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APPLICATION OF PCR FOR DETECTION OF *SALMONELLA* SEROVARS FROM BROILER CHICKENS

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Abstract: A study was carried out for the identification of *Salmonella* serovars prevalent in broilers in and around Bangalore. A total of 260 samples *viz*., 160 tissues each of liver, gall bladder, spleen and heart, 25 fecal samples and 25 feed samples, 10 ovaries and 40 eggs were collected from 5 organized poultry farm and retail outlets in and around Bangalore were screened. Twenty one isolates were subjected for PCR with genus specific *inv*A, serogroup specific *sef*A and serotype specific *fli*C primers. The presumptive identification of isolates was *S.* Gallinarum. All the 21 isolates were amplified with *inv*A and s*ef*A and none from *fli*C. The PCR proved to be the rapid and sensitive test for identification of *Salmonella* serovars.

Keywords: broilers, PCR, *Inv*A, *sef*A, *fli*C, S. Gallinarum.

Introduction

Salmonellosis is an infectious disease of humans and animals caused by organisms of the two species of *Salmonella* (*S.* enterica, and *S.* bongori). Although primarily intestinal bacteria, salmonellae are widespread in the environment and commonly found in farm effluents, human sewage and in any material subject to faecal contamination. *Salmonella* infections in humans occur most commonly as secondary contaminants of food originating from animals and the environment, usually as a consequence of subclinical infection in food animals leading to contamination of meat, eggs, and milk or secondary contamination of fruits and vegetables that have been fertilized or irrigated by faecal wastes.

Poultry enjoys a highly significant position on the sector of livestock whose contribution to the country's GDP is Rs. 260,300 crore (3.6 %) (Ministry of Statistics, Govt. of India, 2012). Poultry products can be major vehicles of food borne salmonellosis because the raw products are initially contaminated with *salmonella* cells (Bryan and Doyle, 1995).

Materials and Methods

Isolation from samples: All the samples were collected in aluminum wrap and were transported to laboratory in refrigerated condition. For isolation OIE recommended procedure

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of pre-enrichment and enrichment followed. Enrichment was done in tetrathionate brilliant green broth and Rappaport-Vassilidias soyabean meal broth with 1ml Buffered Peptone Water (BPW) pre-enriched culture. Plating was done on BGA (M/s Himedia, Mumbai) initially and other selective plates included Xylose Lysine Deoxycholate (XLD), Hektoen Enteric (HE) and MacConkey (MCA). Pinkish round translucent colonies were formed on BGA and XLD, greenish round regular colonies on HE and pale colonies on MCA.

DNA isolation: DNA templates were prepared from isolates by boiling and snap chilling method. In this method, about 1000 µl of the 24 h inoculums from the selective enrichment was centrifuged at 6000 rpm for 5 min and resuspended in 50 µl of molecular grade water. The suspension was then kept in a boiling water bath for 10 min and immediately transferred onto ice, later it was centrifuged at 13000 rpm for 5 min and five µl of supernatant was used as template for PCR technique (Anumolu *et al.,* 2012).

PCR: The primers for detection of *Salmonella* were synthesized at Bioserves Pvt. Ltd., Hyderabad (Table 1).

Table 1. The nucleotide sequences of the primers used in this study for the detection of Salmonella sp.*, *S*. Enteritidis, *S*. Gallinarum and *S*. Pullorum ** and identification of *S*. Typhimurium***

Name of the primer	Nucleotide Sequence 5'— 3'	Amplicon size (bp)
ST-139 *	5'-GTG AAA TTA TCG CCA CGT TCG GGC AA -3'	284
ST-141*	5'-TCA TCG CAC CGT CAA AGG AAC C -3'	
A058**	5'- GAT ACT GCT GAA CGT AGA AGG-3'	488
A01**	5'- GCG TAA ATC AGC ATC TGC AGT AGC -3'	
Fli15***	5'- CGG TGT TGC CCA GGT TGG TAA T -3'	620
Typ04 ***	5'- ACT GGT AAA GAT GGC T -3'	

