Original article

Characterization of antibiotic resistant patterns of *Salmonella* serotypes isolated from beef and chicken samples in Tehran

Mohammad Mehdi Soltan Dallal[1,2,3], Mahnaz Taremi[2], Latif Gachkar[4], Shabnam Modarressi[5], Maryam Sanaei [2], Rounak Bakhtiari[1], Mohammad Kazem Sharifi Yazdi[6], Mohammad Reza Zali[2]

[1]Department of Microbiology, School of Public Health and Institute Health Research Tehran University of Medical Sciences, Tehran, Iran
[2]National Research Department of Food Borne Diseases (NRDFD), Research Center of Gastroenterology and Liver Diseases, Shaheed Beheshti of Medical Sciences, Tehran, Iran
[3]Antimicrobial Resistant Research Center, Iran University of Medical Sciences, Tehran, Iran
[4]Infections Diseases and Tropical Medicine Research Center (IDTMRC), Shaheed Beheshti of Medical Sciences, Tehran, Iran
[5]Islamic Azad University (Science and Research) Tehran, Iran
[6]Department of Medical Laboratory Sciences, Faculty of Para Medicine, Tehran University of Medical Sciences, Tehran, Iran

Received: June 2009 **Accepted:** November 2009

Abstract

Introduction and objective: Infection with *Salmonella* is the most frequently reported cause of bacterial food-borne illness worldwide. Raw meat samples are a common source and, in recent years, much attention has been focused in determining the prevalence of *Salmonella* during the different stages in the poultry and beef production chain. This study was conducted to examine the prevalence of *Salmonella* contamination, and the antibiotic resistance characteristics of isolated strains, from raw samples of packed and unpacked beef and chicken collected randomly from retail stores in Tehran.

Materials and methods: A total of one hundred and thirty three samples were collected from 27 meat providing retail stores in Tehran. *Salmonella* strains were isolated and identified according to the techniques recommended by the International Organization for Standardization (ISO 6579, 1998). Antimicrobial resistance test was performed by disk diffusion method using 13 antibiotics.

Results: Out of one hundred and thirty three samples tested, fifty one (38.3%) were identified as *Salmonella* strains. The percentages of *Salmonella* in chicken and beef samples were 62.7% and 37.3% respectively. The sereotyping results showed that isolated strains belonged to 10 different serotypes, and the most dominated serotype was *Salmonella thompson* (54.9%). Among the variety of antibiotics tested, the highest resistance was found with nalidixic acid followed by tetracycline, trimethoprim, and streptomycin. The percentages resistance of isolates from chicken samples to nalidixic acid, tetracycline, trimethoprim, and streptomycin were 90.6%, 71.9%, 56.6%, and 25%, and the isolates from meat samples were 36.8%, 21%, 26.3%, and 5.3% respectively. About 23.5% of the

Salmonella strains were multiresistant to two or more antibiotic families. Finally, six resistance profiles have been identified. In overall, the degree of resistance of serotypes to nalidixic acid was greater than other tested antibiotics.
Conclusion: Our results indicate that antimicrobial resistant *Salmonella* strains were widely spread among raw chicken and beef meats samples.

Keywords: *Salmonella*, Serotype, Meat, Chicken, Antibiotic resistance

Introduction

The world-wide increase of foodborne infections with antibiotic resistant pathogens is of growing concern and is designated by the WHO as an emerging public health problem [1-4]. Salmonellosis is the major cause of food borne infections, and the second most common food borne illness after *Campylobacter* infection [5]. It constitutes a major public health burden and represents a significant cost in many countries. Millions of human cases are reported worldwide every year and the disease results in thousands of deaths [2,6,7].

It is usually difficult to evaluate the situation of Salmonellosis in developing countries because of the very limited scope of studies and lack of coordinated epidemiological surveillance systems [7]. *Salmonella* infections in humans often result from the ingestion of contaminated foods, such as poultry, beef, pork, eggs, milk, seafood, and fresh produce [1,3,5,8,9]. Direct contact with animals also results in transmission of *Salmonella* to humans [10,11]. Contamination of meat with *Salmonella* in slaughterhouses occurs through excretion of animals, which have no symptoms, contamination of equipments, floor and personnel's [12]. The pathogens can survive in the meat until presented to the market [13,14].

