ABSTRACT

Title	:	**Molecular epidemiology of *Salmonella* of poultry origin and development of rapid diagnostic kit**
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A study was undertaken on molecular epidemiology of *Salmonella* organisms isolated from poultry, poultry related products and environmental samples and to develop a rapid diagnostic kit for early detection of *Salmonella* from samples. A total of 1215 samples were collected from 154 farms and screened for the presence of *Salmonella* organisms. The collected specimens comprised of liver, spleen, ovary, intestinal contents, pooled organs and yolk from poultry, meat, egg, feed, fishmeal, meat and bone meal of poultry products and environmental samples such as water, drag swab, boot swab, muconium and fecal samples. The collected samples were subjected to conventional isolation method involving pre-enrichment in buffered peptone water, selective enrichment in tetrathionate broth and selective plating in brilliant green agar. Pink colour colonies developed in BGA plates were further subjected to biochemical tests such as urease activity, indole production, MR- VP test and lactose fermentation. Twenty one isolates were confirmed as *Salmonella*

enterica subsp *enterica* from the total specimens screened. The *Salmonella* isolates were designated as S2, S44, S87, S99, S100, S102, S184, S185, S186, S255, S380, S381, S585, S586, S711, S714, S759, S791, S828, S931 and S932. The isolates which were confirmed as *Salmonella enterica* subsp *enterica* by conventional method were subjected to molecular identification and further typing.

The overall isolation of *Salmonella* in this study was 1.73 per cent (21/1215). Tissue samples showed highest isolation of 3.06 per cent (12/392) followed by poultry related products 1.52 per cent (7/460) and environmental samples 0.55 per cent (2/363). The incidence of *Salmonella* isolation was high in winter (2.8%) as compared to summer (0.66%). Among the tissue samples collected from affected birds, the isolation was high in chicks below 3 weeks of age (7.27%) followed by layer chicken below 40 weeks (3.6%) and layer chicken above 40 weeks of age (1.78%).

Electron microscopic study of two isolates (S184 and S585) showed the presence of rod shaped morphology with microstructure such as peritrichous flagella and pili external to the cell wall. Antibiotic sensitivity of all the isolates showed cent per cent sensitivity to ciprofloxacin and none of the isolates were sensitive to oxytetracycline.

The molecular tests *viz.,* multiplex PCR, alllele specific PCR, real time PCR were used for the detection of *Salmonella* organisms and ERIC PCR, rRNA spacer region polymorphism, ribotyping and pulse field gel electrophoresis were performed for typing the *Salmonella* isolates.

Multiplex PCR was used to differentiate the *Salmonella* at serotype level by amplifying the fimbrial virulence gene *pef*A and restriction enzyme gene *kpnI.*

Salmonella Typhimurium produced two amplification fragments at 363 bp and 497 bp and *Salmonella* Enteritidis produced one amplification fragment at 497 bp level. Out of 21 isolates, 16 isolates (S2, S44, S87, S99, S100, S102, S184, S185, S186, S255, S380, S381, S711, S714, S931 and S932.) were identified as *Salmonella* Typhimurium and five isolates (S585, S586, S759, S791 and S828) as *Salmonella* Enteritidis.

Allele specific PCR was carried out for differentiating group D *Salmonella* from other *Salmonella* as well as from non–*Salmonella* organisms based on amplification of *rfb*S gene which is present in serogroup D *Salmonella* only. Out of 21 isolates four (S586, S759, S791 and S828) were identified as Group D *Salmonella* in allele specific PCR which produced amplification fragment of 720 bp.

The Real time PCR assay was carried out for the specific detection of *Salmonella* targeting the gene *inv*A. This assay incorporated both primers and hybridization probes based on the sequence of the *Salmonella inv*A gene. All the *Salmonella* isolates produced amplification curve by detecting the *inv*A gene with Ct value ranged from 23.11 $\pm$ 0.104 to 38.61 $\pm$ 0.316.

To identify the variation within the isolates, ERIC PCR was carried out by using the ERIC primers. All the isolates produced different banding pattern ranging from two to six bands with the fragments ranging from 150 bp to 2000 bp. In this study six ERIC profiles were noticed (ERIC 1 to 8).

The variation in the length of the internal transcribed spacer (ITS) region between the 16S and 23S rRNA genes of the *Salmonella* isolates was analyzed by PCR amplification. All the 21 isolates produced two to three fragments with the size ranging from 500 bp to 700 bp.

Ribotyping was used to sequence the *Salmonella* isolates. The 16S rRNA gene of five selected *Salmonella* isolates (S87, S100, S184, S585 and S759) was amplified with universal primers and sequenced. All the five isolates produced amplification of 1500 bp for 16S rRNA gene. The nucleotide sequencing of S87, S100 and S184 had a homology pattern of 93 per cent, 93 per cent and 99 per cent with *Salmonella* Typhimurium and remaining two isolates (S585 and S759) had a homology of 96 per cent and 95 per cent with *Salmonella* Enteritidis respectively.

Pulse Field Gel Electrophoresis was used to study the genetic relationship between the *Salmonella* strains. Out of twenty one isolates, 19 isolates (S2, S44, S87, S99, S184, S185, S186, S255, S585, S586, S380, S381, S711, S714, S759, S791, S828, S931 and S932) generated restriction fragments in PFGE with *Xba*1 enzyme and two isolates (S100 and S102) were untypable. Nineteen *Salmonella* isolates generated up to 13 restriction fragments with molecular size ranging from 40 to 700 kb fragments with 11 PFGE patterns (PFGE1 to PFGE11). Among these 11 different PFGE patterns, there were five PFGE patterns (PFGE1, PFGE 2 PFGE 3, PFGE 4 and PFGE 5) represented by two isolates (10.5%) each, PFGE 6 was represented by four isolates (21%) and remaining 5 PFGE patterns represented by single isolate.

Based on the molecular tests carried out in the present study, a rapid diagnostic kit was developed with multiplex PCR for early detection of *Salmonella* organisms using *Salmonella* specific primers in poultry, poultry related products and environmental samples.