International Food Research Journal 24(2): 881-887 (April 2017)
Journal homepage: *http://www.ifrj.upm.edu.my*

Isolation and identification of *Listeria* spp. in chicken carcasses marketed in northeast of Iran

[1]Zeinali, T., [2*]Jamshidi, A., [3]Bassami, M. and [4]Rad, M.

[1]*Faculty of Health, Birjand University of Medical Sciences, Birjand, Iran*
[2]*Department of Food Hygiene, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*
[3]*Department of clinical sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*
[4]*Department of Pathobiology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran*

Article history

Received: 20 November 2015
Received in revised form: 4 April 2016
Accepted: 15 April 2016

Keywords

Listeria monocytogenes
Antibiotic resistance
Chicken

Abstract

The aim of the present study was to evaluate the contamination of chicken carcasses with *Listeria* spp. Also, the antibiotic susceptibility of the *Listeria monocytogenes* isolates were investigated. In this study, 200 fresh chicken carcasses were examined for the presence of *Listeria* spp. Presumptive isolated *Listeria* colonies were confirmed by m-PCR. From 200 fresh chicken carcasses samples which were collected randomly from different supermarkets and butcheries, 80 samples (40%) were detected as contaminate with *Listeria* spp. and 18% of the isolates identified as Listeria monocytogenes *(L. monocytogenes)* using multiplex PCR assay. Conventional methods were used to differentiate other species of the Listeria genus. The results showed the most prevalent isolates is *L. monocytogenes* (18%). Other isolates were detected as *Listeria innocua* (11.5%), *Listeria grayi* subspp. *murrayi* (8%), *Listeria gray*i subspp. *grayi* (1.5%) and *Listeria welshimeri* (1%). The Majority of the isolates had multidrug resistance to commonly used antibiotics. Most of them were resistant to erythromycin (52.77%), followed by Tetracycline (44.44%), Clindamycin (41.66%), and Trimethoprim (25%). Some of them showed resistance to chloramphenicol (16.66%). The results indicate the noticeable contamination of fresh chicken carcasses with *Listeria* spp. Resistance of the *L. monocytogenes* isolates to antimicrobials commonly used to treat human listeriosis, which could be a potential health hazard for consumers.

Introduction

The genus Listeria consists of a group of Gram-positive bacteria with low G + C content. *Listeria* species are gram positive, non-spore forming bacilli found in a variety of food and environment. The genus *Listeria* contains eight species: *L. monocytogenes, L. innocua, L. welshimeri, L. seeligeri, L. ivanovii, L. grayi, L. marthii* (Graves *et al*., 2010) and *L. rocourtiae* (Leclercq *et al*., 2009). Among these different species, only *L. monocytogenes* is a human pathogen and *L. ivanovii* is an animal pathogen, which is rarely pathogenic in human (Warriner and Namvar, 2009). *Listeria* species are recognized as foodborne organisms, because of their ability to grow across a broad range of temperatures, withstanding osmotic stress and survival under mild preservation techniques (Warriner and Namvar, 2009). *L. monocytogenes* causes a severe foodborne disease with a high mortality rate (20%) in humans which is associated with consumption of contaminated dairy products, raw vegetables, under-cooked meat, seafood and poultry products (Painter and Slutsker, 2007; Todd and Notermans, 2011). Due to the ability of multiplication of Listeria at refrigerator temperature, it could not be act as a safe preservation technique for the contaminated products (Warriner and Namvar, 2009). *Listeria* spp. can contaminate Poultry either environmentally or from healthy carrier birds during breeding in the farm (Skovgaard and Morgen, 1988). Improper cleaning and disinfecting of environment and equipments in poultry abattoir and processing plant; and also mishandling of the products may lead to *Listeria* contamination of poultry carcasses (Loura *et al*., 2005).

