Prevalence and molecular characterization of typhoidal and non-typhoidal Salmonella *isolated from meat and environmental samples of retail shops of Lahore Punjab, Pakistan*

Shakera Sadiq[1*], Mamoona Chaudhry[1], Muhammad Hassan Mushtaq[1], Junaid Sadiq[2], Saima Hasan[1], Lettini Antonia Anna[3]

[1]*Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore, Pakistan.*
[2]*Department of Environmental Sciences, Quaid-i-Azam University, Islamabad, Pakistan*
[3]*Italian National and OIE Reference Laboratory for Salmonella. Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Padua, Italy.*

[*]*Corresponding author at: Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore, Pakistan.*
E-mail: shakera.sadiq@uvas.edu.pk.

Veterinaria Italiana 2022, **58** (4), 425-434. doi: 10.12834/VetIt.2392.14037.1
Accepted: 28.05.2021 | Available on line: 31.12.2022

Keywords
Prevalence,
Zoonotic,
Salmonella,
Retail shops.

Summary
Non-typhoidal *Salmonellae* are important foodborne bacterial pathogens that can cause bacteremia, gastroenteritis, and subsequent infection. The aim of the study was to determine the prevalence of *Salmonella* in the live bird market and retail shops of Lahore (Pakistan). A total of 720 samples of chicken meat, chopping board, cages, hands, and transportation vans were collected. *Salmonella* was recovered from 103 (14.36%) samples. The highest prevalence (33.33%) was found in transportation van samples followed by chicken meat samples (17.26%). In the towns of Lahore, the highest prevalence was found in Samanabad Town (19 %) followed by Data Ganj Bakhsh Town (17%) with the lowest in Gulberg Town (6.9%). *Salmonella* Typhimurium was most common (35.92%) followed by *S.* Enteritidis (25.24%), *S.* Dublin (14.56%), *S.* Gallinarum biovar Gallinarum (8.74%), and untyped *Salmonella* species (15.53%). This was the first baseline study of the prevalence of non-typhoidal *Salmonella* at the live bird market and retail shops of Lahore. Implementation of appropriate control measures is required at both the human side and poultry food production chain to reduce the burden and transmission of the zoonotic *Salmonellae*.

Introduction

Foodborne diseases pose a significant impediment to socio-economic development globally and represent a constant threat to public health (Devleesschauwer, *et al.*, 2018). Almost 1 in 10 people become infected every year due to food-borne diseases. Diarrheal diseases are the most common illnesses from contaminated unsafe food. *Salmonella* is one of the leading causes of bacterial diarrhea worldwide, accounts for one of the four key global causes of diarrheal illnesses (WHO, 2018). Non-typhoidal *Salmonellae* are important foodborne bacterial pathogens that can cause bacteremia, gastroenteritis, and subsequent infection (Acheson and Hohmann, 2001). Every year, non-typhoidal *Salmonella* infections results in approximately 153

Please refer to the forthcoming article as: Sadiq *et al*. 2022. Prevalence and molecular characterization of non-typhoidal *Salmonella* isolated from meat and environmental samples of retail shops of Lahore Punjab, Pakistan. Vet Ital. 10.12834/VetIt.2392.14037.1.

million cases of gastroenteritis and 57,000 deaths globally (Brunette, 2017). According to the data of the global burden of diseases, among the total deaths due to salmonellosis, 38,500 deaths per year occur in children less than five years of age (Delahoy, *et al.*, 2018)

Poultry is considered as the most important reservoir of *Salmonella* and also considered as one of the common sources of human salmonellosis globally (Antunes, *et al.*, 2016; Yang, *et al.*, 2011; Zwe, *et al.*, 2018). Contaminated foods particularly broiler retail chicken meat is considered as the most commonly implicated foods for non-typhoidal salmonellosis in humans (Alali, *et al.*, 2012; Donado-Godoy, *et al.*, 2012; Lamas, *et al.*, 2016; Li, *et al.*, 2017; Shang, *et al.*, 2019). *Salmonella* bacteria own the ability to reside in the healthy chicken without showing any symptoms so it may go unnoticed, therefore poses the risk of human infection through contaminated chicken at retail level (Zwe, *et al.*, 2018).

Salmonella is the leading cause of foodborne disease worldwide especially in the Middle East, Eastern Europe, and Southeast Asia (Ta, *et al.*, 2012). It is also one of the most important zoonotic pathogens both in animals and humans in developing countries (Ejo, *et al.*, 2016)

Important stages for transmission of *Salmonella* to chicken and its products include the status of infection in the host population at the farm after rearing, fecal contamination during transport by vehicles to the slaughtering and processing facility, and cross-contamination between chicken carcasses and equipment during cutting and processing. It is also documented in studies that during transport or shipping, the prevalence of *Salmonella* in positive birds increases due to fecal contamination of feathers and skin by companion birds (McCrea, *et al.*, 2006).

