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Detection of Antibiotic Resistant *Listeria* spp. in Beef Burgers Distributed in Ahvaz City, Iran

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Abstract

Background: *Listeria* spp. are able to be survive in many foods during frozen storage. One particular species, *Listeria monocytogenes*, is one of the most important food-borne pathogens globally. The antimicrobial resistance of pathogenic microorganisms is a worldwide public health concern because of increasing global trade and travel.

Objectives: The aim of this study was to evaluate the occurrence and antibiotic resistance of *Listeria* spp. in the Iranian beef burgers distributed in Ahvaz city.

Materials and Methods: During a five-month period, 150 frozen burgers were purchased from local markets in Ahvaz city, and tested for presence of *Listeria* spp. The experimental procedure consisted of a one-step enrichment in Listeria enrichment broth, followed by plating on Oxford agar. Suspected colonies were subjected to subsequent biochemical tests and a polymerase chain reaction (PCR) assay. The susceptibility of the isolates to various antibiotics was investigated using the Kirby-Bauer disk diffusion method, and the results were analyzed via the chi-square test and Fisher's exact test using SPSS 16.0 software.

Results: Out of 150 samples, only two were contaminated with Listeria innocua, and the statistical analysis showed no significant differences in the prevalence of Listeria between companies ($P > 0.05$). One of the isolates was resistant to tetracycline and the other to co-trimoxazole. Both of the isolates showed an intermediate susceptibility to chloramphenicol; however, they were sensitive to the other tested antibiotics.

Conclusions: *L. innocua* is not a pathogen, but the presence of the bacterium could be an indicator of probable contamination with *L. monocytogenes*. Moreover, there is a potential risk to public health from the consumption of raw or undercooked burgers, which may increase the possibility of the acquisition of resistance to antibiotics.

Keywords: Antibiotic Resistance, Meat Products, Listeria, Polymerase Chain Reaction, PCR

1. Background

Listeria monocytogenes, the most important species of Listeria, is commonly present in food, water, feed, soil, and sewage. This food-borne pathogen causes acute complications such as septicemia, meningitis, and encephalitis in humans, especially infants, pregnant women, elderly people, and immune-compromised individuals (1-4). Listeria can grow at low temperatures, with an optimum pH requirement of 5.4 - 9.6 (5, 6).

The antimicrobial resistance of pathogenic microorganisms is a worldwide public health concern because of increasing global trade and travel (7). However, *L. monocytogenes* is naturally susceptible to various antibiotics targeting Gram-positive bacteria (8), and it has been indicated that clinical strains of *L. monocytogenes* are sensitive to a wide range of antibiotics. Typically, invasive infections have been treated with a combination of ampicillin or amoxicillin and gentamicin (9). Considering the increasing number of new antibiotic resistant strains of *L. monocytogenes* reported worldwide, it seems that this bacterium likely obtains various antibiotic resistance genes by horizontal gene transfer, some of which may come from the commensal microorganisms found in food (10).

According to the high mortality rate of *L. monocytogenes* (about 30%), the presence of this bacterium in food is considered to be a major health problem (11). Moreover, a wide range of food types have been implicated in its transmission, including meat, dairy, fish, and vegetable products. The occurrence of *Listeria* spp. (including *L. monocytogenes*) in meat and raw meat products has been investigated in several countries (12-15). To the best of our knowledge, few studies regarding the prevalence and antimicrobial susceptibility of Listeria in foodstuffs in Iran have been documented (3-5, 16). Therefore, the present study was conducted to determine the occurrence and antimicrobial resistance of *Listeria* spp. isolated from Iranian beef burgers distributed in Ahvaz city.

2. Objectives

The present study was conducted to evaluate the occurrence and antibiotic resistance of *Listeria* spp. in Iranian beef burgers distributed in Ahvaz city.

3. Materials and Methods

3.1. Sample Collection

In this cross-sectional survey, during a five-month period (February - June of 2014), a total of 150 frozen beef burger samples, made by 10 Iranian production companies located in different provinces, were purchased from local markets in Ahvaz. The burger boxes were checked to be completely frozen and within the shelf-life periods. The samples were put in a cold box and transferred to the laboratory. After defrosting, they were microbiologically analyzed on the same day.