Nowadays, the excessive use of antibiotics has led to the emergence of antibiotic-resistant bacteria (Charpentier and Courvalin, 1999). Although, *L. monocytogenes* is usually susceptible to wide range of antimicrobial agents, but several studies have reported

*Corresponding author.
Email: *ajamshid@um.ac.ir*
Tel: +985138805607

multidrug resistance of this species (Charpentier and Courvalin, 1999; Rahimi *et al*., 2012). In recent decades, poultry farmers use antimicrobial agents during poultry breeding to reduce the risk of infectious disease, it may lead to dissemination of antimicrobial-resistant bacteria including resistant strains of *Listeria* in the environment (Filiousis *et al*. 2009). Some commonly used antibiotics in local poultry farms are Felorfenicol, Doxycycline, Linco-Spectin, Tylosin and Thiamolin. The transmission of the resistant strains to human via contaminated poultry products may has a public health consequence (Filiousis et al. 2009). The aim of the present study was to determine the occurrence of *Listeria* spp. in fresh chicken carcasses in northeast of Iran and the resistance profile of *L. monocytogenes* to selected antibiotics.

Materials and Methods

Sampling

A total of 200 fresh chicken carcasses were collected randomly from different supermarkets and butcheries in Mashhad, from August to December, 2013. Each Chicken carcass was placed in a sterile, large plastic bag with 250 ml sterile distilled water and massaged inside the bag for 1 min. The samples were immediately transported to the laboratory inside a portable ice-chest and bacterial analyses started within 1-4 hours.

Isolation and identification of bacteria

After filtration with sterilized cheese cloth and centrifugation at 3000 rpm for 10 minutes of each rinsed fluid, the supernatant fluid was removed. The pellets obtained by centrifugation were unified and resuspended in 9 ml of Listeria enrichment broth (LEB, Merck, Germany) containing 15 mg/L acriflavin, 40 mg/L nalidixic acid and 50 mg/L cycloheximide. All samples were incubated at 30°C for 48 h. After incubation, 0.1 ml of the enriched culture was spread on Oxford agar plate supplemented with Natamycin 25 mg/L, Colistinsulphate 20 mg/L, Acriflavine 5 mg/L, Cefotetan 2 mg/L, and Fosomycin 10 mg/L (Listeria Selectival-SV33 Series-Mast Diagnostic, Germany) which was incubated at 30°C for 48 h (Hitchins and Jinneman, 2013). Colonies that hydrolyzed aesculin were streaked onto another Oxford agar plate and incubated at 30°C for 24 h. Gram positive bacilli that were catalase positive and displayed tumbling motility at room temperature and umbrella motility at 30°C were considered for DNA extraction (Hitchins and Jinneman, 2013).

Duplex-PCR detection of Listeria *genus and* Listeria monocytogenes

In order to extract DNA, typical suspected colonies were harvested and then suspended in 250 µl of deionized and sterilized distilled water. Microtubes containing bacterial suspension were incubated at 100°C for 10 min, and then centrifuged at 14000 rpm for 15 minutes. The supernatants were transferred to sterile nuclease free microtubes and freezed at -18°C until use (Bansal *et al*., 1996).

Two pairs of primers were used for Confirmation of *Listeria* and *Listeria monocytogenes* using duplex PCR assay. The prs primers (Doumith *et al*., 2004; F: 5' GCTGAAGAGATTGCGAAAGAAG 3'; R: 5' CAAAGAAACCTTGGATTTGCGG 3') are specific for the putative phosphoribosil pyrophosphate synthetase gene (370 bp) of *Listeria* spp. and the LM lip1 primers (Wernars *et al*., 1992; F: 5' GATACAGAAACATCGGTTGGC 3'; R: 5' GTGTAATCT TGATGCCATCAG 3') are specific for the *prfA* gene (274 bp) of *Listeria monocytogenes.*

Amplification of bacterial DNA was performed using Cinna-Gene PCR Mastermix (25 µl volumes) (Sinaclon, Iran). Every reaction contained 0.7 and 1 µl of prs and LM lip1 primers, and 2.5 µl of the DNA template. PCR reaction was performed in a thermal cycler (Techne, Germany). The PCR condition was carried out as follows: 5 min at 94°C for initial denaturation, followed by 35 cycles of 94°C for 45 seconds, 54°C for 30 seconds, and 72°C for 1 min and a final extension of 72°C for 10 min (Jami *et al.* 2010). *Listeria monocytogenes* (ATCC 7644) was used as a positive control and deionized distilled water as negative control.

