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Prevalence and Antibiotic Resistance of Listeria Monocytogenes in Chicken Meat Retailers in Yazd, Iran

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ABSTRACT

Introduction: *Listeria monocytogenes* is a pathogen bacteria transmitted by food stuffs. Due to the lack of information about contamination of retail chicken meat to *Listeria monocytogenes* in Iran, this study aimed to determine the prevalence and antibiotic resistance of this bacterium in retail chicken meat in Yazd, Iran.

Materials and Methods: This cross-sectional descriptive study was conducted from January 2018 to June 2018 on 811 randomly selected samples from four districts located in Yazd city, Iran. Isolation of *Listeria monocytogenes* was performed using enrichment and selective culture media as well as biochemical tests. The positive samples were confirmed by PCR assay.

Results: In total, 247 samples (30.5%) were infected with at least one of the *Listeria* spp. Among the 247 samples, the isolates were 68 (27%) *L. monocytogenes*, 155 (63%) *L. innocua*, 5 (2%) *L. seeligeri*, 19 (8%) *L. ivanovii*. In current study, the antibiotic resistance of positive samples was also evaluated that especially *Listeria monocytogenes* were resistant to tetracycline and penicillin.

Conclusions: The presence of this pathogenic microorganism in chicken meat can be a health risk, especially for pregnant women, the elderly, and those with immune deficiency. Considering the amount of contamination with *L. monocytogenes* in chicken and the high mortality rate caused by it, monitoring of the health principles and standards during the production, transportation, and storage as well as training of employees in this industry are necessary.

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Introduction

Listeria is a genus of bacteria with different strains found widely throughout the natural environment, responsible for the contamination of numerous food products. Among these, *L. monocytogenes* as gram-positive intracellular zoonotic pathogen present in the food industry, can cause listeriosis [1] in human. The highest mortality rate resulting by the foodborne pathogens (about 17%) has been observed in relation to this bacterial infection [2]. The bacteria can exist and survive in all conditions, including low temperatures, different pH values, high concentrations of salt or bile, and oxidative stress. Regarding previous studies, some of foodborne diseases caused by contaminated meat and meat products are related to *L. monocytogenes* [3-4]. Accordingly, one of the major problems in the food chain is due to poor management of this widely distributed bacterium which is compatible with various conditions [5].

It is obvious that use of poultry meat and its products have been accounted for many human dietary programs around the world because of high-quality nutrients such as protein, vitamins, and

minerals [6]. Hence, these foods are highly desirable, palatable and digestible, as well as inexpensive rather than other types of meat like beef and mutton. Moreover, the consumption of beef, sheep and poultry meat per Capita has increased nowadays[7]. The poultry industry is a common place that *Listeria spp*. can infect the chicken-meat and products. *Listeria* contamination of poultry carcasses can occur in poultry slaughterhouses and processing plants during various stages, including improper cleaning and disinfecting of environment and equipment, as well as unsanitary transportation of the resulting products [8]. The environment, workers, surfaces, and equipment are possible resources to infect prepared chicken products after cooking [9].

Along with the mentioned problems, globally antimicrobial and multidrug resistance among the pathogens is other serious troublemakers for human beings. The major reason for the current study is the abuse and overuse of antimicrobial agents that may result in the spread of antimicrobial-resistant pathogens, including *Listeria* spp., despite the effect of these agents to control the infections. Public health consequences in human population can be happened due to the spread of bacterial resistant to antimicrobial agents in contaminated food products [10].

According to our knowledge, no investigations have been conducted on *Listeria* spp. contamination in retail poultry meat concerning the prevalence and antibiotic susceptibility in the city of Yazd, Iran. Hence, the main purposes of the current study were to isolate and identify *Listeria* spp. from the chicken as well as to determine antibiotic resistance patterns of the isolates.

