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DEVELOPMENT OF A MULTIPLEX PCR TECHNIQUE FOR DETECTION OF *Salmonella* IN FOOD SAMPLES

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Abstract- We have developed a multiplex Polymerase chain reaction (PCR) assay for detect the *Salmonella* spp. and *Salmonella typhimurium* serotypes. In the present study carried out to report the occurrence *Salmonella* spp. and *Salmonella typhimurium* in chicken and poultry feaces samples. *Salmonella* isolates were identified by a multiplex-PCR using two sets of primers targeting the *inv*A and *Typh* gene sequences from *Salmonella* spp. and *S. typhimurium* respectively. *Salmonella* spp. was detected in 3% (3/100) of the samples, whereas serovars *typhimurium* were identified in 1% (1/100), respectively. The results indicate the need to improve hygiene and sanitary standards in poultry slaughter lines, besides the education of food handlers and information to consumers.
Keywords- *Salmonella*, Polymerase chain reaction (PCR), Food

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Introduction

The genus of *Salmonella* is a gram-negative rod shaped bacteria in the family of *Entrobacteriacea*. Poultry and poultry products have been implicated as a major source of *Salmonella* infections in human. *Salmonella* infections still occur at high frequencies in industrialized [11]. In the other hand they are very important bacterial pathogens of poultry in the all of the world caused an important loss in poultry rearing and food industries. So that prevention of *Salmonella* infection is important for poultry health and for food processing industries [2]. Traditional *Salmonella* detection methods are based on cultures using selective media and characterization of suspicious colonies by biochemical and serological tests. These methods are generally time-consuming. Therefore, a rapid method is necessary for identification of *Salmonella* serotypes from foods. There are several PCR assays to detect *Salmonella* bacteria in feces, but diagnostic PCR is limited by the presence of inhibiting substances in complex biological samples [7,9]. These substances can interfere with cell lysis or inactivate the DNA polymerase, and DNA extraction procedures are usually necessary to remove them [1]. The utility of multiplex PCR as a tool for pathogen detection in clinical and environmental samples is well documented [8,10,14]. The aim of this study was to develop a multiplex PCR assay able to detect *Salmonella* and simultaneously detect the most important serotypes and phage types in Bareilly region.

Material and Method

Take 50 samples each of the chicken and poultry feaces and put in incubator for 24Hrs.in BPW, after that isolate DNA by boiling and snap chilling method. The primer set was chosen to amplify products with lengths similar to those of the 100-bp ladder (Amersham Biosciences, Piscataway, N.J.) bands. A total of two different sequences were amplified in each reaction mixture: a *Salmonella* genus-specific sequence (284bp), a serotype Typhimurium-specific sequence (401bp). PCR assay for duplex gene (*Typh* gene+*invA* gene) was standardized with 2.5 mM $MgCl_2$, 200µM dNTP mix, 10 pmol each of forward and reverse primers of both the *invA* and *Typh* gene and 2 unit Taq DNA polymerase. Primer annealing was done at 55ºC for 1.5 minute. Visualization of PCR amplified product following agarose gel electrophoresis revealed specific amplification of a 284bp and 401bp [Fig-1] nucleotide segments. This duplex gene can be identified all the *Salmonella* strains and differentiate by the *Salmonella typhimurium* serotype.

Result and Discussion

Out of 100 samples there were only three were positive with *inv*A gene from which only one isolate was positive by *Typh* gene. The *invA* gene is essential for full virulence in *Salmonella* and thought to trigger the internalization required for invasion of deeper tissues [20], and is specific for *Salmonella spp.* The *invA* primers amplified 373bp fragment in all seven strains of *S. typhi, S. para A, B,* and *C, S. havana, S. infantis,* and *S. entritidis* [4]. Most of the researchers who have already applied conventional and PCR for detection of *S. enterica* have used often only one gene of this organism in their studies. The serovars other than *Typhi* have been detected in some cases due to cross reaction of single gene directed PCR [12]. Our data indicate that the Multiplex PCR test using two gene

primers was as sensitive as a standard culture method in detecting *Salmonella*.

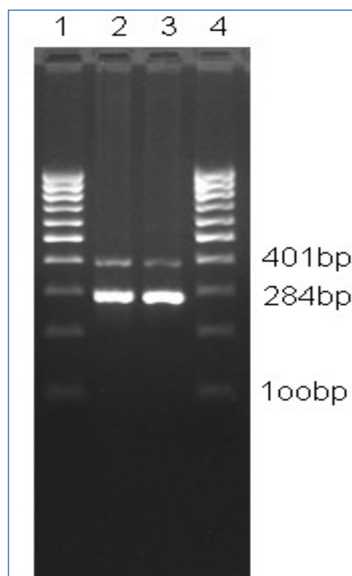

Fig. 1-

Table 1-

S. No.	Primer Sequences	Corresponding gene	Expected Product size
1	F:GTGAAATTATCGCCACGTTCGGGCAA R: TCATCGCACCGTCAAAGGAACC	invA gene	284bp
2	F: TTGTTCACTTTTTACCCCTGAA R: CCCTGACAGCCGTTAGATATT	typh gene	401bp

From all isolates Salmonella was detected in 3% (3/100) of the samples, whereas serovars *typhimurium* were identified in 1% (1/100), respectively. A study on the prevalence of *Salmonella* spp. By *inv*A gene in organs of chickens have reported that 3.23% *Salmonella* spp. [5]. A study made between January 2000 and July 2001 and from July 2001 until December 2003 by Eyigor *et al*. (2005) revealed that *Salmonella* organisms are detected by invA gene in 4.10% and 5.52% of chicken samples, respectively.

In conclusion, we developed and successfully applied a conventional thermocyclers for rapid multiplex PCR detection of *Salmonella* within a much shorter time than even other PCR methods comparable to real time [13]. The specificity and sensitivity were comparable to the currently used standard culture method. We believe that this is the fastest method based on conventional PCR for detection of different *S. enterica* serovars reported so far. This method is simple and rapid, and results obtained in less than 60 min proved to be highly specific and sensitive.

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