Gel electrophoresis

The amplified products were visualized by standard gel electrophoresis using 3 µl of the final reaction mixture with 1 µl of loading dye (Cinna-gene, Sinaclon, Iran) on 1.5% agarose gel in TAE buffer containing 1 µg ml^{-1} ethidium bromide (Gibco, UK) for 45 min at 100 V. A 100 base-pare (bp) DNA ladder molecular weight marker (Fermentas, UK.) was included in each electrophoretic run to allow identification of the amplified products. PCR products were visualized under UV illumination and catalogued with a gel documentation system.

Biochemical identification of other Listeria spp.

Suspected colonies which were confirmed as *Listeria* spp. but not as *Listeria monocytogenes*, in d-PCR assay, were differentiated by the following biochemical tests: β-haemolytic activity, nitrate reduction, and acid production from L-rhamnose,

D-xylose, and, D-manitol (Roberts and Greenwood, 2003). All the isolates identified as *L. monocytogenes* were confirmed by PCR assay.

Antimicrobial susceptibility test

The antimicrobial susceptibility of *Listeria monocytogenes* isolates were examined by use of disc-diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2013) on Mueller-Hinton agar (Merck, Darmstadt, Germany). The following antimicrobial discs were used: ampicillin (10 µg), tetetracyclin (30 µg), erythromycin (15 µg), ciprofloxacin (5 µg), clindamycin (2 µg), penicillin (10 U/IE), chloramphenicol (30 µg), gentamycin (10 µg), vancomycin (30 µg), trimethoprim (5 µg) and rifampin (5 µg). The diameter of growth inhibition zone around each antimicrobial disc was measured after an incubation period of 24 h at 37°C. The results were interpreted according to the Clinical and Laboratory Standards Institute (2013) recommendations, isolates categorized in 3 groups: sensitive, intermediate and resistant. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 were used as reference strains.

Results

Occurrence of Listeria *spp. in chicken carcasses*

In the present study, a total of 200 samples of fresh chicken carcasses were examined for *Listeria* spp. which were isolated from 80 out of 200 (40%) samples. The most *Listeria* isolates determined as *L. monocytogenes* (36 out of 80). Figure 1 shows the m-PCR detection of *Listeria* spp. and *L. monocytogenes*. The second and the third major isolates were *Listeria innocua* and (23 out of 80) *Listeria grayi* subspp. *murrayi* (16 out of 80), respectively. Only 3 and 2 isolates were identified as *Listeria grayi* subspp. *grayi* and *Listeria welshimeri*, respectively.

Antimicrobial susceptibility of L. monocytogenes

Twelve isolates were sensitive to all antimicrobial agents. All of the *L. monocytogenes* isolates were sensitive to Ampicillin and Vancomycin. Overall, 21 of 36 *L. monocytogenes* isolates (58.33%) were resistant to more than one antimicrobial agent. Four isolates were resistant to two antibiotics, but some of the isolates (25%) showed resistance to more than four antimicrobial agents and this defined as multi-drug resistance. Resistance to erythromycin was the most common finding (52.77%) and one isolate had intermediate resistance, followed by resistance to Tetracycline (44.44%), Clindamycin (41.66%), and

