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Detection of *Salmonella* in Food Samples by Culture and Polymerase Chain Reaction Methods

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Abstract

Conventional culture methods for the isolation and identification of food borne bacterial pathogens are rather sensitive and quite inexpensive, but at the same time they are labor-intensive and time-consuming. Molecular techniques are more rapid and highly sensitive for identification of food pathogens. This study was carried out to evaluate a 12 hour PCR method for detection of *Salmonella* in food samples. The results showed that out of 150 food samples, 32 (21.3%) were positive by culture, 35 (23.3%) were positive by PCR, the sensitivity of PCR was 100% while specificity was 97.5%. The study concluded that the 6-h enrichment followed by PCR was rapid, simpler method that allowed the detection of *Salmonella* spp. within a maximum of 12 h.

Keywords: Salmonella; PCR; Enrichment; Culture methods; Food samples

Introduction

Food poisoning is defined as any disease of an infectious or toxic nature caused by the consumption of food or beverages that are contaminated with harmful microorganisms, such as certain bacteria, viruses or parasites [1]. *Salmonella* is a Gram-negative, usually motile, facultative anaerobic, flagellated rod-shaped. *Salmonella enteritidis* has become the most common cause of salmonellosis which is the second major cause of foodborne disease acquired in the United States and leads episodes of hospitalization and death [2,3].

Conventional culture methods have traditionally been considered as the "gold standard" for the isolation and identification of foodborne bacterial pathogens [4]. They consist of a series of steps that include nonselective enrichment, selective enrichment, selective/differential plating and, finally, morphological, biochemical and serological confirmation. This standardized classical culture method is known to be sensitive and inexpensive, but culture methods are labor-intensive and time-consuming, because they require at least, three working days to produce a negative result and five to ten working days for confirmed positive results. Moreover, due to environmental factors, variations in gene expression of microorganisms can occur and may affect the results of biochemical tests. Furthermore, viable but non-cultivable cells are not detected by the conventional methodology [5]. Rapid methods for the detection of Salmonella in food have been developed, for example, electrical techniques, immunoassays and nucleic acid probe analyses [3], but there are still problems with their sensitivity and specificity.

The PCR represents a rapid procedure with high sensitivity and specificity for the immediate detection and identification of specific pathogenic bacteria from different food materials [6-8]. But PCR can be limited by several factors (e.g. food components, humic acid, urine, bile salts, etc) [3]. The low number of pathogens occurring heterogeneously within a relatively large volume of food and inhibition of PCR by the food matrix is a main limitation factor. Moreover, in cases where a pathogen is detected, the conventional method must be used for confirmation [4]. The removal of the inhibitory substances is a major step in the preparation of the samples for PCR based detection of food pathogens. Although these inhibitory substances limit the application of PCR directly to food samples, the application of PCR based assays to enrichment broths has been more successful [9]. This study was carried out to evaluate a rapid (12 hour) method for detection of *Salmonella* in food samples and compare it with the conventional method.

Materials and Methods

One hundred and Fifty food samples of meat, comprising beef (n=64), chicken (n=80), and fish (n=6). Samples were suspected to be contaminated with *Salmonella*. All samples were labeled, recorded and were analyzed as soon as possible. If delayed, samples would be refrigerated on 0-4°C for not more than 24 h after collection. The pre-enrichment of samples was performed according to the method described by Medici *et al.,* [10] with some modification. About 25 g samples were homogenized with 225 mL of Buffered Peptone Water (BPW) medium(Oxoid, CM0509) and then divided into two aliquots. The first aliquot was subjected to pre-enrichment culture for 6 hour while the second one was incubated at 37°C for 24 h. The first aliquot was subjected to DNA extraction by boiling method and the second aliquot was used to confirm the presence of *Salmonella* by standard cultural method, and followed by biochemical and serological confirmatory tests.

Preparation of template DNA samples was performed by boiling method. One mL of the pre-enriched first aliquot sample was transferred to a 1.5 mL micro-centrifuge tube and centrifuged for 10 min at 14,000×g. The supernatant was discarded carefully. The pellet was re-suspended in 300 µL of DNase-RNase-free distilled water by vortexing. The tube was centrifuged at 14,000 × g for 5 min, and the supernatant was discarded carefully. The pellet was re-suspended in 200 µL of DNase-RNase-free distilled water by vortexing. The micro-centrifuge tube was incubated for 15 min at 100°C and immediately

chilled on ice. The tube was centrifuged for 5 min at 14,000 × g at 4°C. The supernatant was carefully transferred to a new micro-centrifuge tube and an aliquot of 10 µL of the supernatant was used as the template DNA in the PCR.