In Pakistan when the broiler flock is ready after rearing in broiler farms, transport vehicles are used to transport live birds either to processing plants, live bird markets or directly to retail shops of the county. Live bird markets or wet markets are common in Asian countries (Nidaullah *et al.*2017) including Pakistan. These live bird markets are served as wholesale markets and used as an important source of chicken consumed by the population on large scale. Live bird markets play an important role in the dissemination of *Salmonella* serovars to the environment and individuals (Sharma, *et al.*, 2019). Many risk factors have been associated with the presence of *Salmonella* spp. at the retail outlets of live bird markets, including the size of live bird market, sources of chicken either from integrated or nonintegrated systems, storage conditions including temperature, geographical location and rearing types of chicken (Alali, *et al.*, 2012; Jarquin, *et al.*, 2015; Khan, *et al.*, 2018; Tabo, *et al.*, 2013; Yang, *et al.*, 2011),

At a worldwide level, foodborne illness is often related to the consumption of poultry meat and its products contaminated with pathogenic bacteria commonly from the retail shop level (Shafini, *et al.*, 2017). In Pakistan, consumers prefer to purchase meat which has been slaughtered in front of them from retail shops. The important risk factors are contaminated knives, chopping boards, and tables and, contaminated hands of retailers. Poor sanitary and hygienic practices are prevalent in these retail outlets in developing Asian countries and are not monitored by regulatory authorities (Nidaullah, *et al.*, 2017; Yang, *et al.*, 2011).

According to the author's knowledge, there is limited data on the prevalence of non-typhoidal *Salmonella* in retail meat shops in Lahore. The present study was conducted to check the prevalence of non-typhoidal *Salmonella* at different steps of the poultry chain including sampling from transport vehicle vans, live bird market, and retail shops of nine towns of Lahore.

Material and methods

A cross-sectional study was conducted starting from February 2018 to August 2018 in District Lahore, Punjab. Samples were collected (n=720) from the live bird market located in Data Gunj Bakhsh Town and retail outlets of Lahore. Sampling was started from the live bird market by initially taking swabs samples (n=45) from transport vehicles. Hand swabs (n=33) were taken after the consent of the drivers. To avoid repetition, the name of the driver and the number of the vehicle were noted on the day of sampling. Sterilized cotton swabs were used for sampling the cages of vans and the hands of van drivers. Hand swabs were taken by swabbing the palms and fingers with sterilized cotton swabs dipped in 0.1% Buffered Peptone Water (BPW, Oxoid) (CM0509) (Okareh and Erhahon, 2015). Swabs were kept in test tubes containing 10 ml buffered peptone water.

Forty (40) retail shops from the live bird market located in Data Ganj Bashkh town and 90 chicken retail outlets from 9 administrative zones of Lahore were selected. A total of 10 shops were selected from each administrative zone of Lahore including 9 towns. Four types of samples including chicken meat (n=262), chopping board swabs (n=130), cage swabs (n=70), and hand swabs of the retailers (n=180) were collected. Meat samples of slaughtered chicken including the thigh, chest meat, and chicken leg were collected. A minimum (approx. 225 gm) of meat was purchased and stored in sterilized polythene bags

from retail shops. For the sampling of chopping boards and cages, sterilized swabs immersed in 0.1% BPW were used. A few samples containing fecal material and debris from the floor of the cages were also collected in sterilized polythene bags. All the samples were properly labeled and packed and immediately transferred to the laboratory of Epidemiology and Public Health within 24 hours for further microbiological processing.

Isolation and identification

For isolation and cultivation of *Salmonella,* ISO 6579 method was used. For meat samples, 25 gr of meat sample were weighed and suspended in 225 ml buffered peptone water for 24 hours at 37°C.

For selective enrichment, two types of *Salmonella* selective broths were used. Firstly, 0.1 ml of BPW was inoculated into 10 ml of Rappaport-Vassiliadis Enrichment Broth (RV broth, Oxoid, CM0669).

The broth was incubated for 48 hours at 42°C. Secondly, one ml of BPW was inoculated in 10 ml of Tetrathionate broth (TT broth, Oxide CM0671). The broth was incubated for 18-24 hours at 37°C. For culturing of *Salmonella,* three selective media were used. Three loops full (one loop for each media) of inoculum was used for inoculating Brilliant Green Agar (BGA, Oxoid, CM 0263), *Salmonella* Shigella agar (SS Agar, Oxoid CM0099) and Xylose Lactose Tergitol™ 4 (XLT 4, Oxoid CM1061). The plates were incubated for 18-24 hours at 37°C. Three presumptive colonies (pink colonies with red background for BGA, round black centered for SS agar, and black centered with the pink background on XLT4) were selected and further streaked for purification on nutrient agar (Oxoid, CM0003). Suspect colonies showing morphological characteristics of *Salmonella* were further confirmed by the Triple sugar iron (Oxoid, CM0277) and urease tests (Oxoid, CM 057).

Molecular identification of non-typhoidal *Salmonella* enterica by multiplex Polymerase Chain Reaction (PCR)

A multiplex PCR was developed for the identification of common serovars of *Salmonella* enterica in Lahore.

