Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol. 21 No.4 Ser. No.73 2020

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Serogroups, and drug resistance of nontyphoidal *Salmonella* in symptomatic patients with community-acquired diarrhea and chicken meat samples in Tehran

Besharati, S.[1]; Sadeghi, A.[1]; Ahmadi, F.[2]; Tajeddin, E.[3]; Mohammad Salehi, R.[4]; Fani, F.[5]; Pouladfar, Gh.[5]; Nikmanesh, B.[6]; Majidpour, A.[7]; Soleymanzadeh Moghadam, S.[8]; Mirab Samiee, S.[9]; Rahnamaye Farzami, M.[9]; Rahbar, M.[9]; Eslami, P.[10]; Rakhshani, N.[11]; Eshrati, B.[12]; Gouya, M. M.[12]; Fallah, F.[13]; Karimi, A.[14]; Owlia, P.[4] and Alebouyeh, M.[14*, **]**

[1]MSc in Microbiology, Department of Microbiology, Faculty of Medicine, Shahed University, Tehran, Iran; [2]MSc Student in Microbiology, Department of Microbiology, Faculty of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran; [3]MSc in Microbiology, Division of Microbiology, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [4]Molecular Microbiology Research Center, Faculty of Medicine, Shahed University, Tehran, Iran; [5]Division of Microbiology, Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; [6]Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Tehran University of Medical Sciences, Tehran, Iran; [7]Division of Microbiology, Anti-Microbial Resistance Research Center, Institute of Immunology and Infectious Diseases (IIID), Iran University of Medical Sciences, Tehran, Iran; [8]MSc in Microbiology, Division of Microbiology, Anti-Microbial Resistance Research Center, Institute of Immunology and Infectious Diseases (IIID), Iran University of Medical Sciences, Tehran, Iran; [9]Health Reference Laboratory, Health Reference Laboratory Research Center, Ministry of Health and Medical Education, Tehran, Iran; [10]MSc in Microbiology, Department of Microbiology, Central Laboratory, Milad Hospital, Tehran, Iran; [11]Pathology Laboratory, Mehr Hospital, Tehran, Iran; [12]Center for Communicable Disease Control, Ministry of Health and Medical Education, Tehran, Iran; [13]Department of Microbiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [14]Pediatric Infections Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[*]***Correspondence:*** *M. Alebouyeh, Pediatric Infections Research Center, Research Institute for Children's Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: masoud.alebouyeh@gmail.com*

[**]These authors contributed equally in this study

(Received 25 Apr 2020; revised version 26 Sept 2020; accepted 20 Oct 2020)

Abstract

Background: *Salmonella* is considered as a main cause of community-acquired diarrhea in humans, however, sources of the multi-drug resistant (MDR) strains and their link with the disease are not well known. **Aims:** This study aimed to investigate the frequency, serogroup diversity, and antimicrobial susceptibility patterns of *Salmonella* strains in poultry meat and stool samples of patients with community acquired diarrhea in Tehran. **Methods:** We compared the frequency of non-typhoidal *Salmonella* serogroups, the similarities of their resistance patterns to 10 antimicrobial compounds, the prevalence of extended spectrum β-lactamase (ESBL) and ampicillinase C (AmpC) genetic determinants, and class 1 and 2 integrons in 100 chicken meat and 400 stool samples of symptomatic patients in Tehran during June 2018 to March 2019. **Results:** *Salmonella* was isolated from 75% and 5.5% of the chicken meats and human stool samples, respectively. The chicken meat isolates mainly belonged to serogroup C (88%, 66/75), while the human stool isolates were mainly related to serogroup D (59.1%, 13/22). The MDR phenotype and the most common rates of resistance to antibiotics, including tetracycline, trimethoprim/sulfamethoxazole (TS) and azithromycin, were detected in 4.5% and 45.3%, 59% and 13.6%, 43% and 9.1%, 42% and 9.1% of the human stool and chicken meat samples, respectively. Carriage of *bla*$_{CTX}$, *bla*$_{SHV}$, and *bla*$_{PER}$ genes in the meat isolate with ESBL resistance phenotype and *bla*$_{ACC}$, *bla*$_{FOX}$, and *bla*$_{CMY-2}$ among the 7 meat strains with AmpC resistance phenotype was not confirmed using polymerase chain reaction (PCR). High prevalence of class 1 and 2 integrons was characterized and showed a correlation with resistance to TS and chloramphenicol. **Conclusion:** These findings showed a lack of association between chicken meats and human isolates due to discrepancy between the characterized serogroups and resistance phenotypes.

Key words: Beta-Lactamases, Diarrhea, Drug resistance, Meat, *Salmonella*

Introduction

Salmonella, a member of *Enterobacteriacea*, is globally considered as a main cause of foodborne diseases. This bacterium exists in the gastrointestinal tract of humans and animals, and could be detected in contaminated food, surface water, sewage water, and river (Da Silva *et al*., 2018). *Salmonella* consists of more than 2500 serotypes, among them some serotypes are host specific (Mietzner *et al*., 2016). Most of the

serotypes have various hosts, but they generally cause no complication in the gastrointestinal tract and do not need medication. However, infants, older- and debilitated patients, pregnant women, and immunodeficient patients are at higher risk of severe infection with this bacterium (Popoff, 1997). The disease occurs as enteric fever (Typhoid, caused by serotypes Typhi and Paratyphi), osteomyelitis, pyelonephritis, empyema, liver necrosis, and meningitis. Bacteremia is commonly linked with serotype Choleraesuis. While consumption of poultry meat and eggs is considered as a source of the infection in more than 50% of the cases, red meats, dairy products, fish, and shrimp also could cause the infection (Threlfall, 2010). Transmission of this bacterium along the food chain is due to its resistance to a variety of environmental stresses as a risk factor for the consumers in the community. *Salmonella enterica*, mainly serotypes Enteritidis and Typhimurium, are among the most prevalent zoonotic pathogens associated with human infections (Mietzner *et al*., 2016; Da Silva *et al*., 2018).

Estimation of the burden of *Salmonella* infection and its link with the rate of food contamination in the community needs well designed pathogen-based epidemiological studies (Kretzschmar *et al*., 2012). This estimation should be performed with usage of standard and validated laboratory methods capable of detecting the bacterium, even the cells damaged (but remaining viable) during the food processing and storage conditions. Application of inappropriate methods could cause underestimation, affecting further interventions for the management of the disease and control of its spread. This is also the case for estimation of antibiotic resistance rate among the isolates when selection of inappropriate methods and reagents could affect therapeutic regimens against severe extraintestinal infections. Resistance to antibiotics could emerge early after first use. In the case of chloramphenicol, the resistance occurred two years after the first use in England (Kaur, 2013). It is believed that the administration of antibiotics in agriculture, as food preservatives or growth promoters, is responsible for the increase in the incidence of multi-drug resistant (MDR) serotypes of *Salmonella* in the community. Also, self-medication and empirical therapy by physicians in outpatient services account as main risk factors for increased rates of resistance to antibiotics (Manyi-Loh *et al*., 2018).