Material and Methods

Sample collection

This cross-sectional descriptive study was conducted from January to June 2018 on 811 randomly selected samples in Yazd city among the most popular and the busiest stores. The samples were taken separately from the stainless steel table (n = 30), poultry moving carts (n = 100), sliced carcasses (n = 170, 85 thighs and 85 chests), packed chicken (n = 100), gloves (n = 60), workers' hands (n = 30), apron (n = 30), knife (n = 45), chicken liver (n = 100), gizzard (n = 90), refrigerator with temperature of 0-4°C (n = 56). All swab specimens were stored in Falcon tubes containing 1 ml of 0.1% peptone water, as well as the meat, liver and viscera samples (25 g) in sterile containers. All samples were transported to the laboratory in the cold box.

Isolation and identification of Listeria spp.

Guidelines proposed by the United States Department of Agriculture (USDA) were applied for isolation, so that 25 g of the sample was aseptically added to 225 ml of University of Vermont-Modified Listeria enrichment broth (UVM I enrichment medium), mixed with a stomacher device, and incubated at 37°C for 24 hours. After 24 hours, 1 ml of the initial enrichment medium was transferred to 9 ml of UVM II medium and incubated at 37°C for 24 hours, and then cultured on palcam agar medium and incubated at 25°C for 48 hours. Three suspicious colonies (black and deep) were cultured on tryptone soy agar with 0.6% yeast extract and incubated at 37°C for 24 hours [11-12]. The isolated *Listeria* species were identified through biochemical tests, including Gram staining, catalase, mobility at 25°C and 37°C, fermentation of glucose, maltose, rhamnose, xylose and mannitol, esculin reduction, nitrate reduction, beta hemolysis, MR/VP, and CAMP test [13].

PCR assays

The isolated bacteria were cultured in Brain Heart Infusion (BHI) medium and incubated at 37°C for 18 hours [14]. The extraction was performed by the boiling method, and then the DNA was extracted. The primers for Listeria genus were related to the *prs* gene to identify all *Listeria* species, including forwarding 01 (5′-GCTGAAGAGATTGCGAAAGAAG-3′) and reverse 02 (5′- CAAAGAAACCTTGGATTTGC GG-3′) to amplify 370-pb DNA fragment. The oligonucleotide primers released from *hly* gene for *Listeria monocytogenes* consisted of DG69 (5'-GTGCCGCCAAGAAAAGGTTA-3') and DG74 (5'-CGCCACACTTGAGATAT-3') to amplify

636-pb DNA fragment. The PCR mixture was used for each sample after optimization of its component concentrations, including the primers, distilled water, Master Mix and Template DNA in 25 µl for each PCR reaction. The mixture was placed in a thermal cycle for gene amplification. Subsequently, the PCR products were electrophoresed on 1.5% agarose gel [15-16].

Antimicrobial susceptibility test

One strain of each positive *Listeria* sample was selected for the antibiotic susceptibility test using the disk diffusion method on Muller Hinton agar supplemented with 5% defibrinated sheep blood in accordance with standard guidelines [17]. The studied antibiotic disks were vancomycin (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), clindamycin (2 µg), erythromycin (15 µg), ampicillin (10 µg), ciprofloxacin (5 µg), tetracycline (30 µg), chloramphenicol (30 µg), gentamicin (10 µg), penicillin G (10U/IE), rifampin (5 µg) and enrofloxacin (5 µg)[18].

Then, each *Listeria monocytogenesis* strain was cultured as scratch method and the disks were placed onto each plate (5 antibiotic disks per plate) and incubated at 35°C for 24 hours. The inhibition zone diameter was measured. The findings were interpreted according to the criteria provided by the CLSI (Clinical and Laboratory Standards Institute) table. *Staphylococcus aureus* ATCC29213 and *Escherichia coli* ATCC25922 were used as quality control organisms in the determination of antibiotic susceptibility.

Statistical analysis

The data were analyzed by SPSS 18 software using descriptive statistics and Chi-square test to compare the prevalence of contamination and bacterial resistance.

Results

The results of *Listeria* contamination in 811 samples, including raw chicken, viscera and poultry retail equipment in Yazd are summarized in Table 1. The PCR result was used as the final confirmation of the identity of presumptive colonies isolated in this study (Figure 1). In total, 247 samples (30.5%) were infected with one of the *Listeria* strains. Among the 247 samples, the isolates were 68 (27%) *L. monocytogenes*, 155 (63%) *L. innocua*, 5 (2%) *L. seeligeri*, and 19 (8%) *L. ivanovii*.

