



Microbial Quality, Biochemical Identification and Molecular Detection of *Salmonella* Targeting His-J Gene in Poultry Meat and Feed in Lahore

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ABSTRACT

Salmonella enterica subsp. *enterica* poses a threat to both human and animal health, with more than 2500 reported serovars. A total of 80 samples, comprising of poultry meat (n=30) from poultry shops and supermarkets, poultry feed (n=30) and swabs from carcasses and muddy (n=20) of poultry shops. The samples were assessed microbiologically for Total Viable Count, Total Coliform Count, and *Salmonella* detection. The mean log values of total viable counts of meat samples of traditional poultry shops, super markets and processed meat were 5.70, 4.65 and 3.60, respectively and significant ($p < 0.05$) results were obtained. The mean log values of total coliform counts in meat samples were 2.7, 2.31 and 2.11, respectively. *E. coli* was predominant 73% in coliform count of all samples. *Salmonella* was found in 3.75% of samples in which retail poultry shops showed 10%, supermarkets showed 10%. While, processed meat was found negative for *Salmonella*. The mean log values of total viable counts of feed samples of store and shed were 7.21 and 7.56, respectively. Results of present study showed absence of *Salmonella* and coliform bacteria in poultry feed samples collected from poultry shed and store-room of poultry farm. Out of 20 swabs only 5% showed *Salmonella* prevalence. Molecular detection of *Salmonella* in collected meat samples through PCR targeting His-J gene showed 6.66% of positive samples previously identified by culturing and biochemical profile. The study showed that poultry meat has highest bacterial load which reflects unsatisfactory sanitation and hygienic conditions in poultry environment that ultimately cause food-borne infections. Besides this, feed also becomes a source of bacterial contamination in animals and humans. This study was helpful in devising strategy to provide safe food for public consumption.

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Authors' Contribution

MR conceived and designed the study. AAS and SF reviewed the study project for final approval. SI executed the experiments and analyzed the study results. AAS and AYS critically revised the manuscript for important intellectual contents and final version was approved by all authors.

Key words

Poultry meat, *Salmonella*, Coliforms, Molecular detection, Bacterial contamination

INTRODUCTION

Poultry is one of the leading industries of Pakistan producing 0.60 million tons of total meat in the country. For the last few years Punjab Food Authority has been strengthened through increased funding, manpower, legal cover and media support. Incidences related to breach of Punjab Pure Food Regulations are commonly reported on print, electronic and social media. Awareness of general public on such issues has been significantly improved

(Hussain *et al.*, 2015).

The poultry meat has been one of the inexpensive and wholesome sources of protein. However, the major health related issues which poultry industry is facing are poor production, management, health, biosecurity, disease diagnostics, prevention, control, transportation, marketing, and processing (Soomro *et al.*, 2011). Availability of high quality, healthy and microbiologically safe broiler meat is of utmost importance (Grepay, 2009). Multiple factors play their role in providing good quality and safe meat. Amongst other factors, 70 percent of the cost of broiler meat production is feed which contains high quantities of proteins (Hossain *et al.*, 2012). If the poultry feed is not of a good microbiological quality and contains pathogens, it is more likely that it may transmit various diseases to poultry itself and food borne diseases to consumers (Aliyu *et al.*, 2012). These pathogens may both be transferred vertically and horizontally (Putturu *et al.*, 2015). Poultry hen environment plays an important role in microbial contamination of poultry feed and meat especially through *Salmonella* infection in poultry and humans. Besides,

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poultry bird droppings, broiler feed, water, litter and aerosol contamination may also occur (Omwandho and Kubota, 2010).

Salmonella spp. are gram negative, rod shaped, motile, non-spore formers and facultative anaerobes. These bacteria are present in everywhere e.g. soil, water and GIT of most animals including humans (Maqsood, 2012). Generally severe type of food poisoning is caused by ingestion of *Salmonella enteritidis* contaminated poultry products. The signs and symptoms seen later 6-72 h after consumption are fever and gastroenteritis in which diarrhea, nausea and vomiting may occur. It is studied that in US in 2005, 45000 cases of non-typhoid *Salmonella* were reported with an estimate of 1.4 million infections and 600 deaths every year (Maqsood, 2012).

