne illnesses and over 10000 deaths occur yearly in North America (Todd, 1989). Stringent quality measures are adopted and severe testing is conducted in the desiccated coconut industry in Sri Lanka as the country almost lost the export of desiccated coconut in 1954 due to *Salmonellae* contamination. *Salmonellae* contamination continues to be a major problem associated with water and foods in Sri Lanka due to poor hygienic practices and ill organized sewage disposal practices. It is, therefore, important for every food industry to test the market products for *Salmonellae.*

In Sri Lanka, the method published by the Sri Lanka Standards Institution is widely used for testing foods for *Salmonella* in the food industry and testing laboratories. However, globalization demands testing laboratories to acquire international accreditation. Therefore, validity of the SLS method used to detect *Salmonellae* in foods plays an important role in

deciding the safety of processed foods for local and export markets. This study was conducted to compare the microbiological test method recommended by Sri Lanka Standards Institution (1992) with that of the method recommended by Food and Agriculture Organization (1991) for *Salmonella* in foods. These two methods are similar in employing the procedural steps of pre-enrichment, selective enrichment, selective agar plating, biochemical screening, and serological identification (Andrews, 1996). Pre-enrichment favours the recovery of microorganisms from the injured state in which some are believed to exist in many foods. Selective enrichment is done for the purpose of increasing *Salmonella* population and inhibiting other organisms in the food. Selective plating helps to differentiate *Salmonella* colonies from non-*Salmonella* colonies. Colonies suspected of being *Salmonella* are then confirmed by submitting cultures from those colonies to biochemical and serological testing. The two methods differ in the media used for pre-enrichment and initial identification of *Salmonella*. The methods also differ in the sample pre-preparation techniques.

MATERIALS AND METHODS

Samples

Six types of foods (black pepper, cinnamon, cardamom, cloves, milk powder, and whole eggs) were selected for the identification and isolation of *Salmonella* according to the procedures published by the Sri Lanka Standards Institution (1992) and Food and Agriculture Organization (1991). All the food samples except milk powder and eggs were sterilized at 121°C for 10 minutes prior to inoculation with the *Salmonella* culture.

Sample preparation

A *Salmonella* serotype H culture was used for inoculation. MacCartney bottles containing 10 ml nutrient broth were inoculated with the maintaining culture, incubated at 37°C for 24 hr and used for inoculation. Black pepper, cinnamon, cardamom, cloves, and milk powder were artificially contaminated by inoculating with the *Salmonella* culture, and then incubated at 37°C for 6 hr. Eggs, which are considered to be frequently contaminated, were not inoculated but crushed with the shell and introduced into the pre-enrichment media instead.

Pre-enrichment

The substrate to enrichment media ratio was maintained as recommended in the two methods. In the SLS method 1:25 sample/broth ratio was maintained for all the foods whereas in the FAO method, the ratio was maintained depending on the food to be tested. According to the FAO method, for black pepper, cardamom, milk powder, and eggs, 1:25 sample/broth ratio was maintained, and for cinnamon and cloves, 1:100 and 1:1000 sample/broth ratios were maintained, respectively. Samples were incubated for pre-enrichment at 37°C for 24 hr. Buffered peptone was used as the pre-enrichment broth for black pepper, cinnamon, cardamom, cloves, and eggs in the SLS recommended method. Trypticase soy broth was used for cloves, cinnamon, cardamom, and black pepper, and lactose broth was used for eggs in applying the FAO method. Sterile distilled water containing brilliant green solution was used as the pre-enrichment media for milk powder tested using both SLS and FAO methods.

Selective enrichment

Selenite cystine broth and Tetrathionate broth are recommended in the FAO method for selective enrichment. Pre-enriched mixture (1 ml) was transferred to above broths and

incubated at 37°C for 24 hr. The SLS method recommends the use of Selenite cystine and Rappaport-Vassiliadis broth for selective enrichment. Pre-enriched media (0.1 ml) was transferred to Rappaport-Vassiliadis broth and (1 ml) Selenite cystine broth. The cultures were incubated at 42 °C for 24 hr.