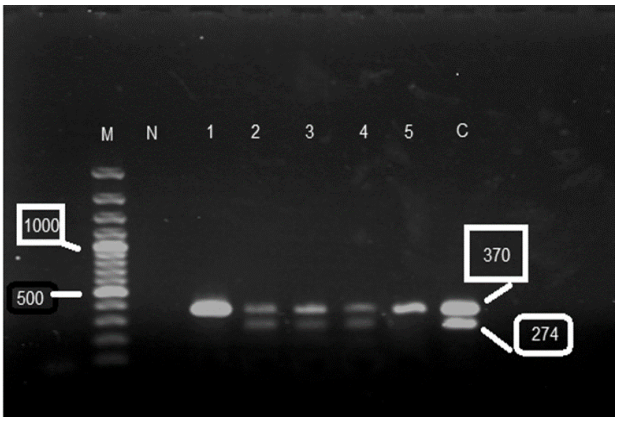

Figure 1. m-PCR detection of *L. monocytogenes*. Lane M: DNA Size Marker (100bp plus); Lane N: Negative Control; Lane C: Positive control; Lane 1 and 5: Positive samples for *Listeria* spp. non-monocytogenes; Lane 2, 3 and 4: Positive samples for *Listeria monocytogenes*

Trimethoprim (25%). Resistance to Gentamycin and Chloramphenicol were observed in six (16.66%) isolates. Five isolates were resistant to Ciprofloxacin and Rifampin. Seven isolates showed intermediate resistance to Tetracycline. 4 isolates had intermediate resistance to Trimethoprim, Clindamycin, and Rifampin. Intermediate resistance to Ciprofloxacin observed in 3 isolates. Only two isolates were resistant to Penicillin.

Discussion

In the present study, *Listeria* spp. was detected in 40% (80) of fresh chicken carcasses in northeast of Iran. Fallah *et al*. (2012) reported the prevalence of *Listeria* from chicken carcasses about 40.7% in Iran. In another study by Jalali *et al*. (2004) *Listeria* spp. was isolated from 3 of 66 fresh poultry meat (Jalali *et al*., 2004). In other studies, the occurrence of Listeria spp. in various poultry products ranged from 8% to 99% (Lawrence and Gilmour 1994; Yücel *et al*., 2005; Chen *et al*., 2009; Pesavento *et al.*, 2010; Osaili *et al*., 2011; Sakaridis *et al.*, 2011).

In our study, *Listeria monocytogenes* was predominant among isolated *Listeria* spp. It was detected in 18% (36) of fresh chicken carcasses. Fallah *et al.* (2012) reported that, 52 out of 402 poultry product samples (12.9%) were positive for *L. monocytogenes*. Otherwise, it was detected in 14.1% of raw poultry products. In another study by Kosek-Paszkowska *et al.* (2005), 43 out of 70 samples of raw poultry meat were contaminated with *Listeria* spp. and 6 of them were defined as *L. monocytogenes*. In other studies, the rate of contamination with *L. monocytogenes* in raw poultry products was found to be 41% in Portugal (Antunes *et al*., 2002), 38.2% in Belgian markets (Uyttendaele *et al*., 1999), 38% in

northern Greece (Sakaridis *et al*., 2011), 34% in Sri Lanka (Ganasena *et al*.,1995), and 22% in the Nordic countries (Gudbjörnsdóttir *et al*., 2004); which are higher than the results of the present study. However, the reported rate of *L. monocytogenes* contamination in Italy (Pesavento *et al*., 2010), Turkey (Yücel *et al.*, 2005), Gauteng, South Africa (Nieropa *et al*., 2005) and Jordan (Osaili *et al*., 2011) are consistent with our results.

In our study, *Listeria innocua* was the second major species among isolated *Listeria* spp. It was detected in 28.75% (23 out of 200) of samples. In another study in Iran, 16.1% of raw poultry meat was contaminated with *L. innocua* (Fallah *et al*., 2012). The rate of contamination with this bacterium in Jalali *et al.* (2004) investigation was as 4.5%, and *L. innocua* was the predominant species, which is different from our results.

In the present study, *Listeria murrayi* (16 out of 200) was detected in 8% of samples. This species was not detected in other studies in Iran (Jalali *et al*., 2004; Fallah *et al*., 2012). Although, we didn't find any Listeria ivanovii in our samples but, Fallah *et al.* (2012) found it in 2 of 22 samples. Also, Jalali *et al.* (2004) didn't find this species in their study, which is in agreement with our results. The difference in the rate of contamination which has been reported in other studies may be due to the methods of sampling and bacterial isolation, and also different geographical aria of sampling.

