Trop Anim Health Prod (2010) 42:893–897
DOI 10.1007/s11250-009-9504-7

Serotype identification and antimicrobial resistance profiles of *Salmonella* spp. isolated from chicken carcasses

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Accepted: 10 November 2009 / Published online: 28 November 2009

Abstract In this study, 32 *Salmonella* strains isolated from 400 chicken carcasses were serotyped, and antibiotic resistance profiles were detected against 12 selected antimicrobial agents using disc diffusion method. Thirty-two isolates were identified as follows; 22 (68.7%) *Salmonella* Enteritidis, five (15.6%) *Salmonella* Virchow, three (9.3%) *Salmonella* Typhimurium and two (6.2%) *Salmonella* Hadar. In all *Salmonella* isolates, antibiotic resistance were detected. Out of 32 *Salmonella* strains, 22 (68.75%) displayed multi-drug resistance. Thirty-two (100.0%) of the isolates were found to be resistant to penicillin G, 20 (62.5%) to nalidixic acid, four (12.5%) to cephalothin, two (6.2%) to streptomycin and two (6.2%) to tetracycline. Fifteen (68.1%) *Salmonella* Enteritidis, one (33.3%) *Salmonella* Typhimurium, two (100.0%) *Salmonella* Hadar and two (40.0%) *Salmonella* Virchow were shown to be resistant to nalidixic acid. Cephalothin resistance was detected in 9.0%, 33.3%, and 20.0% for *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Virchow, respectively. The results indicate that *Salmonella* recovered from chicken carcasses were resistant to multiple antimicrobials and that resistance among these isolates varies by serotype. Also, this emerged as a significant public health problem.

Keywords *Salmonella* · Chicken carcasses · Serotype distribution · Antibiotic resistance · Disc diffusion

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Introduction

Salmonellosis is a worldwide problem of both developed and developing countries, causing health problems, business lost and economic losses for medical treatment (Gareis 1995). Contaminated chicken meat may present the greatest potential hazard of human gastroenteritis caused by salmonellae. The emergence of antimicrobial resistance in *Salmonella*, which has lead to failures in the treatment of human salmonellosis, is also of great concern worldwide (Poppe et al. 1995; Van Duijkeren and Houwers 2000).

Various antimicrobials in intensively managed food animals including chickens are often administered through feed or drinking water either for therapy, prophylaxis, or growth promotion Van Duijkeren and Houwers 2000). The increasing single and multiple antimicrobial-resistant *Salmonella* strains isolated from human cases of salmonellosis has been associated with wide spread use of antimicrobial agents in food animal production. A considerable number of antimicrobials

commonly used in the treatment of salmonellosis and other bacterial infections of humans are also used in veterinary practises. This may entail a public health risk by the transfer of resistant *Salmonella* and other zoonotic bacterial pathogens or the resistant genes from food animals to humans through consumption of contaminated food and food products (Gay et al. 1994; Wegner et al. 1999).

The prevalence of *Salmonella* serotypes and their antimicrobial resistance have been studied in many countries (Beli et al. 2001; Bada-Alambedji et al. 2006; Berrang et al. 2006; Capita et al. 2007; Zewdu and Cornelius 2009) including Turkey (Sarimehmetoglu et al. 1996; Aksakal 2003; Goncagul et al. 2004; Erdem et al. 2005; Kilinc and Aydin 2006).

Very little statistical data on salmonella infections and salmonella serotypes collected from individual studies are available in Turkey. Also, Turkey lacks a national *Salmonella* reference centre; epidemiological data on strains isolated in Turkey can only be collected from the collective findings of individual studies. Therefore, the aim of this study was to highlight the serotype distribution and antibiotic resistance profiles of 32 *Salmonella* strains previously isolated from 400 chicken carcasses between 2003 and 2005 in Ankara, Turkey.

Materials and methods

Bacterial strains

Thirty two *Salmonella* strains previously isolated from 400 chicken carcasses sold in supermarkets in Ankara, Turkey between 2003 and 2005 were used as the material.

