Turkish Journal of Veterinary & Animal Sciences

Volume 32 | Number 2 Article 12

1-1-2008

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Turk. J. Vet. Anim. Sci.
2008; 32(2): 131-135

Short Communication

Prevalence and Antibiotic Resistance of *Listeria* Spp. Isolated from Ready-to-Eat Foods in Ankara*

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Received: 19.07.2007

Abstract: In this study the presence of *Listeria* spp. is tested in 100 ready-to-eat food samples purchased from different stores and traditional food shops in the province of Ankara. The tested materials were 20 each of the following: mayonnaise based salad, kadınbudu köfte (fried meatball), fried liver, rice stuffed mussel, and green salad. Microbiological analyzes showed that 13 of 100 salad samples (13%) were contaminated with *Listeria* spp. while 10 of 100 salad samples (10%) were contaminated with *Listeria monocytogenes*. Among these, 4 of 20 mayonnaise based salad samples (20%), 1 of 20 fried liver (5%), 1 of 20 rice stuffed mussel (5%), 1 of 20 kadınbudu köfte samples (5%), and 6 of 20 green salad samples (30%) were positive for *Listeria* spp. All isolated and identified strains were susceptible to chloramphenicol, trimethoprim, tetracycline, and tobramycin while they were resistant to rifampine.

Key Words: Ready-to-eat food, *Listeria* spp., *Listeria monocytogenes*, antibiotic resistance

Ankara'da Bazı Hazır Gıdalarda *Listeria* Türlerinin Varlığı ve Antibiyotik Dirençliliği

Özet: Bu çalışmada, Ankara'nın farklı market ve pazarlarından satın alınan 100 adet tüketime hazır gıda örneğinde *Listeria* türlerinin varlığı belirlenmiştir. Hazır gıda örneklerini oluşturan, rus salatası, kadınbudu köfte, arnavut ciğeri, midye dolma ve yeşil salata örneklerinden 20'şer adet alınmıştır. Mikrobiyolojik analizlerde 100 salata örneğinin 13'ünün (%13) *Listeria* türleriyle kontamine olduğu, 100 örneğin 10'unun (%10) ise *Listeria monocytogenes*' le kontamine olduğu belirlenmiştir. Bununla birlikte, 20 rus salatası örneğinin 4'ü (%20), 20 arnavut ciğeri örneğinin 1'i (%5), 20 kadınbudu köfte örneğinin 1'i (%5), 20 midye dolma örneğinin 1'i (%5) ve 20 salata örneğinin 6'sı (%30) farklı *Listeria* türleri yönünden pozitif bulunmuştur. İzole ve identifiye edilen tüm suşlar rifampine dirençli olarak saptanırken, kloramfenikol, trimetoprim, tetrasiklin ve tobramisine duyarlı oldukları belirlenmiştir.

Anahtar Sözcükler: Tüketime hazır gıda, *Listeria* spp., *Listeria monocytogenes*, antibiyotik dirençlilik

Ready-to-eat (RTE) food types (red meat, poultry, seafood, and vegetables) vary in countries according to their cultural and social backgrounds. RTE foods, with large variety, widespread availability, no cooking time, and cheaper prices compared to the others, have a very high demand among people. Although it has potential advantages, it may also pose a potential threat for public health (1,2). There is a high health risk caused by *L. monocytogenes* in RTE foods. Major outbreaks of listeriosis, with high morbidity and mortality (25%-30% overall), have been caused by a variety of foods, including soft cheeses, delicatessen meats, and vegetable products (3). Studies performed worldwide have reported the microbiological quality of green salads and some traditional RTE foods (1,2). European Community (EC) and United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) have issued a zero tolerance policy for *L. monocytogenes* in RTE foods (4,5). Antibiotic resistance of *L. monocytogenes* was first reported in 1988 (6). Generally, *Listeria* spp. and *L. monocytogenes* are susceptible to a wide variety of antibiotics except cephalosporin and phosphomycin (7). Due to insufficient information related to the incidence of *Listeria* spp. in RTE foods in Turkey, this study aims to

* The abstract of this research was published in the proceedings of the 3rd Symposium of Agriculture, Veterinary, Forestry and Biotechnology. Sanski Most, September 28th-30th, Bosnia and Herzegovina, 2005.

