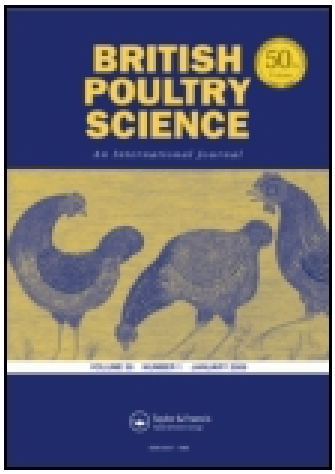

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Incidence and antibiotic resistance of Salmonella spp. in ground turkey meat

Professor O. Iseri [a] & I. Erol [a]
[a] Department of Food Hygiene and Technology , School of Veterinary Medicine, Ankara University , Ankara, Turkey
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Incidence and antibiotic resistance of *Salmonella* spp. in ground turkey meat

O. ISERI AND I. EROL

Department of Food Hygiene and Technology, School of Veterinary Medicine, Ankara University, Ankara, Turkey

Abstract 1. The objectives of this study were to isolate *Salmonella* spp. by conventional culture technique from ground turkey samples, to determine the seasonal distribution of *Salmonella* spp., to verify the isolates by PCR using primers based on *ori*C gene sequence, and to determine the antibiotic susceptibility profiles of the isolates. A total of 240 packaged fresh ground turkey samples marketed in Ankara were analysed between July 2004 and June 2005.

2. One hundred and ten out of 240 (45·8%) samples were positive for *Salmonella* spp. and confirmed by PCR. The distribution of *Salmonella* spp. was determined as 48·3, 55·0, 63·3 and 16·6%, during spring, summer, autumn and winter, respectively. Statistical analysis showed a significant difference for the prevalence of *Salmonella* spp. between winter and the other seasons.

3. Of the isolates, 54 out of 110 (49·0%) were resistant to one or more antibiotics tested. The highest resistance was observed to nalidixic acid (25·4%), followed by streptomycin (17·2%) and tetracycline (15·4%).

4. In conclusion, this is a disturbing finding, both for the high prevalence of *Salmonella* and the extent of antibiotic resistance. Ground turkey should be produced under suitable hygienic and technological conditions and the use of antimicrobials must be controlled by governmental agencies to protect public health from salmonellosis and from the consequences of increased resistance to the antibiotics.

INTRODUCTION

Foodborne infections caused by *Salmonella* other than typhoidal serotypes represent an important public health concern worldwide. Each year, nearly 1·4 million persons are infected with non-typhoidal *Salmonella* in the United States, resulting in 15 000 hospitalisations and 400 deaths (Voetsch *et al*., 2004).

Worldwide, *Salmonella* has been implicated in human illness through the consumption of a variety of processed foods, in particular foods of animal origin. High numbers of human salmonellosis outbreaks have been associated with consumption of raw or undercooked poultry, egg and meat products. Contamination, particularly re- and cross-contamination can occur at multiple points along the food chain including production, processing, distribution, retail marketing, and handling/preparation (Bryan & Doyle, 1995; Nayak *et al*., 2003; Cardinale *et al*., 2005). Because of public health concerns, it is important to test the food products for the presence of *Salmonella* before consumption.

PCR is a rapid procedure with high sensitivity and specificity for the detection as well as verification of *Salmonella* spp. from food and environmental samples (Candrian, 1995). Different PCR procedures have been developed on the basis of gene sequences which are unique for *Salmonella*. Primers used in this study are specific to the origin of DNA replication (*ori*C) on the *Salmonella* chromosome, a potential target

Correspondence: Professor I. Erol, Department of Food Hygiene and Technology, School of Veterinary Medicine, Ankara University, 06110, Diskapi, Ankara, Turkey. E-mail: erol@veterinary.ankara.edu.tr

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for *Salmonella* identification (Widjojoatmodjo *et al.*, 1991; Fluit *et al.*, 1993; Erol *et al.*, 1999).

