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Original Article

Prevalence and Antibiotic Susceptibility of *Listeria Monocytogenes* Isolated from Retail Ready-to-Eat Meat Products in Gorgan, Iran

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ABSTRACT

Background and Objectives: *Listeria monocytogenes* are known as an emerged foodborne pathogen and considered as a severe health risk. In the present study, prevalence and antibiotic resistance of *L. monocytogenes* isolated from ready-to-eat meat products in Iran were assessed.

Materials and Methods: A total of 200 ready-to-eat meat products, including chicken meat (wing, breast, and leg), lamb and fish, were collected, and *L. monocytogenes* was isolated according to ISO 11290–1. All of the isolates were verified using polymerase chain reaction and serotyping methods. Antibiotics susceptibility of *L. monocytogenes* isolates was assessed using the broth microdilution method.

Results: The presence of *L. monocytogenes* was verified in 13% of the samples. The presence of *L. monocytogenes* was reported in 26% of roast chicken meat, 5% of roast fish and 8.33% of cooked beef. Serology showed that Serotype 1/2a (48.13%) was the dominant serotype, followed by 4b (38.4%), 1/2c (6.99%) and 1/2b (6.48%). The result showed that 37 out of 100 *L. monocytogenes* isolates were resistant to all tested antibiotics. Furthermore, eight isolates were intermediately multi-resistant to the antibiotics. The rate of antibiotic resistance in *L. monocytogenes* was 52% in Serotype 1/2a, 39% in Serotype 4b, and 35% in Serotype 1/2c. Isolates of *L. monocytogenes* were mostly resistant to penicillin, ampicillin and erythromycin, but highly susceptible to tetracycline and gentamycin.

Conclusions: The high level of *L. monocytogenes* prevalence and its resistance to antibacterial agents in meat products may result in severe human listeriosis. Therefore, it is necessary to use efficient monitoring protocols for antibiotic administration and further safety management systems in food production units.

Keywords: *Listeria monocytogenes*, Lamb, Chicken, Antibiotic, Fish

Introduction

Listeria monocytogenes, the agent of listeriosis, is a food-borne pathogen that can cause fatal health problems, especially in immunocompromised patients. The *L. monocytogenes* infections are associated with mortality rates of nearly 12%, which is the highest rate within foodborne pathogens (1). This pathogen is found in soil and water as well as infected animals. In general, *L. monocytogenes* is transmitted to humans through contaminated foods (2). The bacterial strains differ in their epidemic potentials and serotypes as Serotypes 4b, 1/2a and 1/2b are responsible for nearly 90% of the human listeriosis (3). other abilities of *L. monocytogenes* are linked to the formation of biofilms, which can grow at refrigerator temperatures and high concentration tolerances to NaCl (4). Several studies reported *L. monocytogenes* from meat products (5,6). The prevalence of *L. monocytogenes* in ready-to-eat (RTE) meat products mostly ranged from 2.60 to 28% in other studies (7–10). Moreover, the contamination rate is different, affected by the food type and geographical area. Naturally, *L. monocytogenes* is sensitive to a wide range of antibiotics (2). For several years, the resistance rate of *L. monocytogenes* to antibiotics was constant (2). Nevertheless, new reports suggest increases in antibiotic resistance of the bacteria isolated from foods such as dairy products and meats (11,12). In the present study, the

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prevalence of *L. monocytogenes* isolates from RTE meats such as chicken meat (wing, breast and leg), lamb and smoked fish was assessed to report their genetic affiliation and antibiotic susceptibility using serological typing methods.

Materials and Methods

Collection of the *L. monocytogenes* isolates

A total of 200 RTE meat product samples were collected from 40 restaurants and retail supermarkets in Gorgan, Iran, from September to November 2018. These samples were transported to the Microbiology Laboratory at Gorgan University of Agricultural Science and Natural Resources under appropriate conditions. Samples were analyzed for *L. monocytogenes*, according to ISO 11290-1 (13). Briefly, 25 g of each sample was homogenized with 225 ml of *Listeria* enrichment broth and incubated at 37 ºC for 24 h. Then 0.1 mL of pre-enrichment media was added into the CHROM agar Listeria and incubated for 24 h at 37 ℃. Four suspicious Listeria colonies were chosen from every selected agar, and then secondary enrichments were streaked into a new CHROM agar and then incubated for 24 h at 37ºC. Confirmation tests were performed using pure culture obtained from BHI (Merck, Germany) agar. Several biochemical tests were used for confirmation, including Gram staining, blood agar test, qualifying the consumption of rhamnose and xylose and mannitol.

