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Prevalence and antimicrobial resistance patterns of *Listeria* species isolated from poultry products marketed in Iran

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ABSTRACT

In the present study, a total of 402 poultry product samples composed of raw, ready-to-cook (RTC) and ready-to-eat (RTE) products were examined for the presence of *Listeria* spp. The total contamination rate with *Listeria* spp. in poultry products was 33.3% with a higher rate of contamination in warm seasons than in cold seasons. The most species recovered was *Listeria innocua* (46.3%); the remaining isolates were *Listeria monocytogenes* (38.8%), *Listeria ivanovii* (9.7%) and *Listeria seeligeri* (5.22%). *L. monocytogenes* was detected in 14.1%, 12.2% and 11.4% of raw, RTC and RTE poultry products, respectively. Serotype 4b (44.9%) was the predominant serotype of *L. monocytogenes* isolates followed by 1/2a (40.8%), 1/2b (10.2%) and 1/2c (4.08%). Considering seasonal variability, 1/2a was the most prevalent serotype in warm seasons, while 4b was predominant in cold seasons. The *Listeria* spp. particularly *L. monocytogenes* isolates were highly resistant to ampicillin, penicillin, fluroquinolones and tetracycline. The results indicate that high prevalence of *Listeria* spp. especially *L. monocytogenes* in poultry products, and resistance of the isolates to the antimicrobials commonly used to treat human listeriosis could be a potential health hazard for consumers. In addition, prevalence of *L. monocytogenes* serotype 4b that involved in the majority of foodborne outbreaks of human listeriosis is a public health concern.

1. Introduction

Listeria species are Gram-positive, non-sporulating and non-acid-fast rods widely distributed in natural environment. The genus *Listeria* contains six species: *Listeria monocytogenes*, *Listeria innouca*, *Listeria ivanovii*, *Listeria grayii*, *Listeria seeligeri* and *Listeria welshimeri*. Among them, *L. monocytogenes* is pathogenic in humans and animals, while *L. ivanovii* is an animal pathogen and rarely pathogenic for humans (Jay, 2000, chap. 25; Razavilar, 1998, chap. 2).

Human listeriosis caused by *L. monocytogenes* is a sporadic disease with a mortality rate of approximately 20%. It has been considered that nearly all cases of human listeriosis are foodborne and associated with consumption of contaminated dairy products, unwashed raw vegetables, and under-cooked meat, seafood and poultry products. The susceptible groups to listeriosis are pregnant women and their unborn children, neonates, immunocompromised individuals and the elderly (Painter & Slutsker, 2007; Todd & Notermans, 2011).

Owing to high prevalence of *L. monocytogenes* in food products and high fatality rate of listeriosis, *L. monocytogenes* has been considered as a public health hazard. Moreover, the presence of any *Listeria* spp. in foods is an indication of microbial contamination (Farber & Peterkin, 1991). Some important characteristics such as the ability of growing across a broad temperature range, withstanding osmotic stress and survival under mild preservation treatment introduce *Listeria* species as foodborne organisms (Rahimi, Ameri, & Momtaz, 2010; Warriner & Namvar, 2009). The unique ability to survive and multiply at refrigeration temperatures may increase the hazard of *Listeria* infection from contaminated foods specially chilled ready-to-eat food products (Hof, Nichterlein, & Kretschmar, 1994).

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Poultry can become contaminated with *Listeria* spp. either environmentally or from healthy carrier birds during production in the farm (Skovgaard & Morgen, 1988). In poultry abattoir and processing plant, improper cleaning and disinfecting of environment and equipments; and also mishandling of the products may lead to *Listeria* contamination of poultry carcasses and the final products (Loura, Almeida, & Almeida, 2005; Uyttendaele, Neyts, Lips, & Debevere, 1997). Contamination of ready-to-eat poultry products can be occurred after cooking by cross-contamination either environmentally or via workers, surfaces and equipments (Osaili, Alaboudi, & Nesiar, 2011).

In recent decades, antimicrobial resistance particularly multi-resistance has been considered as a public health problem worldwide. The excessive and improper usage of antimicrobial agents could be responsible for the emergence of resistant bacteria. Although, the use of antimicrobials in poultry production phase has reduced the risk of infectious disease, it may lead to dissemination of antimicrobial-resistant bacteria including resistant strains of *Listeria* in the environment. The transmission of the resistant strains to human via contaminated food products may have public health consequences (Filiousis, Johansson, Frey, & Perreten, 2009). It has been stated that the antimicrobial resistance of *Listeria* spp. is due to the acquisition of mobile genetic elements such as self-transferable and mobilizable plasmids; and conjugative transposons (Charpentier & Courvalin, 1999).

Referring to the existing scientific literature, no survey has been conducted on the prevalence and antimicrobial susceptibility of *Listeria* spp. in various poultry products in Iran. Therefore, this study aimed to determine the prevalence of *Listeria* species isolated from popular poultry products marketed in Iran; and the resistance profiles of the *Listeria* isolates to the selective antimicrobials. Also, the serotype distribution of *L. monocytogenes* isolates from poultry products during different seasons was investigated.

