Inv A gene specific PCR for detection of Salmonella from broilers

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Abstract

Poultry meat has been identified as one of the principal foodborne source of Salmonella. In this preliminary study the prevalence of Salmonella spp. contamination of broiler carcasses, were determined. Sixty samples were collected from poultry carcasses from the commercial broiler slaughtering facility in Namakkal, Tamil Nadu. The presence of Salmonella spp in collected samples was assessed by performing the pre-enrichment and enrichment culture, followed by PCR assay. The primers were selected from the invA gene specific for the detection of Salmonella spp. In this study 8.3% of poultry carcasses were found to be contaminated with Salmonella spp. In order to provide a more accurate profile of the prevalence of Salmonella spp in broiler carcasses, it is pertinent to use inv A gene specific PCR method that could be considered as an appropriate alternative to conventional culture method.
Key words: Salmonella, broiler, polymerase chain reaction (PCR), invA Gene

Introduction

Poultry and poultry products have been implicated as a major source of salmonella infection in human (Amavisit *et al.,* 2001). Salmonella spp. is the very important bacterial pathogen of poultry in the world cause an important economic loss in poultry rearing and food industries. It has been reported that in addition to mishandling of poultry product and raw poultry carcasses, uncooked poultry meat is also one of the most frequent cause of human infection by Salmonella species (Panisello *et al.*, 2000).

Salmonella enterica serovar Typhimurium and *Salmonella enterica serovar Enteritidis* are the most frequently isolated serovar from food borne outbreaks throughout the world (Herikstad *et al.,* 2002). Established conventional methods to detect and identify Salmonella are time consuming and include selective enrichment and plating followed by biochemical tests (Bennasar *et al.,* 2000; Burtscher *et al.,* 1999; Chiu and Jonathan, 1996). Several techniques have been developed for the improvement in the detection of Salmonella serovars, such as the use of selective culture medium and enzyme-linked immunosorbent assay.

However, because of controversy in interpreting of results, low sensitivity and specificity of these methods, it needs to modify and improve them (Chiu and Jonathan, 1996).

In vitro amplification of DNA by the PCR method is a powerful tool in microbiological diagnostics (Malorny *et al.,* 2003). Several genes have been used to detect Salmonella in natural environ-mental samples as well as food and faecal samples. Virulence chromosomal genes including; invA, invE, himA phoP are target genes for PCR amplification of Salmonella species. (Jamshidi,*et.al.,* 2009). The invA gene of Salmonella contains sequences unique to this genus and has been proved as a suitable PCR target, with potential diagnostic applications (Rahn *et al.,* 1992). In this research, samples from broiler chickens were tested, for isolation of Salmonella, by culturing and biochemical method and then they were confirmed by invA specific PCR methods.

Materials and Methods

Samples from broiler carcasses and culture methods: Sixty samples of broilers were collected from slaughter houses of Namakkal. Samples were aseptically cultured into selenite F broth (Himedia) and incubated at 37^0C for 24 hours. Subsequently, a loopful of each broth was streaked on surface of MacConkey agar plates (Himedia) and Xylose Lysine Desoxycholate agar (Himedia) for further incubation

at 37^{0}C for 24 hrs. The biochemical characters of bacteria from non-lactose fermenting determined using triple sugar iron agar (Himedia). Colonies that show biochemical reactions like Salmonella were transferred to nutrient agar slant (Himedia) and incubated at 37^{0}C for 24 hrs.

An extraction of isolated Salmonella: Bacteria were cultured on nutrient agar for 24 hrs at 37^{0}C. Extraction of DNA was performed by boiling for 10 min and centrifuged at 6000 rpm for 5min. The supernatant were used for amplification by PCR with Salmonella specific primers.