Serotyping

Serotype identification of the *Salmonella* isolates were performed according to the scheme of Kauffmann–White, with lam agglutination and serum neutralisation tests (Popoff 2001). For this purpose, commercial *Salmonella* O and H, phase 1 and phase 2 antisera provided by Difco (Becton Dickinson Co., Franklin Lakes, NJ, USA) were used separately. *Salmonella* strains were formerly serogrouped by the slide agglutination test using O antiserum and were identified by the tube agglutination test using the antiserum H.

Antimicrobial resistance

All *Salmonella* isolates were evaluated for resistance to 12 antimicrobials using the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI; Anon 2006) on Mueller–Hinton agar (OXOID CM0337, Hampshire, UK) using ampicillin (10 μg, OXOID CT0003B), amoxicillin (10 μg, OXOID CT0161B), cephalothin (30 μg, OXOID CT0010B), chloramphenicol (30 μg, OXOID CT0013B), ciprofloxacin (5 μg, Oxoid CT0425B), gentamicin (10 μg, OXOID CT0024B), kanamycin (30 μg, OXOID CT0026B), nalidixic acid (30 μg, OXOID CT0031B), penicillin G (10U, OXOID CT0043B), sulfamethoxazole/trimethoprim (1.25/23.75 μg, OXOID CT0052B), streptomycin (10 μg, OXOID CT0047B) and tetracycline (30 μg, OXOID CT0054B) discs.

The *Salmonella* strains that have been stored at −20°C were transferred to Brain Heart Infusion Broth (OXOID CM0225) and incubated at 35°C for 24 h. After the incubation period, a loop amount of growth transferred into 4–5 ml containing Tryptone Soya Broth (OXOID CM0129). The broth culture was incubated at 35°C until it achieves the turbidity of the 0.5 McFarland (10^8 cfu/ml; 2 to 6 h). Turbidity control was done by comparison to standard McFarland tubes. The suspension was inoculated uniformly on Mueller–Hinton agar (uniform depth of 4 mm) with a sterile cotton swap. After the agar surface dried (3–5 min), antibiotic discs were placed on the plates not closer than 24 mm from centre to centre. The plates were incubated at 35°C for 16–18 h. At the end of the incubation, all plates were examined, and zones around the antibiotic discs were measured by a ruler. Interpretation of the inhibition zones was performed according to the recommendations of the CLSI (Anon 2006), and strains were evaluated as susceptible, intermediate or resistant.

Results

Overall, four different *Salmonella* serotypes were identified. *Salmonella* Enteritidis was the most commonly isolated serotype (22 isolates, 68.7%) in this study. The other ten *Salmonella* isolates were serotyped as follows: *Salmonella* Virchow (five isolates, 15.6%), *Salmonella* Typhimurium (three isolates,

9.3%) and *Salmonella* Hadar (two isolates, 6.2%; Table 1). None of the *Salmonella* isolates showed resistance against the following antimicrobials: ampicillin, amoxicillin, chloramphenicol, gentamicin, kanamycin, and sulfamethoxazole/trimethoprim.

According to the disc diffusion test, 32 (100.0%) *Salmonella* isolates were found to be resistant to one or more antimicrobials. The most common resistance was to penicillin G (100.0%). The prevalences of resistance were 62.5%, 12.5%, 6.2% and 6.2% for nalidixic acid, cephalothin, streptomycin and tetracycline, respectively.