Emergence of resistant strains of *Salmonella* to fluoroquinolones is a major challenge, not only among the invasive strains but also in non-invasive serotypes (Klemm *et al*., 2018a). Reports of infections with extensively drug resistant (XDR) strains, that are resistant to chloramphenicol, ampicillin, and trimethoprim/sulfamethoxazole (TS), as well as fluoroquinolones and third-generation cephalosporins, has increased new worldwide concerns (Klemm *et al*., 2018b); These shows the importance of surveillance study at a global level.

Co-carriage of extended spectrum beta-lactamase (ESBL)-encoding genes, such as $bla_{\text{CTX-M}}$, with *qnr*, a plasmid encoded gene linked to fluoroquinolones resistance, was reported among MDR and XDR-*S. Typhi* human isolates; however, data about their simultaneous spread among non-typhoidal *Salmonella* isolates in humans and food animals is spare (Hendriksen *et al*., 2013; Le Hello *et al*., 2013; Wong *et al*., 2013; Wang *et al*., 2017). The XDR phenotype is mainly conferred through the acquisition of plasmids harboring resistance-determining genes encoding by integrons (Britto *et al*., 2018).

There are reports about rates of disability adjusted life years (DALYs) in human populations for typhoidal and non-typhoidal *Salmonella* infections in different countries (Stanaway *et al*., 2019). In Iran, 114 and 167 DALYs per 100,000 were reported for typhoidal and non-typhoidal infections in 2017, respectively, which are higher than those reported in Qatar (18.1 per 100,000, 2012) (Farag *et al*., 2016), Australia (53.0 per 100,000 in 2013) (Ford *et al*., 2016), Central European countries (55 DALYs per 100,000 in 2017), Central Asia (132.4 DALYs per 100,000 in 2017), North America (103.27 per 100,000 in 2017), but lower than North Africa and Middle East (378.4 and 321.2 DALYs per 100,000, respectively). To reduce disabilities related to these infections and to link them to the sources of contamination, surveillance studies on both patients and food products should be done. European Food Safety Authority and the European Centre for Disease Control and Prevention reported contamination with *Salmonella* in 1.47% of breeding flocks and 2.6% for broiler flocks (Hugas and Beloeil, 2014; Authority *et al*., 2018). A higher rate of the contamination may be predictable for the flocks in Iran, based on the higher rate of the reported human infections. To date, according to our knowledge, no surveillance study was conducted to link the involvement of raw chicken and poultry meat with human infections in Iran. In the current study we aimed to investigate the rate of contamination with *Salmonella* spp. in poultry meat and the rate of infection with this bacterium in a population of patients with diarrhea in 22 regions of Tehran, Iran. Frequencies of antimicrobial drug resistance, similarities of the resistance patterns, homology among the common serogroups, and genetic determinants associated with the resistance phenotypes were also characterized between the chicken meat and human faeces isolates to show possible relationships among the isolates.

Materials and Methods

Study setting, epidemiological design, and sampling

A cross sectional study was designed to determine the contamination rate of *Salmonella* spp. in poultry meat samples distributed across 22 regions of Tehran and their correlations with the human isolates obtained in four hospitals from patients with symptomatic diarrhea. The isolation rates diversity based on the industrial brands and weights of the products was also estimated in each

region. This study was approved by ethical committee of National Institute for Medical Research Development, Islamic Republic of Iran (IR.NIMAD.REC.1396.105). The sampling was done from June 2018 to March 2019. One hundred fresh chicken carcasses were purchased from approved Tehran municipally daily fruit and vegetable markets. The date and region of the sampling were randomly determined. Each sample was packaged in a separate sterile plastic bag, labeled immediately, and was transported in a cool box. Four hundred stool samples were obtained from three general and one pediatric hospitals in Tehran. All samples were provided from symptomatic outpatients with diarrhea with no recent history of hospitalization and antibiotic therapy. Demographic data (age and sex), macroscopic appearance of the samples and excretion of leukocytes, blood, and epithelial cells, and related data for the food of the patients were recorded in a designed questionnaire.

Salmonella isolation from poultry meat and stool samples

Pre-enrichment of meat samples for isolation of *Salmonella* spp. was performed according to a standard method (ISO 6579:2002) (Anonymous, 2002). Twenty-five g of each poultry carcass was taken after cutting the whole carcass into small pieces in sterile conditions. The sections were selected from different parts of the carcass and enriched in 225 ml of buffered peptone water (PBW, pH = 7; Liofilchem, Teramo, Italy) at 37°C for 24 h. Then, 0.1 ml of the enriched sample was transferred into 10 ml of Rappaport-Vassiliadis and Tetrathionate broth media, each incubated at 42°C and 37°C for an additional 24 h, respectively. A loopful of the grown cultures was inoculated on Xylose Lysine Deoxycholate (XLD) agar and Bismuth Sulfite agar plates (Liofilchem, Teramo, Italy).

Fresh stool samples, samples (transportation time <2 h) or swab samples (in Cary Blair transport medium) were inoculated into Selenite F broth medium. A small amount of stool or a swab sample was streaked on MacConkey and XLD agar plates. Growth of suspected colonies was followed after incubation of the cultures at 37°C for 18-24 h. The isolates were purified on Nutrient agar medium and stocked in Trypticase soy broth at -70°C for further analyses. Identity of the colonies with black centers on XLD agar and colorless colonies on MacConkey agar was initially characterized by biochemical tests, including ornithine decarboxylation in Ornithine Decarboxylase (ODC) agar (Liofilchem, Teramo, Italy), sugar fermentation in Triple sugar iron agar (Liofilchem, Teramo, Italy), citrate use in Citrate agar (Liofilchem, Teramo, Italy), H_2S, Indole, and Motility reactions in Sulfide, Indole, motility (SIM) agar (Liofilchem, Teramo, Italy), urease activity in Urea broth (Liofilchem, Teramo, Italy), and Methyl Red/Voges-Proskauer (MRVP) reaction in MRVP broth (Liofilchem, Teramo, Italy) (Mikoleit, 2010; Organization, 2010). *Escherichia coli* strain ATCC 25922 (Molecular Microbiology Research Center, Faculty of Medicine, Shahed University, Tehran, Iran) was used for quality control of the test.

Serogrouping of *Salmonella* isolates

Biochemically confirmed strains of *Salmonella* were serotyped according to the Kaufmann-White scheme (Popoff, 1997; Popoff and Le Minor, 2001). Serogrouping was done by agglutination method using antisera against serogroups A to D (Baharafshan, Iran). Briefly, a loopful of the freshly grown colonies on Nutrient agar was transferred onto a glass slide and mixed with the *Salmonella* antisera, separately for each sample. Agglutination was followed visually for 30 s.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates was tested according to Clinical Laboratory Standards Institute guideline (CLSI, 2018). *Escherichia coli* strain ATCC 25922 was used for quality control of the test. The antimicrobial agents used were cefoxitin (30 µg), azithromycin (15 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg), cefotaxime (30 µg), imipenem (10 µg) and TS (25 µg) (Mast Group Ltd., UK). Multi-drug resistance phenotype was defined as resistance to three or greater classes of antibiotics. Extended spectrum β-lactamase and ampicillinase C (AmpC) resistance phenotypes were determined using double disk synergy test (CLSI, 2018) and AmpC detection Set (Mast group Ltd. Co., UK).