DNA extraction

Four to five pure isolated colonies from fresh culture were first incubated in Lysogeny broth (Luria Bertani medium) (LB broth, Oxoid, CM0996) for 24 hours at 37°C. From LB broth culture, one ml was taken in the Eppendorf tube and centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded, and the pellet was washed by adding 1 ml of ultrapure distilled water and again centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded, and 180 µL of 10X chelex resin (Bio Rad) were added. The suspension was mixed properly by vortexing. Eppendorf tubes were sealed with parafilm and placed in a water bath for 20 minutes at 100°C. Immediately after water bath, samples were chilled on ice for 2 minutes. Samples were centrifuged again at a maximum speed of 14,000 rpm for 10 minutes. The supernatant was used as template DNA for PCR. Template DNA was stored at -20°C until used.

Multiplex PCR

Multiplex PCR was developed for simultaneous identification of *Salmonella* serovar, Enteritidis, Typhimurium, Typhi, Dublin, and *Salmonella* enterica serovar Gallinarum biovar Gallinarum (Table I).

For PCR, 25 µl of the reaction mixture was prepared to contain 1 µl (10 pmol) of each forward and reverse primer, 12.5 µl of Master Mix (Lucigen USA), 2 µl of template DNA and remaining nuclease-free water to make final volume.

Table I. *Primer sequences for multiplex PCR for* Salmonella *serovar Enteritidis, Typhimurium, Typhi, Dublin, and Gallinarum biovar gallinarum.*

Target gene	Sequence	Amplification target	Size bp	Reference
Sdf 1	TGTGTTTTATCTGATGCAAGAGG	Serovar	304	(de Freitas, *et al*., 2010)
	TGAACTACGTTCGTTCTTCTGG	Enteritidis		
Spy	TTGTTCACTTTTTACCCCTGA	Serovar	401	
	CCCTGACAGCCGTTAGATATT	Typhimurium		
ViaB	CACGCACCATCACACCG	Serovar Typhi	738	
	AACAGGCTGTAGCGATTTAGG			
SeD_A1104 gene	ACGCGAAATCTGATGGTCTT	Serovar. Dublin	203	(Stegniy, *et al*., 2014)
	GCCCACCAGTTGTGAAAGGC			
SG0266	CCGCACAACACATCAGAAAG	Serovar	97	
	AGCTGCCAGAGGTTACGCTG	Gallinarum		

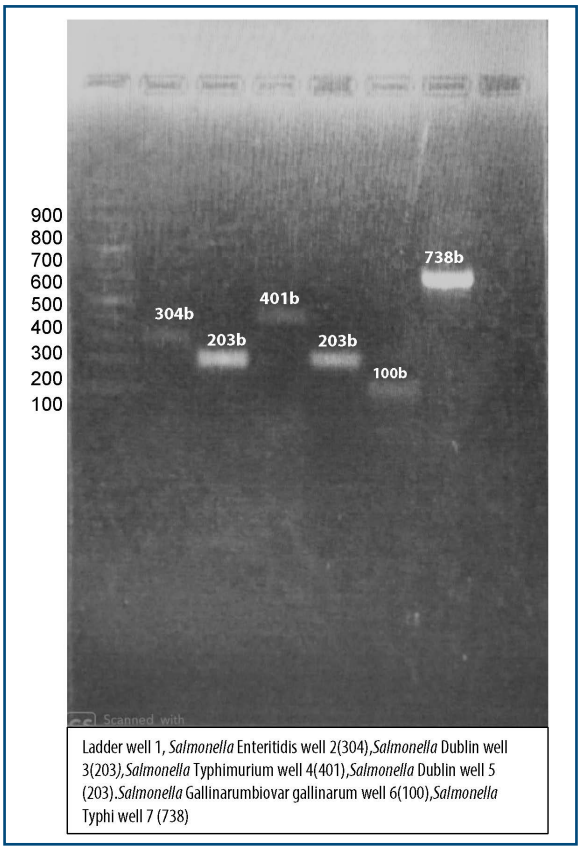

Figure I. *Gel image of multiplex PCR to detect Salmonella.*

Cycling conditions

Cycling conditions of the reaction comprised initial denaturation temperature of 95°C for 2 min; (ii) 30 cycles, with 1 cycle consisting of 1 min at 95°C, 1 min at 57°C, and 2 min at 72°C; and (iii) a final elongation step of 5 min at 72°C (Alvarez, *et al.*, 2004). The PCR products were electrophoresed in 2.5% (wt/vol) D-1 agarose (Oxoid) and stained with 4 µl of ethidium bromide solution. In each PCR run, a non-template control was included to detect possible external DNA contamination. The gel was visualized in the gel Doc system (Syngene, USA).

Results

Figure I showed results of multiplex PCR with five bands of *Salmonella* Enteritidis, Typhimurium, Dublin, Gallinarum and Typhi.

Among the total (n=720) collected samples, 103(14.31%) were found to be positive for *Salmonella* enterica (Genus).