2. Materials and methods

2.1. Sample collection

A total of 402 samples composed of raw, ready-to-cook (RTC) and ready-to-eat (RTE) poultry products were obtained from supermarkets, retail outlets and restaurants in central part of Iran during April 2010 to March 2011 (Table 1). The samples were immediately transported to the laboratory inside a portable ice-chest and examined for the presence of *Listeria* spp. on the day of arrival.

2.2. Isolation and identification of Listeria spp.

Poultry product samples were tested for the presence of *Listeria* species using the selective enrichment and isolation protocol recommended by the United States Department of Agriculture (McClain & Lee, 1988). An amount of 25 g from each sample was homogenized with 225 ml of UVM (University of Vermont) *Listeria* enrichment broth (UVM I; Merck, Darmstadt, Germany) and then incubated at 37 °C for 24 h. A portion of 1 ml of primary enrichment culture was inoculated to 9 ml of UVM II medium (Fraser broth; Merck, Darmstadt, Germany) and incubated at 37 °C for 24 h. The secondary enrichment was streaked on PALCAM agar (Merck, Darmstadt, Germany) and incubated at 37 °C for 48 h. The suspected colonies of *Listeria* were subcultured on tryptone soy agar with 0.6% yeast extract and incubated at 37 °C for 24 h. The isolates were subjected to the following biochemical tests for identification of the genus *Listeria*: Gram staining, urease, catalase, oxidase, voges proskaüer, D-glucose and D-salicin fermentation, aesculin hydrolysis and tumbling motility at 22 °C (Roberts & Greenwood, 2003, chap. 6). Confirmation of the genus (*Listeria*) was performed by a PCR method subsequently described. Then, the confirmed *Listeria* isolates were differentiated by the following biochemical tests: β-haemolytic activity, acid production from D-manitol, D-xylose, L-rhamnose and α-methyl-D-mannoside, nitrate reduction and CAMP test (Roberts & Greenwood, 2003, chap. 6). All the isolates identified as *L. monocytogenes* were confirmed by PCR.

2.3. PCR assays

Bacterial strains were cultured in BHI broth (Merck, Darmstadt, Germany) at 37 °C for 18 h and genomic DNA was extracted according to the method of Gussow and Clackson (1989). Oligonucleotide primers for the PCR assays were selected based on the published nucleotide sequence of the *prs* gene to detect all *Listeria* species (Doumith, Buchrieser, Glaser, Jacquet, & Martin, 2004) and the *hly* gene to detect *L. monocytogenes* (Choi & Hong, 2003). The used pair of primers were 01 (5′-GCTGAAGAGATTGCGAAAGAAG-3′) and 02 (5′-CAAAGAAACCTTGGATTTGCGG-3′) to amplify a 370 bp DNA fragment of the *prs* gene; and DG69 (5′-GTGCCGCCAA-GAAAAGGTTA-3′) and DG74 (5′-CGCCACACTTGAGATAT-3′) to amplify a 636 bp DNA fragment of the *hly* gene. Reaction mixture (25 μL) contained 0.5 μL of each primer at 10 μM (SinaClon Bioscience, Tehran, Iran), 2.5 μL of 10× PCR buffer, 1.5 μL of 50 mM $MgCl_2$, 1 μL of 10 mM dNTPs mix, 0.3 μL of 5 U/μL Taq DNA polymerase (SinaClon Bioscience, Tehran, Iran), and 1 μL of template

Table 1
Prevalence of *Listeria* spp. in different types of poultry products.

Poultry product	Samples tested No.	*Listeria* spp. No. (%)	*L. monocytogenes* No. (%)	*L. innocua* No. (%)	*L. ivanovii* No. (%)	*L. seeligeri* No. (%)
Raw:						
Chicken	54	22 (40.7)	9 (17.6)	10 (18.5)	2 (3.70)	1 (1.85)
Turkey	40	10 (25.0)	5 (12.5)	4 (10.0)	1 (2.50)	0 (–)
Quail	33	7 (21.2)	3 (9.10)	4 (12.1)	0 (–)	0 (–)
Ostrich	21	1 (4.67)	0 (–)	1 (4.76)	0 (–)	0 (–)
Chicken liver	51	29 (56.9)	11 (21.6)	13 (25.5)	2 (3.92)	3 (5.88)
Total	199	69 (34.7)	28 (14.1)	32 (16.1)	5 (2.51)	4 (2.01)
Ready-to-cook:						
Barbecued chicken	45	25 (55.5)	9 (20.0)	11 (24.4)	3 (6.67)	2 (4.44)
Chicken schnitzel	42	8 (19.0)	3 (7.14)	4 (9.52)	1 (2.38)	0 (–)
Chicken nugget	28	5 (17.9)	2 (7.14)	2 (7.14)	1 (3.57)	0 (–)
Total	115	38 (33.0)	14 (12.2)	17 (14.8)	5 (4.35)	2 (1.74)
Ready-to-eat:						
Olovieh salad	32	25 (78.1)	10 (31.2)	11 (34.4)	3 (9.38)	1 (3.13)
Chicken sausage	26	1 (3.85)	0 (–)	1 (3.85)	0 (–)	0 (–)
Chicken burger	30	1 (3.33)	0 (–)	1 (3.33)	0 (–)	0 (–)
Total	88	27 (30.7)	10 (11.4)	13 (14.8)	3 (3.41)	1 (1.14)