Most antimicrobial-resistant *Salmonella* infections are acquired from eating contaminated foods of animal origin (Angulo *et al.*, 2000). It was suggested that the use of antimicrobials for prophylaxis, treatment and growth promotion purposes in animal husbandry has played an important role in antibiotic resistance. Overall, the largest quantities of antimicrobials were used as regular supplements for prophylaxis or growth promoter in the feed of animal herds and poultry flocks (Tollefson *et al.*, 1997), but the European Union banned the use of avoparcin in 1997 and bacitracin, spiramycin, tylosin and virginiamycin in 1999 as growth promoters (Casewell *et al.*, 2003).

Multidrug-resistant phenotypes have been increasingly described among *Salmonella* species worldwide. *Salmonella* Typhimurium definitive phage type 104 (*Salmonella* Typhimurium DT104) is a common multiple-antibiotic-resistant strain that has emerged as a global public health problem (Threlfall *et al.*, 1996; Humphrey, 2001). Studies from different countries revealed that different *Salmonella* serotypes isolated mainly from foods of animal origin have showed multiple antibiotic resistance profiles. A study in Spain, revealed that ampicillin resistance in *Salmonella* species increased from 8 to 44%, tetracycline resistance from 1 to 42%, chloramphenicol resistance from 1·7 to 26%, and nalidixic acid resistance from 0·1 to 11%, in the periods of 1985–1987 and 1995–1998, respectively (Prats *et al.*, 2000). In the US, resistance to tetracycline in *Salmonella* species has increased from 9% in 1980 to 24% in 1990 and ampicillin resistance from 10 to 14% (Lee *et al.*, 1994).

Ground meat has high nutritional value and is useful in cooking. However, it is a suitable medium for growth of many pathogenic and saprophytic microorganisms. Even if ground meat is originally contaminated at a low level with *Salmonella*, growth and/or cross contamination may occur during storage and handling under poor hygienic conditions (Erol, 1999).

The objectives of this study were to isolate *Salmonella* spp. by conventional culture technique from ground turkey samples, to verify the isolates by PCR using primers based on *ori*C gene sequence, to determine the seasonal distribution of *Salmonella* spp., and to determine the antibiotic susceptibility profiles.

MATERIAL AND METHODS

Bacterial strains

Salmonella Typhimurium (ATCC 14028) and *Escherichia coli* (ATCC 25922) were used as control strains for PCR assay and antibiotic susceptibility testing, respectively.

Sample collection

Two hundred and forty packaged fresh ground turkey samples (approximately 450–500 g) marketed in Ankara were collected and tested between July 2004 and June 2005. To determine the seasonal distribution, each month 20 samples were tested. Fresh packaged ground turkey samples were transported to the laboratory under cold conditions and analysed for *Salmonella* spp. within 2 h.

Conventional culture method for the isolation and identification of *Salmonella* spp.

Salmonella was isolated from ground turkey using standard cultivation techniques – Bacteriological Analytical Manual of the Food and Drug Administration (FDA, 2003), the International Organization for Standardization (ISO 6579) (ISO, 2002) and Flowers *et al.* (1992). Ground turkey (25 g) from each sample was weighed in a sterile bag containing 225 ml Buffered Peptone Water (BPW) (Oxoid CM0509, Hampshire, UK) and homogenised for 2 min in a stomacher (AESAP1068-Easy Mix; AES Laboratories, Cambourg, France) and incubated at 37°C for 24 h. After incubation, 0·1, 1 and 1 ml of the pre-enrichment broths were added to 10 ml of Rappaport Vassiliadis (RV) broth (Oxoid CM669), 9 ml of Selenite Cystine (SC) broth (Difco 112534 JC, Detroit, USA) and 9 ml of Tetrathionate (TT) broth (FDA, BAM), respectively. Following 24 h incubation at 43°C for RV broth, at 37°C for SC broth and at 42°C for TT broth, the selectively enriched broths were streaked on to Brilliant-green Phenol-red Lactose Sucrose Agar (BPLS) (Merck 1·07237, Darmstadt, Germany) and Xylose Lysine Deoxycholate (XLD) (Merck 1·05287) Agar and incubated at 37°C for 18–24 h. Up to 5 suspected colonies with typical *Salmonella* morphology grown on BPLS and XLD were confirmed biochemically by inoculation into Triple Sugar Iron Agar (TSIA) (Oxoid CM0277) and Lysine Iron Agar (LIA) (Oxoid CM0381) and incubated at 37°C for 24 h. In addition, a urease test was performed in Urea Broth (Difco 15347 JF) from TSIA positives and incubated at 37°C for 6–24 h. Suspect *Salmonella* colonies tested serologically by agglutination with polyvalent antisera (Difco L840114-1) and isolates that showed agglutination were stored at +4°C on Tryptone Soya Agar (TSA, Oxoid CM0131) and at −85°C (Sanyo MDF-U5186S, Japan) in cryovials for the PCR verification and testing for the antibiotic resistance profiles.

