PREVALENCE AND ANTIMICROBIAL RESISTANCE OF SALMONELLA ISOLATED FROM RETAIL RAW TURKEY, OSTRICH, AND PARTRIDGE MEAT IN IRAN

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Summary

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The objective of this study was to determine the Salmonella prevalence, the serotypes involved and antimicrobial susceptibility patterns of Salmonella isolates recovered from retail raw turkey, ostrich, and partridge meat in Esfahan, Iran. A total of 249 samples of turkey, ostrich, and partridge meat were purchased from 8 randomly selected retail outlets from April 2006 to July 2007. All samples were evaluated for the presence of Salmonella, serotyped and tested for antimicrobial susceptibility. There was an overall Salmonella prevalence of 6.8%. The prevalence of Salmonella was statistically significantly higher in turkey meat (9.7%) than in ostrich meat (4.6%) ($P \le 0.05$). No Salmonella was isolated from partridge meat samples. Salmonella isolates recovered from turkey and ostrich meat samples were of 4 different serotypes including Salmonella enterica ser. Typhimurium, Salmonella enterica ser. Enteritidis, Salmonella enterica ser. Agona, and Salmonella enterica ser. Paratyphi B. The susceptibility of the 17 isolated strains to 12 antimicrobial drugs was determined using the disk diffusion method. Resistance to nalidixic acid was the most common finding (58.8%), followed by resistance to tetracycline (41.2%), streptomycin (29.4%), trimethoprim (23.5%), chloramphenicol (11.8%), and ciprofloxacin (5.9%). Salmonella isolates recovered from ostrich meat samples were susceptible to all 12 antimicrobial agents. To our knowledge, this is the first study on the prevalence of Salmonella in partridge meat and first report of the isolation of Salmonella spp. from retail raw turkey and ostrich meat in Iran.

Key words: antimicrobial resistance, ostrich, partridge, Salmonella, turkey

INTRODUCTION

Salmonellosis is the most frequently encountered food-borne bacterial disease in the world and an important public health concern (Plym Forshell & Wierup, 2006). *Salmonella* is estimated as an annual infectious rate of 21.6 million and approximate death rate of 600 000 with the highest percentage in Africa and Asia (WHO, 2008). There are several transmission routes for salmonellosis, but the majority of human infections are derived from the consumption of contaminated food, especially of animal origin (Plym Forshell & Wierup, 2006). Poultry products are fre-

quently contaminated with *Salmonella* spp. and are consequently thought to be major sources of the pathogen for humans (Uyttendaele *et al.*, 1998).

In recent years, there has been increasing concern regarding the worldwide occurrence of multidrug resistant strains of a number of pathogenic bacteria including Salmonella in foods. The extensive use of antibiotics for therapeutic or preventive purposes in veterinary medicine and as growth promoters in animal feed has contributed to the occurrence of resistant bacteria in animals, including zoonotic pathogens, which can be transmitted to humans via food chain (Tollefson & Miller, 2000; Su et al., 2004). The incidence of Salmonella strains resistant to various antimicrobial agents has increased over the last years, resulting in higher morbidity and mortality rates and higher overall treatment costs (Threlfall et al., 2003). Several studies have shown that antimicrobial drug resistance seen in salmonellosis is a consequence of using antimicrobial drugs in food-producing animals (Threlfall, 2000; Little et al., 2008).

Although a few studies on the prevalence of Salmonella in poultry products have been performed in Iran (Mehrabian & Jaberi, 2007; Jalali et al., 2009; Soltan Dallal et al., 2009), no information regarding the prevalence and antimicrobial susceptibility patterns of Salmonella in retail raw ostrich and partridge meat is currently available and no extensive study has been carrie out on raw turkey meat in Iran. Therefore, this study was conducted to determine the Salmonella prevalence, the serotypes involved and antimicrobial susceptibility patterns of Salmonella isolates recovered from retail raw turkey, ostrich, and partridge meat in Esfahan, Iran.

MATERIALS AND METHODS

Sample collection

From April 2006 to July 2007, 249 meat samples including turkey (n=144), ostrich (n=65), and partridge (n=40) were randomly purchased from 8 retail outlets in Esfahan, Iran. Esfahan, a major industrial centre and the second largest city in Iran is located in the middle of the country. Samples collected in this study included leg (turkey and ostrich) and leg and breast (partridge). All samples were taken by using sterilized utensils, placed in separate sterile plastic bags to prevent spilling and cross contamination, and were immediately transported to the laboratory in a cooler with ice packs.