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determine *Listeria* spp. contamination in some traditional RTE foods and to find out the antibiotic resistance of these micro-organisms.

A total of 100 samples, 20 each of the following: mayonnaise based salad, kadınbudu köfte (fried meatball), fried liver, rice stuffed mussel, and green salad samples collected from different stores and traditional food shops (4-12 °C) in Ankara between April and September of 2004, were tested for the presence of *Listeria* spp. Ingredients and preparation methods of the foods included in the present study are detailed in Table. The samples were taken aseptically and transported to the laboratory under cold chain and tested on the same day for the presence of *Listeria* spp. using the USDA-FSIS method (8). Samples (25 g) were taken and placed in a sterile plastic bag containing 225 ml of Vermont Medium Modified *Listeria* Enrichment Broth (UVM Difco 0223) and homogenized by a stomacher approximately 2 min and incubated at 30 °C for 20-24 h. To 9 ml of Fraser Broth (Difco 0219), 1 ml portions of primary enrichments were transferred and incubated at 35 °C for 24-48 h. Following the enrichment procedure, a loop of homogenate was streaked onto Oxford-*Listeria* Selective Agar (MOX Difco 0225-0218) and the plates were incubated at 35 °C for 48 h. In order to confirm, 5 typical black coloured colonies were selected and streaked onto Trypticase Soy Yeast Extract Agar to purify (TSYEA, Difco 370) and identified by gram stain, catalase, oxidase, motility, β-haemolysis, CAMP, mannitol, L-rhamnose, D-xylose, salicine, dulcit, MR-VP, and nitrate reduction tests (9). The isolate, which was confirmed as *Listeria* spp. in classic culture technique, was identified with Oxoid Microbat *Listeria* 12 L Rapid Identification System® (10) according to the instructions of the manufacturer. All *Listeria* spp. isolates were tested by the standard disc diffusion method (11). Discs containing the following antibiotics were spotted with a 3 cm interval chloramphenicol (30 mg) (Oxoid CT 013), clindamycin (2 mg) (Oxoid CT 064), trimethoprim (5 mg) (Oxoid CT 076), gentamicin (10 mg) (Oxoid CT 024), tetracycline (30 mg) (Oxoid CT 054), rifampine (5 mg) (Oxoid CT 207), and tobramycin (30 mg) (Oxoid CT 056). The isolates were cultured in Trypticase-Soy Broth (TSB) supplemented with 0.6% yeast extract, and transferred to Mueller–Hinton Agar (Oxoid CM 337). The plates were incubated at 37 °C for 48 h.

The results of the present study are presented in Figure. Microbiological analyzes showed that 13 of 100 samples (13%) were contaminated with *Listeria* spp. Among these, 4 of 20 mayonnaise based salad samples

Table. Ingredients and preparation methods of foods sampled for *Listeria* spp.

Samples	Ingredients	Preparation	Environmental Conditions at retail level
Mayonnaise based salad	Boiled potatoes, carrots, boiled peas, mayonnaise	Boiled potatoes and carrots are cubed and mixed with boiled peas and mayonnaise	Presented for sale in fridge* temperature or ambient* temperature
Vegetable salads	Lettuce, tomatoes, cucumber, parsley, green peppers, salt, lemon and oil	Ingredients are mixed in a bowl	Presented for sale in fridge* temperature or ambient* temperature
Kadınbudu köfte	Minced beef, olive oil, chopped onions, rice, parsley, garlic and spices	Ingredients are mixed in a bowl and formed by hand, Fried in vegetable oils	Presented for sale in fridge* temperature or ambient* temperature
Fried liver	Bovine liver and spices	Liver is cubed and mixed with spices Fried in vegetable oils	Presented for sale in fridge* temperature or ambient* temperature
Rice stuffed mussel	Mussel meat, rice, lemon and spices	Boiled mussel and stuffed with cooked rice served with lemon juice	Presented for sale in fridge* temperature or ambient* temperature

