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Isolation and Identification of *Salmonella* spp from Retail Chicken Meat by Polymerase Chain Reaction

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Abstract: A study to isolate and identify *Salmonella* spp from chicken slaughtered under different processing conditions viz., wet market, supermarkets and chicken slaughtered in modern processing units in Karnataka, India, was carried out. In this study, breast and thigh samples were evaluated for presence of *Salmonella* spp. by culture and confirmed by polymerase chain reaction (PCR). A total of 450 (225 breast and 225 thigh muscle) samples were tested. Prevalence of *Salmonella* spp was higher in thigh meat (31.99%) compared to breast muscles (24.88%) irrespective of the processing condition. *Salmonella* spp prevalence was higher in poulterers' shop compared to those obtained from processing units, whereas no significant difference was observed between meat samples from processing units. This study showed a widespread prevalence of *Salmonella* in chicken meat from retail outlets in Bangalore.

Key words: Chicken · Culture · PCR · *Salmonella* spp

INTRODUCTION

Salmonellosis is an important global public health problem causing substantial morbidity, and thus also has a significant economic impact. In spite of the improvement in hygiene, food processing, education of food handlers and information to the consumers, foodborne diseases still dominate as the most important public health problem in most countries [1]. Poultry meat and its derivatives are among the food products that cause the most concern to public health authorities, owing to the associated risks of bacterial food poisoning. The modernization of chicken farms and globalization of the bird breeding trade also have played a role in infection [2].

Salmonella is one of the most important pathogens responsible for human food poisoning in the developed world [3] and chicken products are widely acknowledged to be a significant reservoir for *Salmonella.* They have frequently been incriminated as a source of *Salmonella* contamination and consequently thought to be major sources of the pathogen in humans [4]. Furthermore, one of the commonest causes of *Salmonella* infection reported in humans has been through the handling of raw poultry carcasses and products, together with the consumption of undercooked poultry meat [5]. Established conventional methods to detect and identify *Salmonella* are time consuming and include selective enrichment and plating followed by biochemical tests. Standard culture methods for detecting *Salmonella* spp. in poultry include nonselective pre-enrichment followed by selective enrichment and plating on selective and differential agars. These methods take approximately 4-7 days [6]. Since *Salmonella* is closely related to both public and animal health, more rapid and sensitive methods for the identification of this bacterium are required [7]. Several alternative, faster methods for the detection of *Salmonella* have been developed, the use of the polymerase chain reaction (PCR) being one of the most promising approaches [8]. Hence this study aimed at isolation of *Salmonella spp* from market samples of meat under different processing conditions and confirmation of conventional identification using by PCR identification.

MATERIALS AND METHODS

Samples: Samples of chicken meat (Breast and thigh) was collected from chicken slaughtered at poulterers' shop, and from two retail outlets chicken slaughtered in state of

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art processing units in Karnataka, India. A total of 450 samples were examined. The samples were immediately transported to the laboratories in a cool thermos and were processed for culture.

Culture: *Salmonella* was isolated according to standard methods (ISO 6579, 1993). Twenty five g sample of chicken carcass was added to 225 ml of buffered peptone water (BPW, HIMEDIA). All samples were incubated for 18 h at 37°C and incubated for 18 h at 37°C. One ml of pre-enriched carcass culture was then transferred to Rappaport-Vassiliadis broth (HIMEDIA) and Selenite F broth (HIMEDIA) and incubated at 42°C and 37°C, respectively. After 24 and 48 h of incubation, respectively, one loopful from each of the enriched broths was streaked onto plates of *Salmonella Shigella* (SS) agar (HIMEDIA) and xylose lysine deoxycholate (XLD) agar (HIMEDIA) and incubated at 37°C for 24 h. The plates were examined for the presence of typical colonies of *Salmonella*, i.e. transparent colonies with black centers on SS agar and red colonies with black centers on XLD agar. Suspected colonies were confirmed by conventional biochemical methods [9].

DNA Extraction: A few colonies growth on selective agar was transferred into an Eppendorf tube containing 300 μL sterile distilled water. The tubes were vortexed and incubated at 56°C for 30 min. The suspension was then added in to 300 μL of TNES buffer (20 mM Tris pH 8.0 + 150 mM NaCl + 10 mM EDTA + 0.2% SDS) and 200 μg/ml Proteinase K. Following 30 min boiling, an equal volume of phenol was added to the suspension which was shaken vigorously by hand for 5 min and then, centrifuged at 11600 x g for 10 min. The upper phase was transferred into a new Eppendorf tube. DNA was precipitated with absolute ethanol and 0.3 M sodium acetate at -20°C for 1-2 h. The mixture was then centrifuged at 11600 x g for 10 min and the upper phase was removed. The pellet was washed twice with 90% and 70% ethanol, respectively and each step was centrifuged at 11600 x g for 5 min. Finally, the pellet was dried, resuspended in 50 μL sterile distilled water, and stored at -20°C until further use [10]. *Salmonella* typhimurium (MTCC 1253) obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India was used in PCR tests as a positive control.

Primers: The primers used were: 16SF1 (5'- TGTTGTGGTTAATAACCGCA-3') and 16SIII (5'-CACAAATCCATCTCTGGA-3')(BANGALORE Geni) derived from 16S rRNA gene.

