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Research Article

# Prevalence, Characterization and Detection of *Salmonella spp.* from Various Meat Sources.

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ABSTRACT

This study was designed to determine the presence of *Salmonella* in various meat samples. A total of 400 meat samples from chicken, fish and cattle were collected locally in Bareilly city, Izatnagar, India. The highest prevalence (11.0%) was observed in fish followed by chicken (8.0%) and beef (4.0%) using cultural and PCR methods. Among spiking samples 100% detection of *S. enteritidis* was found using both cultural and PCR method. The results of the study indicate that considerable prevalence of *Salmonella spp.* were observed from various meat samples which is representing use of poor hygienic conditions during slaughtering. Thus the consumer is under potential health threat of Salmonellosis and it is suggested that good hygiene practices should be ensured to maintain good quality of the food in favour of public health.



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## INTRODUCTION

Contaminated food plays one of the most important roles in human health problem such as diseases. It has been considered an important cause of reduced economic productivity (Abubakar et al. 2007). Meat and meat products are consumed all over the world including India. Due to high nutritive value and presence of wide range of micro and macro-nutrients they serve as good medium for the growth of diverse group of bacterial pathogens (Saikia and Joshi, 2010).

Salmonellosis is one of the most important infectious diseases in both humans and animals. *Salmonella* infections are caused by ingestion of contaminated food or water, after which the bacteria are able to colonize the small intestine and subsequently invade intestinal enterocytes. The principal clinical syndromes associated with *Salmonella* infection are enteric (typhoid) fever and gastroenteritis. Enteric fever is a protracted systemic illness that results from infection with exclusively human pathogens such as *Salmonella typhi* (*S. typhi*) and *Salmonella paratyphi* (*S. paratyphi*). Clinical manifestations include fever, abdominal pain, transient diarrhoea or constipation, and occasionally maculo–papular rashes. The pathological hallmarks of enteric fever are mononuclear cell infiltration and hypertrophy of the reticulo–endothelial system, including the intestinal Payer's patches, mesenteric lymph nodes, spleen, and bone marrow. Without treatment, mortality can be 10%–15%. In contrast, many non–typhoidal *Salmonella* strains, such as *S. enteritidis* and *S. Typhimurium*, infect wide range of animal hosts, including poultry, cattle, and pigs (Miller and Pegues, 2000; WHO, 2012). The widespread occurrence of *Salmonella* in natural environment and the intensive husbandry practices used in the meat, and fish industries have been a significant problem in public health (Akbarmehr, 2010).

The conventional identification of *Salmonella* from these foods involves pre–enrichment, selective enrichment, selective plating, biochemical screening and serological confirmation. These generally require 4 to 5 days to confirm the *Salmonella spp.* The PCR based methods have been revealed as rapid and highly specific methods for the detection and identification of the bacteria (Alarcon et al. 2004). The *invasive* (*inv*A) gene based PCR method also has been used for the identification of *Salmonella spp* (Malorny et al. 2009).

In India, studies are available on *Salmonella spp* isolation from various meat sources including beef, fish, poultry and poultry products (Hatha and Lakshmanaperumalsamy, 1997; Agarwal et al. 1999; Selvaraj et al. 2010). However, not much emphasis on the presence of *Salmonella spp.* in these meat sources has been given especially at the consumer point. Therefore, in the present study we characterized *Salmonella spp.* to investigate the prevalence of the pathogen in raw meat samples collected at consumer point in Bareilly, India. Further, the efficiency of standardized PCR assay was also examined and comprised with cultural methods for the rapid detection of *Salmonella spp.* in spiked meat samples,

## MATERIALS AND METHODS

### *Sample collection*

A total of 400 meat samples from beef, chicken and fish were collected from local butchery shops in Bareilly city as per method of Bacteriological Analytical Manual Online, USFDA described by Andrews and Hammack (1998).

Isolation of *Salmonella spp.* from various meat samples was attempted also accordingly to method described by Andrews and Hammack (1998). Briefly, raw meat or milk products (25 g) or raw milk (25 ml) sample were homogenized in 225 ml of



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Lactose broth and incubated at room temperature for 1 h (Pre–enrichment). The pH was adjusted to 6.8 ± 0.2 and incubated mixture at 35°C for 24 h. One ml mixture was transferred to the 10 ml of Tetrathionate (TT) broth and incubated at 35°C for 48 h. After 48 h of enrichment in Tetrathionate broth, inoculum was streaked onto Hektoen enteric (HE) agar and incubated at 35°C for 48 h. Plates were checked for growth of typical colonies after 48h.