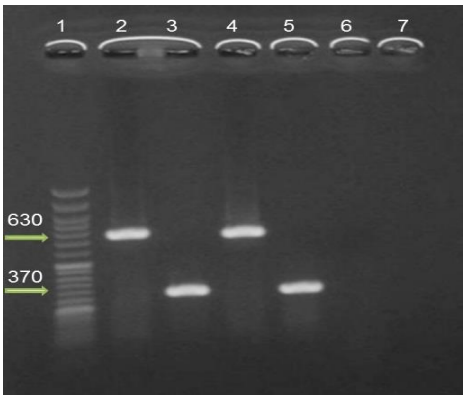

Figure 1: A representative gel of PCR-amplified products of *Listeria* spp.

Lane 1: DNA ladder (50 bp); lane 2: positive control *Listeria* spp.; lanes 3: positive control *L. monocytogenes*; lanes 4: positive *Listeria* spp. isolates showing specific bands at 630 bp; lanes 5 positive *L. monocytogenes* isolates showing specific bands at 370 bp; lanes 6 negative control for *Listeria* spp.; lanes 7: negative control for *L. monocytogenes*

Among the strains, *L. innocua* had the highest incidence. Moreover, during the different stages, the most prevalence was related to the gizzard (45.5%), stainless steel (40%), and liver (40%). The maximum prevalence of *L. monocytogenes* was in the apron (57.2%) and chicken thigh (40%). The lowest prevalence of *L. monocytogenes* was for knife (9%) and workers' hands (14.2%) (Table 1). All 68 isolates of *L. monocytogenes* identified within culturing methods were confirmed using PCR technique.

In current study, all *Listeria* isolates were tested for resistance to 12 different antibiotics by the disk diffusion method. Most isolates were resistant to tetracycline and penicillin. The bacterial resistance to various antibiotics is shown in Table 2. The results obtained from the present study indicated high resistance of *L. monocytogenes* and *L. innocua* to tetracycline and penicillin. Among all samples, 73 samples (29.5%) were resistant to one antibiotic, 76 samples (30.7%) to two antibiotics and 80 samples (32.3%) to more than two antibiotics. Among all samples, 10 (4%) and 15 (6%) samples had the minimum resistance to vancomycin and erythromycin, respectively.

Table 1: Prevalence of *Listeria* spp. by sample types.

Place	Samples No.	*Listeria spp.* No. (%)	*L.monocytogenes* No. (%)	*L.innocua* No. (%)	*L.ivanovii* No. (%)	*L.seeligeri* No. (%)
Stainless steel table	30	12(4)	3(25)	9(75)	1(8)	0**(-)**
poultry meat crates	100	21(21)	6(28)	14(67)	0(-)	1(5)
Raw chicken drumsticks	85	28(32.9)	11(40)	14(49.5)	1(3.5)	2(7)
Raw chicken breast	85	25(29.4)	9(36)	15(60)	0(-)	1(4)
packaged raw chicken	100	40(40)	12(30)	23(57.5)	2(5)	3(7.5)
Gloves	60	8(13.3)	3(37.5)	5(62.5)	0(-)	0**(-)**
Workers hands piece of chicken	30	7(23.3)	1(14.2)	6(85.8)	0(-)	0**(-)**
Aprons workers	30	7(23.3)	4(57.2)	2(28.5)	0(-)	1(14.3)
Knife	45	11(24.4)	2(18)	6(55)	0(-)	3(27)
Liver	100	40(40)	7(17.5)	25(62.5)	2(5)	6(15)
gizzard	90	41(45.5)	9(22)	30(73)	0(-)	2(5)
Refrigerator (0 - 4°C)	56	7(12.5)	2(28.5)	5(71.5)	0(-)	0
Total	811	247(30.5)	69(27.9)	154 (62.4)	5(2)	19(7.7)

Table 2: Antimicrobial resistance patterns of *Listeria* spp. isolated from poultry products.

