Short Communication

Detection of *Salmonella* spp contamination of carcasses slaughtered in poultry abattoir in Mashhad, Iran

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ABSTRACT

A total of 60 neck skin swab samples were taken from 12 different broiler flocks after the chilling stage of processing at a commercial broiler slaughtering facility in Mashhad. The presence of *Salmonella* was assessed by conventional culture method and confirmed by using poly O and poly H antiserum in serotyping. PCR amplification of *invA* gene as a specific method for detection of *Salmonella* was evaluated. In this study *Salmonella* was isolated from 11.66% of samples by conventional culture method, then in serological test 28.6% of them detected as serogroup B and 71.4% as serogroup C. In this investigation all positive results by conventional culture method were confirmed by PCR amplification. Because of rapidity and high specificity and sensitivity of PCR method, by standardization of this method, it could be considered as an alternative to conventional culture method for confirmation of *Salmonella* presence in raw poultry meat.

Keywords: Poultry meat, Salmonella, PCR, *invA* gene, conventional culture method

INTRODUCTION

*Salmonella* spp, cause one of the most important food borne disease in the world called *Salmonellosis* (Malorny et al 2003a). Foods with animal source such as beef, poultry meat, egg and milk have been shown to carry these pathogens (Gillespie et al 2003). Poultry products have been recognized as a major source of human contamination by these pathogens (Corry et al 2002). It is reported that in addition to miss handling of poultry product and raw poultry carcasses, uncooked poultry meat is also one of the most frequent causes of human infection by *Salmonella* (Panisello et al 2000). Conventional culture methods, which use to isolate *Salmonella*, are including non-selective pre-enrichment follow by selective enrichment and plating on selective and differential agars. Then suspect colonies are confirmed by biochemical and serological methods (Van Kessel et al 2003, Manzano et al 1998). Generally these techniques take longer time, since they give only presumptive results after 3-4 days and definitive results after 5-6 days (Manzano et al 1998, Malorny et al 2003b). Although rapid detection methods, such as DNA or
RNA probes, immuno-detection methods and nucleic acid hybridization have been developed, but they have not adequate sensitivity and specificity (Li et al 2000). In vitro amplification of DNA by the PCR is a powerful tool in microbiological diagnostics (Malorny, et al 2003). Several PCR methods for detecting Salmonella strains have been introduced. They utilize specific gene sequences for targeting (Manzano, et al 1998). In 2003 as a part of the major international research project for the validation and standardization of PCR for the detection of five major food borne pathogens including salmonella, the most selective primer set was found to be 139-141, which targets the invA gene, this set of primers were published previously (Rahn et al 1992). This specific PCR assay, which was validated in this project, showed high selectivity on 242 Salmonella strains (inclusivity 99.6%) and 122 non-Salmonella strains (exclusivity 100%) (Malorny et al 2003a). The present study reports isolation of Salmonella from poultry carcasses by conventional culture method and confirmation of this result by PCR assay. This PCR assay can be used as a rapid detection method with high sensitivity and specificity which dose not need the long procedure in conventional culture method.

**MATERIALS AND METHODS**

**Sample collection.** After the chilling stage of the slaughtering process 60 swab samples were taken from the neck skin of broiler’s carcasses from 12 different flocks (5 sample from each flock) in one of the local slaughterhouse in Mashhad (north-west of Iran). Samples were obtained by use of wet and dry swabbing method (Vanderzant et al 1992) then they were placed in the tubes containing 5ml of 0.1% BPW(buffered pepton water) and transported to the laboratory for testing in crashed ice and analysed within four hours.

**Conventional culture.** Swabs which were located in 5ml of BPW were mixed with the same volume of double-concentrated lactose broth and incubated at 37 °C for 24 h, one milliliter of lactose broth were added to 9 ml of each tetrathionate broth and selenit cystine broth, followed by incubation at 42 °C and 37 °C respectively for 24 h. A loopfull of each enriched culture were streaked onto BGA (brilliant green agar) and SS agar (Salmonella Shigella agar) and incubated at 37 °C for 24 h. Suspect colonies were confirmed biochemically by inoculating into lysine decarboxylase broth, urea broth and TSI agar slopes (triple sugar iron agar). Confirmation of presumptive posetive results performed by using of poly O and poly H antisera (Wellcolex colour Salmonella- Remel Inc).

