

Occurrence and Antibiotic Susceptibility of *Listeria* Species in Turkey Meats

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Abstract

The aims of this study were to investigate the occurrence of *Listeria* species in turkey meats and to check the antimicrobial susceptibility of the isolated strains. Hundred and fifteen raw turkey meat samples were randomly collected from the supermarkets, butchers and restaurants. Strain isolation and identification were made according to the ISO11290-1 method. Antimicrobial susceptibility was determined by the standard disc diffusion method. A total of 47 *Listeria* spp. were isolated from 115 (40.9%) raw turkey meat samples. The isolates were distributed between *L. monocytogenes* (25.53%), *L. innocua* (34.04%), *L. grayi* (31.91%) and *L. welshimeri* (8.51%). A total of 55.3 % of *Listeria* spp. isolates were multi-resistant to at least 3 of the antimicrobial agent tested. The level of multi-resistance was higher in *L. monocytogenes* strains (66.7%) than in *L. innocua* (62.5%) and *L. grayi* (53.3%). *Listeria* spp. isolates were highly resistant to ampicillin, cephalothin, penicillin, meticcillin, oxacillin, and trimethoprim-sulfamethoxazole. The isolates particularly *L. monocytogenes* are increasingly resistant to one or more antibiotics and may represent a potential risk for public health because these antibiotics are common used in treatment of listeriosis. The correct and controlled use of antibiotics in veterinary medicine is important to the emergence of resistant strains.

Keywords: *Listeria* spp., *Listeria monocytogenes*, turkey meat, antimicrobial resistance

Received July 22, 2015; Revised August 21, 2015; Accepted August 31, 2015

Introduction

The prevalence of food-borne diseases has been increased in the last decades. *Listeria*, *Campylobacter*, and *Salmonella* species and *Escherichia coli* O157:H7 have been isolated from majority of food-borne outbreaks (Chemburu *et al.*, 2005). *Listeria* species cause listeric infections in a variety of animals including man. They are ubiquitous bacteria widely distributed in the natural environment and in vegetal foods (Low and Donachie, 1997).

The genus of *Listeria* comprises seven species that are *L. monocytogenes*, *L. grayi*, *L. innocua*, *L. ivanovii*, *L. welshimeri*, *L. murrayi* and *L. seeligeri*. The hemolytic species of *Listeria* such as *L. monocytogenes*, *L. seeligeri* and *L. ivanovii* cause infection in human (Lovett and Twedt, 1988). *L. monocytogenes* is considered one of the major foodborne pathogen in human and animals, though *L. ivanovii* and *L. seeligeri* are occasionally isolated from

human diseases (Lovett and Twedt, 1988). Listeriosis may cause meningitis, encephalitis, septicemia, abortion or gastroenteritis in children, pregnant women, the immune-compromised and the elderly (Lorber, 1990). Outbreaks and sporadic cases of listeriosis have been associated with various contaminated foods such as milk, raw meat, cheese, meat products, seafood products, and vegetables (Gomez *et al.*, 2014).

Listeria species are generally susceptible to a wide range of antimicrobials, but the first multiresistant *L. monocytogenes* strain has been isolated in 1988 (Zhang *et al.*, 2007). Since this year, antibiotic-resistant *L. monocytogenes* isolates have been recovered from food, environment, and human listeriosis cases (Zhang *et al.*, 2007). Currently, a β -lactam antibiotic (e.g., ampicillin or penicillin) combined with an aminoglycoside (e.g., gentamicin) is the reference therapy for human listeriosis, while the second choice of treatment is vancomycin, erythromycin and trimethoprim-sulfamethoxazole combination for pregnant women or patients allergic to β -lactams (Hof, 2004). The levels and type of resistance are affected by antibiotic use and regional differences. Thus, investigation and monitoring of the antibiotic susceptibility of *Listeria* species from

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different regions of the world is very important for public health (Wang *et al.*, 2013).

Turkey meat has an important place in poultry meat industry; however, there is little information on the prevalence and antimicrobial susceptibility of *Listeria* species in turkey meats. The aims of this study were to investigate the occurrence of *Listeria* species in turkey meats and to check the antimicrobial susceptibility of the isolated strains.

Materials and Methods

Sample collection

A total of 115 fresh turkey meat samples were randomly collected from the supermarkets, butchers and restaurants between January and September 2014 in Aksaray province, Turkey. All meat samples were immediately placed in sterile plastic bags, cooled on ice during transport to the laboratory, and analyzed on the same day for the detection of *Listeria* species.