Phenotypic and genotypic study of ESBLs and AmpC

Phenotypic identification of ESBLs was performed using double disk method (CLSI, 2018). *Salmonella* strains that showed resistance phenotype to the 3rd generation cephalosporins and cefoxitin, but were not inhibited in the presence of clavulanic acid, were screened for AmpC phenotype using the Mast Group Ltd. AmpC detection set (MAST Group Ltd., UK). All of the strains that were phenotypically positive for ESBL and AmpC were examined genotypically for bla_{TEM}, bla_{CTX}, bla_{SHV}, and bla_{PER} genes (in the case of ESBLs) and bla_{ACC}, bla_{FOX}, and $bla_{\text{CMY-2}}$ (in the case of AmpC) by polymerase chain reaction (PCR).

DNA extraction and PCR for screening of genes related to ESBLs, AmpC, and integrones

Polymerase chain reaction was assigned for *Salmonella* isolates confirmation using specific primers for *invA* gene detection (Table 1). DNA was isolated from freshly grown colonies of *Salmonella* by boiling method. Briefly, a crude suspension was provided in PBS (pH = 8) and after centrifugation at 14000 g (10 min), its pellet boiled for 15 min at 95°C. The supernatant of the treated suspension serves as DNA template for PCR assays, done in the present study using specific primers for bla_{TEM}, bla_{CTX}, bla_{SHV}, and bla_{PER} for ESBLs, and bla_{ACC}, bla_{FOX}, and $bla_{\text{CMY-2}}$ for AmpC β-lactamases, respectively (Table 1). All assays were done

Table 1: Oligonucleotide primers used in this study

Primers	Sequences (5´-3´)	Conditions	Products (bp)	References
InvA	F: GTGAAATTATCGCCACGTTCGGGCAA R: CTGACAGTTACCAATGCTTA	94°C, 5 min, 30 cycles of 94°C, 1 min, 57°C, 30 s, 72°C, 30 s, 72°C, 10 min	285	Tajbakhsh *et al*. (2016)
bla_{TEM}	F: TCAACATTTCCGTGTCG R: CTGACAGTTACCAATGCTTA	94°C, 5 min, 30 cycles of 94°C, 1 min, 57°C, 30 s, 72°C, 30 s, 72°C, 10 min	445	Ghasemi *et al*. (2013)
bla_{CTX-M}	F: ATGTGCAGYACCAGTAARGT R: TGGGTRAARTARGTSACCAGA	94°C, 4 min, 40 cycles of 94°C, 1 min, 57°C, 30 s, 72°C, 40 s, 72°C, 10 min	593	Ghasemi *et al*. (2013)
bla_{SHV}	F: TTTATCGGCCYTCACTCAAGG R: GCTGCGGGCCGGATAACG	94°C, 5 min, 35 cycles of 94°C, 1 min, 57°C, 30 s, 72°C, 57 s, 72°C, 7 min	747	Monstein *et al*. (2007)
ACC	F: AACAGCCTCAGCAGCCGGTTA R: TTCGCCGCAATCATCCCTAGC	94°C, 3 min, 30 cycles of 94°C, 30 s, 61°C, 30 s, 72°C, 1 min, 72°C, 7 min	345	Pérez-Pérez and Hanson (2002)
FOX	F: AACATGGGGTATCAGGGAGATG R: CAAAGCGCGTAACCGGATTGG	94°C, 3 min, 30 cycles of 94°C, 30 s, 64°C, 30 s, 72°C, 1 min, 72°C, 7 min	190	Pérez-Pérez and Hanson (2002)
bla_{CMY}	F: AACACACTGATTGCGTCTGAC R: CTGGGCCTCATCGTCAGTTA	94°C, 3 min, 25 cycles of 94°C, 30 s, 50°C, 30 s, 72°C, 1 min, 72°C, 7 min	1226	Jørgensen *et al*. (2010)
Integron 1	F: CAGTGGACATAAGCCTGTTC R: CCCGAGGCATAGACTGTA	94°C, 3 min; 30 cycles of 94°C, 30 s, 58°C, 30 s, 72°C, 1 min, 72°C, 7 min	160	Fiett *et al*. (2006)
Integron 2	F: CACGGATATGCGACAAAAAGGT R: GTAGCAAACGAGTGACGAAATG	94°C, 3 min, 25 cycles of 94°C, 30 s, 57°C, 30 s, 72°C, 1 min, 72°C, 7 min	789	Derakhshan *et al*. (2014)

separately, except bla_{TEM}, bla_{CTX} for which a multiplex PCR assay was used. Cycling conditions for PCR amplification of the targeted genes were similar: one cycle of initial denaturation at 94°C for 5 min, 35 cycles of 1 min denaturation at 94°C, annealing for 45 s at 55°C, and extension for 30s at 72°C. The final 7 min extension was done at 72°C for one cycle. To show correlations of the resistance phenotypes with carriage of the integrons, all the isolates were screened for class 1 and 2 integrons using PCR (Table 1). All the *Salmonella* isolates were screened for detection of class 1 and 2 integrons by the primers depicted in Table 1. Positive control strains carrying related integrons and β-lactamase genes were kindly provided by Dr. Sh. Nazarian and Dr. K. Amini.

Statistical analysis

Using PRISM software, correlation from contingency table was used for detection of possible correlations between weight of poultry carcasses or age groups of patients and infection rate with *Salmonella*. Correlations between serogroups of *Salmonella* isolates and types of antimicrobial resistance phenotypes were also determined. The P-values ≤0.05 were considered as significant in all the analyses. Homology in resistance phenotypes among the isolates was analyzed by NTSYS 2.02 software.

Results

Poultry meat and patients' samples

A total of 100 chicken meat and 400 patients' stool samples (Male 58%, and Female 42%) were collected during the study period. The poultry carcass samples were from 41 different brands, which their weight ranged from 0.76 kg to 2.71 kg (mean 1.76 ± 0.46 kg). The stool samples were obtained from 4 hospitals in Tehran (three general hospitals and one pediatric hospital). The stool samples were belonged to children (1 month to 9 years old), adolescent (10 to 19 years old), and adults (>19 years old). The samples showed loose (329/400), diarrheal (62/400), and dysenteric (9/400) forms in macroscopic and microscopic analysis.