Bacteriological examination and serotyping

The samples were processed immediately upon arrival using aseptic techniques. Bacteriological examinations for isolation of Salmonella were performed according to the standard conventional culture method (Varnam & Evans, 1991). Briefly, 25 g of each meat sample was homogenized for 2 min with 225 mL of buffered peptone water (Merck, Germany) and incubated at 37 °C for 24 h. After incubation, 0.1 mL of the broth was added to 10 mL of selenite cystine broth (Merck, Germany) and incubated overnight at 42 °C. Then, samples were subcultured onto Salmonella Shigella agar (Merck, Germany) and brilliant green agar (Merck, Germany) and incubated overnight at 37 °C. Identification of presumptive Salmonella isolates was performed using triple sugar iron agar (Merck, Germany), lysine iron agar (Merck, Germany), and urea agar (Merck, Germany) slants overnight at 37 °C.

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Salmonella isolates were further serotyped by direct agglutination method using antisera against O and H antigens (BioRad, France) and the Kauffman– White classification scheme (Brenner, 1998).

Assessment of antimicrobial susceptibility

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (Himedia, M1084), according to the National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2002). The antimicrobial agents tested and their corresponding concentrations were as follows: ampicillin (10 µg), amoxicillin (30 μg), chloramphenicol (30 μg), gentamicin (10 µg), kanamycin (30 µg), streptomycin (10 µg), tetracycline (30 µg), trimethoprim (5 µg), ciprofloxacin (5 µg), nalidixic acid (30 μ g), ceftizoxime (30 μ g), and cefotaxine (30 µg). After incubating the inoculated plate aerobically at 37 °C for 20 h, the susceptibility of the Salmonella spp. to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by NCCLS. When the minimum inhibitory concentration (MIC) of a Sal*monella* isolate for a given antimicrobial was in the intermediate-sensitivity classification for that antimicrobial, it was considered to be not resistant. *Staphylococcus aureus* and *Escherichia coli* were used as quality control organisms in antimicrobial susceptibility determination.

Statistical analysis

Using a statistical software (Analyse-It Software Ltd., Leeds, UK), an analysis was performed to compare the percentages of *Salmonella* isolates recovered from turkey, ostrich, and partridge meat samples. The differences were considered significant at P \leq 0.05.

RESULTS

Of the 249 meat samples, 17 were positive for occurrence of *Salmonella* (overall prevalence of 6.8%). *Salmonella* isolates were recovered from significantly larger number of turkey (9.7%) than ostrich (4.6%) meat samples (P \leq 0.05). No *Salmonella* isolate was recovered from partridge meat samples. *Salmonella* isolates recovered from turkey and ostrich meat samples were of 4 different serotypes (Table 1). The most frequently encoun-

Table 1. Number of turkey, ostrich, and partridge meat samples tested and the distribution of *Salmonella* serotypes in isolates

Salmonella serotypes	Mea	Total		
	Turkey (n=144)	rkey (n=144) Ostrich (n=65) Pa		isolates
S. Typhimurium	5 (3.5%)	1 (1.5%)	_	6 (2.4%)
S. Enteritidis	3 (2.1%)	1 (1.5%)	_	4 (1.6%)
S. Agona	3 (2.1%)	_	-	3 (1.2%)
S. Paratyphi B	1 (0.7%)	1 (1.5%)	_	2 (0.8%)
NC^* serotyped	2 (1.4%)	-	_	2 (0.8%)
Total	14 (9.7%)	3 (4.6%)	_	17 (6.8%)

*NC serotyped – not completely serotyped.