Isolation and identification of *Listeria* species

Strain isolation and identification were made according to the ISO11290-1 method (1996). A 25 g of each meat sample was transferred to sterile plastic bags containing 225 mL of half-Fraser (HF) broth (Oxoid, UK) without supplements and homogenized using a stomacher (Stomacher 400, France) for 1 min. Subsequently, it was incubated 20°C for 1 h. Then, selective supplement SR-166 (Oxoid, UK) was added to HF broth and it was incubated 30°C for 23 h. After this step, 0.1 mL of the HF broth was transferred into 10 mL tubes containing Fraser Broth (Oxoid, UK) supplemented by Fraser selective supplement (SR156, Oxoid, UK) and were incubated at 37°C for 48 h. After enrichment, 0.1 mL of the enriched samples were cultured onto duplicate plates of Oxford agar (Oxoid, UK) supplemented with SR 140E (Oxoid, UK) and Palcam agar (Oxoid, UK) containing supplement SR 150E (Oxoid, UK) and incubated at 37°C for 48 h. The suspected colonies were identified by Gram staining, characteristic colony morphology, hemolytic activity, CAMP test, and biochemical tests. After identification, *Listeria* isolates were stored at -80°C in Brain Heart Infusion broth (Oxoid, UK) containing 15% of glycerol.

PCR assay

Bacterial strains were grown in Brain Heart Infusion Broth (Oxoid, UK) at 37°C for 24 h. After that DNA was extracted using the protocol provided in Promega Wizard

Genomic DNA purification Kit (Promega, USA).

The *Listeria* specific primer pairs were used in PCR for amplification of the *prs* gene of *Listeria* spp. (Doumith *et al.*, 2004). The sequence of forward primer was 5'-GCT-GAAGAGATTGCGAAAGAAG-3' and reverse primer was 5'-CAAAGAAACCTTGGATTGCGG-3'. These primers showed a 370 bp amplicon. The PCR mixture was prepared in a total volume of 50 µL containing 5 µL of 10 X PCR buffer, 1.5 mM MgCl₂, 250 µM each of the four dNTPs (Fermentas, Lithuania), 1.25 U of Taq DNA polymerase (Fermentas, Lithuania), 0.5 µM of each primer (IDT, USA), and 5 µL of template DNA. The amplifications were performed in a thermalcycler (Eppendorf, Mastercycler *gradient*, Germany) using the protocol reported by Doumith *et al.* (2004). The PCR product were showed by electrophoresis on a 2% agarose gel.

Antimicrobial susceptibility testing

Susceptibility to the following antibiotics was determined for all the isolates of *Listeria* by the standard disc diffusion method (National Committee for Clinical Laboratory Standards, 1988). Briefly, bacterial cultures were grown at 37°C for 48 h in tryptic soy broth (TSB, Oxoid, UK) and transferred to Mueller-Hinton agar (Oxoid, UK). Then, antimicrobial susceptibility test discs were applied and plates incubated at 37°C for 24 h. Discs containing the following antibiotics were spotted with a 3 cm interval: ampicillin (10 µg), cephalothin (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), chloramphenicol (30 µg), gentamicin (10 µg), levofloxacin (5 µg), moxifloxacin (5 µg), meticillin (5 µg), oxacillin (1 µg), penicillin G (10 µg), rifampicin (30 µg), trimethoprim-sulfamethoxazole (1.5-23.5 µg), tetracycline (30 µg) and vancomycin (30 µg).

Results and Discussion

A total of 47 *Listeria* spp. were isolated from 47 of 115 (40.9%) raw turkey meat samples. All *Listeria* isolates were confirmed as *Listeria* spp. by PCR assay. The isolates were distributed between *L. monocytogenes* (12 isolates), *L. innocua* (16 isolates), *L. grayi* (15 isolates) and *L. welshimeri* (4 isolates). In this study, approximately 41% of meat samples were contaminated with *Listeria* spp. The percentage of *Listeria* contamination in various poultry meat samples ranged from 8% to 99% in previous studies (Chen *et al.*, 2009; Fallah *et al.*, 2012; Lawrence and Gilmour, 1994; Osaili *et al.*, 2011; Pasavento *et al.*, 2010; Sakaridis *et al.*, 2011; Yücel *et al.*, 2005). Yücel *et al.*

al. (2005) reported that 23 (42.6%) *Listeria* spp. isolated from chicken meat samples in Turkey. Pasavento *et al.* (2010) isolated *Listeria* spp. in 21.6% of poultry meat samples in Italy. Additionally, *Listeria* spp. was identified in 134 of 401 poultry product in Iran by Fallah *et al.* (2012) and in 76 of 160 fresh broiler chicken samples in Jordan by Osaili *et al.* (2011). The presence of *Listeria* in raw meats could be the result of environmental contamination by food handlers or fecal contamination during evisceration (Fenlon *et al.*, 1996; Yücel *et al.*, 2005).