3.2. Microbial Culture

In the first step, the samples were analyzed for the detection of *Listeria* spp. using enrichment, selective, and isolation protocols, as recommended by the US food and drug administration (17). For each individual sample, 25 g was aseptically removed, blended in 225 mL of Listeria enrichment broth (Merck, Germany), and incubated at 35°C for 48 hours. The enrichment cultures were streaked onto Oxford agar (Merck, Germany) plates, incubated at 35°C for 48 hours, and examined for *Listeria* (black colonies with black halos). All of the suspected *Listeria* colonies were subjected to standard biochemical tests, including Gram staining, catalase, and motility testing at 25°C. For further confirmation, other biochemical reactions showing acid production from maltose, mannitol, rhamnose, and xylose, as well as β-hemolytic activity on 5% sheep's blood agar (Merck, Germany) and MRVP testing were performed.

3.3. Polymerase Chain Reaction Procedures

In the next step, for the identification and confirmation of *L. monocytogenes*, the suspected colonies were tested via PCR assay. The template DNA was obtained by boiling from a pure culture of the suspected isolate, which was grown in Tripticase Soy Broth (TSB; Merck, Germany) at 30°C overnight. Briefly, the overnight culture was centrifuged, the pellet was re-suspended in 1 mL of deionized water (dH_2O), and the sample was boiled at 100°C for 10 minutes. After heating, the obtained suspension was centrifuged at 14,000 rpm for 10 minutes, and then the supernatant was used as the PCR template. In addition, the DNA of standard *L. monocytogenes* (ATCC 7644) and distilled water were used as positive and negative controls, respectively. Each PCR tube contained 50 μL of the reaction mixture, consisting of the PCR buffer (10 X, 5 μL), $MgCl_2$ (50 mM, 1.5 μL), Taq DNA polymerase (5 U/μL, 0.5 μL), dNTPs Mix (10 mM, 1 μL), primers (100 pmol/μL, 1 μL each), ddH_2O (35 μL), and 5 μL (100 ng) of the template DNA. The primers used (Table 1) were from the P60-protein-coding gene (iap-P60), according to Kim et al. (18).

The cycling conditions used in the thermal cycler (Bioer, China) were as follows: first, denaturation at 94°C (3 minutes, 1 cycle), denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and extension at 72°C for 1 minutes. After 35 cycles, a final cycle comprised of a 5 minutes extension step at 72°C was conducted. The amplified PCR products were detected by agarose gel electrophoresis (Paya Pajoohesh Pars, Iran), stained, and visualized under UV light illumination (Kiagen, Iran).

3.4. Antimicrobial Susceptibility Testing

According to the methods of the Clinical and Laboratory Standards Institute (CLSI) (19), antimicrobial susceptibility tests were carried out using the Kirby-Bauer disk diffusion method. The tested antimicrobial agents were ampicillin, amikacin, erythromycin, streptomycin, penicillin, tetracycline, gentamicin, chloramphenicol, co-trimoxazole, and vancomycin. A swab was taken from each bacterial suspension (1×10^7 CFU/mL) and stroked on Mueller-Hinton agar (Merck, Germany), and then antibiotic discs (Padtan Teb, Iran) were placed on the agar. After incubation at 35°C for 24 hours, the diameter of the inhibition zone was measured for each antibiotic based on the appropriate schedule. Then, the isolates were classified as resistant, intermediate (reduced susceptibility), or sensitive.

3.5. Statistical Analysis

The results were analyzed via the chi-square test and Fisher's exact test using SPSS 16.0 software. The mean values were considered to be statistically different at 95% confidence levels.

4. Results

Out of 150 examined beef burger samples, on Oxford agar, black colonies were observed on 23 culture plates, with similarity to *Listeria* spp. colonies. Using biochemical tests, two samples (1.33%) were positive for *L. innocua*, and the rest were negative. Moreover, none of the suspected colonies were *L. monocytogenes* positive via the PCR assay (Table 2).