* fridge temperature (4-12 °C) and ambient temperature (20-25 °C)

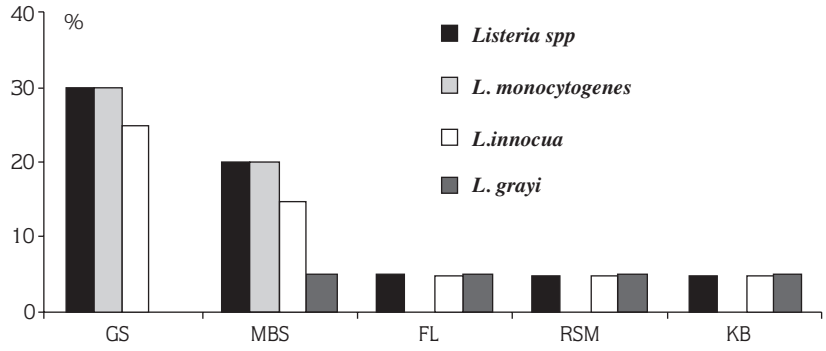

Figure. Prevalance of *Listeria* spp. samples in RTE foods.
GS: Green Salad, MBS: Mayonnaise Based Salad, FL: Fried Liver, RSM: Rice Stuffed Mussel, KB: Kadınbudu Köfte.

(20%), 1 of 20 rice stuffed mussel samples (5%), 1 of 20 kadınbudu köfte samples (5%), 1 of 20 fried liver samples (%5), and 6 of 20 green salad samples (30%) were positive for *Listeria* spp. *L. innocua* was isolated from 1 of 4 positive mayonnaise based salad samples while *L. grayi* was found in one of the other positive mayonnaise based salad. Both *L. innocua* and *L. monocytogenes* were isolated from 2 of 4 positive mayonnaise based salad samples. One rice stuffed mussel samples; one fried liver samples, and one kadınbudu köfte samples were contaminated with *L. innocua* and *L. grayi*. One of 6 positive green salad samples was contaminated with *L. monocytogenes* while the others were contaminated with both *L. innocua* and *L. monocytogenes*. The predominant species of the positive samples was *L. innocua* and followed by *L. monocytogenes* and *L. grayi* (Figure). In some cases, *L. innocua* was isolated together with *L. monocytogenes* or *L. grayi* from the same samples. Besides, in this study the results of *L. monocytogenes* isolates, which were already isolated and identified by classical culture technique, were confirmed by Oxoid Microbak 12 L Rapid Identification System.

Antibiotic resistance evaluation of all *Listeria* spp. by zone diameter around the antibiotic discs showed that all isolates were sensitive to chloramphenicol, trimethoprim, tetracycline and tobramycin but all were resistant to rifampine. *L. innocua* isolated from fried liver, *L. monocytogenes* isolated from salad samples and *L. grayi* isolated from mayonnaise based salad were found resistant to clindamycine whereas *L. innocua* and *L. monocytogenes* isolated from salad samples were found resistant to gentamicine.