In the present work, the incidence of *L. monocytogenes* was found to be 10.43% in turkey meat samples. This contamination rate is similar to the rates of 12.5% and 12.7% from two previous studies (Bilir Ormancı *et al.*, 2008; Fallah *et al.*, 2012). In some previous studies, high incidences of *L. monocytogenes* were detected in raw turkey meat samples (Ojeniyi *et al.*, 2000; Wesley *et al.*, 2002; Wong *et al.*, 1990). In USA, Wesley *et al.* (2002) reported that approximately 38% of turkey meat samples were contaminated with *L. monocytogenes*. In another study, the prevalence of *L. monocytogenes* in raw turkey product was found as 17.4% in Denmark (Ojeniyi *et al.*, 2000). In Taiwan, Wong *et al.* (1990) isolated *L. monocytogenes* in 38% of turkey parts. On the other hand, the prevalence of *L. monocytogenes* in other works ranged from 9.4% to 38% in chicken meat samples (Fallah *et al.*, 2012; Osaili *et al.*, 2011; Pasavento *et al.*, 2010; Sakaridis *et al.*, 2011; Wang *et al.*, 2013; Yücel *et al.*, 2005). This difference in the prevalence rates could be because of the sizes and varieties of samples, the used methods, and the sampling times (Hansen *et al.*, 2006). Additionally, the presence of *L. monocytogenes* in raw meats threatens the public health and it may cause severe disease in children, pregnant women, the immune-compromised and the elderly (Lorber, 1990).

L. innocua was isolated from 14.8% of raw turkey meat samples. It was the predominant species among *Listeria* spp. isolated from turkey meats in our study. Other studies indicated that *L. innocua* was the most common species in poultry meats (Fallah *et al.*, 2012; Osaili *et al.*, 2011; Pasavento *et al.*, 2010; Yücel *et al.*, 2005). *L. innocua* and *L. monocytogenes* are closely related species and they are genetically very similar. It has been suggested that *L. innocua* is nonpathogenic bacterium, but it become a transferable reservoir of antibiotic resistance for *L. monocytogenes* (Bertrand *et al.*, 2005). It is interesting to note that 13% of isolates were identified as *L. grayi* in our work and it was the second common species in raw turkey meats.

The resistance to one or more antibiotics has been detected in *L. monocytogenes* isolates from foods, environment and human cases since the first strain with antimicrobial resistance was isolated in 1988 (Gomez *et al.*, 2014). In the last years, increasing number of antimicrobial-resistant *L. monocytogenes* isolates has been detected around the world. This indicates that *L. monocytogenes* rapidly obtains a wide range of antibiotic resistance genes (Lungu *et al.*, 2011). It is known that antimicrobial resistance in *L. monocytogenes* is mainly caused by mobile genetic elements which are self-transferable plasmids, conjugative transposons, and mobilizable plasmids of other bacterial species such as *Staphylococcus* spp. and *Enterococcus* spp. (Pourshaban *et al.*, 2002).

In this study, the resistance to 15 antibiotics of 47 strains of *Listeria* spp. isolated from raw turkey meats were investigated (Table 1). Six isolates (14.9%) were susceptible to all used antimicrobials. 55.3% (27) of *Listeria* spp. isolates were multi-resistant to at least 3 of the antimicrobial agent tested. The level of multi-resistance was higher in *L. monocytogenes* strains (66.7%) than in *L. innocua* (62.5%) and *L. grayi* (53.3%). These results represent that *Listeria* spp. particularly *L. monocytogenes* strains are slowly occurring resistant to antibiotics (Conter *et al.*, 2009).

In the present study, a high resistance of *L. monocytogenes*, *L. innocua*, *L. grayi*, and *L. welshimeri* to ampicillin and penicillin that are used to treatment of listeriosis was showed. Similarly, ampicillin and penicillin resistant strains were reported in various turkey and chicken meats (Ayaz and Erol, 2010; Bilir Ormancı *et al.*, 2008; Fallah *et al.*, 2012; Yücel *et al.*, 2005). On the other hand, the some researchers have announced a high susceptible of *L. monocytogenes* isolates to these antibiotics (Gomez *et al.*, 2014; Kovacevic *et al.*, 2013; Pasavento *et al.*, 2010; Sakaridis *et al.*, 2011; Wang *et al.*, 2013). In our work, 44.7% and 85.1% of the isolates was resistant to meticcillin and oxacillin, respectively. In Italy, Pasavento *et al.* (2010) found that a high number of *Listeria* spp. isolates from raw meats were resistant to meticcillin and oxacillin. *Listeria* species can either transfer or acquire meticcillin resistance's genes to *Enterococcus* spp. Besides, meticcillin is routinely used in treatment of infections of *Enterococcus* (Pasavento *et al.*, 2010).