Over the 36 *Listeria monocytogenes* isolates from fresh chicken carcasses, twelve isolates were sensitive to all antimicrobial agents. The acquisition of mobile genetic elements such as self-transferable and mobilizable plasmids; and conjugative transposons is the cause of emergence of antimicrobial resistance of *Listeria* spp. (Charpentier and Courvalin, 1999). In our study, all of the isolates were sensitive to Ampicillin and Vancomycin. Fallah *et al*. (2012) and Ayaz and Erol (2010) observed resistance to Ampicillin, but all of their isolates were sensitive to Vancomycin.

Resistance to erythromycin was the most common finding (52.77%), and one isolate had intermediate resistance to this antibiotic but, in a study by Fallah *et al.* (2012), this resistance was reported in 15.2% of the isolates. Resistance to erythromycin is conferred by a broad-host-range plasmid pAMb1 of Enterococcus faecalis, which could be transferred successfully by conjugation (Flamm *et al*., 1984). This plasmid is able to replicate in the new host and conjugative transferred between strains of *L. monocytogenes.* Plasmid pAMb1 was also compatible with two cryptic plasmids of *L. monocytogenes* (Flamm et al., 1984).

In our study, only 2 isolates were resistant to penicillin while in the report of other researchers, a high resistance to this agent was reported (Ayaz and Erol, 2010; Fallah *et al.*, 2012). In contrast, the other researchers (Davis and Jackson 2009; Sakaridis *et al.*, 2011; Alonso-Hernando *et al.*, 2012) reported a high susceptibility of *L. monocytogenes* to ampicillin and penicillin, which is in agreement with the results of present study. Dhanashree *et al.* (2003) in India reported that all the isolates of *L. monocytogenes* were susceptible to ampicillin and penicillin.

In this study, resistance to Tetracycline was 44.44% which was consistent with the other reports (Fallah *et al*., 2012; Dharmendra *et al.*, 2013). Diverse species of *Enterococcus* and *Streptococcus* harboring conjugative plasmids and transposons are also present at very high numbers in the digestive tract in humans and animals; where *L. monocytogenes* is frequently found (Khachatourarians, 1998). The results of high antimicrobial resistance rate to tetracycline in this study could be explained by the frequent use of this antibiotic in order to treatment of infection in Iranian poultry farms. Moreover, use of antibiotics including tetracycline in human may lead to discharge of contaminated waste water in the environment. Also, agricultural residual of antibiotics (e.g., as growth promoting and prophylactic agents in animals) could lead to the emergence of antibiotic resistance bacteria (Khachatourarians, 1998). This observation reinforces the notion that the intestinal tract represents an ecosystem most favorable to direct exchange of genetic information between these two bacterial genera (*Enterococcus-Streptococcus* and *Listeria*).

In our study resistance to Gentamycin and Chloramphenicol were observed in six (16.66%) isolates. Conter *et al.* (2009) indicated that *L. monocytogenes* isolated from food and environmental samples were highly sensitive to ampicillin and gentamicin. Moreover, resistance of some isolates to chloramphenicol could be due to the illegal use of this antimicrobial in some poultry farms. Plasmid pIP501 has a broad host range and confers resistance to chloramphenicol, macrolides, lincosamides, and streptogramins (Evans and Macrina, 1983).

Administration of ampicillin or penicillin G combined with an aminoglycoside as like as gentamicin is the standard therapy for human listeriosis. At the second line of therapy is combination of trimethoprim (TMP) and sulfamethoxazole (SMX) (Carpentier and Courvalin, 1999). The results of the present study indicate a high susceptibility of *L. monocytogenes* to ampicillin and penicillin, the drugs

of choice for treatment of listeriosis. Resistance to Trimethoprim was observed in 25% of the isolates. Moreover, 4 isolates had intermediate resistance to this agent. A 3.7-kb plasmid (pIP823) containing a gene *(dfrD)* coding for a high-level trimethoprim-resistant dihydrofolate reductase was correspondence to resistance to trimethoprim in *L. monocytogenes* (Charpentier and Courvalin, 1997). PIP823 is a broad host range plasmid, and conjugative mobilization of pIP823 was obtained by self-transferable plasmids between different species (Charpentier *et al*., 1999).