Selective plating

Bismuth sulfite (BS) agar and Xylose lysine desoxycholate (XLD) agar were used for plating of samples tested by FAO method. After incubation, a loopful from the culture in the Tetrathionate broth was streaked on the surface of plates containing two each of BS agar and XLD agar plates. Similar procedures were followed using incubated Selenite cystine medium. In testing by the SLS method, loopfuls from Selenite cystine and Rappaport-Vassiliadis broths were streaked on the surface of XLD agar and Brilliant green (BG) agar plates. They were incubated at 37 °C for 24 hr and examined for the presence of colonies suspected to be *Salmonella*.

Typical *Salmonella* colonies on BS agar may appear brown, gray, or black, with a metallic sheen appearance. On XLD agar typical *Salmonella* colonies appear as pink colonies with or without black centers. Pink colonies with bright red surrounding medium are considered to be the typical colonies on BG agar.

Biochemical confirmation

Colonies considered to be typical were taken from each plate, streaked on the surface of pre-dried nutrient agar plates and incubated for 24hr at 37 °C. By using these colonies biochemical tests were performed with TSI agar, Urea agar, Lysine decarboxylation, β-galactosidase, Voges-proskauer, and Tryptone-tryptophan reagents.

Serological confirmation

The colonies were confirmed by a serological test. Auto-agglutinable strains were eliminated by using a part of a colony from 18 hr to 24 hr old culture dispersed in a drop of saline. The colonies recognized as non-auto agglutinable were then examined for O-antigens and H-antigens by using O-antiserum and H-antiserum respectively.

RESULTS AND DISCUSSION

Black pepper, cardamom, and milk powder

In these three samples typical colonies were observed in the plates tested by both SLS and FAO methods. They gave positive reactions for all the biochemical tests and serotype H was identified from the serology test.

Cloves and cinnamon

There were no colonies observed from cloves by SLS method whereas typical colonies were observed for the test carried out by FAO method. In the test carried out for cinnamon by SLS method no colonies were observed in the plates streaked using Rappaport-Vassiliadis broth, but there were typical colonies in the media used in the FAO method. These typical colonies were identified to be of serotype H. According to the FAO method for detection of *Salmonella* in spices such as cloves and cinnamon, it is specified that toxicity of these spices need to be neutralized prior to testing. Therefore, they were diluted beyond their toxic levels to examine them in the FAO method. The anti-microbial substances in cloves and cinnamon appear to inhibit the growth of *Salmonella* in the test when applying SLS recommended

method. In examining food it is important to pre-enrich samples in highly specific media as the *Salmonella* in foods may already be debilitated due to food processing treatments, dehydration or presence of anti-microbial substances, especially in spices and spicy foods.

Eggs

No colonies were observed in Brilliant green agar plates for the fresh egg sample tested by SLS method. However, there were colonies in XLD agar plates, which showed typical *Salmonella* colony characteristics. In the FAO method typical *Salmonella* colonies were observed in both XLD agar and BS agar plates. The reason for these discrepancies may be the inhibition of growth of *Salmonella* by Brilliant green agar. Therefore it may not be possible to detect the presence of *Salmonella* if only one plating media (e.g. Brilliant green agar) was used. Many reference methods (FAO, AOAC) recommend the use of two or more selective plating media to facilitate the recovery of strains of *Salmonella* that were inhibited by a particular plating media. Some serotypes of *Salmonella* grow poorly or fail to grow on certain selective agars, for example, *Salmonella typhi* is inhibited by Brilliant green agar (Silliker, 1986).

In the control tests with sterile uninoculated samples of black pepper, cardamom, cinnamon, and cloves no colonies were observed with both methods. In milk powder, although colonies were observed by both SLS and FAO methods, these colonies did not give positive reactions for the serological test. Milk powder sample was not sterilized, and it was used as it is for inoculation and control, because during sterilization, the texture of the product gets denatured.

CONCLUSIONS

The SLS method provides a general guidance for sample pre-enrichment whereas the FAO method specifies different pre-enrichment media and procedures depending on the food to be tested. SLS test method provides too general guidance for microbiological examination of foods, and owing to the number and variety of the products, it may be necessary to make changes to this method or even to use other methods depending on food components. In view of regular use of spices containing anti-microbial substances in foods prepared in Sri Lanka, the reliability of SLS method needs detailed investigation. The discrepancy observed in the results for *Salmonella* in eggs, which is very frequently contaminated, also leave room to suspect the validity of the test results by SLS method. The use of FAO method for detection of *Salmonella* is recommended to the food industry.

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