The highest prevalence was found in transportation vehicle samples (33.3%), chicken meat samples (17.56%), chopping board samples (11.54%), hand swabs of retailers (9.6%) and cage samples (8.57%) (Table II).

As the live bird market of Lahore is located in Data Gunj Bakhsh Town, the samples collected from Tollinton market with samples collected from retail shops in streets of Data Gunj Bakhsh Town were combined.

Table III and Figure II present the area wise prevalence in different towns of Lahore. The highest prevalence was found in Samanabad Town (19%) Data Gunj Bakhsh Town (17.0%), Ravi Town (16%), Shalimar town (15.0%), Wahga town (15%), Alama Iqbal Town (12%), Aziz Bhatti Town (9%), and Gulberg Town (7%). Table IV shows the serovar distribution

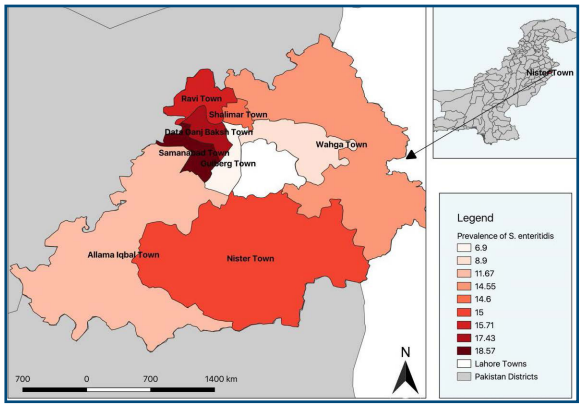

Figure II. *Map showing prevalence of* Salmonella *in different towns of Lahore.*

Table II. *Overall prevalence of* Salmonella *in chicken meat, environmental samples, and hand swabs.*

Sample type	No of samples tested	No of positive samples	Percentage
Chicken meat samples	262	46	17.56
Hand swabs of retailers	213	21	9.86
Cages of birds	70	6	8.57
Chopping board samples	130	15	11.54
Transportation van sample	45	15	33.33
Total	**720**	**103**	**14.31**

Table III. *Area prevalence of* Salmonella *in retail shops of towns of Lahore (Pakistan).*

Location of retail outlets	Total number of shops tested	*Salmonella* positive shops	No of samples collected	No of positive samples	Percentage of positive samples
Ravi Town	10	6	70	11	15.71
Shalimar Town	10	7	82	12	14.6
Aziz Bhatti Town	10	4	90	8	8.9
Data Gunj Bakhsh Town	*50(10+40)	30	§195(75+120)	¶34(15+19)	17.43
Samanabad Town	10	8	70	13	18.57
Gulberg Town	10	2	58	4	6.90
Wahga Town	10	5	55	8	14.55
Alama Iqbal Town	10	6	60	7	11.67
Nishter Town	10	4	40	6	15

* Total number of shops selected from live bird market and retail shops of Data Ganj Bukhsk Town.

§ Total number of samples selected from live bird market and retail shops of Data Ganj Bukhsk Town.

¶ Total number of *Salmonella* positive samples from live bird market and retail shops of Data Ganj Bukhsk Town.

Table IV. Salmonella *serovar identification in retail shops of Lahore (Pakistan).*

Salmonella serovars	Total number identified	*Percentages*
Salmonella Typhimurium	37	35.92
Salmonella Enteritidis	26	25.24
Salmonella Dublin	15	14.56
Salmonella Gallinarum biovar gallinarum	9	8.74
Salmonella Typhi	0	0
Untyped	16	15.53
Total	**103**	**100**

among the total isolated samples with the most common serovar being *Salmonella* Typhimurium (35.92%) then Enteritidis (25.5%), Dublin (14.56%), Gallinarum (8.74%), Typhi (0%), and untyped (15.53%).

Discussion

The present study was conducted for the first time to assess the burden of non-typhoidal *Salmonella* at the retail shops of Lahore (no study published earlier). Lahore is the largest city of Punjab province, Pakistan, and the fifth-largest city in South Asia. The land area of Lahore is 1772 square kilometer with a total population of approximately 12.18 million (2019 statistics). To meet consumer demands, there are more than 9,000 retail shops for red and white meat (Nisar*, et al.*, 2018). According to the survey (Jalil*, et al.*, 2013), the daily chicken meat consumption in Lahore is approx 361,000 kilograms. The chicken is supplied to the whole city through two major markets including the Tollinton market (shares 55% of total supply) and the Sheranwala market (shares 30% of total supply). The remaining 15% of chicken meat is sold by direct suppliers.

The presence of *Salmonella* is a major hindrance to the export of poultry and poultry products from Pakistan as well as its zoonotic threat (http://www.livestockpunjab.gov.pk/LiveStockAdmin/uploads/editor_files/performance_report_-_directorate_of_poultry_research_institute_oslln.pdf). A limited number of studies on non-typhoidal *Salmonella* in poultry have been reported from Pakistan. The purpose of this research was to determine the presence of non-typhoidal *Salmonella* in the major live bird market (Tollinton market) and retail shops of nine administrative towns of Lahore.