In the last 20 years, the worldwide emergence of multidrug-resistant *Salmonella* serotypes has become of a great concern. Since the beginning of the 1990s, strains of *Salmonella* which are resistant to a range of antimicrobials, including first-choice agents for the treatment of humans, have emerged and are threatening to become a serious public health problem [1,14-16]. This resistance results from the use of antimicrobials in both humans, and animal husbandry. Multi-drug resistance to critically important antimicrobials is compounding the problems [17-19]. Emerging resistance in these pathogens is mainly because of increasing usage of antimicrobial agents in clinics and slaughterhouses and this is becoming a global problem [12,20,21].

The increase isolation of single or multiple resistant *Salmonella* from human infections is due to abundant use of antimicrobial agents in food production [22,23]. Remarkable numbers of antimicrobial agents, which are used in treatment of salmonellosis and other bacterial infections in human, are also used in slaughterhouses [12,15,17,19,24]. This study was undertaken to fully characterize the levels of resistance to a variety of antibiotics in non-typhoid *Salmonella* serotypes in Tehran.

Materials and methods

Collection, isolation, identification and serotyping of Salmonella

A total of 133 meat samples (66 from beef including 26 packaged beef and 40 unpackaged beef, 67 from chicken including 26 packaged chickens and 41 unpackaged chickens) were tested. Among them, 51 (38.3%) were positive for *Salmonella*. The samples were analyzed for *Salmonella* according to ISO- 6579 [25]. Twenty five gram food samples were placed

in sterile stomacher bag and 225ml of buffered peptone water (BPW, Merck, Germany) was added to each sample. They were homogenized using a stomacher for two minutes, followed by incubation for 24 hours at 37˚C. Then, 0.1ml of the pre-enriched broth was transferred into 10ml of Rappaport-Vasisiliadis medium (RV) (Oxoid CM 669) and incubated for another 24 hours at 42˚C.

The enrichment samples were then applied onto Hekton agar (Hi Media M647) plate and incubated for 24 hours at 37˚C. Suspicious colonies were identified with biochemical tests (Oxidase reaction, acid production from manitol, O-nitrophenyl-β-D-galactopyranoside (ONPG) test, H_2S and indol production as well as proofs of urease and lysine decarboxylase). *Salmonella* strains were affirmatively identified and serotype at Razi vaccine and serum investigation institute at Tehran, with slide agglutination tests as described by Ewing [26] and flagellar antigens were detected by a technique of utilizing microtitre plates [27].

Testing for antimicrobial susceptibility

Antibiotic resistances of putative *Salmonella* isolates were tested by standard disk diffusion method [28]. To do this, first 1-3 colonies were transferred into BHI (Heart Infusion Broth, Difco) and incubated for 24h at 37°C, then according to the 0.5 McFarland tube, the turbidity of broth culture was determined and each was streaked onto Mueller Hinton Agar (Merck) with a sterile swab, finally the antibiotic disks (HiMedia) were placed on the culture. The following disks were used: Gentamicin (10µg), Trimethoprim (5µg), Nalidixic acid (30µg), Ciprofloxacin (5µg), Cephotaxime (30µg), Imipenem (10µg), Colistin (10µg), Ceftazidim (30µg), Amoxicillin (30µg), Ampicillin (10µg), Chloramphenicol (30µg), Streptomicin (10µg) and Tetracycline (30µg). After incubation at 35˚C for 24 hours, zone size was measured. Standard and reference strains were used and interpretation of the strains as susceptible, intermediate or resistant was made following the recommendations of the Clinical Laboratory and Standards Institute (CLSI). Reference strains included *Salmonella typhi* (PTCC 1639).