PCR: The reaction mixture was prepared in a total volume of 50 μL containing 5 μL of 10x PCR buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 5 μL of 25 mM MgCl2, 250 μM of each deoxynucleoside triphosphate, 2 U of Taq DNA Polymerase, 10 pg of each primers and 5 μL samples of extracted bacterial DNA. PCR involved 35 cycles of denaturation (94°C, 1 min), primer annealing (58°C, 1 min) and primer extension (72°C, 1 s). The primer extension step (72°C, 10 min) followed the final amplification cycle. For all experiments, a Touchdown Thermocycler was used.

Electrophoresis of PCR Products: The amplified DNA products from *Salmonella* specific-PCR were analyzed with electrophoresis on 1.2% agarose w/v gels stained with ethidium bromide and visualized by UV illumination. A current of 120 V was applied to each gel. Eight micro liter of PCR product mixed with 3 micro liters of 6 X loading dye were loaded on to agarose gel. A 100 bp DNA ladder was used as a marker for PCR products.

RESULTS AND DISCUSSION

Suspected isolates were biochemically identified as *Salmonella* spp. [9]. Samples of chicken carcasses (breast and thigh) from different processing conditions and% prevalence of *Salmonella* spp. isolated are presented in Table 1. In the PCR examination, positive results with the molecular size of 572-bp were obtained from all *Salmonella* spp. suspicious isolates (Figure 1).

The need for the development of rapid and accurate detection methods for *Salmonella* spp. has been increased in recent years due to the higher incidence of salmonellosis in industrialized countries over the past decades [11, 12] since the conventional methods for the isolation and identification of salmonellae require up to 4-7 days. Recently, the PCR has become a powerful and increasingly popular tool in microbial identification [13]. The primers 16SF1 and 16SIII were proved to be specific for the PCR detection of all *Salmonella* isolates with various serogroups [14]. For these reasons, we used the primers 16SF1 and 16SIII derived from the 16S rRNA gene and found that all *Salmonella* isolates identified by conventional tests gave positive bands with PCR

Table 1: % prevalence of *Salmonella* spp in chicken carcasses (breast and thigh) from different processing conditions

	Samples Evaluated		Positive Samples		% Prevalence	
Source	Breast	Thigh	Breast	Thigh	Breast	Thigh
Local Road Side shops	75	75	24	29	32.00	38.66
Super markets	75	75	19	23	22.66	28.00
Processing Unit II	75	75	15	22	20.00	29.33
Average					24.88	31.99

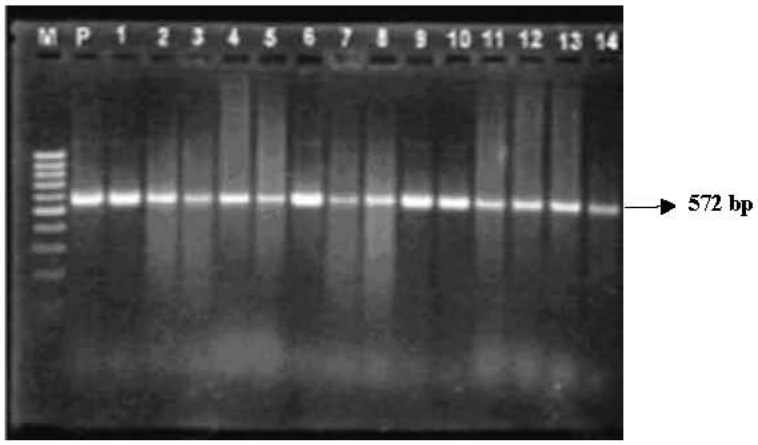

Fig. 1: An agarose gel stained with ethidium bromide, with PCR products of Salmonella isolate (M: 100 bp DNA ladder, P: positive control, 1-7: Salmonella isolates from breast, 8-14: Salmonella isolates from thigh).

Our findings of contamination rates with *Salmonella* were lower than those observed by Waltman *et al* [15]- 65.4%, Machado and Bernardo [16] - 57%, but higher than Brown *et al*. [17]- 1.2%. Mead [18] has observed that 70% of broiler carcasses are contaminated with *Salmonella*. Not much literature has been available on the prevalence of *Salmonella* in chicken carcasses from India, few researches reported negligible prevalence as low as 5% [19, 20].

In this study, the *Salmonella* detection rate was the highest in the road side shops compared to that of those obtained from processing unit indicating high level of hygiene in place in state of art processing facilities, which can control the presence of *Salmonella* in chicken carcasses [21]. The results also indicated that the thigh muscle had higher contamination rates (31.99%) compared to that of breast muscle (24.88%) irrespective of the processing condition, which might be due to the fact that during evisceration process the thigh / legs because of its proximity to point of evisceration are highly prone for contamination from the gut contents in case of improper procedure [22]. This finding is in agreement with the results of Carraminana *et al*.[23] who reported higher contamination rates in chicken carcasses (55%) compared to offals (livers and hearts) (40%). When comparing our results to those of other authors, several factors must be taken into consideration, such as differences in origin, time period and age of the samples, sampling procedure, contamination level of animals, slaughterhouse sanitation, level of processing and cross contamination of the products, and differences in methodology applied to detect the pathogen [24, 25].

CONCLUSION

This study showed that *Salmonella* spp. was widespread among the chicken carcasses and internal organs of slaughtered chickens in Bangalore. It may be due to insufficient hygiene, during slaughtering and processing of the flocks in the region. Further studies are needed to improve surveillance strategies to decrease the prevalence of *Salmonella* spp. in chicken population of Bangalore.

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