Extraction of DNA from the *L. monocytogenes* isolates

In the following, the genomic DNAs were separated from 1 mL of the tested bacterial culture mediums, using a "Bacterial Genomic DNA Purification Kit" (DENA Zist Asia, Iran). DNA samples were re-suspended in 50 mL ddH_2O and then were stored at -20 C.

Polymerase chain reaction (PCR) assay

The PCR reactions were proceeded under optimized conditions using *L. monocytogenes* PTCC 1298 as control. The PCR was carried out in a 25-µl volume of the mixture containing ten mM of tris–HCl, 50 mM of KCl, 1.5 mM of $MgCl_2$, 0.2l M of each primer, 0.2 mM of each dNTP, 1 µl of DNA template and 0.5 unit of Taq polymerase (Takara, Japan). The forward primer included 5′-TTGCGCAACAAACTGAAGC-3′ and the reverse primer included 5′-GCTTTTACGAGAGCACCTGG-3′. The PCR condition respectively included 35 cycles of denaturation, annealing and extension at 95 ºC for 30 s, 56 ºC for 30 s and 72 ºC for 1 min. The final extension was carried out at 72 ºC for 7 min. The PCR products were electrophoresed to verify expected bands of 346 bp (5).

Serotyping of the *L. monocytogenes* isolates

All of the isolates were serotyped based on a protocol by Seeliger and Hohne (14). The isolates were analyzed using *Listeria* SEIKEN Antisera Kit (set code: 294616) for O and H antigens according to the kit manufacturer's instruction (Denka-Seiken, Japan).

Antibiotic resistance of the *L. monocytogenes* isolates

Antibiotics susceptibility of the *L. monocytogenes* isolates was assessed using the broth microdilution method (15). The antibiotics included enrofloxacin (5 µg), ciprofloxacin (5 µg), ampicillin (10 µg), erythromycin (15 µg), penicillin (5 µg), clindamycin (2 µg), tetracycline (30 µg), gentamycin (10 µg) and vancomycin (30 µg). The minimum inhibitory concentration (MIC) was calculated using broth microdilution susceptibility and serial microdilution assays using Sensititre Vizion System (Trek, UK). Antibiotic susceptibility of the isolates was assessed according to instructions by the Clinical and Laboratory Standards Institute (CLSI) (16).

Statistical analysis

Rates of *L. monocytogenes* contamination in various RTE meat products were compared with each other using the chi-square test of the SPSS Software v.9.0 (SPSS Inc., Chicago, IL, USA). Furthermore, distributions of various serotypes of *L. monocytogenes* in each season were compared with each other using the same test. Differences were considered significant at $P < 0.05$.

Results

Prevalence and serotype distribution of the *L. monocytogenes* isolates in RTE meat products

From a total of 200 RTE meat product samples, 29 samples (14.5%) were contaminated with *L. monocytogenes* (Table 1). Roasted chicken meats included the highest *L. monocytogenes* contamination

rate with 13 (26%) out of 50 positive samples. Moreover, 5% of the roasted fish and 8.33% of the cocked beef were contaminated. Results of the serology showed that Serotype 1/2a was the most common serotype (45.4%) in the present study, majorly similar to other studies (17–19), followed by Serotypes 4b (38.4%), 1/2c (6.99%) and 1/2b (6.58%).

Table 1. Prevalence of *Listeria monocytogenes* in RTE meat products collected from retail markets in Iran

Sample	Food item	*L. monocytogenes* (%)
1	Cooked beef	8.33 (5/60)
2	Roast chicken	26.25 (21/80)
3	Roast fish	5 (3/60)

Antibiotic susceptibility

A total of 100 *L. monocytogenes* isolated from RTE meat products were tested for resistance against nine antibiotics (Table 2). Results showed that 37 isolates (37%) were resistant to all antibiotics. Moreover, all isolates were resistant to at least one (40%), two (18.3%), three (12.4%), four (4.20%) or five (3.25%) antibiotics. The bacterial resistance to ampicillin (64.71%) was the most common finding. Resistances to erythromycin, vancomycin, enrofloxacin, and ciprofloxacin were detected in 52.94, 35.29, 29.41 and 16.64% of the *L. monocytogenes* isolate, respectively (Table 2). The *L. monocytogenes* Serotypes 1/2a was the most common serotype in the present study, mostly resistant to ampicillin, penicillin and tetracycline. Serotype 4b was mostly resistant to vancomycin and penicillin, Serotype 1/2b to erythromycin and penicillin and Serotype 1/2c to ampicillin (Table 2).