Salmonella prevalence in chicken meat and human stool samples

Salmonella was isolated from 75% (75/100) of the chicken meet samples carcasses. Comparison of the culture media for isolation of the chicken samples showed a relative preference for RVS *vs* Tethrathinate broth and XLD *vs* Bismuth sulfite agar. However, the contamination rate showed different frequencies in carcasses >1.5 kg (56/72; 77.7%) compared to those with a weight of ≤1.5 kg (19/28; 67.8%). Higher frequency in weight of the samples (19/28, 67.8% *vs* 56/72, 77.7% in the products with ≤1.5 kg [28/100] and >1.5 kg [72/100], respectively). Different frequencies were also observed during summer (15/21; 71.4%) and autumn (60/79; 76%). However, these differences were not statistically significant (P=0.3 and P=0.7, respectively for the weight groups and the season). Higher frequencies of *Salmonella* contamination were detected in regions 3, 5, 6, 9, 13, 17, 21, and 22 (contamination rate: 100%). In other regions (4, 7, 18, 19) the isolation rates were 0 to 40% (Fig. 1).

In the case of stool samples, *Salmonella* was isolated from 5.5% (22/400) of the total samples. As shown in Table 2, the frequency was higher in children and infants than in young adults. The difference was not statistically significant (P=0.63).

Table 2: Diversity in the frequency of *Salmonella enterica* in patients at different age groups

Age groups	*Salmonella* spp.		Total
	Negative	Positive	
Adolescent	15	2	17
Adult	274	15	289
Child	35	1	36
Infant	49	4	53
Unknown	5	0	5
Total	378	22	400

Diversity of *Salmonella* serogroups among the human and chicken samples

The chicken meat isolates mainly belonged to serogroup C (88%, 66/75), while the remaining isolates were related to serogroup B (2.6%, 2/75), serogroup D serogroup C 77.3% *vs* 22.7%; and serogroup D 75% *vs* 25%). The difference was statistically significant (P=0.021). The human faecal isolates mainly belonged to serogroup D (59.1%, 13/22), while other strains were related to serogroup B (31.8%, 7/22), serogroup C (4.5%, 1/22), and non-A to D serogroups (4.5%, 1/22). Investigation of the link between common serogroups of *Salmonella* isolates in the chicken meat and those isolated from stool of patients with diarrhea did not show statistically significant association.

Antimicrobial resistance

The characterized resistance phenotypes in the chicken meat isolates were in the following order: tetracycline (59%), TS (43%), azithromycin (42%), chloramphenicol (27%), cefoxitin (7%), ciprofloxacin (4%), gentamicin (3%), ceftriaxone (1%), imipenem (1%), and cefotaxim (0%). The human isolates showed lower frequencies of the resistance patterns: tetracycline (13.6%), TS (9.1%), azithromycin (9.1%), cefotaxim (0%), chloramphenicol (0%), cefoxitin (0%), ciprofloxacin (0%), gentamicin (0%), ceftriaxone (0%), and imipenem (0%). Multi-drug resistant phenotype was detected in 4.5% (1/22) of the *Salmonella* isolates from the human stool samples, while it was more frequent among the chicken meat isolates (45.3%, 34/75). Concurrent resistance to 3 classes of antibiotics (3DR) was the most common MDR pattern among the chicken (5.3%, 4/75), and non-A to D serogroups (4%, 3/75). All the serogroups, except non-A to D serogroups (100 *vs* 0%), were more frequently isolated from chicken carcasses with >1.5 kg weights *vs* those with ≤1.5 kg weight, respectively (serogroup B 100% *vs* 0%; isolates (76.4%, 26/34; Table 3). Azithromycin/ tetracycline/trimethoprim/sulfamethoxazole (26.4%, 9/ 34), azithromycin/chloramphenicol/tetracycline/ trimethoprim/sulfamethoxazole (17.6%; 6/34) and azithromycin/chloramphenicol/tetracycline (11.7%; 4/34) were among common characterized MDR phenotypes in the chicken isolates. The only human isolate with MDR phenotype showed azithromycin/ tetracycline/ticarcillin pattern, which was detected in 1 chicken meat sample, similarly. Statistical analysis showed no association between resistance phenotypes detected in the chicken meat and the human isolates. No correlation was found between the characterized resistance phenotypes in *Salmonella* strains and the brands of chicken meat or the time of production.

Comparison of homology based on the phenetic drug resistance patterns

Homology of the resistance patterns between the human and chicken meat samples was analyzed. As shown in Fig. 2, different isolates of *Salmonella* from faecal and chicken samples were placed in different clusters. The largest cluster (cluster A) contained the human and chicken strains sensitive to all antibiotics. Five isolates of poultry meat and 6 isolates of human stool in this cluster belonged to a common sampling period. Comparison of the poultry isolates based on their phenetic resistance patterns, sampling dates, and the

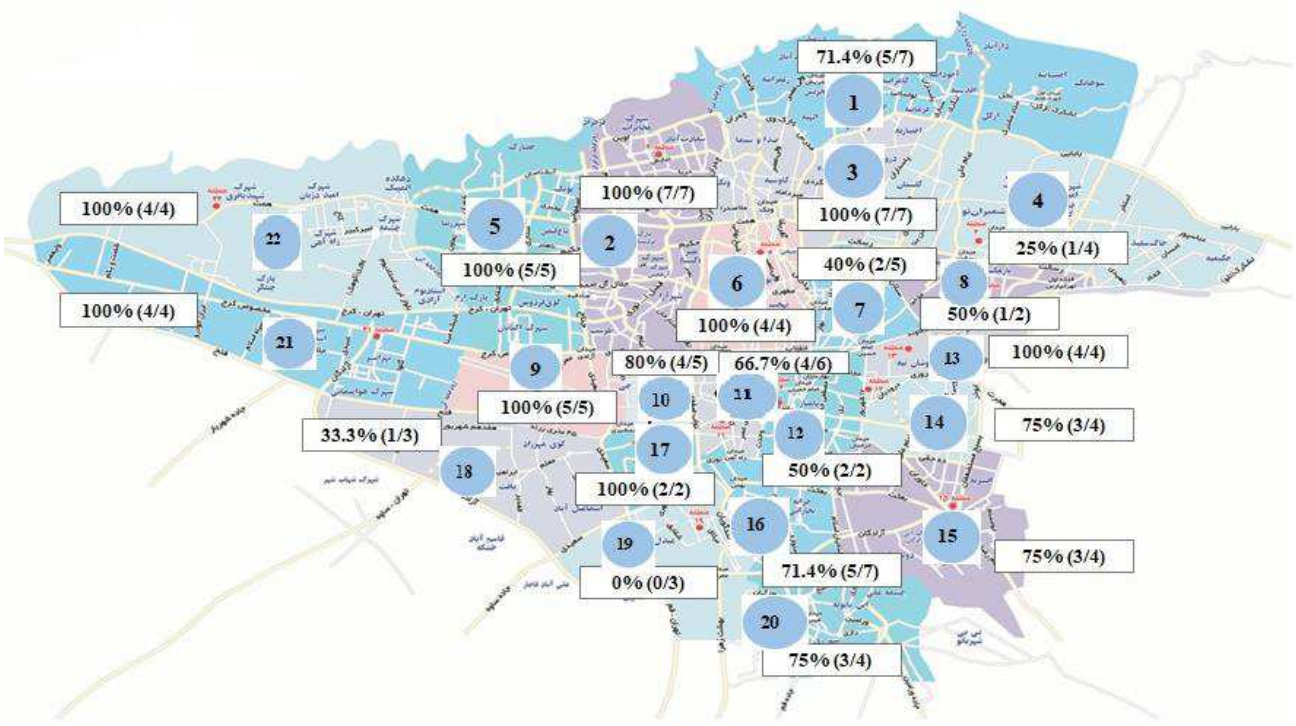

Fig. 1: Frequency of *Salmonella* in poultry meat samples distributed among Tehran municipally daily fruit and vegetable markets in different regions of Tehran