PCR was done as follows: A 50 µl PCR mixture contained a 5 µl of DNA template, 1 µl (100 pmol) of each primer and a 25 µl of Taq PCR Master Mix polymerase containing 100 mM Tris-HCl, 500 mM KCl at pH 8.3 at 20°C, 1.5 mM $MgCl_2$, 200M each deoxyribonucleoside triphosphate and 0.025U Taq polymerase (Qiagen, USA). Amplification of DNA was performed using Mastercycler personal PCR machine. heat denaturation at 95°C for 5 min, followed by 35 cycles (90 s at 95°C, 60 s at 62°C, and 90 s at 72°C), and an elongation step of 7 min at 72°C. The primers used were Salm3 (5′GCTGCGCGCGAACGGCGAAG-3′) and Salm4 (5′TCCCGGCAGAGTTCCCATT-3′), which amplify a 389-bp fragment within the conserved invA gene sequence of *Salmonella* spp. [11]. Data of culture and PCR results were tested for correlation (spearman, s rho) and analyzed by computer using Statistical Package for Science (SPSS) version 21 program.

Results

The results showed that out of 150 food samples, 32 (21.3%) were positive by culture, 15⁄64 (23.4%) were beef meat while 15⁄80 (18.6%) were chicken meat and 2⁄6 (33.3%) were fish meat. Also out of 150 food samples, 35 (23.3%) were positive by PCR, 16⁄64 (25%) were beef meat while 17⁄80 (21.3%) were chicken meat and 2⁄6 (33.3%) were fish meat as shown in Figure 1. When culture was considered as golden standard, the sensitivity of PCR was 100% while specificity was 97.5% with positive predictive value (PPV) and negative prdictive value (NPV) 91.4% and 100% respectively. Statistically there was strong correlation (0.954) between PCR and culture. Correlation is significant at the p-value (0.01) level.

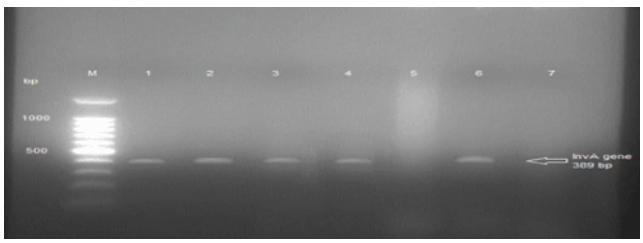

Figure 1: The *invA* gene after PCR on 2% agarose gel electrophoresis. Lane M: 100-bp DNA ladder. Lane 1: Control positive. Lanes 2, 3, 4, and 6: positive *invA* gene of *Salmonella* strains (389 bp). Lane 5: negative *invA* gene of *Salmonella* strains. Lane 7: control negative.

Discussion

The increased frequency of food-borne *Salmonella* has been causing recurring outbreaks, sometimes with fatal infections. Most infections result from the ingestion of foods of animal origin contaminated with *Salmonella* species such as beef, chicken, shellfish, eggs, and milk [12-14]. The routine detection of *Salmonella* in foods is a important part of public health programs because the presence of *Salmonella* in food can cause health problems. In this study 150 food samples (suspected to be contaminated with *salmonella*) were tested for presence of *Salmonella* by two different methods, culture and PCR. The culture results showed that 21.3% of the total samples were positive; the contamination of beef meat by *Salmonella* was 23.4% while chicken meat was 18.6% and fish meat was 33.3%. Cultural methods are based on nutrient acquisition, biochemical characteristics, and metabolic products unique to *Salmonella* spp [15]. However, the cultural methods require multiple sub-culturing stages followed by biochemical and serological confirmatory tests, which can take up to 7 days to get a confirmed positive result. Molecular screening methods have been also used to detect nucleic acids. In this study, we used a rapid and simpler method proposed by Ferretti et al. [16] that relying on a 6-h nonselective enrichment in BPW followed by cell breaking and PCR to detect *Salmonella* spp. within a maximum of 12 hour from the receipt of food samples. Pre-enrichment culture has been done in order to increase the viable number of *Salmonella* in the samples prior to the detection of *Salmonella* by PCR technique. We found that the number of samples detected by PCR using genomic DNA obtained by boiling method from contaminated food samples had been raised to 23.3%., 16⁄64 (25%) were beef meat while 17⁄80 (21.3%) were chicken meat and 2⁄6 (33.3%) were fish meat. When culture was considered as golden standard, the sensitivity of PCR was 100% while specificity was 97.5% with PPV and NPV of 91.4% and 100% respectively. This result proved the specificity of 12 h pre-enrichment-PCR. The 12 h pre-enrichment-PCR procedure could offer a rapid and good diagnostic tool for the routine monitoring of detection of *Salmonella* in food samples compared to the conventional culturing method. Other studies have also reported that the use of a PCR assays were more sensitive than the culture method for detecting *Salmonella* in food, especially in poultry, meat, and poultry related products [17-21]. In this study we used a method proposed by Ferretti et al. [16] who applied a PCR method without Internal Amplification Control (IAC), and although similar studies have been published previously [17-21], but PCR could be improved by introduction of a general IAC to prevent the occurrence of false negative results and to control the effects of inhibiting agents on amplification efficiency [22,23].

Finally our findings indicated that the 6-h enrichment followed by PCR was rapid and simple method that allowed the detection of *Salmonella* spp. within a maximum of 12 h from the receipt of food samples.

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