**Isolation of Salmonella Typhimurium from poultry eggs and meat of Tarai region of Uttaranchal**

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Presence of *Salmonella* in samples, 100 each of chicken eggs and meat, collected from various retail outlets of the Tarai region of Uttaranchal, was detected by polymerase chain reaction (PCR) using InvA gene primer, a rapid and sensitive DNA based method. The results were further validated by established conventional method of *Salmonella* isolation and biochemical tests, including slide agglutination test (poly “O” antiserum specific for *Salmonella*). Of 200 samples analyzed, 4 (one meat and 3 egg) were found to be contaminated with *Salmonella enterica* var Typhimurium. Further, the antibiogram of the isolates revealed sensitivity in the decreasing order to Chloromphenicol, Colistin, Polymixin, Enrofloxacin, and Ciprofloxacin.

**Keywords:** antibiotic sensitivity, eggs, meat, PCR, poultry, *Salmonella*

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*Salmonella* needs special concern in the developing world because of the poor hygienic conditions that favour its spread. There are more than 2,435 known serotypes of *Salmonellae* of which 209 serovars have been documented in India¹ and many of these serotypes are well documented human pathogens². For the effective prevention and control of this food-borne zoonosis, rapid and sensitive detection methods are required. The isolation and identification of *Salmonella* using conventional cultural method is time consuming and laborious. Many different conventional media and enrichment regimes have been proposed for isolating *Salmonella* species from the food and environmental samples³⁴. Even then these organisms are still considered difficult to culture, detect or enumerate in the complex microbial communities⁵. The rapid development in the field of molecular biology has paved way for formulating number of DNA based techniques that are in current use and have overcome the difficulties associated with the conventional methods. The DNA based techniques that are presently used include plasmid profiling, chromosomal characterization (ribotyping, RFLP, IS200 profiling and PFGE) and use of PCR with complementary approaches (RAPD, AP-PCR and ERIC-PCR). Most widely used DNA based technique is PCR, utilizing genus specific primers targeting various genes. For instance, InvA gene⁶ have been introduced for the effective, rapid and accurate detection of *Salmonella* in foods of animal origin.

Salmonellosis is a direct occupational anthropozoonotic disease of great economic and public health concern. *Salmonellae* are widely distributed in nature and cause a spectrum of diseases in man, animal and birds. Poultry eggs, meat and their products are the commonest vehicles of *Salmonella* to humans. In India Salmonellosis is hyper-endemic and there is an urgent need to strengthen the monitoring and surveillance of salmonellosis using suitable diagnostic tools so as to prevent and control its occurrence¹. The present study was designed with an objective to know the status of Salmonellosis in poultry products in the Tarai region of Uttaranchal, India and to validate a sensitive technique for its rapid detection in poultry eggs, meat and meat products.

To analyze the presence of *Salmonellae*, samples were obtained from different locations of Tarai region of Uttaranchal. As many as 200 samples (100 each of chicken eggs and meat) were collected from retail markets in and around Pantnagar (District Udham Singh Nagar of Uttaranchal, India). About 25 g of sample was triturated aseptically and pre-enriched in 250 mL of buffered peptone water (BPW). After incubation at 37°C for 18 h in BPW, 2 mL of culture was taken in microfuge tube and centrifuged at 14,000 rpm for 10 min at 4°C. The supernatant was discarded and the pellet was re-suspended in 100 µL distilled water. The hot-cold lysis of the bacterial cells was performed and centrifuged at 14,000 rpm for 10 min at 4°C. 10 µL of cell lysate was added to a mixture (40 µL) consisting of 5 µL 10 × PCR-reaction-buffer with 15 mM MgCl₂ (Banglore GENEI), 5 µL 2 mM dNTP mix (Banglore GENEI), 10 pmol of each InvA