Antimicrobial	Number (%) [95% CI ^c] of isolates resistant to antimicrobial agents ^d					
agent	<i>Salmonella</i> Typhimurium	Salmonella Enteritidis	<i>Salmonella</i> Paratyphi B	Untypable Salmonella	Total	
Chloramphenicol	1 (5.9%) [6.6–18.4 %]	1 (5.9%) [6.6–18.4 %]	0	0	2 (11.8%) [5.3–28.8%]	
Streptomycin	3 (17.6 %) [2.6–37.9%]	1 (5.9%) [6.6–18.4 %]	1 (5.9%) [6.6–18.4 %]	0	5 (29.4%) [5.3–53.6%]	
Tetracycline	4 (23.5%) [1.0–46.0%]	2 (11.8%) [5.3%–28.8%]	1 (5.9%) [6.6–18.4 %]	0	7 (41.2%) [15.1–67.3%]	
Trimethoprim	3 (17.6 %) [2.6–37.9%]	1 (5.9%) [6.6–18.4 %]	0	0	4 (23.5%) [1.0–46.0%]	
Ciprofloxacin	0	1 (5.9%) [6.6–18.4 %]	0	0	1 (5.9%) [6.6–18.4 %]	
Nalidixic acid	5 (29.4%) [5.3–53.6%]	4 (23.5%) [1.0–46.0%]	0	1 (5.9%) [6.6–18.4 %]	10 (58.8%) [32.4–84.9%]	

Table 2. Antimicrobial resistance of 17 Salmonella strains isolated from turkey and ostrich samples^a

^aSalmonella isolates recovered from ostrich meat samples were susceptible to all 12 antimicrobial agents; ^bAll isolates were susceptible to ampicillin, amoxicillin, gentamicin, kanamycin, ceftizoxime and cefotaxine; ^cCI: confidence interval; ^d Salmonella Agona strains were susceptible to all 12 antimicrobial agents.

tered serotypes were *S*. Typhimurium and *S*. Enteritidis. Two isolates were not completely serotyped.

Table 2 summarizes the resistance patterns of 17 Salmonella isolates to 12 antimicrobial agents tested in this study. All 17 isolates were susceptible to ampicillin, amoxicillin, gentamicin, kanamycin, ceftizoxime, and cefotaxine. Salmonella isolates recovered from ostrich meat samples were susceptible to all 12 antimicrobial agents. A high proportion of the Salmonella isolates was resistant to nalidixic acid (58.8%; 95% confidence interval [CI]: 32.4% to 84.9%) and tetracycline (41.2%; 95% CI: 15.1% to 67.3%) and to a lesser extent to streptomycin (29.4%; 95% CI: 5.3% to 53.6%) and trimethoprim (23.5%; 95% CI: 1.0% to 46.0%). Only 1 isolate (S. Enteritidis) was resistant to ciprofloxacin (5.9%; 95% CI: 6.6% to 18.4 %) and two isolates (*S*. Enteritidis and *S*. Typhimurium) were resistant to chloramphenicol (11.8%; 95% CI: 5.3% to 28.8%). *Salmonella* Agona strains were susceptible to all 12 antimicrobial agents.

DISCUSSION

The contamination rate of turkey meat samples observed in this study (9.7%) was in agreement with those reported by Beli *et al.* (2001) in Albania (8.2%), Jordan *et al.* (2006) in Ireland (3.1%), and Little *et al.* (2008) in the UK (5.6%). In one study conducted in Austria, only one *Salmonella* isolate was recovered form 262 turkey meat samples (Mayrhofer *et al.*, 2004). However, Bentley (1984) and Lammerding *et al.* (1988) reported higher prevalence of *Salmonella* in turkey carcasses in Canada (68.8% and 69.1%, respectively).

key carcasses (Clouser *et al.*, 1995). In this study, 3 *Salmonella* isolates were recovered from 65 ostrich meat samples. There has been limited research on the prevalence of *Salmonella* in ostrich. In one study conducted in the US, only 1 of 152 carcass samples was found positive for *Salmonella* (Ley *et al.*, 2001). Using a DNA probe, ostrich products including meat, bone meal, and ostrich fillet were negative for *Salmonella* in Zimbabwe (Gopo & Banda, 1997). These data suggest that contamination of ostrich meat

with Salmonella may be rare; however, more research is needed to establish the

prevalence of *Salmonella* in ostrich meat. Due to relative increase in the consumption of partridge meat in Iran, we included 40 partridge meat samples in this study; however, no *Salmonella* was isolated from these samples. Rare studies have been reported on prevalence of *Salmonella* in free-flying partridge (Sharma *et al.*, 1980) and no extensive study was found on the prevalence of *Salmonella* in commercial products from farm-raised partridge.