Foodborne infections caused by *Salmonella* represent an important public health issue and it is due to consumption of poultry products contaminated with *Salmonella* and improper cooking. Poultry feed should also be safe from pathogenic microorganisms otherwise cause diseases in birds which ultimately cause infections in humans due to consumption of food of animal origin. Keeping in view the importance of topic, the present study was designed to check microbial quality in poultry meat and feed having public health significance and to compare quality of meats available in different management conditions: (1) Street level slaughter shops with poor hygiene, (2) Clean meat shops having chilling facilities with apparently good hygienic conditions, and (3) Branded and processed poultry meat sold at super stores.

MATERIALS AND METHODS

Sample collection

A number of 80 samples were collected including 30 samples of meat (10 meat samples available in retail market shops, 10 from meat shops which were clean and having chilling facilities and 10 from processed meat, 20 samples/swabs from environment of poultry meat shops such as 10 swabs from drums (used to put slaughter birds) and 10 from wooden cutting board. Beside these samples, 30 samples of feed from store and shed were also collected. All these samples were transported to Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore under appropriate conditions. All the samples were subjected to microbiological analysis including total viable counts, total coliform counts and detection of *Salmonella*.

Microbiological analysis

One gram of each feed and meat sample was separately ground and minced using sterile micro-pestle

and mortar, respectively. The samples were separately processed for 10-fold serial dilutions. Then, 0.1 mL of appropriate dilutions were inoculated onto nutrient agar plate and MacConkey's agar plate and incubated for 18-24 h at 37°C. Thereafter, the plates having any bacterial growth (30-300 colonies) were selected and counted. Then multiplied the number of colonies with dilution factor and correction factor to determine the colony forming unit per gram of feed and meat (Aliyu *et al.*, 2012).

Isolation and identification of *Salmonella*

For the pre-enrichment of samples, pouring of 25 g of sample (feed/meat) in 225 mL of buffered peptone water was done and incubation was given aerobically at 37°C for 18-24 h. A 0.1 mL of inoculum from buffered peptone water was added to a tube containing 10 mL of the Rappaport Vassiliadis Soy Broth for enrichment and followed by incubation at 41.5°C for 24 h. A loop full culture from the enriched culture was inoculated onto selective media such as *Salmonella Shigella* agar and Brilliant Green agar and give incubation at 37°C for 18-24 h for isolation and purification. The plates having colorless colonies with black centered on *Salmonella Shigella* agar and whitish pink colonies with red halos on brilliant green agar were isolated and purified (Waghmare *et al.*, 2017). First of all, Gram staining was performed and then biochemical tests such as indole production, methyl red, voges prausker, citrate utilization, urease test, triple sugar iron test, catalase and oxidase test were performed for further identification following Bergey's manual of determinative Bacteriology.

DNA extraction

Genomic DNA of *Salmonella* was extracted by using GF-1 Vivantis Bacterial DNA extraction kit method. Extracted DNA was stored at 4°C or -20°C for further processing. The quantification and purity of extracted DNA was done by Nano drop method using Thermo Scientific NanoDrop™ 2000/2000c Spectrophotometer.

PCR amplification of *hisJ* gene

For conventional PCR analysis, primers pair was used against *his-J* having amplicon size of 496-bp and this is most conserved region among *Salmonella* species. This gene codes for histidine transport operon. The specificity of pair of primers was evaluated by nucleotide similarity searched with the BLAST algorithm at the NCBI website (<http://www.ncbi.nlm.nih.gov>) (Cohen *et al.*, 1993).

A reaction mixture of 25 µL was prepared as 12.5 µL master mix, 1 µL forward primer, 1 µL reverse primer, 2 µL DNA and 8.5 µL nuclease free water. The conditions of PCR was as follows; initial denaturation of 5 min at 95°C followed by denaturation of 30 seconds at 94°C, annealing

at 60°C for 30 seconds, extension at 72°C for 45 seconds followed by final extension at 72°C for 10 min. Finally, 3 µL of loading buffer was mixed into 7 µL of PCR product and electrophoresed on 1% gel at current and voltage of 150 Amp and 100 volts, respectively. A 100 bp DNA ladder was also used for PCR amplicons and positive and negative controls were also run along the samples. After 30 min, gel was seen on UV transilluminator to see the bands.