In the present study, the prevalence rate of *Salmonella* in raw chicken meat is 17.56% which is lower than that reported from all other studies conducted in different areas of Pakistan. The reported area wise prevalence of *Salmonella* in chicken meat was 38% in Hyderabad (Soomro*, et al.*, 2010); (34%) in raw chicken meat in Kohat (Asif*, et al.*, 2017); (30%) in chicken meat in Faisalabad (Akhtar*, et al.*, 2010), and (18%) in raw poultry meat in Swat (Uddin*, et al.*, 2018). The lower prevalence of *Salmonella* in Lahore (Punjab) as compared to other areas may be due to the role of the Poultry Research Institute (PRI, Punjab) in surveillance and

control of poultry diseases at regional levels. The reported prevalence in raw chicken meat (17.56%) is also lower as compared to other Asian countries: 21-65.3% in Vietnam (Huong, *et al.*, 2006; Nguyen, *et al.*, 2016; Phan, *et al.*, 2005; Ta, *et al.*, 2014; Van, *et al.*, 2007); 52.2% in China (Yang, *et al.*, 2011); 48% in Malaysia (Abatcha, *et al.*, 2018). The difference in the prevalence rate of *Salmonella* in Asian countries may be due to different farming practices, transport vehicles, slaughtering and processing environment, unhygienic practices at retail shops, and also could be due to differences in isolation methods (Sharma, *et al.*, 2019).

Moreover, in developing nations, the chicken meat is kept at room temperature as no cooling system is installed in wet meat shops and therefore it provides optimum survival conditions for *Salmonella*.

The lack of strict hygiene-related laws in retail meat shops and unawareness among butchers are considered the two most important reasons for the increased prevalence of *Salmonella*.

The purpose of hand swabbing was to determine the level of hygienic practices of the retailers and their involvement in the dissemination of pathogen to an uninfected chicken carcass.

The contamination of the retailer's hand can impose a greater risk for self-infection as well as the dissemination of pathogens to the family. In the present study, the frequency of the occurrence of *Salmonella* on the retailer's hands was (9.86%) which is not comparable locally as no study has been conducted before to analyze the level of non-typhoidal *Salmonella* contamination on hands. Our reported prevalence varies from that reporte in other studies: 0%-14.29% on meat shop retailers hands in India (Sharma, *et al.*, 2019; Suresh, *et al.*, 2004); 23.3%-33.3% on hands in Ethiopia (Abdi, *et al.*, 2017; Garedew, *et al.*, 2015).

Personal hygiene may be the reason for discrepancies in prevalence. Poor hand washing practices and use of single cloth for drying hands after every single slaughter are very common in retail shops of Lahore. Normally, there is one large bowl of water which is used repeatedly for washing of hands without any soap or sanitizer. A large piece of cotton cloth is also commonly seen which is used for drying hands.

The prevalence of *Salmonella* in environmental samples was highest in transportation vans samples: 33.3% followed by chopping board samples; 11.54%, and cages; 8.57%.

Transportation trucks are used to transport live birds from broiler farms to live bird markets and retail shops. These vehicles can be a continuous source of contamination to healthy flocks (Beach, *et al.*, 2002; Ejo, *et al.*, 2016; Gallegos Robles, *et al.*, 2009; Hald, *et al.*, 2000).

The reported prevalence in Lahore (33.3%) is lower than reported in Korea (62.5%) from vehicles (Ha, *et al.*, 2018), where the *Salmonella* prevalence in broiler transporting vehicles was highest in the whole food chain. The difference could be due to uncleanliness of floors of trucks and how frequently these vehicles are washed to reduce contamination.

Chopping boards are used for cutting and chopping of meat after de-feathering and evisceration process. Retailers mostly clean the chopping board with a wet cloth after cutting off one carcass and this cloth is used repeatedly after every carcass cutting. The prevalence of *Salmonella* on chopping boards in the present study was 11.54%, which is higher than the 5.6% reported from Ethiopia (Garedew, *et al.*, 2015) and lower than the 18.75% reported from India (Suresh, *et al.*, 2004).

The difference in prevalence could be due to the method of taking samples as the chopping board surface is porous and bacteria sometimes reside inside surfaces which is difficult to collect during swabbing (Ak, *et al.*, 1994). Studies reported that uncleaned chopping boards are a potential source *Salmonella* cross-contamination (Zwe, *et al.*, 2018).

Cages are compartments mostly placed on one side or the backside of the shop.

The floor surface of the cages is covered with droppings and it is cleaned occasionally before the entrance of the next batch of birds.

According to previous studies, levels of shedding dramatically increased, while birds were confined in cages before slaughter (Corry, *et al.*, 2002; Ha, *et al.*, 2018; Hald, *et al.*, 2000).