Table 3: Frequency of multi-drug resistant *Salmonella enterica* isolates from poultry meat and patients' stool samples

MDR phenotypes	(%) In chicken meat (n=75)				(%) In patients' stool (n=22)			
	1 DR (%)	2 DR (%)	3 DR (%)	4 DR (%)	5 DR (%)	1 DR	2 DR	3 DR
ATH/C		2 (2.6)						
ATH/C/CIP/T				1 (1.3)				
ATH/C/T			4 (5.3)					
ATH/C/T/TC			1 (1.3)					
ATH/C/T/TS			6 (8)					
ATH/C/TS		2 (2.6)						
ATH/CIP/T/TS				1 (1.3)				
ATH/CRO/TS			1 (1.3)					
ATH/FOX		1 (1.3)						
ATH/FOX/C/T/TS					2 (2.6)			
ATH/FOX/T			1 (1.3)					
ATH/FOX/T/TS				1 (1.3)				
ATH/T		6 (8)						
ATH/T/TC			1 (1.3)					1 (4.54)
ATH/T/TS			9 (12)					
ATH/TS		3 (4)						
C/CIP/GM/IMP/T					1 (1.3)			
C/CIP/GM/T					1 (1.3)			
C/T		2 (2.6)						
C/T/TS			2 (2.6)					
C/TS		1 (1.3)						
FOX/C/T			1 (1.3)					
FOX/C/T/TS				1 (1.3)				
GM/T		1 (1.3)						
T	5 (6.6)					1 (4.54)		
T/TS		11 (14.6)						
TS	1 (1.3)							
T/TC							1 (4.54)	
ATH						1 (4.54)		
Susceptible	6 (8)					18 (81.8)		

MDR: Multidrug resistance, DR: Drug resistance, ATH: Azithromycin, C: Chloramphenicol, CIP: Ciprofloxacin, T: Tetracycline, TC: Ticarcillin, TS: Trimethoprim/Sulfamethoxazole, CRO: Ceftriaxone, FOX: Cefoxitin, GM: Gentamicin, and IMP: Imipenem

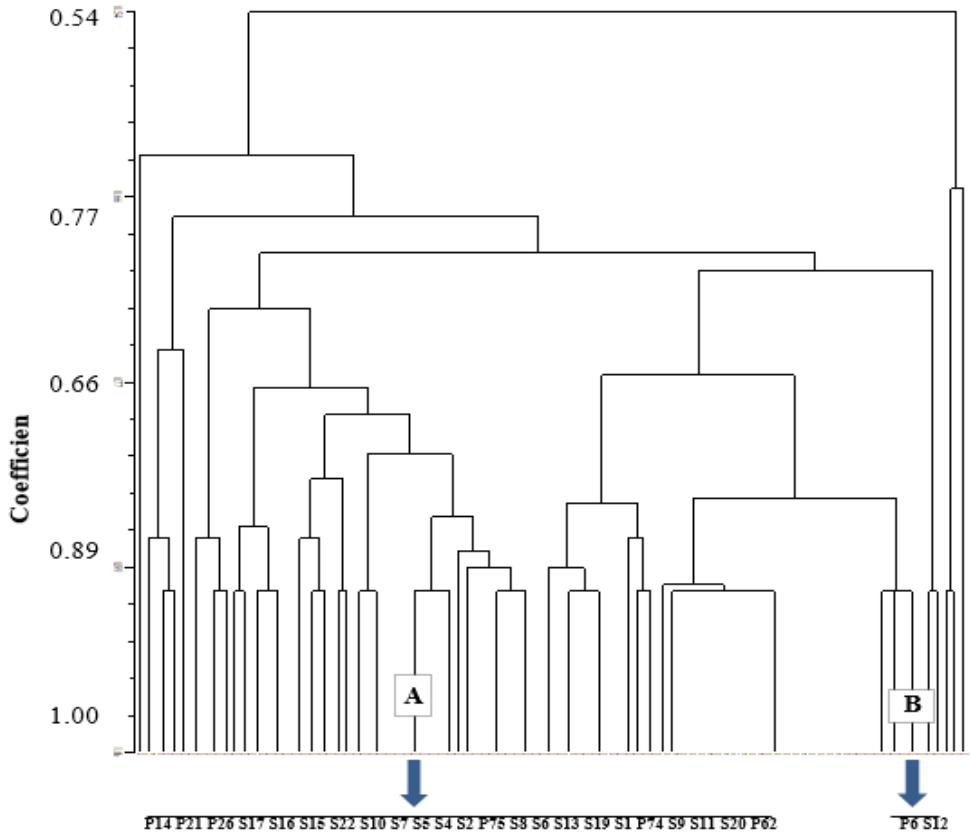

Fig. 2: Homology in resistance phenotypes among the *Salmonella* isolates from stool of symptomatic patients with diarrhea and poultry meat samples. Scale bar shows percentage of homology. Arrows show homology of the isolates among human and meat samples in clusters A and B. P and S refer to poultry and stool samples, respectively

urban sampling areas showed spatial and temporal homology among some of the isolates. Other isolates with 100% homologies showed no specific temporal and spatial affinities.

Frequency of ESBL and AmpC phenotypes among the *Salmonella* strains

Out of 75 chicken meat and 22 human stool isolates, resistance to the 3rd generation cephalosporines was detected in 1 and 0 of the *Salmonella* isolates, respectively. Resistance to cefoxitin was detected in 7 strains of the chicken isolates. Screening of the resistant isolates with β-lactamase inhibitor, cefotaxime (30µg), cefotaxime-clavulanic acid (10 µg-30 µg) (MAST UK product), and AmpC detection set (Mast Group, UK) confirmed the existence of AmpC phenotype among 14.2% (1/7) of these strains. The PCR results showed that none of the relevant genes, except bla_{TEM}, were detected in the isolates with related resistance phenotypes. bla_{TEM} was detected in 4 isolates of the chicken meat samples that were resistant to cefoxitin.