RESULTS AND DISCUSSION

Food production and safety has been major concern now a days (Shareef *et al.*, 2009). Poultry meat is very good source of proteins for humans in the form of egg and meat (Maqsood, 2012). Many food borne pathogens like *Salmonella* spp. and *E. coli* present in feed and then from here transfer to meat and other animal commodities which ultimately results in food poisoning. Poultry meat, eggs and other food products are known sources of *Salmonella* contamination (Sanchez *et al.*, 2002). Foodborne salmonellosis affects public health badly worldwide. It was estimated that non typhoidal *Salmonella* causes almost 93.8 million infections and 155,000 deaths every year worldwide (Antunes *et al.*, 2016).

Foodborne infections are mostly caused by *Salmonella* (Waghamare *et al.*, 2017) while *Salmonella enterica* serovar *Enteritidis* and *typhimurium* are involved in salmonellosis (Modarressi and Thong, 2010). The former one is of medical significance in humans (Roy *et al.*, 2002) and also significant infectious agent for existence of gastrointestinal complications (Schrack *et al.*, 2001). So there is need to study on evaluation of microbiological analysis of meat and feed to reduce microbial challenges and infectious diseases. Therefore, this study was planned to evaluate microbiological quality of feed and meat mainly *Salmonella* spp.

Total viable counts and total coliform contents in meat sample

In our study, the mean log values of total viable counts of meat samples in different management conditions such as traditional poultry shops, popular super markets and processed meat are 5.70, 4.65 and 3.60, respectively, and results showed significant difference between processing of meat in different management conditions ($p < 0.05$) (Table I) and the results agreed with Cohen *et al.* (2007) and Kozačinski *et al.* (2006) for raw poultry shops and supermarkets. The results of total viable counts of meat sold at popular super markets was not more than the results of study in Eglezos *et al.* (2008). The mean log values of total coliform counts in meat samples of retail poultry

shops, popular super markets and processed meat were 2.7, 2.31 and 2.11, respectively showed no significance and agreed with that reported for poultry in studies of Adu-Gyamfi *et al.* (2012). From coliform bacteria, *E. coli* occurrence was 73% of all samples examined in which meat sold at retail poultry shops and popular super markets showed 80% prevalence and processed meat showed 60%. This showed high percentage as comparable to findings of Iman *et al.* (2015) except processed meat.

The highest bacterial load and coliform count was found in raw poultry shops and lowest in processed meat such as chicken nuggets of different brands show that poor hygienic and unsatisfied sanitary conditions in retail poultry shops and due to chilling facility the bacterial load was lower in popular super markets and processed meat than retail poultry outlets. The processed poultry (chicken nuggets) showed lowest aerobic plate count as compared to other due to processing, adding spices, packaging and freezing as these all activities disturbed the growth of bacteria.

Molecular detection of Salmonella in poultry meat

Black centered, colorless colonies of *Salmonella* were observed on Salmonella Shigella agar while, pinkish white colonies were appeared on Brilliant green agar. His-J gene is the most conserved region in genome of *Salmonella* and present in almost all species of *Salmonella*. The association between the results of selective culturing and PCR was same as there was no difference between their outcomes. Only the samples that were identified for *Salmonella* occurrence through selective plating and biochemical tests were confirmed by polymerase chain reaction targeting *hisJ* gene. In 6.66% of all meat samples, retail poultry shops showed 10%, meat from supermarkets showed 10% and no sample of processed poultry meat was positive for *Salmonella*. The prevalence of *Salmonella* in raw poultry meat was more than that reported by Razzaq *et al.* (2013) which was 2% and less than with the findings of Soomro *et al.* (2011) with 38% occurrence. The results of *Salmonella* detected in meat of supermarkets was almost agreed with results of Kozačinski *et al.* (2006) and more than reported by Cohen *et al.* (2007). Out of 2 *Salmonella* positive samples in meat (Fig. 1) and out of 20 environmental swabs from poultry shops, only 1 sample showed positive results by PCR for *Salmonella* having percentage occurrence of 5% (Fig. 2). The results showed no significant differences between swabs of cutting board/muddy and carcasses. From this, swabs from muddy/wooden cutting board showed 10% prevalence which is less than reported by Upadhyaya *et al.* (2012) and the swabs which were taken from carcasses showed negative result for *Salmonella* less than the findings of Waghamare *et al.* (2017). As odd ratio value

Table I. Results of total viable counts and total coliform counts in meat (traditional meat shapes, popular super market, processed meat) and feed (store, shed) samples.