The presence of antimicrobial-resistant *Listeria* in chicken meat has an important public health implication, since listeriosis is transmitted primarily via contaminated foods, especially in developing countries, where there is frequent and uncontrolled use of antibiotics. Further study on genotypic characterization of all *Listeria* isolates by molecular techniques will be needed to determine whether antimicrobial resistance observed in this study is associated with particular genotypes. Therefore, the result of the present study indicates the prevalence of multi-drug resistant isolates of *L. monocytogenes* in poultry samples, and underlines the need for broad surveillance of their antibiotic resistance for selection of appropriate treatment, especially for those cases of food-borne listeriosis with severe or prolonged symptoms or in immunocompromised patients.

Conclusion

In conclusion, presences of multidrug resistance of *L. monocytogenes* in the chicken meat indicate the potential risk of infection with these bacteria, especially peoples consuming chicken meat barbecue, which is a popular meal in Northeast of Iran.

Acknowledgement

The authors would like to thanks veterinary College of Ferdowsi University of Mashhad for financial support of this study (Grant No. 3/28199).

References

Alonso-Hernando, A., Prieto, M., García-Fernández, C., Alonso-Calleja, C. and Capita, R. 2012. Increase over time in the prevalence of multiple antibiotic resistance among isolates of *Listeria monocytogenes* from poultry in Spain. Food Control 23: 37-41.

Antunes, P., Réu, C., Sousa, J.C., Pestana, N. and Peixe, L. 2002. Incidence and susceptibility to antimicrobial agents of *Listeria* spp. and *Listeria monocytogenes* isolated from poultry carcasses in Porto, Portugal. Journal of Food Protection 65(12): 1888-1893.

Ayaz, N.D. and Erol, I. 2010. Relation between serotype distribution and antibiotic resistance profiles of *Listeria monocytogenes* isolated from ground Turkey. Journal of Food Protection 73: 967-972.

Bansal, N. S., Mcdonell, F. H., Smith, A., Arnold, G. and Ibrahim, G. F. 1996. Multiplex PCR assay for the routine detection of *Listeria* in food. International Journal of Food Microbiology 33: 293–300.

Charpentier, E. and Courvalin, P. 1997. Emergence of the trimethoprim resistance gene dfrD in *Listeria monocytogenes* BM4293. Antimicrobial Agents and Chemotherapy 41: 1134-1136.

Charpentier, E., Gerbaud, G. and Courvalin, P. 1999. Conjugative mobilization of the rolling-circle plasmid pIP823 from *Listeria monocytogenes* BM4293 among Gram-positive and Gram-negative bacteria. Journal of Bacteriology 181: 3368-3374.

Charpentier, E. and Courvalin, P. 1999. Antimicrobial resistance in *Listeria* spp. Antimicrobial Agents and Chemotherapy 43: 2103-2108.

Chen, J., Zhang, X., Mei, L., Jiang, L. and Fang, W. 2009. Prevalence of Listeria in Chinese food products from 13 provinces between 2000 and 2007 and virulence characterization of *Listeria monocytogenes* isolates. Foodborne Pathogens and Disease 6: 7-14.

CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria isolated from Animal; approved standard- fourth Edition. CLSI document Vet01-A4. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.Vol. 33 No. 7

Conter, M., Paludi, D., Zanardi, E., Ghidini, S., Vergara, A. and Ianieri, A. 2009. Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*. International Journal of Food Microbiology 128: 497–500.

Davis, J.A. and Jackson, C.R. 2009. Comparative antimicrobial susceptibility of *Listeria monocytogenes, L. innocua,* and *L. welshimeri.* Microbial Drug Resistance 15: 27-32.