Frequency of class 1 and 2 integrons among the *Salmonella* strains

The PCR assay was performed to identify class 1 and 2 integrons among 97 *Salmonella* isolates from the chicken meat (75/97) and stool samples (22/97). The prevalence of class 1 and 2 integrons for human stool specimens was 40.9% (9/22) and 27.2% (6/22), respectively. Prevalence of class 1 and 2 integrons for chicken samples was 50.6% (38/75) and 29.3% (22/75), respectively. The frequency of class 1 integrons were higher than that of class 2 integrones in the *Salmonella* isolates. Multi-drug resistance phenotype among the integron encoding isolates was detected in 63.8% (23/36) of the chicken meat and 44.4% (16/36) and human stool samples. Resistance to TS was significantly higher in class 2 integron-bearing isolates in the poultry meat samples compared with integron-negative isolates (P=0.008). Moreover, resistance to chloramphenicol was about 2-fold higher in the integron positive strains (50% *vs* 25.6%). This association was also seen in the human stool isolates, where resistance to TS was only detected in the strains that carried class 1 and class 2 integrons.

Discussion

An increase in population together with globalization of food supply, an increase in travel and tourism, and a tendency to consume ready-to-eat and outdoor foods have brought up foodborne diseases as global health problems. The longer timeframe of transmission could be considered as a possible reason for the increased microbial load of the collected samples from some areas. Raeisi and Ghiamirad (2015) in a study in Ardabil, Iran, showed that 10% of poultry samples were contaminated with *Salmonella*. This rate was lower than the results we obtained in our study, which can be due to the method of isolation of *Salmonella* (Anonymous, 2002). In similar studies, infection rates of 8.3% in Poland (Zdrodowska *et al*., 2014), 14.3% in China (Zeng *et al*., 2019), 17.9% in Ethiopia (Tibaijuka *et al*., 2003), and 34% in Egypt (Abd-Elghany *et al*., 2015) were reported.

In the case of infection rates of *Salmonella* in patients with community-acquired infection, Soltan Dallal and Moezardalan (2004) showed a prevalence of 2.6% in Tehran. In a study by Konaté *et al*. (2019) from 2013 to 2015 in Africa, 16.8% (53/315) of patients with gastrointestinal symptoms showed *Salmonella* infection.

In this study, it was shown that the frequency of serogroup D, including *Salmonella enterica* serotype Enteritidis, was higher in the human cases, consistent with other studies in Europe. The most reported cases of *Salmonella* in Europe were related to serogroups D, C, and B, in agreement with our results (Authority *et al*., 2018).

The observed difference in the frequency of dominant serogroups of *Salmonella* isolates between the human (Serogroup D) and chicken meat samples (Serogroup C) was similarly reported by Noda *et al*. (2010) from Japan.

The frequency of MDR phenotype was higher among the chicken isolates compared with the human ones. The MDR strains were resistant to tetracycline, TMP/SMX, and azithromycin. Concurrent resistance to 3DR was the most common type of MDR phenotype among the chicken isolates. In a similar study by Raeisi and Ghiamirad (2015) done on chicken meat samples in Ardabil, 100% of the isolates were resistant to at least two antibiotics. In the present study, the only human isolate with MDR phenotype was resistant to azithromycin/tetracycline/ticarcillin, similar to one of the chicken isolates. Possible relationship of these isolates was not confirmed due to their difference in serogroups. All of the chicken and human isolates were susceptible to cefotaxime and almost all of them were susceptible to imipenem and ceftriaxone. Regarding the prohibition of the use of chloramphenicol in poultry industry, the high rate of resistance to this antibiotic in poultry may indicate its illegal use (White *et al*., 2000). Results of our study showed that quinolones (e.g. ciprofloxazine) and cephalosporins (e.g. ceftriaxone) have good *in vitro* effects against the human isolates, in consistent with the studies in Saudi Arabia (Ramadan *et al*., 1992) and the USA (Travers and Michael, 2002).

Despite the increased incidence of resistance to ciprofloxacin among *Salmonella* strains in some countries, this resistance phenotype was detected only in 4% of poultry meat and none of the human isolates in our study Prohibition of the usage of some antibiotics, including gentamicin and ciprofloxacin as feed additives in Iran, as implemented by many countries, could explain these results (Gehring *et al*., 2006; Davis *et al*., 2009). The observed resistance phenotype in some of the strains could be due to the illegal use of antibiotics in some poultry farms or through the evolutionary genetic changes that can lead to the emergence of the resistant strains (Hur *et al*., 2011).

The human isolates with tetracycline and TS resistance phenotypes showed statistically a correlation

with carriage of class 1 and class 2 integrons. Moreover, resistance to tetracycline, TS and chloramphenicol in *Salmonella* isolates from poultry meat samples was correlated with carriage of these integrons. These results highlighted the role of the integrons in the distribution of MDR pattern across *Salmonella* strains. The role of integrons in generating multiple drug resistance phenotypes was established by studies of Hur *et al*. (2011), Firoozeh *et al*. (2014), and Miriagou *et al*. (2006). In our study, the prevalence of class 1 and 2 integrons was higher than that of the other studies in Iran (varying between 20% to 60% and 15% to 30%, respectively) (Amini, 2016). In the study of Raeisi and Ghiamirad that performed on *Salmonella* isolates, resistance to tetracycline, chloramphenicol, TS, ampicillin, and amoxicillin was reported higher in integron positive strains than in negative ones (Raeisi and Ghiamirad, 2015). The present study showed that the frequency of class 1 integrons was higher than that of class 2. This study also showed a significant relationship between the presence of bla_{TEM} gene and MDR phenotype in chicken isolates (P=0.04). The presence of class 1 integrons showed the highest association with azithromycin resistance among the chicken isolates. Confirmation of this link needs further investigation.

Our study revealed the prevalence of ESBL and AmpC producing strains among 4% and 1% of the isolates, respectively. Although sequencing of bla_{TEM} product, as the only gene associated with ESBL phenotype, was not done in our study, its dominance in poultry isolates has been confirmed by others. In a study by Chuma *et al*. (2013), 24.7% of bla_{TEM} genes were found in *Salmonella* isolates with ESBL phenotype. The low frequency of AmpC among the chicken meat isolates was similarly reported in the study of Jeon *et al*. (2018) in the Republic of Korea. Among 336 chicken meat samples that was analyzed by these authors, AmpC positive *Salmonella* was detected in only one isolate (1.8%), while ESBL genes detected in 14.8% of them (Jeon *et al*., 2018).

Results of the present study confirmed *Salmonella* as a cause of community-acquired diarrhea in Tehran. Although high frequency of *Salmonella* in chicken meat samples proposed its potential risk of infection for consumers, such a correlation was not confirmed in our study due to the discrepancy between the characterized serogroups and the resistance phenotypes between the meat and human isolates. To find the main sources of the infection in humans, its other sources including livestock and vegetables should also be investigated in future studies. Our results showed a higher frequency of MDR and ESBL/AmpC phenotypes among the chicken isolates compared with the human ones. The higher rate of resistance to some antibiotics in the poultry isolates may indicate illegal use of these drugs during the rearing period, showing the need for special consideration. The high frequency of class 1 and class 2 integrons among the MDR *Salmonella* strains in chicken meat and the human stool samples indicated their possible involvement in the transmission of resistance genetic determinants.