PCR method

Primers

The selected primers used are specific to the origin of DNA replication (*ori*C) on the *Salmonella* chromosome, amplifying a 163-bp sequence (Primer-I: 5′-TTA TTA GGA TCG CGC CAG GC-3′; Primer-II: 5′-AAA GAA TAA CCG TTG TTC AC-3′) (Widjojoatmodjo *et al.*, 1991).

DNA extraction

All isolates, stored at −85°C (Sanyo MDF-U5186S) in cryovials, were resuscitated in Brain Heart Infusion broth (BHI, Oxoid CM 0225) at 37°C for 24 h. One ml of each enrichment culture was then separately transferred to a micro-centrifuge tube. All tubes were centrifuged (Eppendorf Centrifuge 5417R, Hamburg, Germany) at 10°C at 5000×*g* for 15 min. The supernatant was removed and the pellet was suspended in 1 ml sterile bidistilled water and mixed well. This suspension was centrifuged at 10°C at 5000×*g* for 5 min, the supernatant was removed and the pellet was resuspended in 200 µl sterile bidistilled water. These suspensions were mixed and boiled in a water bath (Memmert WB/OB 7-45, WBU45, Schwabagh, Germany) at 95°C for 20 min, and then cooled on ice (Widjojoatmodjo *et al.*, 1991; Fluit *et al.*, 1993; Erol *et al.*, 1999).

PCR amplification

The PCR was performed with a final volume of 50 µl containing incomplete 1×PCR Buffer, 1·5 mM $MgCl_2$, 2 U *Taq* DNA Polymerase (Fermentas EP0402, Lithuania), 100 µM dNTP mix (Fermentas R0181), 0·5 µM of each primer (Integrated DNA Technologies, IDT, USA) and 10 µl sample DNA. Amplification was carried out with a Thermal cycler (Biometra Personal Cycler, Goettingen, Germany) for 35 cycles. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, and extension at 72°C for 1 min. A final extension was carried out at 72°C for 10 min. The resultant PCR product was further analysed by agarose gel (1·5%, Agarose-Basica LE, Prona, Spain) electrophoresis (Biometra, Agagel-Maxi-System B15359) stained with 0·1 µg/ml ethidium bromide (Merck 111608) for 1 h at 100 V. Amplicon visualisation and documentation were performed using gel documentation and analysis system (Sygene Ingenius, Cambridge, UK) (Fluit *et al.*, 1993; Erol *et al.*, 1999).