Out of the 32 *Salmonella* isolates, 22 (68.7%) were multi-resistant. Fifteen (68.1%) *Salmonella* Enteritidis, one (33.3%) *Salmonella* Typhimurium, two (100.0%) *Salmonella* Hadar and two (40.0%) *Salmonella* Virchow were resistant to nalidixic acid. It was detected that two (9.0%) *Salmonella* Enteritidis, one (33.3%) *Salmonella* Typhimurium and one (20.0%) *Salmonella* Virchow were found to be resistant to cephalothin, while two (100.0%) *Salmonella* Hadar were resistant to streptomycin and tetracycline (Table 1). Among 22 (68.7%) resistant *Salmonella* isolates, 18 (56.25%) had a multiple resistance pattern to two antimicrobials (16 of them to nalidixic acid and penicillin G, two of them to cephalothin and penicillin G), while two (6.25%) isolates were resistant to four antimicrobials (nalidixic acid, penicillin G, streptomycin and tetracycline). Although most of the isolates had multi-drug resistance to two antimicrobials (15 *Salmonella* Enteritidis, two *Salmonella* Typhimurium, and one *Salmonella* Virchow), just four *Salmonella* isolates had multi-drug resistance to three or four antimicrobials (one *Salmonella* Enteritidis and one *Salmonella* Virchow to cephalothin, nalidixic acid and penicillin G; two *Salmonella* Hadar to nalidixic acid, penicillin G, streptomycin and tetracycline; Tables 1 and 2).

Discussion

In this study, *Salmonella* isolates were identified as *Salmonella* Enteritidis (68.7%), *Salmonella* Virchow (15.6%), *Salmonella* Typhimurium (9.3%) and *Salmonella* Hadar (6.2%). *Salmonella* Enteritidis was found to be the most prevalent serotype. *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Paratyphi B and *Salmonella* Typhi are the most common serotypes isolated from humans in Turkey (Erdem 2001). Erdem et al. (2005) have investigated 53 *Salmonella enterica* group C isolates obtained from various human samples in ten provinces of Turkey during a 2-year period. They have identified the isolates as *Salmonella* Virchow and *Salmonella* Hadar. Also, Willocks et al. (1996) have reported that human salmonellosis caused by *Salmonella* Virchow is related to consumption of poultry products. Our results are in agreement with these data. The high prevalence of *Salmonella* Enteritidis observed in present study is comparable to the situation described by other investigations (Beli et al. 2001; Kilinc and Aydin 2006; Capita et al. 2007). It reflects the presence of this serotype in the intestinal tract of live chickens (Aksakal 2003), contaminating carcasses during slaughter and processing (Sarimehmetoglu et al. 1996).

In the present study, isolates found to be resistant 100% to penicillin G, 62.5% to nalidixic acid, 12.5% to cephalothin, 6.2% to streptomycin and 6.2% to tetracycline. Also, multiresistant isolates were 68.7% in our study. Similarly, Kilinc and Aydin (2006) have

Table 1 Serotypes and antimicrobial resistance patterns of 32 *Salmonella* isolates from chicken carcasses

Serotypes	Number of serotypes (%)	Number of multiresistant isolates (%)	Resistance patterns (n)
Salmonella Enteritidis	22 (68.70)	16 (50.00)	CEF (2), NAL (15), PEN (22)
Salmonella Typhimurium	3 (9.30)	2 (6.25)	CEF (1), NAL (1), PEN (3)
Salmonella Virchow	5 (15.60)	2 (6.25)	CEF (1), NAL (2), PEN (5)
Salmonella Hadar	2 (6.20)	2 (6.25)	NAL (2), PEN (2), STR (2), TET (2)
Total	32 (100.00)	22 (68.70)	CEF (4), NAL (20), PEN (32), STR (2), TET (2)

CEF cephalothin, *NAL* nalidixic acid, *PEN* penicillin G; *STR* streptomycin, *TET* tetracycline (Anon 2009).

Table 2 Multiple antimicrobial resistances of *Salmonella* serotypes isolated from chicken carcasses

Number of resistant antibiotics	Antimicrobial resistance pattern (n)	n (%)
One	PEN (10)	10 (31.25)
Two	NAL, PEN (16)	18 (56.25)
Two	CEF, PEN (2)	2 (6.25)
Three	CEF, NAL, PEN (2)	2 (6.25)
Four	NAL, PEN, STR, TET (2)	2 (6.25)

n number of isolates, Table 1

reported that 100% of tested *Salmonella* isolates were resistant to penicillin and multi-resistance rate was high. Penicillins are commonly used antimicrobials for the treatment, controlling and prevention of animal diseases in veterinary medicine and have been widely used for decades in animal husbandry (Kowalski and Konieczna 2007).