In this study, all *L. monocytogenes* isolates were sensitive to fluoroquinolones (ciprofloxacin, levofloxacin, and moxifloxacin) and tetracycline, but *L. innocua* strains were highly resistant to these antibiotics. The tetracycline and ciprofloxacin sensitive *L. monocytogenes* isolates were

Table 1. Antimicrobial resistance patterns of *Listeria* spp. isolated from turkey meats

| Antimicrobial | <i>L. monocytogenes</i> | <i>L. innocua</i> | <i>L. welshimeri</i> | <i>L. grayi</i> |
|-------------------------------|-------------------------|-------------------|----------------------|-----------------|
| | No. (%) | No. (%) | No. (%) | No. (%) |
| Ampicillin | 9 (75) | 9 (56.2) | 3 (75) | 5 (33.3) |
| Cephalothin | 8 (66.7) | 10 (62.5) | 1 (25) | 12 (80) |
| Ciprofloxacin | 0 (-) | 4 (25) | 0 (-) | 3 (2) |
| Clindamycin | 2 (16.6) | 3 (18.8) | 1 (25) | 5 (33.3) |
| Chloramphenicol | 1 (8.3) | 3 (18.8) | 0 (-) | 0 (-) |
| Gentamicin | 1 (8.3) | 2 (12.5) | 0 (-) | 7 (46.7) |
| Levofloxacin | 0 (-) | 1 (6.3) | 0 (-) | 0 (-) |
| Moxifloxacin | 0 (-) | 6 (37.5) | 0 (-) | 4 (26.7) |
| Meticillin | 4 (33.3) | 4 (25) | 1 (25) | 12 (80) |
| Oxacillin | 9 (75) | 14 (87.5) | 3 (75) | 14 (93.3) |
| Penicillin G | 8 (66.7) | 0 (-) | 2 (50) | 11 (73.3) |
| Rifampicin | 1 (8.3) | 3 (18.8) | 0 (-) | 0 (-) |
| Trimethoprim-sulfamethoxazole | 6 (50) | 0 (-) | 0 (-) | 0 (-) |
| Tetracycline | 0 (-) | 2 (12.5) | 0 (-) | 0 (-) |
| Vancomycin | 0 (-) | 0 (-) | 0 (-) | 0 (-) |

reported in chicken meat samples by some authors (Bilir Ormancý *et al.*, 2008; Gomez *et al.*, 2014; Osaili *et al.*, 2011; Wang *et al.*, 2013; Yücel *et al.*, 2005). However, high antimicrobial resistance rate to fluoroquinolones and tetracycline was detected in meat samples in Iran by Fallah *et al.* (2012). The sensitivity of *L. monocytogenes* isolates to some antibiotics which mostly used to treat of listeriosis (rifampicin, gentamicin, and clindamycin) was in agreement with the papers of Pesavento *et al.* (2010) and Fallah *et al.* (2012). In addition, all our *Listeria* spp. strains were vancomycin susceptible and this drug is used as the last therapeutic options for treatment of human listeriosis. The percentage of chloramphenicol resistant strains of *L. monocytogenes* and *L. innocua* were 8.3% and 18.8%, respectively. Similar results have been reported in poultry products in Iran by Fallah *et al.* (2012). The resistance for this antibiotic could be due to the illegal use of it in some poultry farms (Fallah *et al.*, 2012). *Listeria* species displayed high resistance to cephalothin in this study. It is not surprising that *Listeria* spp. have been announced to be naturally resistant to cehalosporins (Troxler *et al.*, 2000).

The number of papers about antibiotic resistances of *Listeria* species has been increased in the last years. In 2005 Yücel *et al.*, in 2008 Bilir Ormancý *et al.*, and in 2010 Ayaz and Erol reported that all *L. monocytogenes* strains from turkey or chicken meats in Turkey were susceptible to gentamicin and chloramphenicol, but in this study we found resistance of 8.3% to gentamicin and 8.3% to chloramphenicol. Besides, same authors (Ayaz and Erol, 2010; Bilir Ormancý *et al.*, 2008; Yücel *et al.*, 2005) announced that the percentage of ampicillin resis-

tant *L. monocytogenes* was 66%, 73.9%, and 67.9%. However, in our work, 75% of the *L. monocytogenes* isolates was resistant to ampicillin. Results of our study comparing to the findings of others works in Turkey (Ayaz and Erol, 2010; Bilir Ormancý *et al.*, 2008; Yücel *et al.*, 2005) indicate that there is an increasing percentage of multiresistance in *Listeria* isolates in the last decade.

In conclusion, our results represent information about the high contamination status of raw turkey meats with *Listeria* spp. in Turkey. The isolates particularly *L. monocytogenes* are increasingly resistant to one or more antibiotics and may represent a potential risk for public health because these antibiotics are common used in treatment of listeriosis. The correct and controlled use of antibiotics in veterinary medicine is important in order to the emergence of resistant strains.

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