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gene primer (ST-139, 5′-GTG AAA TTA TCG CGT TCG GGC AA-3′ and ST-141, 5′-TCA TCG CAC CGT CAA AGG AAC C-3′; Life Technology), 0.5 U Taq DNA polymerase (MBI Fermentas Inc.) and made to final volume using sterile triple glass distilled water. Then PCR was conducted as per the standard protocol with slight modifications, i.e., initial denaturation at 94°C for 5 min, followed by 30 cycles with denaturation at 94°C for 1 min, annealing at 60°C for 2 min, extension at 72°C for 2 min with a final extension at 72°C for 5 min. Amplified (10 µL) product was mixed with 2 µL of 6 × loading dye and loaded on 1.5% agarose gel pre-stained with ethidium bromide (0.5 µg/mL) using electrophoresis buffer (1 × TAE). The samples were run @ 5 V.cm⁻¹ for 30 min. Bands were visualized by UV transilluminator and were photographed with gel documentation system.

The PCR results were further confirmed by conventional cultural method described for the isolation of Salmonella. After incubation at 37°C for 18 h about 0.1 mL of the pre-enriched culture was inoculated into 10 mL of RV-10 (Rappaport-Vassidias broth) and incubated at 43°C for 18-24 h. Thereafter, a loop full of culture was streaked on brilliant green agar (BGA) and McConkey lactose agar (MLA) for the selective isolation of Salmonellae. Representative colonies were then further tested by using a battery of biochemical tests such as TSI, urease, nitrate reduction, oxidase, indole, methyl red, Voges Proskauer, citrate and sugar (adonitol, dulcitol, glucose, inositol, lactose, mannitol, salicin, sorbitol and sucrose) fermentation tests. The putative isolates were confirmed by slide agglutination test using polyclonal “O” antiserum specific for genus Salmonella (procured from Indian Veterinary Research Institute, Izatnagar). The confirmed isolates were sent to National Salmonella Centre, IVRI, Izatnagar (Bareilly), for serotyping. Simultaneously, PCR was performed as described above on the samples after pre-enrichment to assess its sensitivity with respect to rapid detection. Further, in vitro antibiotic sensitivity of these isolates was assessed using 14 different antibiotics by employing Kirby-Bauer’s single disc diffusion method.

Presence of Salmonella was documented by the appearance of an amplified PCR fragment of 284 bp. Out of 200 samples analyzed, 4 samples were found to be contaminated with Salmonella enterica var Typhimurium (one meat and three eggs). Further, all the four isolates were found to be sensitive to Chloromphenicol and Colistin, followed by Polymixin, Enrofloxacin and Ciprofloxacin in the decreasing order. However, all the isolates were resistant to Cotrimazole and Cephalothin (Table 1). 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).

Use of PCR has revolutionized the diagnostic tools for the detection of various food-borne pathogens. The present study validates the application of PCR in the rapid detection of Salmonellae. Several PCR methods for the detection of Salmonella have been published utilizing a specific DNA sequence for the amplification. Several sets of primers targeting different fragments of genomic DNA of Salmonella have also been evaluated for the specific detection of Salmonella by PCR. PCR based rapid diagnosis of Salmonella in the field samples is of utmost importance in the prevention and control of Salmonellosis. In the present study, detection of

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<th>Antibiotic</th>
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<th>Isolate-3 (Egg)</th>
<th>Isolate-4 (Egg)</th>
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S—Sensitive, I—Intermediate, R—Resistant.
Salmonella serovars from the foods of animal origin

Fig. 1—Agarose gel electrophoresis of PCR product of InvA gene of Salmonella Typhimurium: Lane M, Marker; lane N: Negative control (Triple glass distilled water); lane P: Positive control (known S. Typhimurium); lane 1: S. Typhimurium isolated from chicken meat; & lane 2-4: S. Typhimurium isolated from chicken eggs.

was carried out by enrichment broth cultivation–PCR procedure. The primer selected was from sequences of InvA gene, which produced a fragment of 284 bp. The sensitivity and specificity PCR was in accordance with the conventional cultural method (Fig. 1). The results obtained in the present study were in corroboration with other investigators\(^\text{16}\) and the PCR can be used as an alternative tool to the conventional isolation and identification methods for the rapid detection of Salmonellae in the poultry.

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References