A prevalence of 8.57% was reported from cages in the present study. Cage samples are considered under environmental samples and prevalence is in the range of that reported from the neighboring country India [7.94% from environmental samples (Sharma, *et al.*, 2019)].

In our study, the area wise highest prevalence was found in Samanabad town (19%), followed by Data Gunj Bukh Town (17%), and Gulberg (6.9%). The reason behind the high prevalence in Samanabad and Data Gang Bukhsh is the high population congestion rate (22.72 and 25.33 persons/square meter, respectively). Conversely, Gulberg is a newly established area of Lahore with high literacy rate and elite class residents with congestion rate of 20 person/square meter (Joshua and Ali, 2011; Nisar, *et al.*, 2018).

The most prevalent serovar in our study was *Salmonella* serovar Typhimurium (35.92%), followed by *S*. Enteritidis (25.24%), *S*. Dublin (14.56%), *S.* Gallinarum biovar gallinarum (8.74%). All the samples were negative for *Salmonella* Typhi (0%) while the untyped isolates were 15.53%.

As both, *Salmonella* Dublin and Gallinarum are host specific serovars, the possible chances of occurrence may be due to infection of birds at farms or the environmental contamination of samples at retail shops. In our study, the predominant serovar was Typhimurium, a recent study reported a prevalence of 28.45% from farm samples from Faisalabad (Punjab) (Wajid*, et al.*, 2019). No other study in Pakistan reported the prevalence of *S.* Typhimurium. The prevalence of *Salmonella* Enteritidis (25.24%) reported in our study was simlar to that reported from Kohat (23.3% (Asif*, et al.*, 2017), but lower than reported in different districts of Punjab (31.34%), (Yasmin*, et al.*, 2019).

Conclusions

This is the first baseline study in Lahore, Punjab for the prevalence of different *Salmonella* serovars. The high prevalence of zoonotic serovars in the retail shops of major city of Punjab is a major threat to public health. Food and Health authorities of Pakistan should implement appropriate control strategies to minimize the spread food borne zoonotic pathogens to consumers

Ethical permission

This study was approved by Ethical Review Committee for Animals and humans, University of Veterinary and Animal Sciences, Lahore.

Acknowledgments

Authors acknowledge the poultry retailers for their participation in study. The authors will also like to Mr. Mubashar Hanif laboratory assistant for help in laboratory work and Clerk, Miss Fakhra Sarwar for help in formatting of write up.

Funding Statement

The current research study was funded by project entitled "Molecular detection of emerging and remerging meat borne bacterial pathogens and evaluation of various control strategies" and HEC Indigenous PhD Scholarship (Awarded to Shakera Sadiq). The funding agency had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Abatcha M.G., Effarizah M.E. & Rusul G. 2018. Prevalence, antimicrobial resistance, resistance genes and class 1 integrons of Salmonella serovars in leafy vegetables, chicken carcasses and related processing environments in Malaysian fresh food markets. *Food Control*, **91**, 170-180.

Abdi R.D., Mengstie F., Beyi A.F., Beyene T., Waktole H., Mammo B., Ayana D. & Abunna F. 2017. Determination of the sources and antimicrobial resistance patterns of Salmonella isolated from the poultry industry in Southern Ethiopia. *BMC Infect Dis*, **17**, 352.

Acheson D. & Hohmann E.L. 2001. Nontyphoidal Salmonellosis. *Clin Infect Dis*, **32**, 263-269.

Ak N.O., Cliver D.O. & Kaspar C.W. 1994. Cutting boards of plastic and wood contaminated experimentally with bacteria. *J Food Protect*, **57**, 16-22.

Akhtar F., Hussain I. & Rahman S. U. 2010. Prevalence and Antibiogram Studies of Salmonella Enteritidis Isolated from Human and Poultry Sources. *Pakistan Vet J*, **30** (1), 25-28.

Alali W. Q., Gaydashov R., Petrova E., Panin A., Tugarinov O., Kulikovskii A., Mamleeva D., Walls I. & Doyle M. P. 2012. Prevalence of Salmonella on retail chicken meat in Russian Federation. *J Food Protect*, **75**(8), 1469–1473.

Alvarez J., Sota M., Vivanco A. B., Perales I., Cisterna R., Rementeria A. & Garaizar J. 2004. Development of a multiplex PCR technique for detection and epidemiological typing of salmonella in human clinical samples. *J Clin Microbiol*, **42**(4), 1734–1738.

Antunes P., Mourão J., Campos J. & Peixe L. 2016. Salmonellosis: the role of poultry meat. *Clin Microbiol Infect*, **22**, 110-121.

Asif M., Rahman H. & Qasim M. 2017. Molecular detection and antimicrobial resistance profile of zoonotic Salmonella enteritidis isolated from broiler chickens in Kohat, Pakistan. *Journal Chinese Medical Association*, **80**, 303-306.

Beach J.C., Murano E.A. & Acuff G.R. 2002. Prevalence of Salmonella and Campylobacter in Beef Cattle from Transport to Slaughter. *J Food Protect*, **65**, 1687-1693.