The statistical analysis showed no significant difference in the prevalence of *Listeria* spp. between the different companies ($P > 0.05$).

The data revealed that one of the isolates was resistant to tetracycline, and the other was resistant to co-trimoxazole. Both of the isolates showed intermediate susceptibility to chloramphenicol; however, they were sensitive to the other tested antibiotics.

Table 1. The Target Gene, Sequence of Primers, and Product Size (bp) Used for the Detection of *L. monocytogenes*

Target Gene	Primer Sequence	Product Size, bp	Reference
iap		454	(18)
Forward	5'-CTGGCACAAAATTACTTACAACGA-3´		
Reverse	5'-AACTACTGGAGCTGCTTGTTTTTC-3´		

Table 2. Number of Samples; Suspected and Positive *Listeria* spp

Company	Sample Number	Number of Suspected *Listeria*	*Listeria* spp. Positive	Confirmed Species
1	17	4	0	-
2	17	0	0	-
3	16	4	1	*L. innocua*
4	12	2	0	-
5	18	3	0	-
6	16	4	0	-
7	10	2	0	-
8	14	0	0	-
9	17	1	1	*L. innocua*
10	13	3	0	-
Total	150	23	2	2

5. Discussion

Many incidences of listeriosis have resulted from the consumption of contaminated foodstuffs, such as dairy, meat, vegetables, and seafood (3, 4). The presence of *L. monocytogenes* in raw meat increases the risk of listeriosis in people who consume undercooked meat or meat by-products, such as burgers. Therefore, to ensure food safety, this pathogen should be absent in 25 g of foodstuff (20). Overall, 1.33% (2 of 150) of all of the beef burger samples in the present study were contaminated with *Listeria* spp., while *L. monocytogenes* was not detected. It is worth noting that *L. innocua* is not a pathogen, but since both species share ecological niches, the presence of the bacterium could be an indicator of probable contamination with *L. monocytogenes* (21). Thus, when looking for sources of *L. monocytogenes*, the presence of other species, especially *L. innocua*, could be managed as equally significant.

There have been several reports regarding the isolation of *Listeria* spp. and *L. monocytogenes* from raw meat and meat products worldwide. For example, in Bulgaria, 786 samples (containing 505 samples of fresh meat and 281 samples of raw-dry and raw-smoked sausage) were analyzed for *Listeria* spp. From the beef and pork samples, 7.7%, 0.6%, 4.6%, and 0.8% were contaminated with *L. monocytogenes*, *L. ivanovii*, *L. innocua*, and *L. welshimeri*, respectively (22). In another study in Malaysia, *L. monocytogenes* was detected in 8.57% of the meat product samples (13). According to Wieczorek et al. (15), it was determined that 44 out of 406 hide samples (10.8%) were contaminated with *L. monocytogenes*, whereas 10 (2.5%) corresponding bovine carcasses were positive for this pathogen in Poland.

There are limited data regarding the prevalence of *Listeria* spp. in beef products consumed in Iran. For instance, Jalali and Abedi (5) found that *Listeria* spp. were present in 6.7% of the meat and meat product samples, 1.3% of the dairy samples, 1.2% of the vegetable samples, and 12% of the ready-to-eat samples in Isfahan, Iran. Moreover, Rahimi et al. (4) reported that out of 1,107 different meat samples collected in Iran, 141 samples (12.7%) were positive for *Listeria* spp. The most commonly recovered species was *L. innocua* (75.9%), followed by *L. monocytogenes* (19.1%). In contrast, a low incidence of *Listeria* spp. (2.7%) and *L. monocytogenes* (0.66%) in minced beef in Ahvaz city has been reported (16).

Our findings are not in agreement with the high prevalence of *Listeria* spp. in meat products reported by previous Iranian researchers. This could be due to the season, geographic conditions, sanitation in the meat production chain supply, or methodological differences. The low number of *Listeria* isolates in this survey may also be due to the actual low incidence in the products, or the presence of live-injured bacterial cells (LIBC) which cannot grow properly on culture media. This is a big concern in public health with regard to the presence of LIBC, because they may be undetectable via regular culture methods, but are potentially pathogenic under favorable conditions (13).