In the present study, 100% of the *Salmonella* isolates were found resistant to one or more antimicrobials. Berrang et al. (2006) have found that *Salmonella* isolates from chicken carcasses were resistant to nalidixic acid, streptomycin and tetracycline. The results of present study are in agreement with those of Berrang et al. (2006). Capita et al. (2007) isolated 60 *Salmonella* strains from chicken carcasses and determined 24 of the strains as multi-resistant (*Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Virchow). They have stated that tetracycline (36.67%), enrofloxacin (23.33%) and ciprofloxacin (21.67%) resistances were the most frequently encountered resistances. Goncagul et al. (2004) have investigated antimicrobial resistance profiles of *Salmonella* Enteritidis isolates from chicken meat and found that four of the isolates had multi-drug resistance to ampicillin, sulphamethoxazole/trimethoprim and chloramphenicol. Molla et al. (2003) have reported that 51 (63.7%) of the 80 *Salmonella* strains isolated from chicken carcasses, and giblets were resistant to one or more antimicrobials including sulfisoxazole, spectinomycin, amoxicillin/clavulanic acid, ampicillin, tetracycline, chloramphenicol, florfenicol and streptomycin. The high levels of resistant *Salmonella* isolates to one or more antimicrobials in present study and previous studies may be due to the worldwide overuse of antimicrobials in different fields.

Cui (2004) has isolated 27 (44%) *Salmonella* from 61 chicken samples and found that the 100% *Salmonella* Typhimurium were resistant to amoxicillin/clavulanic acid, ampicillin, cephalothin, ceptiofur and cefoxitin. On the contrary, all *Salmonella* isolates were susceptible to ampicillin, amoxicillin, ciprofloxacin, chloramphenicol, gentamicin, kanamycin and sulfamethoxazole/trimethoprim in the present study. This may be explained by the limited availability and high cost of the antimicrobials that would reduce their frequent utilisation in veterinary practise in Turkey.

In a previous study, 80 *Salmonella* isolates were recovered from chicken carcasses and identified 15 serotypes including *Salmonella* Hadar (9%), *Salmonella* Typhimurium (4%) and *Salmonella* Enteritidis (1%). It was shown that 43.75% of the isolates were resistant to one or more antimicrobial, and 36% were determined as multi-drug resistant. Moreover, 100% of *Salmonella* Hadar had resistance to streptomycin and sulfamethoxazole/trimethoprim while *Salmonella* Typhimurium and *Salmonella* Enteritidis had no resistance (Berrang et al. 2006). These results are in agreement with our results for antimicrobial resistance pattern of *Salmonella* Hadar, *Salmonella* Typhimurium, and *Salmonella* Enteritidis.

The results of the present study are supported by some previous studies. Zewdu and Cornelius (2009) have investigated 29 *Salmonella* isolates from 208 chicken carcasses, identified 32.7% of them resistant to one or more antimicrobials and determined 100% of *Salmonella* Hadar as multiresistant to streptomycin and tetracycline. Similarly, antimicrobial resistance patterns of *Salmonella* Enteritidis and *Salmonella* Hadar isolated from chicken carcasses reported by Bada-Alambedji et al. (2006) in Senegal. Their isolates were resistant to a variety of the antimicrobials including cephalothin, nalidixic acid and tetracycline.

The high prevalence of antimicrobial resistance of *Salmonella* spp. is probably due to wide spread uses of the common antimicrobials in the veterinary medicine. Also, the accessibility to various antimicrobials without a prescription can be another possible underlying reason together with the unconscious usage. Differences of the resistance percentage between the present study and the previous ones can be possibly attributed to the use of different antimicrobials as therapheutics, prophylactic agents or growth promoters in Turkey.

The results of this study indicated that the resistance of *Salmonella* spp. to commonly used antimicrobials is

relatively high in chicken carcasses and has emerged as a significant public health problem in Turkey. The use of antimicrobials as prophylactics or growth promoter agents must be limited and monitoring systems should be continued regularly.

Acknowledgement The authors are grateful to Yildiz AYAZ from Etlik Central Veterinary Control and Research Institute for the serotype identification of the *Salmonella* isolates.

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