The typical *Salmonella* colonies were examined for their size, colour, consistency, shape and microscopic examination after Gram's staining. For the conformation of *Salmonella*, the biochemical characterization of the suspicious colonies was determined by lactose and sucrose fermentation, indole production, methyl red, voges proskauer, citrate utilization, $H_2S$ production, lysine decarboxyllation, and urease production assay as described by Andrews and Hammack (1998).

PCR reaction for *inv*A gene based identification of *Salmonella spp.* was standardized as per recommendation by Cocolin et al. (1998) with some modifications. The DNA extraction method was adopted as described earlier by Sambrook and Russell (2001). The primers used in this study were synthesized by Agile Life Sciences, Mumbai, India. The PCR reaction for amplification of *inv*A gene (389 bp) was optimized as follows: 5 µl of 10X PCR buffer (20 mM Tris HCl, pH 8.0 at 25°C, 100 mM KCl, 0.1 M EDTA, 1 mM DTT, 50% glycerol, 0.5% Tween 20 and 0.5% Nonidet–P40), 1.5 mM $MgCl_2$, 0.2 mM of each dATP, dCTP, dGTP and dTTP, 1 µl of each primer (10 pmol), 1 U (unit) of *Taq* DNA polymerase (Biogene, USA), 5 µl of DNA as template and final volume made upto 50 µl using nuclease free water. The cycling conditions include initial denaturation step at 95°C for 5 min followed by 35 subsequent cycles of heat denaturation of 95°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min. A final extension was performed at 72°C for 5 min to complete the synthesis of all strands. The PCR products were separated in 1.5% agarose gel electrophoresis, stained by ethidium bromide (Sigma–Aldrich, USA), and visualized under UV light.

The specificity of PCR assay was determined using reference/standard cultures of *S. typhimurium* (MTCC 98), *S. enteritidis* (E 2094), and *E. coli* (MTCC 443), as positive and negative controls.

For determining the efficiency of PCR in spiked meat samples, the meat samples were collected and subjected to isolation and detection of *Salmonella spp.* using cultural method as described above. The samples found negative for the presence of *Salmonella* were selected for spiking with *S. enteritidis* (E 2094). A total of 50 meat samples were prepared for the spiking studies.

The preparation and spiking of samples were performed as per Alarcon et al. (2004) with slight modifications. Briefly, meat (2 g) sample was mixed in 18 ml of Buffered Peptone Water separately, in a sterile plastic bag with lateral filter. Then samples were homogenized in a stomacher for one min, separately. The 1.8 ml of resulting mixture from filtered and homogenized sample was taken and inoculated with 0.2 ml of brain heart infusion broth cultures ($10^8$ cfu/ml) of standard strains of *S. enteritidis* (E 2094). The spiked samples were incubated at 37°C for 18 h.

The DNA extraction was performed using heat lysis (Snap chill method) method as described earlier by Arora et al. (2006) with required modifications. Briefly, 100 µl of broth culture (pure) was centrifuged at 5,000 rpm for 5 min. The pellet was resuspended in 100 µl of PBS (Fermentas, Lithuania, USA) in a microcentrifuge tube. This step was repeated twice and resulting pellet, after proper mixing, was kept in a boiling water bath for 10 min. After heat treatment, the cell lysate was kept into ice immediately and after 10 min, centrifuged at 5,000 rpm for 5 min. The PCR reaction standardized as described earlier in this section to detect the *Salmonella* by using supernatant (5 µl) as template.

Simultaneously, the spiked samples were also subjected for the detection of *Salmonella* by cultural methods.

## RESULTS AND DISCUSSION

Salmonellosis is an important food borne infective disease worldwide, occurring mostly as sporadic cases in families or as outbreaks. Poultry and poultry products have been the most commonly implicated foods to cause infection in human (Loongyai et al. 2010). Although meat and meat products, milk and milk products, and water have also been associated with large outbreaks of Salmonellosis (Bansal et al. 2006; Bhunia et al. 2009; Nicolay et al. 2010).