Testing for antimicrobial resistance profiles

The antibiotic resistance tests on *Salmonella* isolates were carried out by the disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2003) on Mueller Hinton agar (Oxoid CM0337) with ampicillin (10 µg, Oxoid CT0003B), ciprofloxacin (5 µg, Oxoid CT0425B), chloramphenicol (30 µg, Oxoid CT0013B), gentamicin (10 µg, Oxoid CT0024B), kanamycin (30 µg, Oxoid CT0026B), nalidixic acid (30 µg, Oxoid CT0031B), tetracycline (30 µg, Oxoid CT0054B), trimethoprim (5 µg, Oxoid CT0076B), trimethoprim/sulfamethoxazole (25 µg, Oxoid CT0052B), and streptomycin (10 µg, Oxoid CT0047B). The *Salmonella* isolates stored at −85°C were transferred to Tryptone Soya Broth (Oxoid CM0129) and the broth culture was incubated at 35°C until it reached the turbidity of the 0·5 McFarland standard (approximately 6 h) to ensure that the suspension contains 10^8 cfu/ml cells. The correct density of the turbidity standard was verified by NanoDrop spectrophotometer (NanoDrop ND-100, DE, USA). The suspension was distributed evenly, using a sterile cotton swab, on to Mueller Hinton Agar (uniform depth of 4 mm) and after the agar surface dried (3–5 min) antibiotic discs were placed on the plate not closer than 24 mm from the centre. The plates were incubated at 35°C for 16–18 h and the inhibition zones were measured to interpret the results. *Escherichia coli* (ATCC 25922) was used as a control strain according to the NCCLS.

Statistical analysis

A chi-square test was performed to determine significance of the seasonal distribution of *Salmonella* spp. isolated (SPSS, 2007).

RESULTS

In this study, *Salmonella* spp. were detected in 110 out of 240 (45·8%) ground turkey samples. In all, *Salmonella* spp. was isolated from 114 ground turkey samples using conventional culture technique. However, the isolates of 4 samples were not verified by PCR and these 4 samples recorded as false negative. Therefore, 110 out of 114 (96·4%) *Salmonella* spp. isolated by culture method, were confirmed using PCR and the PCR results are shown in the Figure.

The distribution of *Salmonella* spp. was determined as 48·3, 55·0, 63·3 and 16·6%, during spring, summer, autumn and winter, respectively. According to the statistical analysis, there was no significant difference for the seasonal prevalence of *Salmonella* spp. ($P > 0{\cdot}05$) between spring, summer and autumn but the difference was significant ($P < 0{\cdot}001$) between winter and other seasons.

According to the disc diffusion test, 49·0% of the isolates (54/110) were resistant to at least one, and 12·7% (14/110) of the isolates to more than one antibiotic. The highest resistance rate was observed against nalidixic acid (25·4%). Data are in the Table.

Multiple resistance occurred in some isolates. One of the isolates (0·9%) showed multiple resistance to tetracycline, trimethoprim/sulfamethoxazole, trimethoprim, chloramphenicol, nalidixic acid, ciprofloxacin and streptomycin, 4 isolates (3·6%) to tetracycline, trimethoprim/sulfamethoxazole, trimethoprim, chloramphenicol, nalidixic acid and streptomycin, one isolate (0·9%) to kanamycin, nalidixic acid, gentamicin, streptomycin and ampicillin, 5 isolates (4·5%) to kanamycin, gentamicin, streptomycin and ampicillin, one isolate (0·9%) to tetracycline and kanamycin, and one isolate (0·9%) to nalidixic acid and streptomycin.

DISCUSSION

In recent years, many studies have been conducted on the incidence of *Salmonella* spp. in raw and processed meat and poultry. This study has shown the importance of *Salmonella* contamination (45·8%) in ground turkey. Poultry meat can be contaminated at the different stages of the production including flock, slaughterhouse, processing, distribution and retail marketing, but cross-contamination plays an important role throughout the processing. Our results are similar to those reported by Erol *et al.* (2006) for the years of 2005–2006, when 30·5% of turkey meat samples were found to be contaminated with *Salmonella* spp., suggesting that ground turkey and turkey meat may a potential vehicle of transmission of *Salmonella* spp. As in the present study, the prevalence of *Salmonella* in ground turkey and turkey meat in the USA was 49·9 and 49·4%, respectively (Rose *et al.*, 2002; Fakhr *et al.*, 2006). The prevalence of *Salmonella* spp. in turkey carcases tested by Lammerding *et al.* (1988) was higher (69·1%) in Canada than the value reported in the present study.