Antimicrobial agent	*Listeria* spp. No. (%)	*L. monocytogenes* No. (%)	*L. innocua* No. (%)	*L. ivanovii* No. (%)	*L. seeligeri* No. (%)
Ampicillin	92(10.4)	33.7(33.7)	60(65.2)	1(1.1)	0(-)
Chloramphenicol	71(8.1)	18(25.4)	45(63.4)	3(4.1)	5(7)
Ciprofloxacin	72(8.2)	20(27.8)	49(68.1)	1(1.4)	2(2.8)
Clindamycin	23(2.6)	2(8.7)	19(82.6)	0(-)	2(8.7)
Enrofloxacin	61(6.9)	18(29.5)	37(60.7)	0(-)	6(9.8)
Erythromycin	15(1.7)	2(13.3)	12(80)	1(6.7)	0(-)
Gentamycin	25(2.8)	2(8)	22(88)	0(-)	1(4)
Penicillin	114(12.9)	40(35.1)	67(58.8)	4(3.5)	3(2.6)
Rifampin	21(2.4)	10(47.6)	11(52.4)	0(-)	0(-)
Tetracycline	118(13.4)	36(30.5)	73(61.9)	6(5.1)	3(2.5)
Trimethoprim/ Sulfamethoxazole	30(3.4)	9(30)	21(70)	0(-)	0(-)
Vancomycin	10(1.1)	5(50)	5(50)	0(-)	0(-)
Resistance to 1 antimicrobial	73(8.3)	21(28.8)	48(65.8)	1(1.4)	3(4.1)
Resistance to 2 antimicrobials	76(8.6)	26(34.2)	43(56.6)	3(3.9)	4(4.2)
Resistance to > 2 antimicrobials	80(9.1)	33(41.3)	42(52.5)	2(2.6)	3(3.8)

Discussion

Poultry meat centers are environments with suitable temperature and humidity for the growth of *Listeria* bacteria. Therefore, the bacterium can remain in the environment for few months or even years, which leads to food contamination. Sustainability of *Listeria* in the environment is due to its ability to create a biofilm on different levels. The first comprehensive study on the prevalence of *listeria* in Iran was reported by Jalali and Abedi in Isfahan. In their study, from the total 617 samples achieved from different foods, 4.6% had *Listeria*. The infection rate in fresh poultry meat was 4.5% and all positive samples were related to *Listeria innocua*; none of the samples were contaminated with *Listeria monocytogenes* [19]. Fallah et al. reported a prevalence of 34% for raw poultry meat at the supply level in center of Iran [20]. The amounts of contamination in current study for *Listeria monocytogenes, Listeria innocua, Listeria ivanovii, and Listeria seeligeri* were 14.1%, 16.1%, 2.51%, and 2.01%, respectively. Jamali et al. also reported that the prevalence of *Listeria* in the chicken retail centers of Tehran was 16.1%. The infection rates related to *L. ivanovii*, *L. monocytogenes*, *L. innocua*, and *L. seeligeri* were respectively 9.8%, 3.6%, 2.1%, and 0.7%. In another study conducted in 5 large cities of Iran [21], the mean prevalence of *Listeria* in meat retail centers was 15.8%. In present research, the prevalence of *Listeria* in Yazd was 17.7%. The prevalence of *L. monocytogenes*, *L. innocua*, and *L. welshimeri* was reported as 2.1%, 14.6%, and 1%, respectively. The prevalence of *Listeria* achieved from the current study was higher than the previous studies carried out in Iran, but it was consistent with the results reported from different countries; 34% in Canada [22], 28% in Japan [23], and 32.7% in Turkey [24].

In order to compare the results of the present study with those of other studies, the country, the separation technique, and the investigated type of tissue should be considered. The actual incidence of *L. monocytogenes* is generally higher than the reported results through the traditional isolation method, which relies on selective enrichment medium (UVM) and Fraser broth to purify *L. monocytogenes*. At temperatures below 40 ° C, *L. innocua* grows faster than *L. monocytogenes* and this is one of the reasons for higher prevalence of *L. innocua* than *L. monocytogenes* which was reported in the current study because the conventional enrichment method is more in favor of *L. innocua* spp. growth. In previous studies, *L. innocua* was more prevalent than the other species [20-21, 25-27]. The prevalence of *L. monocytogenes* in the northern European countries was 22% [28], in Italy 21.8% [26], and in Sri Lanka was 34% [29] that is compatible with the current results.