Due to consumer trends, public demand is increasing for RTE foods, which need no additional preparation or cooking before consumption. Common consumption of RTE foods poses a potential risk for public health. Lack of adequate food safety issues during production, storage, serving, and cross contaminations are the most important factors contaminating RTE foods (1). In the present study, some traditional RTE foods were collected from retail stores in Ankara and were analysed for the presence of *Listeria* spp. Results indicated that contamination levels of *Listeria* spp. (13%) and *L. monocytogenes* (10%) for 5 different RTE food samples were above the microbiological criteria of US Department of Agriculture Food Safety and Inspection Services (FSIS) and European Community (EC), both of which have "zero tolerance" policy (4,5). Results are in agreement with Monge and Arias-Echandi (12) who reported *Listeria* spp. contamination in 32% of fresh green salad samples, but they found *L. welshimeri* along with *L. monocytogenes* in samples. However, results of the present study differ from those of Kaneko et al. (2) who found *L. welshimeri* and Soriano et al. (13) who isolated and identified *L. ivanovii* and *L. Seeligeri*. In the present study, the incidence of *L. monocytogenes* was 30% in green salads. However, Monge and Arias-Echandi (12) reported 20% and Soriano et al. (13) 10% *L. monocytogenes* contamination in green salads whereas Kaneko et al. (2) did not detect any *L. monocytogenes* and Gombas et al. (14) detected *L. monocytogenes* in 4.7% of the packed seafood salad samples. In addition, the high incidence rate of *L. monocytogenes* in green salads (30%) found in this study might be explained by the use of contaminated raw materials, insufficient hygienic conditions, mixing by hand

during preparation, inadequate heat treatments, and extended storage duration. Nørrung et al. (15) found *Listeria* spp. (5%) and *L. monocytogenes* (2.9%) from RTE heat-treated meat products whereas Soriano et al. (13) did not detect any *L. monocytogenes* in these types of foods. The findings of the present study indicated 5% *Listeria* spp. contamination in heat-treated fried liver, kadınbudu köfte, and rice stuffed mussel, which is in agreement with those of Nørrung et al. (15). However *L. monocytogenes* prevalance rates found in this study are different from those reported by Nørrung et al. (15) but in agreement with the results obtained by Soriano et al. (13). This can be explained by different forms of products, differences in food production and handling practices, use of poultry, red meat, cheese, or vegetables that may possibly contaminated with *L. monocytogenes* during preparation (16) and different heating and cooking procedures used. Moreover, the incidence of *Listeria* spp. contamination in this study is lower than that obtained by Hartemink and Georgsson (17), who found 32% of mayonnaise based seafood salads contaminated. This high contamination rate indicated by these authors most likely come from the seafood components, lack of hygienic conditions and recontamination during preparation and extended storage duration of salad. On the other hand, results indicating the presence of *Listeria* spp. contamination in mayonnaise based salads in this study are in line with the results obtained by Nørrung et al. (15), who analysed the same types of foods stored at the same temperatures. As the critical limit maintaining microbial safety of mayonnaise against *L. monocytogenes* is reported to be pH < 4.0 (3), contamination could still occur because of the other components of salad and/or from the improper hygienic conditions during preparation.

The inappropriate use of antibiotics may contribute to the development of resistance in foods. The findings of Franco Abuin et al. (18) were similar in determining the antibiotic susceptibilities of *Listeria* spp. isolated from RTE foods. *L. monocytogenes*, an important foodborne pathogen (16), found to be resistant to more than one antimicrobial agent (rifampin and gentamicin) in this study. Similarly, Facinelli et al. (19) indicated that *L. monocytogenes* isolated from chicken and turkey frankfurter samples were resistant to gentamicin and rifampin. Results of this study agree with those of Franco Abuin et al. (18) who reported that *L. monocytogenes* were resistant to tetracycline. Although Facinelli et al. (19) reported high resistance to both tetracycline and chloramphenicol, no resistance was observed to chloramphenicol for any *Listeria* spp. during this study. *L. grayi* isolated from mayonnaise based salads displayed resistance to clindamycine.

In conclusion, it is suggested that rigid policies, such as "zero tolerance", applied for *L. monocytogenes* in RTE foods in most countries (4,5) must be adapted into microbiological criteria of Turkish Food Codex (20). RTE foods could pose an important public health risk if contaminated with *L. monocytogenes* during preparation under poor conditions such as poor environmental and personal hygiene and lack of sanitary facilities. Thus we suggest that preventative measures including the implementation of good hygiene practise and good manufacturing practise should be applied during preparation. In addition, ensuring cold chain during storage, preventing cross contaminations, thorough washing of raw materials, and consuming RTE foods in 1 or 2 days are important factors to limit the risk of listeriosis.

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