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Presence and Antimicrobial Resistance Profiles of *Salmonella* spp. in Retailed Sausages in Kayseri, Turkey

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This study was carried out to investigate the presence and antimicrobial susceptibilities of *Salmonella* spp. isolates from sausage obtained from retail outlets in Kayseri, Turkey. A total of 100 samples were analyzed in the study. The method proposed by ISO 6579 with minor modifications, for isolation of *Salmonella* spp. was used and the isolates were confirmed by PCR. Antibacterial susceptibility testing of the isolates to ampicillin, enrofloxacin, cefazolin, danofloxacin, gentamicin, nalidixic acid, neomycine, oxytetracycline and trimethoprim-sulfamethoxazol was performed by disc diffusion method. *Salmonella* spp. was isolated from 4 (4%) out of 100 samples tested. All isolates, were resistant only to neomycine and were susceptible to other antimicrobial agents except for gentamicin. Only one *Salmonella* isolate was found to be susceptible (intermediate level) to gentamicin. These results demonstrate that sausage samples may be sporadically contaminated with *Salmonella* spp. and therefore present a potential risk for public health.

Key Words: *Antibiotic, Salmonella spp., sausage, PCR.*

Kayseri'de Satışa Sunulan Sucuklarda *Salmonella* spp. Varlığı ve Antimikrobiyel Direnç Profilleri

Bu çalışmada Kayseri'de satışa sunulan sucuklarda *Salmonella* spp.'nin varlığı ve elde edilen izolatlarda antimikrobiyel duyarlılığının araştırılması amaçlandı. Toplam 100 numune incelendi. *Salmonella* spp. izolasyonu için ISO 6579 sayılı standardında bildirilen kültür tekniği modifiye edilerek kullanıldı ve izolatlar PCR ile doğrulandı. İzolatların ampisilin, enrofloksasin, sefazolin, danofloksasin, gentamisin, nalidiksik asit, neomisin, oksitetrasiklin ve trimetoprim-sülfametaksazol antibiyotiklerine duyarlılıkları disk difuzyon metodu ile belirlendi. İncelenen 100 örneğin 4'ünden (%4) *Salmonella* spp. izole edildi. Bütün izolatlar sadece neomisine dirençli ve test edilen diğer antibiyotiklerden gentamisin haricindeki antibiyotiklere duyarlı olarak belirlendi. Sadece bir adet *Salmonella* spp. izolatı gentamisine orta derecede duyarlı bulundu. Elde edilen sonuçlar sucukların *Salmonella* spp. ile sporadik olarak kontamine olabileceğini ve halk sağlığı için risk oluşturabileceğini göstermektedir.

Anahtar Kelimeler: *Antibiyotik, Salmonella spp., sucuk, PCR.*

Introduction

Salmonellosis is one of the most frequently reported food-borne diseases worldwide (1). According to the WHO Surveillance Programme for Control of Food-borne Diseases in Europe, incidences of Salmonellosis in Europen have increased dramatically in the period from 1985 to 2000. Several European countries still demonstrate a significant increasing trend, proving that continuous efforts for prevention and control are still necessary (2). Among more than 2500 *Salmonella* serotypes, *Salmonella typhimurium* and *Salmonella enteritidis* accounted for 46% and 24% outbreaks caused by *Salmonella* and bacteria, respectively (CDC 2009). In the United States, these two serotypes were also the two most frequently reported serotypes (33% of isolates) from human sources (3). Likewise, *Salmonella* spp detected in Turkish sausages served for consumption and with poor hygienic quality, was reported in some studies (4-7). The emergence of antimicrobial-resistant bacterial pathogens has become a major public health concern. A contributing factor in the development of resistance is the using of antimicrobials in human medicine, veterinary medicine, animal husbandry, as well as agricultural and aquaculture practices (8). The utilizing of antimicrobials for various purposes including disease treatment and growth promotion in domestic livestock, can potentially cause widespread dissemination of antimicrobial-resistant bacteria (9). In recent years, *Salmonella* spp. isolated from the US and other countries have demonstrated an increasing rate in multidrug resistance (10, 11).

The objectives of this study were to determine the prevalence and the antimicrobial resistance profiles of *Salmonella* spp. in retailed sausages in Kayseri, Turkey and to investigate their potential risks for public health.

Materials and Methods

Samples: In this study, a total of 100 Turkish sausages samples that received heat treatment during production were analyzed between April and June 2013 in Kayseri in

Turkey. The sausage samples were purchased from different retail markets periodically. The samples were immediately transported to the laboratory in a cool box and analysed within 1-2 h.