Table 2. Number and proportion of the *Listeria monocytogenes* isolates resistant to nine antimicrobial agents

Antimicrobial agent	*L. monocytogenes* (*n* = 100)				Total no. (%)
	1/2a no. (%)	1/2b no. (%)	1/2c no. (%)	4b no. (%)	
Vancomycin	26 (27.4)	0 (0.0)	8 (16.66)	1 (18.8)	35 (35)
Ampicillin	43 (39)	12 (9.09)	7 (18.18)	2 (18.18)	64 (64)
Erythromycin	41 (37.8)	8 (8)	1 (11.11)	2 (22.22)	52 (52.94)
Enrofloxacin	13 (8.2)	0 (0.0)	2 (20)	3 (20)	18 (29.41)
Ciprofloxacin	2 (1.91)	0 (0.0)	0 (0.0)	1 (33.33)	3 (16.64)
Tetracycline	1 (0.96)	0 (0.0)	0 (0.0)	3 (54.3)	4 (5.78)
Penicillin	5 (4.83)	6 (20)	0 (0.0)	3 (30)	14 (58.82)
Clindamycin	2 (50)	0 (0.0)	1 (25)	1 (25)	4 (24.52)
Gentamycin	6 (50)	0 (0.0)	0 (0.0)	3 (26.32)	9 (11.76)

Discussion

Results of prevalence and serotype distribution of *L. monocytogenes* in the current study are mostly similar to those in other studies. Manios et al. (2015) isolated *L. monocytogenes* from 28% of RTE meat samples. The contamination level of *L. monocytogenes* in roasted chicken could be linked to fecal contamination during slaughtering or contact with hands (22). The high rate of *L. monocytogenes* contamination in roasted chicken were demonstrated in previous studies. For example, *L. monocytogenes* was isolated in 6.9% of the samples (9/131) in China (23) and 24% of the samples in Turkey (5). Although other studies reported a lower prevalence of *L. monocytogenes*. For example, bacteria were detected in 5.3% of meat products in China (24), 4% in Egypt (25) and 0.7% in Estonia (26). In the current study, the prevalence of *L. monocytogenes* in roasted fish, roasted chicken meat and cocked beef were similar to that in Shi et al. study (23); in which, *L. monocytogenes* was recovered from 6.9% of Chinese roast chicken meats and 6.5% of Chinese cocked beef. Results were also similar to results by Fallah et al. (7), where *L. monocytogenes* was recovered from 4% of Iranian RTE fish products.

In this study, resistance to penicillin was the most common resistance in *L. monocytogenes* isolates. Issa et al. (2011) showed high levels of resistance to penicillin and ampicillin in *L. monocytogenes*. Other researches (10,28,29) reported susceptibility of *L. monocytogenes* to ampicillin and penicillin. Ampicillin and penicillin are the most common antibiotics in listeriosis treatment (30,31). The current report on *L. monocytogenes* sensitivity to tetracycline and gentamycin is in agreement with the report of Gomez et al. for isolates from meats and animal-derived foods (10,32). Reports are available on relationships between serotype distribution and antibiotic susceptibility of the *L. monocytogenes* isolates. Lemes-Marques et al. (2007) report that the human *L. monocytogenes* serotypes (1/2a, 1/2b and 4b) were resistant to ampicillin, vancomycin, gentamycin and trimethoprim (33). In Turkey, isolates of *L. monocytogenes* Serotype 1/2c were highly resistant to antibiotics, more than isolates of Serotypes 1/2a, 1/2b and 4b (34). The results of this study showed differences in antibiotic susceptibility of *L. monocytogenes* isolated from Iran. Furthermore, the results of the current serology study were similar to those of other serology studies (17–19).

Conclusion

The high rate of *L. monocytogenes* contamination in RTE meat products can be linked to the fact that these meats were not cooked thoroughly, which may cause severe health risks to consumers. Furthermore, great resistance of the *L. monocytogenes* isolates to antibiotics can lead to serious health problems and needs public awareness of meat producers and consumers.

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