Primers set and PCR amplification program: Salmonella specific primers, S139 and S141 (Rahn *et al.*, 1992) have respectively the following nucleotide sequence based on the invA gene of Salmonella 5´ GTG AAA TTA TCG CCA CGT TCG GGC AA -3´and 5´ TCA TCG CAC CGT CAA AGG AAC C -3´. Reaction with these primers were carried out in a 50 micro liters amplification mixture consisting of 25 microliters of PCR Master mix (Genei, Bangalore), 2 micro liter of each primer, 19 micro liters of molecular grade water and 2 micro litres of extraction for each isolate were used in the reaction.

Amplification was conducted in Master-gradient Thermocycler (Eppendorf). The cycle conditions were as follow: An initial incubation at 94^{0}C for 60^{0}sec. Followed by 35 cycles of denaturation at 94^{0}C for 60 sec, annealing at 64^{0}C for 30 sec and elongation at 72^{0}C for 30 sec, followed by 7 min final extension period at 72^{0}C.

Electrophoresis of PCR products: The amplified DNA products from Salmonella specific-PCR were analyses with electrophoresis on 1.2% agarose w/v gels stained with ethidium bromide and visualized by UV illumination. A current of 120 V was applied to each gel. Eight micro liter of PCR product mixed with 3 micro liters of 6 X-loading dye (Genei, Bangalore), were loaded on to agarose gel. A 100 bp DNA ladder was used as a marker for PCR products.

Results and Discussion

In recent years most investigators (Guo *et al.*, 1999; Ferretti *et al.*, 2001; Schneder *et al.*, 2002) try to establish a method, which can reduce the periods of Salmonella identification procedures from various samples. In an 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 specific PCR assay, which was validated in that project, showed high selectivity on 242 Salmonella strains (sensitivity 99.6%) and 122 non-Salmonella strains (specificity 100%). Amplification of invA gene now has been recognized as an international standard for detection of Salmonella genus (Malorny et al., 2003). This gene encodes a protein in the inner membrane of bacteria which is responsible for invasion to the epithelial cells of the host (Darwin and Miller, 1999).

Salmonella specific PCR with primers for invA is rapid, sensitive, and specific for detection of Salmonella in many clinical samples (Lampel *et al.*, 2000). The present study supports the ability of these specific primer sets to confirm the isolates as Salmonella. In the present study we used S139 and S141 primers for specific detection of Salmonella at the genus level. A total of five Salmonella isolates were found in chicken samples (8.3%), by conventional culturing and confirmed by PCR. All strains were subjected to Salmonella-specific gene (invA) and were confirmed as Salmonella positive by the predicted product a 284-bp DNA fragment. The results obtained in the present study were in corroboration with Nagappa *et al.,* (2007).

The ability of *Salmonella* specific primers to detect *Salmonella* species rapidly and accurately in the present study is primarily due to the primer sequences that are selected from the gene *invA* of *S. typhimurium as reported earlier by* Darwin and Miller, 1999. In other similar studies, *Salmonellae* have been recovered from poultry meat (*S.* Typhimurium,*S.* Saint Paul, *S.* Indiana, *S.* Stanley, *S.* Derby and *S.* Newport) and fresh buffalo meat (*S.* Typhimurium) (Sharma, *et. al.,* 1995 & 1989).

Studies in other countries have reported that the prevalence of Salmonella in poultry carcasses, with contamination percentages ranging from 3% to 66% (Zhao *et al.,* 2001; Uyttendaele *et al.,* 1998), although our results were in this range indicate the need to improve hygiene and sanitary standards in poultry slaughter lines, besides the information to consumers.

Culture techniques are universally recognized as the standard methods for the detection of bacterial pathogens, such as Salmonella in food stuffs (White *et al.,* 2002). These techniques generally take longer time (Malorny *et al.,* 2003) and are less sensitive compared to PCR based methods (Oliveira *et al.,* 2002). The use of inv A Gene specific PCR method in most diagnostic and research laboratories is possible and through the molecular basis Salmonella identification techniques, this method is the simplest and less expensive.

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