Dhanashree, B., Otta, S.K., Karunasagar, I., Goebel, W. and Karunasagar, I. 2003. Incidence of *Listeria* spp. in clinical and food samples in Mangalore, India. Food Microbiology 20: 447–453.

Doumith, M., Buchrieser, C., Glaser, P., Jaquet, C. and Martin, P. 2004. Differentiation of the major *Listeria monocytogenes* serovars by Multiplex PCR. Journal of Clinical Microbiology 42: 3819-3822.

Evans, R.P. and Macrina, F.L. 1983. Streptococcal R plasmid pIP501: endonuclease site map, resistance determinant location, and construction of novel derivatives. Journal of Bacteriology 154: 1347–1355.

Fallah, A.A., Saei-Dehkordi S. S., Rahnama M., Tahmasby H. and Mahzounieh M.R. 2012. Prevalence and antimicrobial resistance patterns of *Listeria* species isolated from poultry products marketed in Iran. Food Control 28: 327-332.

Filiousis, G., Johansson, A., Frey, J. and Perreten, V. 2009. Prevalence, genetic diversity and antimicrobial susceptibility of *Listeria monocytogenes* isolated from open-air food markets in Greece. Food Control 20:

314-317.

Flamm, R. K., Hinrichs, D. J. and Thomashow, M. F. 1984. Introduction of pAMb1 into Listeria monocytogenes by conjugation and homology between native *L. monocytogenes* plasmids. Infection and Immunity 44: 157–161.

Ganasena, D. K., Kodikara, C. P., Ganepola, K. and Widanapathirana, S. 1995. Occurrence of *Listeria monocytogenes* in food in Sri Lanka. Journal of National Science Council of Sri Lanka 23: 107-114.

Graves, L.M., Helsel, L.O., Steigerwalt, A.G., Morey, R.E., Daneshvar, M.I., Roof, S.E., Orsi, R.H., Fortes, E.D., Millilo, S.R., Den Bakker, H.C., Wiedmann, M., Swaminathan, B. and Sauders, B.D. 2010. *Listeria marthii* spp. isolated from the natural environment, Finger Lakes National Forest. International Journal of Systematic and Evolutionary Microbiology 60: 1280-1288.

Gudbjörnsdóttir, B., Suihko, M. L., Gustavsson, P., Thorkelsson, G., Salo, S., Sjöberg, A. M. et al. 2004. The incidence of *Listeria monocytogenes* in meat, poultry and seafood plants in the Nordic countries. Food Microbiology 21: 217-225.

Harakeh, S., Saleh, I., Zouhairi, O., Baydoun, E., Barbour, E. and Alwan, N. 2009. Antimicrobial resistance of *Listeria monocytogenes* isolated from dairy-based food products. Science of Total Environment 407: 4022–4027.

Hitchins, A.D. and Jinneman, K. 2013. Detection and Enumeration of Listeria monocytogenes in Foods. In Bacteriological Analytical Manual. Available online at: *http://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm071400.htm*)

Jalali, M. and Abedi, D. 2008. Prevalence of Listeria species in food products in Isfahan, Iran. International Journal of Food Microbiology 122: 336–340.

Jami, S., Jamshidi, A. and Khanzadi, S. 2010. The presence of *Listeria monocytogenes* in raw milk samples in Mashhad, Iran. Iranian Journal of Veterinary Research 11(4): 363-367.

Khachatourarians, G.G. 1998. Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. Canadian Medical Association Journal 159: 1129–1136.

Kosek-Paszkowska, K., Bania, J., Bystroń, J., Molenda, J. and Czerw, M. 2005. Occurrence of *listeria* spp. in raw poultry meat and poultry meat products. Bulletin of Veterinary Institute in Pulawy 49: 219-222.

Lawrence, L.M. and Gilmour, A. 1994. Incidence of *Listeria* spp. and *Listeria monocytogenes* in a poultry processing environment and in poultry products and their rapid confirmation by multiplex PCR. Applied and Environmental Microbiology 60: 4600-4604.