**PCR amplification.** Suspected colonies from selective media were cultured on LB agar and incubted at 37 °C for 24 h, and then a loopful of bacteria was mixed with 300 µl of distilled water, and boiled for 10 minutes, then centrifuged at 6000rpm for 5 minutes. The amount of 1.5µl of supernatant was used for PCR amplification test using Salmonella specific primers based on the invA gene. The sequence of S139 and S141 primers was respectively as follow: 5´- GTG AAA TTA TCG CTA CGT TCG GGC AA -3´ and 5´- TCA TCG CAC CGT CAA AGG AAC C -3´ (10). Reactions with these primers were carried out in a 25µl amplification mixture consisting of 2.5 µl 10x PCR buffer (500mM KCl, 200 mM Tris HCl), 1.25 µl dNTPs (10 mM), 1.6 µl Mg Cl2, 0.5 µl of each primer, 0.5µl of Taq DNA polymerase (fermentase) and 1.5µl of boiled bacteria supernatant. Amplification was performed in thermocycler (Techneh, TC512). The cycling condition was as follow: an initial incubation at 94 °C for 60 seconds, followed by 35 cycles of denaturation at 94 °C for 60 seconds, annealing at 64 °C for 30 seconds, elongation at 72 °C for 30 seconds, and final extention priod for 7 minutes at 72 °C. Amplified products from Salmonella specific-PCR were electrophoresed in 1.2% agarose gel and a 100bp DNA ladder was used as a size reference. After
staining with ethidium bromide the gel was documented with a video camera. A positive response was defined by the presence of a visible band at the expected size. Deionised distilled water and E. coli O2 K12 were used as negative control and S. typhymurium (ATCC: 25923) were used as positive control.

RESULTS AND DISCUSSION

A total of 60 samples were analysed by conventional culture method for the detection of Salmonella. Based on this method 7 samples (11.66%) were found positive and in serological test two samples were detected as serogroup B (28.6%) and five samples as serogroup C (71.4%). In PCR assay, using S139 and S141 primers that amplifies a 284 bp sequence of the invA gene, all positive samples in conventional culture method, generated a 284 bp amplified DNA fragment (Figure 1).

![Figure1. Electrophoretic analysis of 284 bp amplification product from invA gene of Salmonella spp isolated from raw poultry meat. M: 100bp marker. Lane 1: negative control (water), 2: positive control (S. typhymurium ATCC: 25923). Lanes 3, 4, 6, 7, 8, 9, 10: samples.](image)

Prevalence of Salmonella spp, has been investigated in poultry carcasses in Iran (Shiraz), using specific primers for invA gene in PCR method (Zahraei Salehi et al 2005).

Culture techniques are universally recognised as the standard methods for detection of bacterial pathogens, such as Salmonella in food stuffs (White et al 2002). These techniques generally take longer time, because obtaining definitive results take 5-6 days (Manzano et al 1998, Malorny et al 2003b). Background microorganisms in the food matrix can negatively influence the growth of the target pathogen (Bulte et al 1995). The degree and type of sublethally injured or stressed Salmonella cells can influence the bacterial recovery and consequently limiting detection of this pathogen (Malorny et al 2003b). The presence of growth inhibitory factors in the selective media may also limit the growth of Salmonella on the laboratory media (White et al 2002). In this study we used preenrichment and enrichment stage for recovery of injured or stressed bacteria. It has been reported that higher sensitivity of PCR methods compare to culture techniques can be use for the detection of Salmonella (Gibson 1998). PCR assay for the detection of Salmonella which was evaluated in an international collaborative study, showed an analytical accuracy of 98% (Malorny et al 2003a). Based on these studies, the PCR based methods can be an alternative to the traditional culture method (Malorny et al 2003b).

Some PCR amplification methods for detection of Salmonella has been recommended such as amplification of fimA gene (Cohen et al 1996), 16s rRNA gene (Lin et al 2004), agfA gene (Hashimoto et al 1995), virulence associated plasmid (Rexach et al 1994), viaB gene (Hashimoto et al 1995), and sefA gene (Medici et al 2003). It has been suggested that the most selective primer set, uses the invA gene as the target (Oliveira et al 2002, Malorny et al 2003b). In this study we used primers targeting the invA gene of Salmonella to confirm the isolated colonies. Although in this study all of the isolated colonies were also positive in PCR amplification. But it has been reported that some strains of Salmonella such as Salmonella litchfield and Salmonella senftenberg could not be detected by these primers (Rahn et al 1992). In addition some
Salmonella species also could not be detected by other PCR methods (Van Kessel et al 2003). It is obvious that the use of either conventional or PCR method in isolation and identification would have resulted in the failure to detect Salmonella in a number of positive samples (White et al 2002). Therefore, it may be pertinent to use a combination of the PCR and conventional culture methods in order to maximise the detection of Salmonella in naturally contaminated poultry carcasses. In this study we used PCR method as an alternative to conventional confirmation methods.

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References