Statistical analysis

Statistical analysis of results was performed with SPSS/PC software (SPSS Chicago, IL). The chi-squared test and Fisher's exact two-tailed test were used for statistical analysis. A p value <0.05 was used for statistical significance.

Results

From a total of 133 samples, 67(50.4%) chicken meat and 66(49.6%) beef meat, 51(38.3%) of isolates were identified as *Salmonella* (19 from beef samples and 32 from chicken samples). The number of positive *Salmonella* in unpacked and chicken samples was more frequent than packed and red meat samples, as it is summarized in table 1. Of the 51 *Salmonella* isolates, 10 different serotypes were identified. *S. thompson* accounted for 54.9% of total isolates (43.2% and 11.8%) from chicken meat and red meat samples, followed by *S. enteritidis* (9.8%), *S. paratyphi* (7.8%), *S. veyle* (7.8%) and *S. meleagridis* (3.9%) respectively. Other isolated serotypes were *S. virginia*, *S. typhimurium*, *S. group Π*, *S. harardt* and *S. anatum* with percentage of 1.9% each, and three isolates were un-typable. The distributions of serotypes are shown in table 2.

Number of nalidixic acid resistant were 29(90.6%) of isolates from chicken meat and 7(36.8%) from beef meat, followed by tetracycline 23(71.9%), 4(21%), and trimethoprim, 21(65.6%), 5(26.3%) respectively. All isolates were susceptible to colistin, ceftazidim,

imipenem, cephotaxime, chloramphenicol, ciprofloxacin and gentamicin, table3. Statistical analysis found significant difference in the rate of contamination among the different sources of the chicken and beef meat *Salmonella* isolates ($p<0.05$). 23.5% were resistant to at least two antibiotics nalidixic acid+ tetracycline, tetracycline+trimethoprim or tetracycline+ streptomycin, table 4.

Table 1: Distribution of *Salmonella* in red meat and chicken samples by their sources

Samples	Positive		Negative		Total	
	No	%	No	%	No	%
Packaged beef	8	30.8	18	69.2	26	100
Unpackaged beef	11	27.5	29	72.5	40	100
Packaged chicken	15	57.7	11	42.3	26	100
Unpackaged chicken	17	41.5	24	58.5	41	100
Total	51	38.3	82	61.7	133	100

$\chi^2= 6.91$ df= 3 p = 0.75

Table 2: Distribution of *Salmonella* serotypes in beef and chicken samples

Serotype	Chicken		Beef		Total
	Packaged	Unpackaged	Packaged	Unpackaged	
S. thompson	11 (21.6%)	11 (21.6%)	2 (3.9%)	4 (7.8%)	28 (54.9%)
S. paratyphi C	1 (2%)	2 (3.9%)	0 (0.0%)	1 (2%)	4 (7.8%)
S. meleagridis	0 (0.0%)	0 (0.0%)	2 (3.9%)	0 (0.0%)	2 (3.9%)
S. enteritidis	1 (2%)	3 (5.9%)	0 (0.0%)	1 (2%)	5 (9.8%)
S. virginia	1 (2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2%)
S. groupΠ	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2%)	1 (2%)
S. haardt	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2%)	1 (2%)
S. anatum	0 (0.0%)	0 (0.0%)	1 (2%)	0 (0.0%)	1 (2%)
S. veyle	0 (0.0%)	0 (0.0%)	1 (2%)	3 (5.9%)	4 (7.8%)
S. typhimurium	0 (0.0%)	1 (2%)	0 (0.0%)	0 (0.0%)	1 (2%)
S. untypable	1 (2%)	0 (0.0%)	2 (3.9%)	0 (0.0%)	3 (5.9%)
Total	15 (29.4%)	17 (33.3%)	8 (15.7%)	11 (21.6%)	51 (100%)

Table 3: Distribution of antimicrobial resistance in chicken and beef samples

Antibiotics	Chicken		Beef	
	No	%	No	%
Nalidixid acid	29	90.6	7	36.8
Tetracycline	23	71.9	4	21
Trimethoprim	21	65.6	5	26.3
Streptomicin	8	25	1	5.3
Ampicillin	1	3.1	-	-
Amoxicillin	1	3.1	-	-