Reference Strain: *Salmonella typhimurium* (ATCC 13311) reference strain was used as positive control for the isolation of *Salmonella* spp. The reference strain was provided by Department of Microbiology, Faculty of Medicine, University of Erciyes, Kayseri, Turkey.

Isolation of Salmonella spp.: For the isolation and the characterization of *Salmonella* spp. from sausage samples, the method proposed by ISO 6579 with minor modifications was used (12). In brief, 25 g sausage samples were added to 225 mL volumes of buffer peptone water (BPW CM1049, Oxoid, Basingstoke, UK). The samples were homogenised for 2 min and incubated for 24 h at 37 ºC. Then, dropping of 0.1 mL of pre-enrichment aliquots were inoculated into tubes containing 10 mL Rappaport Vassiliadis (RV) broth and incubated for 48 h at 42 °C. From each of the RV broths were inoculated onto Xylose Lysine Deoxycholate (XLD) agar plates and incubated for 18-24 h at 37 °C. Up to two suspect colonies with typical *Salmonella* morphology were tested biochemically. Serological tests were carried out using specific *Salmonella* O and H agglutinating antisera (Difco 2537-47).

DNA Extraction and PCR Amplification: Total genomic DNA was extracted from strains by using a commercial AxyPrep™ Bacterial Genomic DNA extraction kit (Axygen, Bioscience, USA) as described by the manufacturer. The species specific primers were used for the detection of the 23S rRNA gene as described by Aabo et al. (13). (ST11: 5'AGC CAA CCA TTG CTA AAT TGG CGC A3′ and ST15: 5′GGT AGA AAT TCC CAG CGG GTA CTG 3′). PCR was performed in a reaction mixture of 50 µL final volume containing 5 µL template DNA, 5 µL 10XPCR buffer (Vivantis), 1.5 U Taq polymerase (Vivantis), 500 µM dNTP Mix (Vivantis), 3 mM $MgCl_2$ (Vivantis) and 25 pmol of each primer. PCR amplification was carried out with an initial denaturation of 95 °C for 1 min followed by 30 cycles, each consisting of 94 °C for 15 s, 57 °C for 15 s and 72 °C for 30 s. The final extension cycle was consisted of 8 min at 72 °C (Techne TC-512). All amplification products were determined by agarose gel (1.5%) electrophoresis at 100 V for 45 min (EC250-90, Thermo, USA). The gels were stained with ethidium bromide and visualized under a UV transilluminator (Vilber Lourmat, Marne La Vallee, France).

Determination of the Antimicrobial Sensitivity: The antibacterial susceptibility testing of isolates to ampicillin (AMP, 10 µg), cefazolin (KZ, 30 µg), danofloxacin (DFX, 5µg), enrofloxacin (ENR, 5µg), gentamicin (CN, 10 µg), nalidixic acid (NA, 30 µg), neomycine(N, 10 µg), oxytetracycline (T, 30 µg) and trimethoprim-sulfamethoxazol (SXT, 23.7 µg-1.25 µg) was performed by disc diffusion method (14). The antimicrobial discs were purchased from Oxoid (UK) except for enrofloxacin and danofloxacin, which were obtained from Bayer (Germany).

Antimicrobial susceptibility test was carried out using the disc diffusion method described by Bauer et al. (14). Briefly, the isolates were grown on Blood Agar (Merck, 1.10886) at 37 °C for 24 h. Then, a suspension of each organism adjusted to McFarland 0.5 by using physiological saline. The suspensions were spread onto Mueller Hinton Agar (Merck, 1.05437). Antibiotic discs were placed onto the agar and incubated at 37 °C for 24 h aerobically. After 24 h of incubation, the diameter of the inhibition zones were measured with callipers and the results were interpreted according to the CLSI standarts (15).

Results

In this study, 4 (4%) out of 100 sausage samples were found to be positive for *Salmonella* spp. through standard culture technique, on the other hand. All these four isolates obtained from positive samples were comfirmed by PCR.

All isolates, were resistant only to neomycine (100%). These isolates were susceptible to ampicillin, enrofloxacin, cefazolin, danofloxacin, nalidixic acid, oxytetracycline and trimethoprim-sulfamethoxazol. One *Salmonella* isolate was found to be susceptible to gentamicin at intermediate level (25%). The diameter of the zone of inhibition for *Salmonella* spp. obtained from sausage samples ranged from 33±0.03 mm to 10±0.00 mm. The antibacterial susceptibility testing results of *Salmonella* spp. isolates against 9 different antibacterial agents are exhibited in Table 1.