Brunette G.W. 2017. CDC Yellow Book 2018: health information for international travel. Oxford University Press.

Corry J. E., Allen V. M., Hudson W. R., Breslin M. F. & Davies R. H. 2002. Sources of Salmonella on broiler carcasses during transportation and processing: modes of contamination and methods of control. *J Appl Microbiol*, **92**(3), 424–432.

de Freitas C.G., Santana Â.P., da Silva P.H.C., Gonçalves V. S., Barros MdeA., Torres F. A., Murata L. S. & Perecmanis S. 2010. PCR multiplex for detection of Salmonella Enteritidis, Typhi and Typhimurium and occurrence in poultry meat. *Int J Food Microbiol*, **139**, 15-22.

Delahoy M.J., Wodnik B., McAliley L., Penakalapati G., Swarthout J., Freeman M. C. & Levy K. 2018. Pathogens transmitted in animal feces in low- and middle-income countries. *Int J Hyg Environ Health*, **221**, 661-676.

Devleesschauwer B., Haagsma J. A., Mangen M.-J. J., Lake R. J. & Havelaar A. H. 2018. The Global Burden of Foodborne Disease. *In* Food Safety Economics : Incentives for a Safer Food Supply (T.Roberts, Ed.). Springer, 107–122,

Donado-Godoy P., Clavijo V., León M., Tafur M. A., Gonzales S., Hume M., Alali W., Walls I., Lo Fo Wong D. M. & Doyle M. P. 2012. Prevalence of Salmonella on retail broiler chicken meat carcasses in Colombia. *J Food Protect,* **75**(6), 1134–1138.

Ejo M., Garedew L., Alebachew Z. & Worku W. 2016. Prevalence and Antimicrobial Resistance of Salmonella Isolated from Animal-Origin Food Items in Gondar, Ethiopia. *BioMed Res Int*, **8**, 4290506.

Gallegos-Robles M.A., Morales-Loredo A., Alvarez-Ojeda G., Osuna-García J. A., Martínez I. O., Morales-Ramos L. H. & Fratamico P. 2009. PCR detection and microbiological isolation of Salmonella spp. from fresh beef and cantaloupes. *J Food Sci*, **74**(1), M37–M40.

Garedew L., Hagos Z., Addis Z., Tesfaye R. & Zegeye B. 2015. Prevalence and antimicrobial susceptibility patterns of Salmonella isolates in association with hygienic status from butcher shops in Gondar town, Ethiopia. *Antimicrob Resist Infect Control,* **4**, 21.

Ha J. S., Seo K. W., Kim Y. B., Kang M. S., Song C. S. & Lee Y. J. 2018. Prevalence and characterization of Salmonella in two integrated broiler operations in Korea. *Ir Vet J,* **71**, 3.

Hald B., Wedderkopp A. & Madsen M. 2000. Thermophilic Campylobacter spp. in Danish broiler production: a cross-sectional survey and a retrospective analysis of risk factors for occurrence in broiler flocks. *Avian Pathol*, **29**, 123-131.

Jalil H., Hussain S. & Siddiqi A. 2013. An empirical study of meat supply chain and prices pattern in Lahore (Pakistan): A case study. *Journal of Supply Chain Management Systems*, **2**, 1-10.

Jarquin C., Alvarez D., Morales O., Morales A. J., López

B., Donado P., Valencia M. F., Arévalo A., Muñoz F., Walls I., Doyle M. P. & Alali W. Q. 2015. Salmonella on raw poultry in retail markets in Guatemala: Levels, antibiotic susceptibility, and serovar distribution. *J Food Protect*, **78**, 1642-1650.

Joshua G. & Ali Z. 2011. Avian diversity with the varying urban congestions of Lahore. *J Anim Plant Sci*, **21**, 421-482.

Khan A.S., Georges K., Rahaman S., Abdela W. & Adesiyun A. A. 2018. Prevalence and serotypes of Salmonella spp. on chickens sold at retail outlets in Trinidad. *PloS one*, **13**, e0202108.

Lamas A., Fernandez-No I.C., Miranda J.M., Vázquez B., Cepeda A. & Franco C. M. 2016. Prevalence, molecular characterization and antimicrobial resistance of Salmonella serovars isolated from northwestern Spanish broiler flocks (2011–2015). *Poultry Sci*, **95**, 2097-2105.

Li K, Ye S, Alali WQ, Wang Y., Wang X., Xia X. & Yang B. 2017. Antimicrobial susceptibility, virulence gene and pulsed-field gel electrophoresis profiles of Salmonella enterica serovar Typhimurium recovered from retail raw chickens, China. *Food Control* , 72, 36-42.

Luu Q. H., Fries R., Padungtod P., Tran T. H., Kyule M. N., Baumann M. P. & Zessin K. H. 2006. Prevalence of Salmonella in retail chicken meat in Hanoi, Vietnam. *Ann N Y Acad Sci,* **1081**, 257-261.