Table 4: Distribution of multi drug resistance *Salmonella* spp. isolated from chicken and beef samples

Antibiotics	Multi drug resistance
Nalidixic acid, Tetracycline, Trimethoprim, Streptomicin	1
Nalidixic acid, Tetracycline, Trimethoprim	3
Nalidixic acid, Tetracycline,	5
Tetracycline, Trimethoprim	2
Tetracycline, Streptomicin	1
Total	12

Discussion

The high prevalence of *Salmonella* spp. in raw chicken and beef meats samples found in this study agrees with data from other studies [5,12,19,29]. *Salmonella* infections are usually caused by handling or consuming contaminated foods, especially those of animal origin or through contact with farm animals, reptiles, and pets [10,13,14,22,30]. The isolation of invasive *Salmonella* serotypes such as *S. typhimurium* and other pathogenic *Salmonella*s in our study indicate the public health significance of these serovars as contaminated chicken meat and meat products may pose health hazards. This risk may further be higher if chicken meat or giblets are consumed undercooked or cross contamination in the kitchen with *Salmonella* during meal preparation [8,13,25].

Salmonella thompson is a strain of *Salmonella* that can cause symptoms of diarrhea, nausea, and vomiting in humans and may cause serious illnesses in immune-compromised individuals [31,32]. In this study *S. thompson* was dominated serotype (68.7% in chicken samples and 31.6% in beef meat samples), this might be due to improper handling of product, and possible contamination of product during production or cross contamination in meat shop with the contaminated pet. This report also highlight that *Salmonella* contaminated pet poses threat to human, and public health practitioners should consider pet threats a potential source for *Salmonella* transmission. The level of contamination in beef samples was lower than chicken samples, this might be due to specific tissue, which becomes acidic when the animal is dead and this leads to reducing the pH. Few studies had been demonstrated that bacteria grow slowly on the meat products with low pH [19].

Although most intestinal *Salmonella* infections don't require treatment, antimicrobial may be lifesaving in persons with immune-suppressing conditions or invasive illness, such as bacterimia and meningitides [1,2]. According to an investigation in Spain, spread of *Salmonella* in chicken meat during slaughtering and preparing is more common [19]. The results of this study showed that more than 50% of isolate were resistant to nalidixic acid. This needs special attention since nalidixic acid is a common antibiotic for the treatment of salmonellosis [21,33].

The level of resistance of *Salmonella* to different antibiotics should be alarming to the food processing, distribution and handling of food product [12,14]. Therefore, it is necessary to inform people involved in the food industry as well as distributors to take care in handling the food products. Antibiotics have been successfully used in poultry and farming for different purposes such as growth promotion, prophylaxis, or therapeutics.

However, their indiscriminate use caused an increased bacterial resistance, mainly in *Salmonella* strains [34].

The emergence of antimicrobial-resistant *salmonella* is associated with the use of antibiotics in animals raised for food; resistant bacteria can be transmitted to humans through foods, particularly those of animal origin [14,15,32,33]. A part of *S. thompson*, which accounted 54.9% of total isolates (43.2% and 11.8%) for from chicken meat and red meat samples, the presence of nine other serotypes were relatively low. This is because potential relationships, associations, correlations and interaction of microbial species found throughout the beef and chicken production chain are not well known, and therefore, presence or absence of a specific microorganism should not be used as an index or indicator of presence or absence of others, including pathogens.

The development of antimicrobial resistance in zoonotic bacteria (e.g. *Salmonella*) constitutes a public health risk, as it may potentially affect the efficacy of drug treatment in humans [17-19,32-34]. The differences between the results of the present paper and those of other researchers may be explained when several factors, such as differences in origin, period of collection and sampling procedure. These results indicate that the presence of *Salmonella* resistances to antimicrobial drugs is common in chicken and beef. Further studies are needed to identify the sources and causes of this drug resistance.