Isolates were subjected for three sets of primers *viz.,* genus specific *inv*A, serogroup D1 specific *sef*A and serotype specific *fli*C. The PCR protocol, annealing temperatures and cycling conditions initially standardized by (Siddique *et al*., 2009) for *inv*A and for *sef*A (Oliveira *et al*., 2002) and *fli*C were applied. *sef*A and *fli*C cycling conditions were prolonged to 1 min each as the conditions described (Oliveira *et al*., 2002) did not give satisfactory results. The reaction mixture consisted of 5µl of the template, 2.5 µl of 10x assay buffer for *Taq* polymerase containing 1.5 mM $MgCl_2$, 1 µl of 25 µM each dNTP mix, 1 µl each of

forward and reverse primer (4 pmol) and 0.9 U/ µl of *Taq* DNA polymerase made up to 25 µl using molecular grade water. Routinely, master mix was prepared and 20 µl each was distributed to the PCR tubes, to which 5 µl of the template was added. Amplification was carried out with initial denaturation at 94°C for 5 min, followed by 35 cycles each of denaturation at 94°C for 1 min, annealing at 45.1^{o}C for 30 sec and extension at 72°C for 38 sec with a final extension period of 72°C at 7 min. The amplification products were analyzed by agarose gel electrophoresis using 1.5% agarose gel containing 0.5 µg/ml ethidium bromide at constant voltage 5 V/cm in 1x TAE.

PCR targeting genus specific *inv*A gene that codes invasive protein of Salmonella, serogroup specific *sef*A that encodes fimbrial protein and serotype specific *fli*C gene that encodes flagellar protein of *S.* Typhimurium (Oliveria *et al.,* 2002).

Amplification for *inv*A gene primers was carried out using, initial denaturation at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 68°C for 1 min, extension at 72°C for 1 min followed by a final extension at 72°C for 5 min. Conditions for *sef*A were Initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min followed by a final extension at 72°C for 7 min.

Amplification products were separated by electrophoresis on 1.5% agarose gel containing 5 mg/ml ethidium bromide with 100 bp ladder as molecular weight marker. Bands were visualized under UV transilluminator and photographed by gel documentation system

Results and Discussion

Colonies suggestive of *Salmonella* were obtained based on morphology and Grams staining. Twenty one isolates were positive for *Salmonella* with the biochemical pattern. Biochemically isolates were negative for Indole, VP, Urea Hydrolysis and Oxidase and positive for Catalase, MR, lysine decarboxylation, and reduced nitrate**.** The biochemical profile was in accordance with traditionally accepted results (Mdegela *et al*., 2000), however there was variation associated with citrate utilization and hydrogen sulphide production on TSI.

Salmonella specific PCR with primers for *inv*A 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 genus level. A total of twenty one Salmonella isolates were found in chicken samples (8.07%), by

conventional culturing and confirmed by PCR. All strains were subjected to Salmonella-specific gene (*inv*A) and were confirmed as Salmonella positive by predicted product a 284-bp DNA fragment (Fig. 1). The results obtained in the present study were in corroboration with Nagappa *et al*., (2007).

All 21 isolates were amplified with an amplicon size of 284 bp with *inv*A primers with thermal cycling conditions confirming *Salmonella* genus. All 21 also were amplified with *sef*A serogroup specific primers with amplicon size of 488 bp (Fig. 2) and none of isolates were amplifies with *fli*C *S.* Typhimurium specific primers.

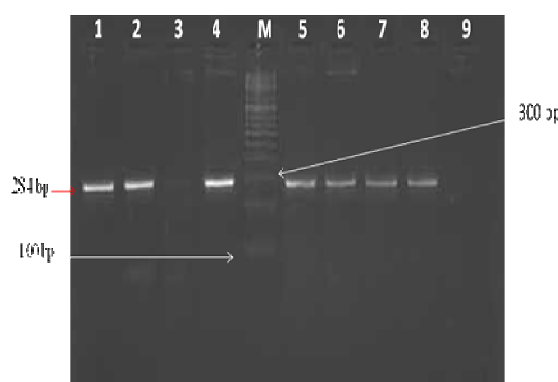

Fig. 1: PCR amplification of 284 bp *inv*A gene of *Salmonella* genus isolated from poultry.

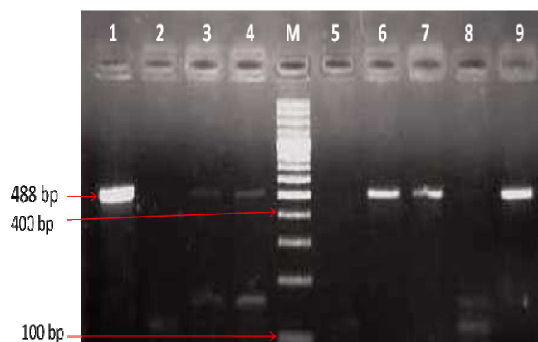

Fig. 2: PCR amplification of 488 bp *sef*A gene of *Salmonella* serogroup D1 isolated from poultry.

Conclusion

Culture techniques are universally recognized as gold standard methods for the detection of bacterial pathogens such as Salmonella in clinical samples, however these techniques take longer time and are less sensitive compared to PCR based methods. Salmonella specific PCR

methods with primers for *inv*A gene at genus level and *sef*A and *fli*C gene at serotype level are rapid, sensitive and specific for the detection of Salmonella in clinical samples.

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