In the present study, a total of 22 (11.0%), 12 (8.0%) and 02 (4.0%) isolates of *Salmonella spp.* were isolated from various meat samples viz. fish, chicken and beef using cultural and biochemical methods (Table 1). A wide range of *Salmonella spp.* prevalence from 0.9 to 90.0% from various meat sources has been reported by some other investigators (Bouchrif et al. 2009; Kumar et al. 2010). Soltan Dallal et al. (2009) reported close prevalence (6.7%) in ground beef whereas Selvaraj et al. (2010) also reported a very close prevalence (4.5%) of *Salmonella spp.* from chicken samples.

**Table 1.** Prevalence (%) of *Salmonella spp.* in various meat sources by cultural and PCR methods.

| Source (number of samples tested) | Cultural method (%) | PCR method (%) |
|---|---|---|
| Beef (50) | 02 (4.0%) | 02 (4.0%) |
| Fish (200) | 22 (11.0%) | 22 (11.0%) |
| Chicken (150) | 12 (8.0) | 12 (8.0) |

The invasion of intestinal epithelium cells is one of the earliest and important steps in the pathogenic cycle of the *Salmonella spp.* The genetic locus, inv, allows *Salmonella spp.* to enter epithelial cells and *inv*A is a member of this locus (Galan et al. 1992) that codes inner membrane protein of bacteria, which is necessary for the invasion into epithelial cells (Darwin and Miller, 1999). The *inv*A 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; Ginocchio and Galan, 1994; Shanumugasamy et al. 2011). The amplification of this gene now has been recognized as an international standard for detection of *Salmonella* genus (Malorny et al. 2003). The *inv*A gene has been the most frequently targeted gene for primer selection in PCR based detection (Gado et al. 2000; Ueda et al. 2000; Chen and Griffiths, 2001; Zahraei et al. 2007; Jamshidi et al. 2008; Amini et al. 2010) and has been reported in all the serovars of *Salmonella* (Galan and Curtis, 1991; Swamy et al. 1996). Therefore, in this study, PCR was utilized for rapid identification of *Salmonella species* from meat sources, targeting *inv*A gene and found all the 36 *Salmonella* isolates characterized biochemically were positive for *inv*A gene by standardized PCR assay (Table 1). Numerous reports available on incidence of *inv*A gene in *Salmonella spp.* are supporting our findings (Hong et al. 2003; Rivera–Betancourt et al. 2004; Karns et al. 2005; Kumar et al. 2008; Upadhyay et al. 2010).


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The primer sequences used in this study for the *inv*A gene identification have been described in literature earlier. The *inv*A gene specific PCR assay used in this study generated a PCR product of 389 bp only from *S. typhymurium* (MTCC 98) and *S. enteritidis* (E 2094) (Fig. 1). Similar amplified products were also obtained from the *Salmonella* isolates obtained in our study (Fig. 2). The amplification of the specific product of 389 bp with these primers also from *Salmonella* isolates suggested that these sequences of *inv*A gene are highly conserved among *Salmonella spp*. The results obtained in this study by using these primers are in agreement with the earlier work described (Malorny et al. 2003). Therefore, our results also favour this gene as suitable marker for the identification of *Salmonella spp.*

The results obtained from spiking study found that cultural and PCR methods were equally reliable for detection of *Salmonella spp.* contamination at the level of $10^8$ cfu/g after 18 h enrichment of meat samples. The detection of 100% was noticed and no significant difference was observed by using both methods (Table 2). However, due to poor competent nature of the *Salmonella spp.*, the identification at the low level of cell concentration might be difficult. Moreover, the detection of bacterial contamination below the 100 cfu/g