Acknowledgements

This study was supported by a grant from the National Institute for Medical Research Development, Islamic Republic of Iran (Code: 958101). The Molecular Microbiology Research Center, Faculty of Medicine, Shahed University, Tehran, Iran, provides technical facility and equipment. The authors would like to thank all colleagues in National Institute for Medical Research Development, Islamic Republic of Iran; The Center for Communicable Diseases Control, Ministry of Health and Medical Education, Tehran, Iran; Reference Health Laboratory, Ministry of Health and Medical Education, Tehran, Iran; staff of Molecular Microbiology Research Center, Faculty of Medicine, Shahed University, Tehran, Iran; and Technicians of four laboratories in hospitals that the stool samples were collected. We also would like to thank Dr. K. Amini and Dr. Sh. Nazarian for providing control samples.

References

Abd-Elghany, S; Sallam, K; Abd-Elkhalek, A and Tamura, T (2015). Occurrence, genetic characterization and antimicrobial resistance of *Salmonella* isolated from chicken meat and giblets. Epidemiol. Infect., 143: 997-1003.

Amini, K (2016). Prevalence of antibiotic resistance genes in *Salmonella enteritidis* isolated from animal and human and determining their antibiotic resistance patterns. J. Comp. Pathobiol. Iran., 12: 1733-1740.

Anonymous (2002). EN ISO 6579. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization, Geneva, Switzerland.

Britto, CD; Wong, VK; Dougan, G and Pollard, AJ (2018). A systematic review of antimicrobial resistance in *Salmonella enterica* serovar Typhi, the etiological agent of typhoid. PLoS Negl. Trop. Dis., 12: e0006779.

Chuma, T; Miyasako, D; Hesham, D; Takayama, T; Nakamoto, Y; Shahada, F; Akiba, M and Okamoto, K (2013). Chronological change of resistance to β-lactams in *Salmonella enterica* serovar Infantis isolated from broilers in Japan. Front Microbiol., 4: 113.

CLSI (2018). *Performance standards for antimicrobial susceptibility testing*. 28th Edn., Wayne, Pennsylvania. PP: 1-258.

Da Silva, N; Taniwaki, MH; Junqueira, VC; Silveira, N; Okazaki, MM and Gomes, RAR (2018). Microbiological examination methods of food and water: a laboratory manual. 2nd Edn., Taylor and Francis Group, UK, CRC Press. PP: 1-632.

Davis, JL; Smith, GW; Baynes, RE; Tell, LA; Webb, AI and Riviere, JE (2009). Update on drugs prohibited from extralabel use in food animals. J. Am. Vet. Med. Assoc., 235: 528-534.

Derakhshan, S; Najar Peerayeh, S; Fallah, F; Bakhshi, B; Rahbar, M and Ashrafi, A (2014). Detection of class 1, 2, and 3 integrons among *Klebsiella pneumoniae* isolated from children in Tehran hospitals. Arch. Pediatr. Infect. Dis., 2: 164-168.

European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC) (2018). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. EFSA J., 16: e05500.

Farag, E; Garcell, HG; Ganesan, N; Ahmed, SNN; Al-Hajri, M; Al Thani, SMHJ; Al-Marri, SA; Ibrahim, E and Al-Romaihi, HE (2016). A retrospective epidemiological study on the incidence of salmonellosis in the State of Qatar during 2004-2012. Qatar Med. J., 2016: 3.

Fiett, J; Baraniak, A; Mrówka, A; Fleischer, M; Drulis-Kawa, Z; Naumiuk, Ł; Samet, A; Hryniewicz, W and Gniadkowski, M (2006). Molecular epidemiology of acquired-metallo-β-lactamase-producing bacteria in Poland. Antimicrob. Agents Chemother., 50: 880-886.

Firoozeh, F; Zahraei-Salehi, T and Shahcheraghi, F (2014). Molecular clonality and detection of class 1 integron in multidrug-resistant *Salmonella enterica* isolates from animal and human in Iran. Microb. Drug Resist., 20: 517-524.

Ford, L; Glass, K; Veitch, M; Wardell, R; Polkinghorne, B; Dobbins, T; Lal, A and Kirk, MD (2016). Increasing incidence of *Salmonella* in Australia, 2000-2013. PLoS One. 11: e0163989.

Gehring, R; Baynes, R and Riviere, J (2006). Application of risk assessment and management principles to the extralabel use of drugs in food-producing animals. J. Vet. Pharmacol. Ther., 29: 5-14.

Ghasemi, Y; Archin, T; Kargar, M and Mohkam, M (2013). A simple multiplex PCR for assessing prevalence of extended-spectrum β-lactamases producing Klebsiella pneumoniae in Intensive Care Units of a referral hospital in Shiraz, Iran. Asian Pac. J. Trop. Med., 6: 703-708.

Harish, B and Menezes, G (2011). Antimicrobial resistance in typhoidal *Salmonellae*. Indian J. Med. Microbiol., 29: 223-229.

Hendriksen, RS; Joensen, KG; Lukwesa-Musyani, C; Kalondaa, A; Leekitcharoenphon, P; Nakazwe, R; Aarestrup, FM; Hasman, H and Mwansa, JC (2013). Extremely drug-resistant *Salmonella enterica* serovar Senftenberg infections in patients in Zambia. J. Clin. Microbiol., 51: 284-286.

Hugas, M and Beloeil, P (2014). Controlling *Salmonella* along the food chain in the European Union-progress over the last ten years. Eurosurveillance. 19: 20804.

Hur, J; Kim, JH; Park, JH; Lee, YJ and Lee, JH (2011). Molecular and virulence characteristics of multi-drug resistant *Salmonella* Enteritidis strains isolated from poultry. Vet. J., 189: 306-311.

Jeon, HY; Seo, KW; Kim, YB; Kim, DK; Kim, SW and Lee, YJ (2018). Characteristics of third-generation cephalosporin-resistant *Salmonella* from retail chicken meat produced by integrated broiler operations. Poult. Sci., 98: 1766-1774.

Jørgensen, RL; Nielsen, JB; Friis-Møller, A; Fjeldsøe-Nielsen, H and Schønning, K (2010). Prevalence and molecular characterization of clinical isolates of *Escherichia coli* expressing an AmpC phenotype. J. Antimicrob. Chem., 65: 460-464.

Kaur, J (2013). Increasing antimicrobial resistance and narrowing therapeutics in typhoidal *Salmonellae*. J. Clin. Diagn. Res., 7: 576.