S. No	Sample code	Total viable counts (cfu/g)	Mean log values	Total coliform counts (cfu/g)	Mean log values
Traditional meat shops					
1	A	3.0×10^6		9.6×10^3	
2	B	6.0×10^6		2.8×10^3	
3	C	8.6×10^8		7.3×10^3	
4	D	3.6×10^6		1.2×10^3	
5	E	1.5×10^7		1.1×10^4	
6	F	3.0×10^4		3.0×10^2	
7	G	2.2×10^4		No count	
8	H	2.5×10^4		No count	
9	I	1.24×10^6		4.0×10^2	
10	J	2.92×10^4	5.70	3.5×10^3	2.70
Popular super markets					
11	HYP	2.7×10^3		1.0×10^2	
12	EMP	1.7×10^5		1.0×10^3	
13	CF	1.8×10^4		1.15×10^3	
14	ZN	2.6×10^4		No count	
15	GV	2.5×10^5		7.0×10^2	
16	ZB	1.21×10^4		1.4×10^2	
17	SW	2.26×10^5		2.0×10^3	
18	JS	6.9×10^3		No count	
19	FC	7.9×10^5		2.1×10^3	
20	MT	5.0×10^5	4.65	3.0×10^3	2.31
Processed meat					
21	KN1	1.0×10^4		No count	
22	KN2	2.8×10^4		3.0×10^3	
23	SF1	2.14×10^3		No count	
24	SF2	1.16×10^3		No count	
25	MN1	2.32×10^3		1.5×10^4	
26	MN2	2.5×10^4		1.27×10^3	
27	BB1	3.2×10^4		4.0×10^2	
28	BB2	1.36×10^3		1.3×10^3	
29	SB1	2.45×10^3		5.0×10^2	
30	SB2	2.7×10^2	3.60	No count	2.11
Store					
1	A1	5.5×10^5		Negative	
2	A2	3.2×10^5		Negative	
3	A3	3.1×10^3		Negative	

S. No	Sample code	Total viable counts (cfu/g)	Mean log values	Total coliform counts (cfu/g)	Mean log values
4	A4	4.8×10^5		Negative	
5	A5	2.0×10^5		Negative	
6	A6	1.5×10^6		Negative	
7	A7	1.18×10^3		Negative	
8	A8	1.04×10^3		Negative	
9	A9	1.85×10^4		Negative	
10	A10	8.0×10^5		Negative	
11	A11	8.0×10^4		Negative	
12	A12	3.5×10^3		Negative	
13	A13	2.7×10^4		Negative	
14	A14	2.0×10^4		Negative	
15	A15	7.1×10^3	7.21	Negative	NR
Shed					
16	B1	6.2×10^3		Negative	
17	B2	5.0×10^5		Negative	
18	B3	1018×10^6		Negative	
19	B4	1.12×10^3		Negative	
20	B5	9.2×10^5		Negative	
21	B6	9.6×10^5		Negative	
22	B7	4.2×10^5		Negative	
23	B8	1.3×10^5		Negative	
24	B9	3.8×10^5		Negative	
25	B10	1.8×10^4		Negative	
26	B11	2.2×10^3		Negative	
27	B12	6.8×10^6		Negative	
28	B13	4.1×10^2		Negative	
29	B14	2.0×10^6		Negative	
30	B15	3.4×10^3	7.56	Negative	NR

is 1.11 which indicates a positive association and high risk estimate among the risk related factors and the prevalence of *Salmonella*. Out of above positive results, no *Salmonella enteritidis* was found. It depicts the occurrence of other *Salmonella* serovars except *S. enteritidis* as reported by Cohen *et al.* (2007).

Total viable counts and total coliform counts in feed samples

The mean log values of total viable counts of feed samples of store and shed were 7.21 and 7.56 respectively showed no significant difference (Table II). The results of total viable counts showed similarity and accordance with the results of studies in Ukaegbu-Obi *et al.* (2017) and Obi and Ozugbo (2007). There were no coliform bacteria present in feeds of store and shed means there

was no fecal contamination and not agreed with results of *Enterobacteriaceae* counts reported by Kukier and Kwiatek (2011) and Sultana *et al.* (2017) but higher bacterial loads in shed as compared to store showed pitiable processing, variation in weather conditions, production, contaminated feed ingredients, storage and poor management of poultry industry and farms. There were no significant differences between total viable counts and total coliform counts of feeds of store and shed.