It is known that meat and meat products may be infected at the slaughterhouse due to cross-contamination, which

occurs during evisceration, slicing, mincing, and other processing stages. Meat and meat products are stored under refrigeration or freezing, and the absence of competitive microorganisms, along with the suitable water activity and pH values of the food, allow this psychrotolerant pathogen to grow at high levels (23). In preparing minced meat, the release of blood and meat juices during cutting, deboning, and grinding also favor the growth of *Listeria*, and may cause an increase in contamination during the processing of raw meat products (4, 23).

The results of the antimicrobial susceptibility tests have indicated intermediate susceptibility of the isolates to chloramphenicol. In addition, one of the isolates was resistant to tetracycline and another to co-trimoxazole, and both strains showed susceptibility to the other tested antibiotics. These results are in agreement with previous works (9, 24), which reported that *L. innocua* isolates from meat products were resistant to tetracycline and co-trimoxazole. According to previous studies, the *L. innocua* isolated from meat and foodstuffs, and their antibiotic resistance, are indicated in Table 3.

Table 3. Antibiotic-resistant *L. innocua* Isolated From Meat Products in Previous Studies

Antibiotic/Reference	Resistant	Intermediate susceptibility
Ciprofloxacin		
(12)	+	
(24)		+
Clindamycin		
(12)	+	
(9)	+	
Tetracycline		
(9)		+
Cefoxitin		
(12)	+	
Chloramphenicol		
(9)		+
Oxacillin		
(9)	+	
Co-trimoxazole		
(24)	+	+
Erythromycin		
(24)	+	+
Nalidixic acid		
(12)	+	
Penicillin G		
(9)		+

With the increasing number of antibiotic resistant strains of *L. monocytogenes* reported worldwide, it seems that antibiotic resistance in this bacterium can be acquired or transferred by genes from plasmids and transposons of commensal microorganisms, such as other *Listeria* spp. and Gram-positive bacteria which may found in foods (10), or mutational events in chromosomal genes (8, 25, 26). The newly acquired resistance protects the bacteria from being disrupted during antibiotic treatment (11).

As previously mentioned, *L. innocua* and *L. monocytogenes* are closely related species, and thus genetically very similar. Although *L. innocua* is not a pathogenic species, it has been reported that the bacterium could be a transferable reservoir of antibiotic resistance for *L. monocytogenes* (27). Therefore, it could be suggested that the isolation of resistant *L. innocua* in foodstuffs could be a potential risk to public health.

Although *L. monocytogenes* strains with resistance to streptomycin, penicillin, and tetracycline have been isolated from food sources (11, 28, 29), resistance to those antibiotics commonly used to treat listeriosis, such as ampicillin, amoxicillin (with or without gentamicin), and trimethoprim-sulfamethoxazole (co-trimoxazole), has rarely been observed (9).

5.1. Conclusions

Our findings showed the presence of antibiotic resistant *Listeria* strains in Iranian beef burgers, indicating the possible presence of *L. monocytogenes*. Thus, it is possible for the pathogenic bacterium to obtain various antibiotic resistance genes by horizontal gene transfer in the meat production supply chain. Additionally, there is a potential risk to public health from the consumption of raw or undercooked burgers, which may increase the possibility of the acquisition of resistance to antibiotics.

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Footnotes

Authors' Contributions: Siavash Maktabi: study design, study management, supervision, and writing; Mehdi Pourmehdi: statistical calculations and advising; Mehdi Zarei: advising; Amir Ali Fooladgar: sampling, processing, and performing the conventional and molecular.

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References

1. Boughattas S, Salehi R. Molecular approaches for detection and identification of foodborne pathogens. *J Food Qual Hazard Cont.* 2014;**1**(1):1–6.