McCrea B. A., Tonooka K. H., VanWorth C., Boggs C. L., Atwill E. R. & Schrader J. S. 2006. Prevalence of Campylobacter and Salmonella species on farm, after transport, and at processing in specialty market poultry. *Poultry Sci,* **85**(1), 136–143.

Nguyen D.T.A., Kanki M., Nguyen P.D., Le H. T., Ngo P. T., Tran D. N., Le N. H., Dang C. V., Kawai T., Kawahara R., Yonogi S., Hirai Y., Jinnai M., Yamasaki S., Kumeda Y. & Yamamoto Y. 2016. Prevalence, antibiotic resistance, and extended-spectrum and AmpC β-lactamase productivity of Salmonella isolates from raw meat and seafood samples in Ho Chi Minh City, Vietnam. *Int J Food Microbiol*, **236**, 115-122.

Nidaullah H, Abirami N, Shamila-Syuhada AK, Chuah L.O., Nurul H., Tan T. P., Abidin F. W. Z. & Rusul G. 2017. Prevalence of Salmonella in poultry processing environments in wet markets in Penang and Perlis, Malaysia. *Vet World*, **10**, 286-292.

Nisar M., Ahmad M. U. D., Mushtaq M. H., Shehzad W., Hussain A., Nasar M., Nagaraja K. V. & Goyal S. M. 2018. Occurrence of Campylobacter in retail meat in Lahore, Pakistan. *Acta Trop,* **185**, 42–45.

Okareh O. & Erhahon O. 2015. Microbiological assessment of food and hand-swabs samples of school food vendors in Benin City, Nigeria. *Food Public Health*, **5**, 23-28.

Phan T. T., Khai L. T., Ogasawara N., Tam N. T., Okatani A. T. Akiba M. & Hayashidani H. 2005. Contamination of Salmonella in retail meats and shrimps in the Mekong Delta, Vietnam. *J Food Prot*, **68**(5), 1077–1080.

Shafini A., Son R., Mahyudin N., Rukayadi Y. & Tuan Zainazor T.C. 2017. Prevalence of Salmonella spp. in chicken and beef from retail outlets in Malaysia. *Int Food Res J*, **24**, 437.

Shang K., Wei B., Jang H.-K. & Kang M. 2019. Phenotypic characteristics and genotypic correlation of antimicrobial resistant (AMR) Salmonella isolates from a poultry slaughterhouse and its downstream retail markets. *Food Control*, **100**, 35-45.

Sharma J., Kumar D., Hussain S., Pathak A., Shukla M., Prasanna Kumar V., Anisha P.N., Rautela R., Upadhyay A.K. & Singh S.P. 2019. Prevalence, antimicrobial resistance and virulence genes characterization of nontyphoidal Salmonella isolated from retail chicken meat shops in Northern India. *Food Control*, **102**, 104-111.

Soomro A.H., Khaskheli M., Bhutto B., Shah G. , Memon A. & Dewan P. 2010. Prevalence and antimicrobial resistance of Salmonella serovars isolated from poultry meat in Hyderabad, Pakistan. *Turk J Vet Anim Sci*, **34**(5), 455-460.

Stegniy B., Gerilovych A., Arefyev V., Glebova K. & Potkonjak A. 2014. A method for detecting and typing of Salmonella by multiplex PCR. *Arhiv Veterinarske Medicine*, **7**, 47-56.

Suresh T., Hatha A.M., Srinivasan D., Srinivasan S. & Lakshmanaperumalsamy P. 2004. Salmonella cross-contamination in retail chicken outlets and the efficacy of spice extracts on Salmonella enteritidis growth inhibition on various surfaces *et al.*, 2004. Salmonella cross-contamination in retail chicken outlets and the efficacy of spice extracts on Salmonella enteritidis growth inhibition on various surfaces. *Microbes Environments*, **19**, 286-291.

Ta Y.T., Nguyen T.T. & To P.B. 2012. Prevalence of Salmonella on Chicken Carcasses from Retail Markets in Vietnam. *J Food Prot*, **75**, 1851-1854.

Ta Y.T., Nguyen T.T. & To P.B. 2014. Quantification, serovars, and antibiotic resistance of Salmonella isolated from retail raw chicken meat in Vietnam. *J Food Prot*, **77**, 57-66.

Tabo D.A., Diguimbaye C.D. & Granier S.A. 2013. Prevalence and antimicrobial resistance of non-typhoidal Salmonella serotypes isolated from laying hens and broiler chicken farms in N'Djamena, Chad. *Vet Microbiol*, **166**, 293-298.

Uddin M.N., Farooq M.M. & Waqas M. 2018. Antibiotic assays of Salmonella isolated from poultry chicken of various locations in districts Swat. *Pure Appl Biol*, **7**, 78-84.

Van T.T.H., Moutafis G., Istivan T., Tran L. T. & Coloe P. J 2007. Detection of Salmonella spp. in retail raw food samples from Vietnam and characterization of their antibiotic resistance. *Appl Environ Microbiol*, **73**, 6885-6890..