Table1. Antibacterial resistance profiles of *Salmonella* spp. isolated from sausage samples

Antibiotics	Diameter of the inhibition zones *of Salmonella* spp according to CLS (mm)			Zone of inhibition (mm) in this study (n= 4)		
	Susceptible	Intermediate	Resistant	Susceptible / (%)	Intermediate/ (%)	Resistant/ (%)
Ampicillin	≥17	14-16	≤13	21±0.00 (% 100)	-	-
Enrofloxacin	≥21	16-20	≤15	33 ±0.03 (% 100)	-	-
Cefazolin	18	15-17	≤14	22±0.05 (% 100)		-
Danofloxacin	≥21	16-20	≤15	31±0.00 (% 100)		-
Gentamicin	15	13-14	≤12	-	13±0.00 (%25)	-
Nalidixic Acid	19	14-18	≤13	21±0.03 (% 100)	-	-
Neomycine	15	13-14	≤12	18±0.00 (% 100)	-	10±0.00 (%100)
Oxytetracycline	15	12-14	≤11	18±0.00 (% 100)	-	-
Trimethoprim-Sulfamethoxazol	16	11-15	≤10	22±0.00 (% 100)	-	-

Discussion

In this study, the presence and the antimicrobial resistance patterns of *Salmonella* spp. isolated from sausage were evaluated. Comparing to our results (%4), similar results were presented by Ozbey et al. (16) and Siriken et al. (7). Ozbey at al. (16) examined 100 camel sausage samples for the presence of *Salmonella* spp. by culture method and PCR and 7 (7%) of the samples were identified as positive for *Salmonella* spp. Similarly, Kok et al. (17) and Oksuztepe et al. (18) isolated *Salmonella* spp from 5 (5%) and 3 (3%) of 100 fermented sausage samples, respectively. However, our isolation rates were higher than that of Erdogrul et al. (4) and Little et al. (19). Erdogrul et al. (4) tested 60 sausage and found *Salmonella* spp. contamination only in one (1.6%) sample while Little et al. (19) tested 2283 sausage and detected in 2 (0.08%) samples. In addition, no *Salmonella* spp. in the analyzed sausage samples were reported by Sancak et al. (20) and Kalantari et al (21). In addition, Mattick et al. (22), detected *Salmonella* spp. at a rate of 7.5% in frozen and 9,1% in chilled sausages (8,6% overall) out of 162 samples. In earlier studies the prevalence of *Salmonella* spp. in sausage samples were found to the range between 8-50% (25-30). Risk factors for human *Salmonella* infection include the consumption of contaminated meats, improper handling of contaminated raw meats and cross-contamination to other ready-to-eat products (19).

Nowadays, in order to achieve a good structure and texture and to obtain microbiologically safe product, the central temperature of sausages is applied at between 57 °C and 69 °C for certain times. These temperatures and process time were reported to be effective for inhibiting of *Salmonella* spp. (22, 23). *Salmonella* spp. isolation rate of sausage samples examined in the study is low compared to the previous studies, due to the fact that these samples may be heat-treated (24-27).

All isolates were resistant only to neomycine and were susceptible to other antimicrobial agents tested, except for gentamicin. One *Salmonella* isolate was found to be susceptible to gentamicin at intermediate level. Similarly, Cetinkaya et al. (28) reported that two *Salmonella* strains isolated from cig kofte samples were found to be susceptible to all of the tested antibiotics (AMP, CN, SXT, NA). In contrast, Ghozzi et al. (29) stated that 16 (20.0%) *Salmonella* strains, isolated from raw meat, were found to be resistant to AMP. Furthermore, Thong and Modarressi (30) found that 88 *Salmonella* isolates from 300 meat samples were resistant to NA (44.3%), AMP (17.0%), SXT (19.3%) and CN (2.2%) whereas Samaxa et al. (27) stated that 65 (21.7%) *Salmonella* isolates of meat origin were resistant to CN.

In the present study, 4 (4%) out of 100 sausage samples found to be contaminated with *Salmonella* spp. The Turkish Food Codex recommended zero tolerance for *Salmonella* spp. in 25 g sausage samples (31). Therefore, the sausage samples harboring *Salmonella* spp. are not suitable for consumption. For this reason, the safe consumption of sausage is principally ensured by controlling at the source, product design and control stages, and the application of Good Hygienic Practices during production. In addition, necessary precautions should be taken such as hygiene and sanitation education.

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