2. Drevets DA, Bronze MS. Listeria monocytogenes: epidemiology, human disease, and mechanisms of brain invasion. *FEMS Immunol Med Microbiol.* 2008;**53**(2):151–65. doi: 10.1111/j.1574-695X.2008.00404.x. [PubMed: 18462388]
3. Rahimi E, Momtaz H, Sharifzadeh A, Behzadnia A, Ashtari MS, Zandi Esfahani S, et al. Prevalence and antimicrobial resistance of Listeria species isolated from traditional dairy products in Charar Mahal & Bakhtiary, Iran. *Bulgarian J Veterin Med.* 2012;**15**(2):115–22.
4. Rahimi E, Yazdi F, Farzinezhadizadeh H. Prevalence and antimicrobial resistance of listeria species isolated from different types of raw meat in Iran. *J Food Prot.* 2012;**75**(12):2223–7. doi: 10.4315/0362-028X.JFP-11-565. [PubMed: 23212021]
5. Jalali M, Abedi D. Prevalence of Listeria species in food products in Isfahan, Iran. *Int J Food Microbiol.* 2008;**122**(3):336–40. doi: 10.1016/j.ijfoodmicro.2007.11.082. [PubMed: 18221811]
6. McLauchlin J, Mitchell RT, Smerdon WJ, Jewell K. Listeria monocytogenes and listeriosis: a review of hazard characterisation for use in microbiological risk assessment of foods. *Int J Food Microbiol.* 2004;**92**(1):15–33. doi: 10.1016/S0168-1605(03)00326-X. [PubMed: 15033265]
7. Doyle MP, Loneragan GH, Scott HM, Singer RS. Antimicrobial Resistance: Challenges and Perspectives. *Comprehens Rev Food Scin Food Safe.* 2013;**12**(2):234–48. doi: 10.1111/1541-4337.12008.
8. Charpentier E, Courvalin P. Antibiotic resistance in Listeria spp. *Antimicrob Agents Chemother.* 1999;**43**(9):2103–8. [PubMed: 10471548]
9. Gomez D, Azon E, Marco N, Carraminana JJ, Rota C, Arino A, et al. Antimicrobial resistance of Listeria monocytogenes and Listeria innocua from meat products and meat-processing environment. *Food Microbiol.* 2014;**42**:61–5. doi: 10.1016/j.fm.2014.02.017. [PubMed: 24929718]
10. Lungu B, O'Bryan CA, Muthaiyan A, Milillo SR, Johnson MG, Crandall PG, et al. Listeria monocytogenes: antibiotic resistance in food production. *Foodborne Pathog Dis.* 2011;**8**(5):569–78. doi: 10.1089/fpd.2010.0718. [PubMed: 21166580]
11. Pesavento G, Ducci B, Nieri D, Comodo N, Lo Nostro A. Prevalence and antibiotic susceptibility of Listeria spp. isolated from raw meat and retail foods. *Food Control.* 2010;**21**(5):708–13. doi: 10.1016/j.foodcont.2009.10.012.
12. Kovacevic J, Mesak LR, Allen KJ. Occurrence and characterization of Listeria spp. in ready-to-eat retail foods from Vancouver, British Columbia. *Food Microbiol.* 2012;**30**(2):372–8. doi: 10.1016/j.fm.2011.12.015. [PubMed: 22365350]
13. Marian MN, Sharifah Aminah SM, Zuraini MI, Son R, Maimunah M, Lee HY, et al. MPN-PCR detection and antimicrobial resistance of Listeria monocytogenes isolated from raw and ready-to-eat foods in Malaysia. *Food Cont.* 2012;**28**(2):309–14. doi: 10.1016/j.foodcont.2012.05.030.
14. Ozbey G, Icyeroglu A, Muz A. Prevalence of Listeria species in raw hamburger meatballs and chicken burgers in eastern Turkey. *African J Microbiol Res.* 2013;**7**(31):4055–8.
15. Wieczorek K, Dmowska K, Osek J. Prevalence, characterization, and antimicrobial resistance of Listeria monocytogenes isolates from bovine hides and carcasses. *Appl Environ Microbiol.* 2012;**78**(6):2043–5. doi: 10.1128/AEM.07156-11. [PubMed: 22247138]