Salmonella serotypes recovered in this study included S. Typhimurium, S. Enteritidis, S. Agona, and S. Paratyphi B. All these serotypes are common isolates from turkeys and ostriches (Gopo & Band, 1997; Beli *et al.*, 2001; Jordan *et al.*, 2006), S. Enteritidis and S. Typhimurium are the predominant serotypes of the genus Salmonella found in human salmonellosis in many developed countries including Japan, Australia, New Zealand, and many countries in Europe (Fischer, 2004). There have been reports of human salmonellosis caused by consumption of poultry meat contaminated by S. Typhimurium (National Disease Surveillance Center, 2004). S. Agona has been reported as the cause of an outbreak of salmonellosis associated with consumption of precooked turkey meat in human (Synnott *et al.*, 1998). Salmonella Paratyphi B (a manadapted serotype) isolated from one turkey meat sample in this study could be due to cross-contamination during the slaughter process and handling.

In this study, Salmonella isolates recovered from turkey meat samples showed high resistance rate to nalidixic acid, tetracycline, streptomycin, and trimethoprim (range from 23.5% to 58.8%). Antunes et al. (2003) reported a high antimicrobial resistance of Salmonella isolates recovered from poultry products including chicken and turkey to nalidixic acid, tetracycline, and streptomycin (range from 36% to 50%) but a low resistance rate to trimethoprim (3%) in Portugal. Higher rate of multidrug resistance has been reported in Salmonella isolates from turkey (55.6%) as compared to chicken (20.9%) and duck (13.6%) meat samples (Little et al., 2008). Although it has been suggested that resistance to nalidixic acid may be an indicator of decreased susceptibility to ciprofloxacin (Hakanen et al., 1999, Kapil et al., 2002; Asna et al., 2003; Threlfall et al., 2003), in our study only 1 of 17 Salmonel*la* strains (5.9%; CI: 6.6% to 18.4%) was resistant to ciprofloxacin. Susceptibility to ciprofloxacin among Salmonella strains resistant to nalidixic acid has been also reported by other investigators (Heurtin-Le Corre et al., 1999; Chang et al., 2005). In our study, all S. Agona isolates were susceptible to the 12 tested antimicrobial agents. A resistance to antibiotics, including amoxicillin, ampicillin, streptomycin, tetracycline, and trimethoprim has been however reported in S. Agona isolated from turkey meat samples (White et al.,

2001). Further study on genotypic characterization of all *Salmonella* isolates using molecular techniques such as pulsed-field gel electrophoresis (PFGE) and polymerase chain reaction (PCR) will be needed to determine whether antimicrobial resistance observed in this study is associated with specific genotypes.

The widespread use of antibiotics as supplements for prophylaxis and growth promotion has promoted the selection of antimicrobial-resistant Salmonella strains at the farm level during poultry production (Antunes et al., 2003). Crosscontamination between carcasses during processing might transfer resistant Salmonella strains to the final product (Navak et al., 2004). Therefore, contaminated poultry products may serve as vehicles for the transmission of antimicrobial-resistant strains to humans (Antunes et al., 2003; Carramiñana et al., 2004). Most countries require that Salmonella is absent from ready-to-eat food products and numerous programmes to eliminate Salmonella, especially S. Enteritidis and S. Typhimurium, from the poultry chain have been established in several countries (Uyttendaele et al., 1998). Since salmonellosis is transmitted primarily through food of animal origin, the presence of antimicrobial - resistant Salmonella in raw meat products has important public health implications especially in developing countries, where the use of antibiotics is widespread and uncontrolled (Hart & Kariuki, 1998). Therefore, the use of in vitro susceptibility testing of Salmonella may take on greater importance in ensuring rapid and appropriate management of patients with food-borne salmonellosis.

In summary, we recovered 17 Salmonella isolates from turkey and ostrich meat samples including S. Typhimurium, S. Enteritidis, S. Agona, and S. Paratyphi B. These isolates showed high resistance rate to nalidixic acid, tetracycline, streptomycin, and trimethoprim. To our knowledge, this is the first study on prevalence of *Salmonella* in partridge meat and first report of the isolation of *Salmonella* spp. from retail raw turkey and ostrich meat in Iran.

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