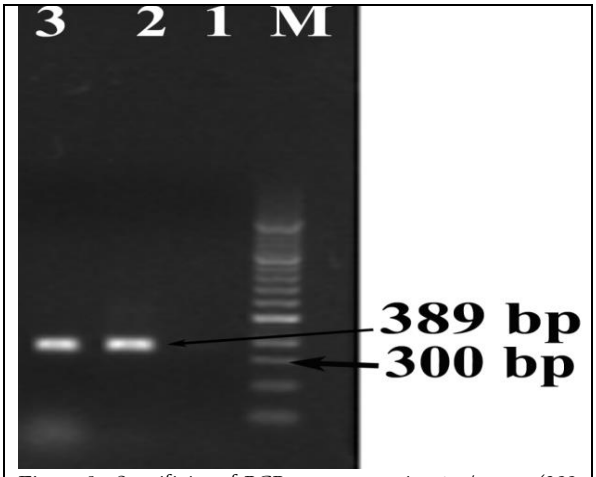


**Figure 1.** Specificity of PCR assay targeting *inv*A gene (389 bp) for the detection of *Salmonella spp.* Lane M– DNA ladder (100 bp); Lane 1– *E. coli* (MTCC–443); Lane 2– *S. typhimurium* (MTCC 98); Lane 3–*S. enteritidis* (E 2094).

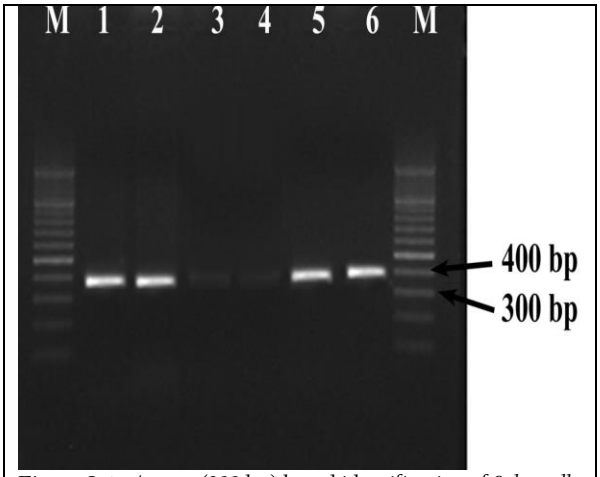


**Figure 2.** *inv*A gene (389 bp) based identification of *Salmonella* isolates by PCR from various meat samples.
Lane M–DNA ladder (100 bp); Lane 1–6–isolates positive for *Salmonella spp.*

**Table 2.** Detail of cultural and PCR based detection of *S. enteritidis* in spiked meat samples.

| Sample No. | Detection by cultural method | Detection by PCR method | Sample No. | Detection by cultural method | Detection by PCR method |
|---|---|---|---|---|---|
| SM1 | + | + | SM26 | + | + |
| SM2 | + | + | SM27 | + | + |
| SM3 | + | + | SM28 | + | + |
| SM4 | + | + | SM29 | + | + |
| SM5 | + | + | SM30 | + | + |
| SM6 | + | + | SM31 | + | + |
| SM7 | + | + | SM32 | + | + |
| SM8 | + | + | SM33 | + | + |
| SM9 | + | + | SM34 | + | + |
| SM10 | + | + | SM35 | + | + |
| SM11 | + | + | SM36 | + | + |
| SM12 | + | + | SM37 | + | + |
| SM13 | + | + | SM38 | + | + |
| SM14 | + | + | SM39 | + | + |
| SM15 | + | + | SM40 | + | + |
| SM16 | + | + | SM41 | + | + |
| SM17 | + | + | SM42 | + | + |
| SM18 | + | + | SM43 | + | + |
| SM19 | + | + | SM44 | + | + |
| SM20 | + | + | SM45 | + | + |
| SM21 | + | + | SM46 | + | + |
| SM22 | + | + | SM47 | + | + |
| SM23 | + | + | SM48 | + | + |
| SM24 | + | + | SM49 | + | + |
| SM25 | + | + | SM50 | + | + |


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in food samples by using cultural method is difficult due to the presence of a high level of background microflora and competitor organisms (Khan et al. 2011). The superiority of PCR over cultural methods for the detection of *Salmonella spp.* in food samples has been reported (Jenikova et. al. 2000). The detection of *Salmonella spp.* even upto 1 cell/25 g after 30 h is reported by Robel et al. (2009). However, enrichment time play an important role for the detection of pathogen. The PCR assay described in this study can be used to identify *Salmonella spp.* in food laboratories within 24 h and thus can improve identification efficiency, by replacing cultural methods which required 4 to 5 days.

In conclusion, results indicated that the beef, fish and chicken meat sold at retail butcheries were contaminated with *Salmonella spp.* in Bareilly city that may cause severe *Salmonella* infections. Therefore, it is suggested that implementation of Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) should be ensured to maintain the good quality of the food and food products.

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