16. Maktabi S, Pourmehdi M, Zarei M, Moalemian R. Occurrence and Antibiotic Resistance of Listeria monocytogenes in Retail Minced Beef Distributed in Ahvaz, South-West of Iran. *J Food Qual Hazard Cont.* 2015;**2**(3):101–6.
17. Lovett J. Isolation and enumeration of Listeria monocytogenes. *Food Technol Biotechnol.* 1988;**42**:172–5.
18. Kim JS, Lee GG, Park JS, Jung YH, Kwak HS, Kim SB, et al. A novel multiplex PCR assay for rapid and simultaneous detection of five pathogenic bacteria: Escherichia coli O157:H7, Salmonella, Staphylococcus aureus, Listeria monocytogenes, and Vibrio parahaemolyticus. *J Food Prot.* 2007;**70**(7):1656–62. [PubMed: 17685339]
19. Jorgensen JH. *Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria: Approved Guideline.* National Committee for Clinical Laboratory Standards; 2010.
20. Rantsiou K, Alessandria V, Urso R, Dolci P, Cocolin L. Detection, quantification and vitality of Listeria monocytogenes in food as determined by quantitative PCR. *Int J Food Microbiol.* 2008;**121**(1):99–105. doi: 10.1016/j.ijfoodmicro.2007.11.006. [PubMed: 18061295]
21. K.C. J MM, W ME. In: R ET, M EH. *Incidence and behaviour of L. monocytogenes in fish and seafood products.* New York, USA: Marcel Dekker; 1999:631-55.
22. Karakolev R. Incidence of Listeria monocytogenes in beef, pork, raw-dried and raw-smoked sausages in Bulgaria. *Food Cont.* 2009;**20**(10):953–5. doi: 10.1016/j.foodcont.2009.02.013.
23. Vitas AI, Garcia-Jalon VA. Occurrence of Listeria monocytogenes in fresh and processed foods in Navarra (Spain). *Int J Food Microbiol.* 2004;**90**(3):349–56. [PubMed: 14751690]
24. Dhanashree B, Otta SK, Karunasagar I, Goebel W, Karunasagar I. Incidence of Listeria spp. in clinical and food samples in Mangalore, India. *Food Microbiol.* 2003;**20**(4):447–53. doi: 10.1016/s0740-0020(02)00140-5.
25. Harakeh S, Saleh I, Zouhairi O, Baydoun E, Barbour E, Alwan N. Antimicrobial resistance of Listeria monocytogenes isolated from dairy-based food products. *Sci Total Environ.* 2009;**407**(13):4022–7. doi: 10.1016/j.scitotenv.2009.04.010. [PubMed: 19427675]
26. Pourshaban M, Ferrini AM, Mannoni V, Oliva B, Aureli P. Transferable tetracycline resistance in Listeria monocytogenes from food in Italy. *J Med Microbiol.* 2002;**51**(7):564–6. doi: 10.1099/0022-1317-51-7-564. [PubMed: 12132772]
27. Bertrand S, Huys G, Yde M, D'Haene K, Tardy F, Vrints M, et al. Detection and characterization of tet(M) in tetracycline-resistant Listeria strains from human and food-processing origins in Belgium and France. *J Med Microbiol.* 2005;**54**(Pt 12):1151–6. doi: 10.1099/jmm.0.46142-0. [PubMed: 16278428]
28. Evrim Gunes A, Deniz K, Serap C, Kamuran A, Vijay K J, Luis M. Antibiotic and bacteriocin sensitivity of Listeria monocytogenes strains isolated from different foods. *Food Nutrit Sci.* 2012;**2012**.
29. Walsh D, Duffy G, Sheridan JJ, Blair IS, McDowell DA. Antibiotic resistance among Listeria, including Listeria monocytogenes, in retail foods. *J Appl Microbiol.* 2001;**90**(4):517–22. [PubMed: 11309061]