Conclusion

In conclusion, the present study demonstrates the need for education on the sanitary handling of chicken and beef meat, which are possible infectious sources of these *Salmonella* serotypes. Therefore it is necessary to avoid abundant usage of antimicrobial agents in ranches which leads to resistant strains and can be passed to human through food products.

Acknowledgment

We would like to thank the research team of NRDFD (National Research Department of Food borne Diseases); Azimi Rad, Moezardalan, Golkar for their sincere help in performing technical parts of the project. This study was funded by the Research Center for Gastroenterology and Liver Diseases, Shaheed Beheshti University of Medical Sciences.

References

1) Centers for disease control and prevention (CDC). Preliminary food net data on the incidence of infection with pathogen transmitted commonly through food 10 state/2005. *MMWR Morb Mortal Wkly Rep.* 2006; 55(14): 392-5.
2) Lynch M, Painter J, Woodruff R, Braden C. Centers for disease control and prevention. Surveillance for foodborne-disease outbreaks United States, 1998-2002. *MMWR Surveill Summ*. 2006; 55(10): 1-42.
3) Dalton CB, Gregory J, Kirk MD, *et al*. Foodborne disease outbreaks in Australia, 1995 to 2000. *Commun Dis Intell*. 2004; 28(2): 211-4.
4) Much P, Pichler J, Allerberger F. Foodborne infectious outbreaks, Austria 2005. *Wien Klin Wochenschr*. 2007; 119 (5-6): 139-41.
5) Meldrum RJ, Wilson IG. *Salmonella* and *Campylobacter* in United Kingdom retail raw chicken in 2005. *J Food Protect.* 2007; 70: 1937-9.
6) Mead PS, Slutsker L, Dietz V, *et al*. Food-related illness and death in the United States. *Emerging Infect Dis.* 1999; 5: 607-25.
7) Oosterom J. Epidemiological studies and proposed preventive measures in the fight against human salmonellosis. *Int J Food Microbial.* 1991; 12: 41-52.
8) Gomez TM, Motarjemi Y, Miyagawa S, Kaferstein FK, Stohr K. Foodborne