Klemm, EJ; Shakoor, S; Page, AJ; Qamar, FN; Judge, K; Saeed, DK; Wong, VK; Dallman, TJ; Nair, S and Baker, S (2018a). Emergence of an extensively drug-resistant *Salmonella enterica* serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. mBio. 9: e00105-00118.

Konaté, A; Guessennd, N; Kouadio, F; Dembélé, R and Kagambèga, A (2019). Epidemiology and resistance phenotypes of *Salmonella* spp. strains responsible for gastroenteritis in children less than five years of age in Ouagadougou, Burkina Faso. Arch. Clin. Microbiol., 10: 90.

Kretzschmar, M; Mangen, MJJ; Pinheiro, P; Jahn, B; Fevre, EM; Longhi, S; Lai, T; Havelaar, AH; Stein, C and Cassini, A (2012). New methodology for estimating the burden of infectious diseases in Europe. PLoS Med., 9: e1001205.

Le Hello, S; Harrois, D; Bouchrif, B; Sontag, L; Elhani, D; Guibert, V; Zerouali, K and Weill, FX (2013). Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: a microbiological study. Lancet Infect. Dis., 13: 672-679.

Manyi-Loh, C; Mamphweli, S; Meyer, E and Okoh, A (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. Molecules. 23: 795.

Mietzner, TA; Carroll, KC; Hobden, JA; Miller, S; Morse, SA; Mitchell, TG; Sakanari, JA; McKerrow, JH and Detrick, B (2016). *Jawetz, Melnick & Adelberg's medical microbiology*. 27th Edn., Chapter 15, Enteric Gram-Negative Rods (Enterobacteriaceae). McGraw-Hill Education. PP: 231-238.

Mikoleit, M (2010). Laboratory protocol: biochemical identification of *Salmonella* and *Shigella* using an abbreviated panel of tests. Geneva, Switzerland: WHO Global Foodborne Infections Network. http:// antimicrobialresistance.dk/CustomerData/Files/Folders/6-pdf-protocols/63_18-05-isolation-of-salm-220610.pdf.

Miriagou, V; Carattoli, A and Fanning, S (2006). Antimicrobial resistance islands: resistance gene clusters in *Salmonella* chromosome and plasmids. Microbes Infect., 8: 1923-1930.

Monstein, HJ; ÖStholm-Balkhed, Å; Nilsson, MV; Nilsson, M; Dornbusch, K and Nilsson, LE (2007). Multiplex PCR amplification assay for the detection of bla_{SHV}, bla_{TEM} and bla_{CTX}-M genes in *Enterobacteriaceae*. APMIS. 115: 1400-1408.

Noda, T; Murakami, K; Ishiguro, Y and Asai, T (2010). Chicken meat is an infection source of *Salmonella* serovar Infantis for humans in Japan. Foodborne Pathog. Dis., 7: 727-735.

Pérez-Pérez, FJ and Hanson, ND (2002). Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J. Clin. Microbiol., 40: 2153-2162.

Popoff, MY (1997). Antigenic formulas of the *Salmonella* serovars. WHO Collaborating Center for Reference and Research on *Salmonella*. https://www.pasteur.fr/sites/ default/files/veng_0.pdf.

Popoff, M and Le Minor, L (2001). Antigenic formulas of the *Salmonella* serovars, WHO Collaborating Centre for Reference and Research on *Salmonella*. World Health Organization, Geneva, Switzerland.

Raeisi, E and Ghiamirad, M (2015). Survey on prevalence of *Salmonella* serogroups and antibiotics susceptibility pattern in chicken meat in Ardabil, Iran. J. Ardabil Univer. Med. Sci., 15: 320-329.

Ramadan, F; Unni, A; Hablas, R and Rizk, M (1992). *Salmonella*-induced enteritis. Clinical, serotypes and treatment. J. Egypt. Public Health Assoc., 67: 357-367.

Soltan Dallal, MM and Moezardalan, K (2004). *Aeromonas* spp associated with children's diarrhoea in Tehran: a case-control study. Ann. Trop. Paediatr., 24: 45-51.

Stanaway, JD; Reiner, RC; Blacker, BF; Goldberg, EM; Khalil, IA; Troeger, CE; Andrews, JR; Bhutta, ZA; Crump, JA and Im, J (2019). The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect. Dis., 19: 369-381.

Tajbakhsh, M; Avini, MY; Alikhajeh, J; Tajeddin, E; Rahbar, M; Eslami, P; Alebouyeh, M and Zali, MR (2016). Emergence of *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{TEM-169}}$ and *bla*$_{\text{PER-1}}$ extended-spectrum β-lactamase genes among different *Salmonella enterica* serovars from human faecal samples. Infect Dis. (London)., 48: 550-556.

Threlfall, EJ (2010). *Salmonella*. In: *Topley & Wilson's microbiology and microbial infections*. (Systemic Bacteriology) 10th Edn., Vol. 2, Hoboken, NJ, John Wiley & Sons, Ltd.

Tibaijuka, B; Molla, B; Hildebrandt, G and Kleer, J (2003). Occurrence of *Salmonellae* in retail raw chicken products in Ethiopia. Berl. Munch Tierarztl Wochenschr., 116: 55-58.

Travers, K and Michael, B (2002). Morbidity of infections caused by antimicrobial-resistant bacteria. Clin. Infect. Dis., 34: S131-S134.

Wang, W; Peng, Z; Baloch, Z; Hu, Y; Xu, J; Zhang, W; Fanning, S and Li, F (2017). Genomic characterization of an extensively-drug resistance *Salmonella enterica* serotype Indiana strain harboring *bla*$_{\text{NDM-1}}$ gene isolated from a chicken carcass in China. Microbiol. Res., 204: 48-54.

White, DG; Hudson, C; Maurer, JJ; Ayers, S; Zhao, S; Lee, MD; Bolton, L; Foley, T and Sherwood, J (2000). Characterization of chloramphenicol and florfenicol resistance in *Escherichia coli* associated with bovine diarrhea. J. Clin. Microbiol., 38: 4593-4598.

WHO (2010). Laboratory Protocol. In: *Isolation of Salmonella spp. from food and animal faeces*. 5th Edn., June 2010. PP: 4-8, 13.

Wong, MHY; Zeng, L; Liu, JH and Chen, S (2013). Characterization of *Salmonella* food isolates with concurrent resistance to ceftriaxone and ciprofloxacin. Foodborne Pathog. Dis., 10: 42-46.

Zdrodowska, B; Liedtke, K and Radkowski, M (2014). Post-harvest *Salmonella* spp. prevalence in turkey carcasses in processing plant in the northeast part of Poland. Pol. J. Vet. Sci., 17: 181-183.

Zeng, YB; Xiong, LG; Tan, MF; Li, HQ; Yan, H; Zhang, L; Yin, DF; Kang, ZF; Wei, QP and Luo, LG (2019). Prevalence and antimicrobial resistance of *Salmonella* in pork, chicken, and duck from retail markets of China. Foodborne Pathog. Dis., 16: 339-345.