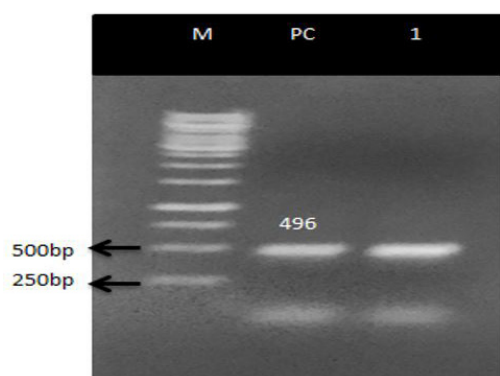


Fig. 1. Agarose gel electrophoresis of product of polymerase chain reaction of *hisJ* gene (496 bp) in swabs of poultry environment. Lane M, DNA marker of 1kb; Lane PC, Positive control; Lane 1 *Salmonella* positive sample in mucky of poultry house environment.

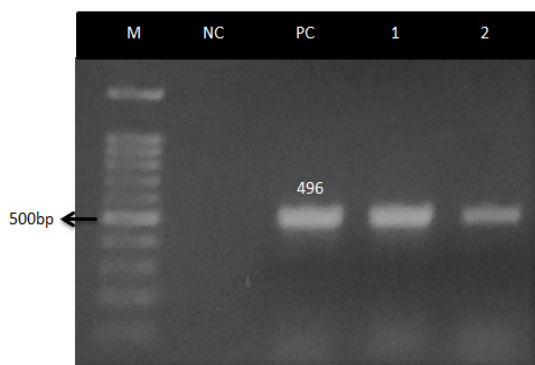


Fig. 2. Agarose gel electrophoresis of product of polymerase chain reaction of *hisJ* gene (496 bp) in meat samples Lane M, DNA marker of 100 bp; NC, Negative control; Lane PC, Positive control; Lane 1 and Lane 2, *Salmonella* positive in meat samples.

The high bacterial contamination in feed is not suitable and safe for the consumption of poultry being a part of its tissue and also not good for human consumption. Results of PCR for confirmation of *Salmonella* revealed that no sample was found positive in feed samples that was less than reported in Kukier *et al.* (2012) which found

0.84% prevalence in his study. In the study of Okonko *et al.* (2010) he found 3% prevalence of *Salmonella* which is higher than our results. Our findings indicate somewhat better processing and production of poultry feed especially heat treatment to kill the pathogenic bacteria if present in raw feed material which ultimately reduce the risk of contamination in feed processing units, feed handlers, and also in the environment. The pathogenic bacteria such as *Salmonella* spp. in broiler feed is also a source of infection in poultry birds and causes different diseases such as fowl typhoid and salmonellosis. The presence of these pathogenic microbes shows that these pathogens consume feeds as nutrition for their growth and metabolic reactions (Ukaegbu-Obi *et al.*, 2017). The low recovery rate of *Salmonella* was observed in current study which might be due to good management practices adopted at farm level, use of *Salmonella* free chicken feed, rearing of *Salmonella* free chicks, improved biosecurity practices at farm level during poultry production. The exertions should be applied which decrease the number of bacteria in feed as much as possible so our objective is not to sterile feed but feed with safe contamination level.

Table II. Detection of *Salmonella* in poultry related samples.

Samples	Number of samples	Positives samples	Percentage occurrence
Meat			
Traditional shops	10	1	10
Supermarkets	10	1	10
Processed	10	0	0
Swabs of poultry environment			
Carcasses	10	0	0
Wooden cutting board	10	1	10
Feed	30	0	0
Total	80	3	3.75

The prevalence of *Salmonella* in chicken meat estimated the poor quality of poultry meat which is a source of food borne infections in animals and humans. The traditional slaughtering procedure and warm temperatures favour the growth of bacteria. There is need to implement HACCP to detect and control the hazards in poultry products. It was indicated that various risk factors are involved in transmission of *Salmonella*. There is need to adopt strict guidelines and recognized potential bio risks which are involved in dispersal of food borne diseases to provide safe food for public consumption. This also showed possible risks and source of infection in humans.

CONCLUSION

This study was intended for the estimation of total viable counts, total coliform counts, and *Salmonella* detection in meat and feed to provide safe food for public consumption and devised strategy to determined risk factors which would be guiding for policy intervention. It is concluded that poultry feed and processed chicken are free from *Salmonella*, however, the presence of *Salmonella* in retail chicken meat could be because of post slaughter contamination and unhygienic practices opted at retail meat shops.

Statement of conflict of interest

The authors have declared no conflict of interest.

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