In contrast, lower incidences have been reported by other researchers**.** *Salmonella* prevalence in ground turkey in the USA varied from 16·8% (Fratamico, 2003) to 24·0% (White *et al.*, 2001). In Albania, 8·2% of turkey meat samples were found to be contaminated with *Salmonella* spp. (Beli *et al.*, 2001). When comparing our results to those of other authors, several factors should be taken into account, such as slaughter hygiene, processing of the samples, cross contamination of the products at different stages throughout the food chain, differences in the methodology applied to detect the *Salmonella* and seasonal differentiation.

In 110 (96·4%) of 114 *Salmonella* spp. isolated by conventional culture technique, *ori*C

Table. *Antibiotic resistance profiles of* Salmonella *spp. isolated from ground turkey*

Antibiotics	Number of the isolates (%)		
	Resistant	Intermediate	Susceptible
Nalidixic acid	28 (25·4)	6 (5·4)	76 (69·0)
Streptomycin	19 (17·2)	87 (79·0)	4 (3·6)
Tetracycline	17 (15·4)	3 (2·7)	90 (81·8)
Ampicillin	8 (7·2)	2 (1·8)	100 (90·9)
Kanamycin	7 (6·3)	94 (85·4)	9 (8·1)
Gentamicin	6 (5·4)	22 (20·0)	82 (74·5)
Trimethoprim/ sulfamethoxazole	5 (4·5)	2 (1·8)	103 (93·6)
Trimethoprim	5 (4·5)	–	105 (95·4)
Chloramphenicol	5 (4·5)	–	105 (95·4)
Ciprofloxacin	2 (1·8)	6 (5·4)	102 (92·7)

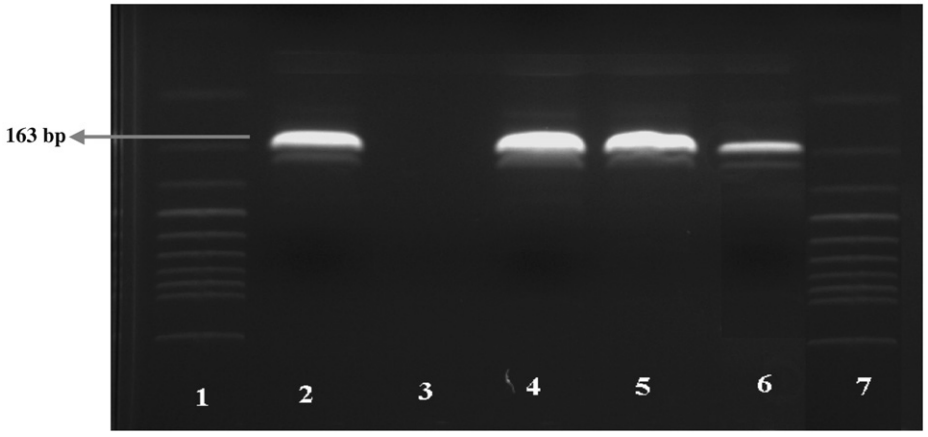

Figure. *ori*C *gene detected* Salmonella *spp. isolates. (1 and 7: 100 bp DNA marker. 2: Positive control (*Salmonella Typhimurium *ATCC 14028). 3: Negative control. 4-6:* ori*C positive* Salmonella *spp. isolates).*

gene were detected and verified by PCR. Various researchers have reported that *ori*C gene based primers were highly sensitive for the detection of *Salmonella* (Widjojoatmodjo *et al.*, 1991; Fluit *et al.*, 1993; Erol *et al.*, 1999). Cross reaction of antisera was detected by agglutination test for 4 *Salmonella* isolates. Our data showed that *Salmonella* may exhibit some cross reactivity with some isolates of other *Enterobacteriaceae*. Thus, the presence of cross-reacting bacteria is likely to cause a false-positive result by conventional culture (Metzler and Nachamkin, 1988).