In Loura et al. research, the chicken liver samples were investigated and 10% and 20% of infections were reported by *L. innocua* and *L. grayi*, respectively. None of the samples were contaminated with *L. monocytogenes* [30]. A study conducted in Bulgaria showed that from 112 samples of goose liver, 35.7% were contaminated with *L. monocytogenes* [31]. The prevalence of *L. monocytogenes* in the chicken liver in Iran was 21.6 [20], in Brazil 23.3% [32], in Malaysia 25% [33], which was consistent with the results achieved form the current study. Chicken's liver and gizzard are not contaminated during slaughter; their most important source of contamination is after slaughter and in processing by cross-contamination between other parts of the poultry and table, equipment, and the work environment [33-34].

Laura et al. found that the knife used to cut chicken was 80% and 50% contaminated with *L. monocytogenes* and *L. innocua*, respectively, while no contamination with *L. monocytogenes* was observed in workers' gloves [30]. Barbalho et al. [35] reported that contamination of workers' hands and gloves by *L. monocytogenes* and *L. innocua*, were 11.8% and 40.5%, respectively which are consistent with the current study. Kerr [36] and Yuewei [37] found 11.8% of contamination with *L. monocytogenes* in workers' hands and gloves that were in line with the current results. High contamination in equipment shows that conventional disinfection methods are inefficient [30].

Studies represent that the antibiotic resistance of *L. monocytogenes* isolated from foods is increasing

worldwide [38]. In a study on chicken meat offered in Ankara, Turkey[27], *L. monocytogenes*, *L. welshimeri*, and *L. innocua* species were very sensitive to chloramphenicol (88-100%), but they showed resistance to ampicillin (66-100%). In present study, the bacteria were most resistance to tetracycline and penicillin, while the least rate of resistance was to vencomycin and erythromycin. Antibiotic resistance results in present study showed high resistance of *L. monocytogenes* to ampicillin, tetracycline, chloramphenicol, and penicillin antibiotics. Antibiotic resistance of *Listeria* sp. is due to the acquisition of genetic elements such as moving plasmids and conjugate transposons [39].

Osaili et al. [25], in Jordan, observed that from 10 antibiotic tests of chicken meat samples, the highest resistance was related to tetracycline. Furthermore, Fallah et al. [20], studied poultry products in the central part of Iran and reported that the highest antibiotic resistance was for ampicillin, penicillin, and tetracycline that are consistent with the results of the present study. Walsh et al. [40], showed that the highest antibiotic resistance of *L. monocytogenes* is related to tetracycline and penicillin antibiotics with 6.7% and 3.7% of prevalence, respectively. The antibiotic resistance of *L. innocua* in current study was more than that of *L. monocytogenes.* Although the genetic basis for these differences is not known, the presence of plasmids encoded antibiotic resistance was reported in *L. innocua* [40]. In the current study, *L. innocua* had the highest resistance to antibiotics spectrum, which is consistent with the above study. There are different factors causing the antibiotic resistance of various bacteria, such as excessive consumption of antibiotics in the poultry industry. The conducted studies in the field of antibiotic resistance of *Listeria* bacteria have indicated that this bacterium has a growing resistance to different antibiotics, which can increase the risk of infection for children, pregnant women, the elderly, and those with immune deficiency [41].

Conclusion

The results of present study provide information about the contamination state of raw poultry meat and retailers' equipment in Yazd, Iran. The presence of this pathogenic microorganism in chicken meat can be a health risk, especially for pregnant women, the elderly, and those with immune deficiency. Considering the amount of contamination with *L. monocytogenes* in chicken and the high mortality rate caused by it, monitoring of the health principles and standards during the production, transportation, and storage as well as training of employees in this industry are necessary.

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Conflict of interests

All authors declare that they have no competing interests.

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