Salmonellosis. *World Health Stat. Q* 1997; 50: 81-9.
9) Braden CR. *Salmonella enterica* serotype *Enteritidis* and eggs: a national epidemic in the United States. *Clin Infect Dis.* 2006; 43: 512-7.
10) Acha PN, Szyfree B. Zoonoses and communicable diseases common to man and animals. Third Edition, Washington DC. *Pan American Health Organization* 2001; 1: 233-46.
11) Bagcigil AF, Ikiz S, Dokuzeylu B, Basaran B, Or E, Ozgur NY. Fecal shedding of *Salmonella spp*. in dogs. *J Vet Med Sci.* 2007; 69: 775-7.
12) Molla B, Alemayehu D, Salah W. Sources and distribution of *Salmonell*a serotypes isolated from food animals, slaughterhouse personel and retail meat products in Ethiopia.1997-2002. *Ethiopian J Health Develop.* 2003; 17: 63-70.
13) Redmond EC, Griffith CJ. Consumer food handling in the home: a review of food safety studies. *J Food Protect.* 2003; 66: 130-161.
14) Kimura AC, Palumbo MS, Meyers H, Abbott S, Rodriguez R, Werner SB. A multi-state outbreak of *Salmonella* serotype *thompson* infection from commercially distributed bread contaminated by an ill food handler. *Epidemiol Infect.* 2005; 133: 823-8.
15) Fey PD, Safranek TJ, Rupp ME, *et al*. Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. *New Engl J Med.* 2003; 42: 1242-9.
16) Threlfall E J, Ward L R, Frost J A, Willshaw GA. The emergence and spread of antibiotic resistance in food-borne bacteria. *Int J Food Microbial.* 2000; 62: 1-5.
17) Angkititrakul S, Chomvarin C, Chaita T, Kanistanon K, Waethewutajarn S. Epidemiology of antimicrobial resistance in *Salmonella* isolated from pork, chicken meat and humans in Thailand. *Southeast Asian J Trop Med Public Health.* 2005; 36: 1510-5.
18) Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *New Engl J Med.* 1998; 338: 1333-8.
19) Carraminana JJ, Rota C, Augutin I, Herrera Am. High prevalence of multiple resistance to antibiotics in *Salmonella* serovars isolated from poultry slaughterhouse in Spain. *Veter Microbiol.* 2004; 104: 133-9.
20) Adesiyon AA, Oni OO. Prevalence and antibiograms of *Salmonella* in slaughtered Cattle, slaughter areas and effluents in Zaria abattoris, Nigeria. *J Food Protect.* 1989; 52: 232-5.
21) Van TT, Moutafis G, Istivan T, Tran LT, Coloe PJ. Detection of *Salmonella spp.* in retail raw food samples from Vietnam and characterisation of their antibiotic resistance. *Appl Environ Microbiol.* 2007; 73(21): 6885-90.
22) Aarestrup FM. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. *Int J Antimicrob Agents.* 1999; 12: 279-85.
23) Zhao S, McDermott PF, Friedman S, *et al*. Antimicrobial resistance and genetic relatedness among *Salmonella* from retail foods of animal origin: NARMS retail meat surveillance. *Foodborne Pathog Dis.* 2006; 3(1): 106-17.
24) Cardoso MO, Ribeiro AG, Ruschel dos Santos M, *et al*. Antibiotic resistance in *Salmonella entertidis* isolated from broiler carcasses. *Brazil J Microbiol.* 2006; 37: 368-71.
25) International Organization for Standardization (ISO) 6579. Microbiology of food and animal feeding stuff-horizontal method for the detection of *Salmonella*, *ISO*, Geneva, 1998.
26) Ewing WH. Serologic identification of *Salmonella.* In: Ewing WH. (ed.): Edwards and Ewings identification of Enterobacteriaceae, 4th ed., Elsevier Science Publishing Co., New York, 1986.
27) Shipp CR, Rowe B. A mechanized micro-technique for *Salmonella* serotyping. *J Clin Pathol.* 1980; 33: 595-7.
28) Clinical and laboratory standard institute. Performance standards for antimicrobial

disk susceptibility tests. *NCCLS documents* M100. USA, 2005.
29) Mehrabian S, Jaberi E. Isolation, identification and antimicrobial resistance patterns of *Salmonella* from meat products in Tehran**.** *Pakistan J Biol Sci*. 2007; 10(1): 122-6.
30) www.cdc.gov/fuatures/ Reptiles and *Salmonella;* 2007.
31) Linares AP, Cohen SH, Goldstein E, Kelley ADK, Eisenstein TK. Febrile gastroenteritis due to *Salmonella thompson*, report of an outbreak. *Western J Med*. 1984; 141(2): 203-5.
32) Nygård K, Lassen J, Vold L, *et al*. Outbreak of *Salmonella thompson* infections linked to imported rucola lettuce. *Foodborne Pathog Dis*. 2008: 5(2): 165-73.
33) Wegner HC, Arestrup FM, Gerner- Smidt P. Transfer of antibiotic resistant bacteria from animal to man. *Acta Veterinaria Scandinavica Supple.* 1999; 92: 51-7.
34) Abdellah C, Filali Fouzia R, Abdelkader C, Bencheikh Rachida S, Mouloud Z. Prevalence and anti-microbial susceptibility of *Salmonella* isolates from chicken carcasses and giblets in Meknès, Morocco. *Afr J Microbiol Res*. 2009; 3(5): 215-9.

Address for correspondence:
Mohammad Mehdi Soltan Dallal, Department of Microbiology, School of Public Health and Institute Health Research, Tehran University of Medical Sciences, Tehran, Iran
Tel: +9821 66462268; Fax: +9821 66462267
Email: soltanirad34@yahoo.com