Leclercq, A., Clermont, D., Bizet, C., Grimont, P., Le Flèche-Matéos, A., Roche, S., Buchrieser, C., Cadet-Daniel, V., Le Monnier, A., Lecuit, M. and Allerberger, F. 2009. *Listeria rocourtiae* spp. International Journal of Systematic and Evolutionary Microbiology 60: 2210-2214.

Loura, C.A.C., Almeida, R.C.C. and Almeida, P.F. 2005. The incidence and level of *Listeria* spp. and *Listeria monocytogenes* contamination in processed poultry at a poultry processing plant. Journal of Food Safety 25: 19-29.

Nierop, W. V., Duse, A.G., Marais, E., Aithma, N., Thothobolo, N., Kassel, M., Stewart, R., Potgieter, A., Fernandes, B., Galpin, J.S. and Bloomfield, S.F. 2005. Contamination of chicken carcasses in Gauteng, South Africa, by *Salmonella, Listeria monocytogenes* and *Campylobacter.* International Journal of Food Microbiology 99: 1– 6.

Osaili, T. M., Alaboudi, A. R. and Nesiar, E. A. 2011. Prevalence of *Listeria* spp. and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw chicken and ready-to-eat chicken products in Jordan. Food Control 22: 586-590.

Painter, J. and Slutsker, L. 2007. Listeriosis in humans. In Ryser, E. T. and Marth, E. H. (Eds). *Listeria*, listeriosis, and food safety, p. 85-110. London: CRC Press.

Pesavento, G., Ducci, B., Nieri, D., Comodo, N. and Lo Nostro, A. 2010. A. Prevalence and antibiotic susceptibility of *Listeria* spp. isolated from raw meat and retail foods. Food Control 21: 708-713.

Rahimi, E., Ameri, M. and Momtaz, H. 2010. Prevalence and antimicrobial resistance of *Listeria* species isolated from milk and dairy products in Iran. Food Control 21: 1448–1452.

Roberts and Greenwood. 2003. chap. 6. In Downes F. P. and Ito, k. (Eds). Compendium of methods for microbiological examination of foods. American Public Health Association.

Sakaridis, I., Soultos, N., Iossifidou, E., Papa, A., Ambrosiadis, I. and Koidis, P. 2011. Prevalence and antimicrobial resistance of *Listeria monocytogenes* isolated in chicken slaughterhouses in northern Greece. Journal of Food Protection 74: 1017-1021.

Skovgaard, N. and Morgen, C. A. 1988. Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. International Journal of Food Microbiology 6: 229-242.

Soni, D.K., Singh, R.K., Singh, D.V. and Dubey, S.K. 2013. Characterization of *Listeria monocytogenes* isolated from Ganges water, human clinical and milk samples at Varanasi, India. Infection, Genetics and Evolution 14: 83–91.

Todd, E. C. D. and Notermans, S. 2011. Surveillance of listeriosis and its causative pathogen, *Listeria monocytogenes*. Food Control 22: 1484-1190.

Uyttendaele, M., De Troy, P. and Debevere, J. 1999. Incidence of *Salmonella, Campylobacter jejuni, Campylobacter coli,* and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. Journal of Food Protection 62(7): 735-740.

Warriner, K. and Namvar, A. 2009. What is the hysteria with *Listeria*? Trends in Food Science and Technology 20: 245-254.

Wernar, K., Heuvelman, K., Notermans, S., Domann, E., Leimeister-Wachter, M. and Chakraborty, T. 1992. Suitability of the *prfA* gene, which encodes a regulator

of virulence genes in *Listeria monocytogenes*, in the identification of pathogenic Listeria spp. Applied and Environmental Microbiology 58: 765–768.

Yücel, N., Çitak, S. and Önder, M. 2005. Prevalence and antibiotic resistance of *Listeria* species in meat products in Ankara, Turkey. Food Microbiology 22: 241-245.