Widjojoatmodjo *et al.* (1991) showed that using *ori*C gene sequence-based PCR in combination with IMS was useful for detection of *Salmonella* in pure and mixed cultures. Using the same primers, Fluit *et al.* (1993), reported that the detection limit of *Salmonella* in spiked chicken samples was 0·1 cfu/g, when a 6- and 24-h pre-enrichment was performed prior to PCR. Gooding and Choudary (1999) compared 5 different primer pairs on 14 *Salmonella* isolates obtained from various sources. Their results showed that all isolates except two, gave accurate results when using *ori*C gene sequence.

The incidence of *Salmonella* spp. in ground turkey samples was higher during spring (48·3%), summer (55·0%) and autumn (63·3%) than winter (16·6%), suggesting a positive correlation between environmental temperature and *Salmonella* isolation rate. Although in some reports *Salmonella* prevalence of red and poultry meats were found higher in the warm months (Erol, 1999; Logue *et al.*, 2003), others found no seasonal effects on *Salmonella* prevalence in meat products (Wedderkopp *et al.*, 2001; Jordan *et al.*, 2006).

Using antibiotics for a long period as prophylactic or growth promoter in food animals may be one of the most important factors for developing resistance to different bacterial strains (Tollefson *et al.*, 1997). In studies performed worldwide, antibiotic resistance profiles of *Salmonella* spp., isolated from poultry and poultry products were showed different distributions depending on the countries and regions. In our study, higher resistance was found against nalidixic acid at 25·4%, followed by streptomycin at 17·2% and tetracycline at 15·4%. Fluoroquinolones are effective for treating a variety of clinical and veterinary infections including salmonellosis, urinary tract infections, gastrointestinal infections and respiratory tract infections (Reid, 1992; Chen and Lo, 2003). Resistance of *Salmonella* spp. to nalidixic acid, tetracycline and streptomycin has been reported by different authors (Antunes *et al.*, 2003; Yazıcıoğlu *et al.*, 2005; Ayaz *et al.*, 2006; Erol *et al.*, 2006). According to Ayaz *et al.* (2006) 62·5% of the isolates from poultry carcasses, to Antunes *et al.* (2003) 50·0% isolates from chicken and turkey carcasses, and to Yazıcıoğlu *et al.* (2005) 48·1% isolates from chicken neck and wing samples were resistant to nalidixic acid. Likewise, Erol *et al.* (2006), reported resistance to nalidixic acid in isolates from turkey meat as 41·8%. Lower rates between 5·2 and 7·0% (White *et al.*, 2001; Yazıcıoğlu *et al.*, 2005; Ayaz *et al.*, 2006) and higher rates between 35 and 93% (Duffy *et al.*, 1999; Antunes *et al.*, 2003; Logue *et al.*, 2003; Erol *et al.*, 2006) to tetracycline and streptomycin were reported.

In the present study 7·2, 6·3, 5·4, 4·5, 4·5, 4·5 and 1·8% of the *Salmonella* isolates were found to be resistant to ampicillin, kanamycin, gentamicin, trimethoprim/sulfamethoxazole, trimethoprim, chloramphenicol and ciprofloxacin, respectively. Similarly, *Salmonella* spp. from turkey meat carcases and chicken meats showed resistance to the antibiotics mentioned above (Antunes *et al.*, 2003; Logue *et al.*, 2003; Goncagül *et al.*, 2004; Erol *et al.*, 2006).

Most of the isolates showed higher susceptibility rates between 69·0 and 95·4% to nalidixic acid, gentamicin, tetracycline, ampicillin, ciprofloxacin, trimethoprim/sulfamethoxazole, trimethoprim and chloramphenicol.

In conclusion, ground turkey may be a potential health risk, because a large number of the samples tested were contaminated with *Salmonella* spp. and the isolates were resistant to various antibiotics. Therefore, ground turkey should be produced under appropriate hygienic and technological conditions and the use of antimicrobials must be controlled by governmental agencies.

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