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Molecular Identification of *Listeria monocytogenes* in Raw Hamburgers from Kerman, South-East of Iran

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HIGHLIGHTS

- Three out of 100 (3%) hamburger samples were biochemically diagnosed as *Listeria* contamination.
- Two isolates were confirmed by molecular identification assay to be *L. monocytogenes*.
- Risk assessment surveys are required for *L. monocytogenes* in other food products in the country.

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Acronyms and abbreviations
PCR=Polymerase Chain Reaction

ABSTRACT

Background: *Listeria monocytogenes* is a Gram-positive and facultative anaerobic food-borne bacterium which is capable of intra and extra cellular growth. *L. monocytogenes* usually can exist on different surfaces and instruments at production and processing sites of food products with animal origin. In Iran, the consumption of burger has increased recently, but its safety is still of great concern. Despite few reports from some areas of Iran, there is limited information about burger contamination in Kerman province. Therefore, this research was set to molecular identification of *L. monocytogenes* in hamburgers distributed in Kerman, Iran.

Methods: A total of 100 raw hamburgers were collected from 20 fast food/sandwich shops in Kerman city, Iran during summer 2014. The hamburgers stored in ice box and transported to the food hygiene laboratory. The samples were microbiologically analyzed for the presence of *L. monocytogenes*. The isolated bacteria were confirmed by molecular assay.

Results: Three out of 100 (3%) hamburger samples were biochemically diagnosed as *Listeria* contamination; however molecular identification assay confirmed that two of them were *L. monocytogenes*.

Conclusion: Although the prevalence rate of *L. monocytogenes* was not high in hamburger samples of Kerman, the risk of human listeriosis must not be ignored or underestimated. Further surveys are required in future for risk assessment of this pathogenic bacterium in other food products distributed in the country.

Introduction

Listeria monocytogenes is a Gram-positive and facultative anaerobic food-borne bacterium which is capable of intra and extra cellular growth. It is one of the most important bacterial agents with high dispersion in the environment. The agent is found in soil, water, human/animal feces, vegetables, raw meat, fish, meat products, milk, etc. that may be pathogenic for human beings. This psychrophilic bacterium grows easily at the 0-45 °C and 4.4-4.9 pH levels (Buchanan et al., 2017; Jarvis et al., 2016; Zamani-Zadeh et al., 2011). *L. monocytogenes* usually can exist on different surfaces and instruments at production and processing sites of food products with animal

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origin (Carpentier and Cerf, 2011). In contrast to its low prevalence, the mortality rate of *L. monocytogenes* is relatively high (Jay et al. 2005). The Polymerase Chain Reaction (PCR)-based methods with high specificity and sensitivity are efficient for detection and identification and are applicable when antibiotic therapy decreases the isolation sensitivity. After one stage of selective enrichment, the direct PCR diagnosis is feasible which implies the necessity of enriching substances to achieve reliable results. The virulence genes of *Listeria* is *actA* gene which develops the polymerization of host cell skeletal actin and make feasible movements of bacteria among host cells; and *iap* gene which encodes the P60 protein that promotes the cell invasion. These genes are specifically used to confirm the identification of *L. monocytogenes* isolates (Liu, 2008; Longhi et al., 2003; Manzano et al., 1998; Travier and Lecuit, 2014; Zunabovic et al., 2011).

L. monocytogenes causes different diseases and symptoms in human like abortion, newborn septicemia, uterine granulomatosis, encephalitis, etc. The risk of this pathogen exists in the unheated as well as chilled stored food products such as hamburger (Chen et al., 2017; Friedly et al., 2008; Jalali and Abedi, 2008; Jay et al., 2005; Wong et al., 2012; Yucel et al., 2005). In Iran, the consumption of burger has increased recently, but its safety is still of great concern. Despite few reports from some areas of Iran, there is limited information about hamburger contamination in Kerman province. Therefore, this study was set to molecular identification of *L. monocytogenes* in hamburgers distributed in Kerman, Iran.

Materials and methods

Sampling

A total of 100 raw hamburgers were collected from 20 fast food/sandwich shops (5 samples per shop) in Kerman city, Iran during summer 2014. The samples stored in ice box and transported to the food hygiene laboratory.

Diagnosis

Culturing was done with adding 25 g of each sample to *Listeria* enrichment broth enriched media for *Listeria* (Merck, Germany) and incubated at the temperature of 30 ºC for 48-72 h. Then, the colonies were transported to the specific media of PALCAM agar (Himedia, India) containing Oxford additive (Microgen bioproducts Ltd, Cambridge, UK) and incubated for 48 h at 37 ºC. The confirmative biochemical tests, including oxidase and catalase were also carried out according to the standard method described by Ozbey et al. (2013). *L. monocytogenes* ATCC7644 strain was used as standard strain.

DNA extraction

The boiling method was applied to extract the nucleic acid. For this purpose, 3-4 pure colonies of bacteria were homogenized in 1 ml sterilized sodium chloride (0.85%) and centrifuged at 14000 rpm for 1 min. Washing was repeated with 500 µl sterilized sodium chloride (0.85%). The sediment was homogenized with 250 µl sterilized distilled water and boiled at 100 ˚C, and then stored immediately on ice. Finally, the supernatant contained the extracted nucleic acid was used for multiplex PCR.

Molecular identification

To identify the virulence genes of *iap* and *actA*, the specific primers which shown in Table 1 were used through multiplex PCR method (Manzano et al., 1998). The total mixture volume of 25 µl contained 100 ng DNA template, 10 pmol each primer, 0.2 mM dNTP (Cinnagen, Iran), 1.5 mM $MgCl_2$, 2.5 µl 10X PCR buffer, one unit *Taq* DNA polymerase, and distilled water up to the ultimate volume. The reactions were done using thermal cycler (Biorad, USA) in which the primary denaturation was performed for 5 min at 95 ˚C and 35 cycles (90 s at 95 ˚C for denaturation, 80 s at 46 ˚C for annealing, 120 s at 72 ˚C for extension. Final extension was done for 7 min at 72 ˚C. PCR products were assessed using 1.5% agarose gel electrophoresis alongside with 100 bp DNA ladder (Cinnagen, Tehran, Iran). *L. monocytogenes* strain of ATCC 7644 and distilled water were used as positive and negative controls, respectively (Mansouri Najand et al., 2015).

Results

Three out of 100 (3%) hamburger samples were biochemically diagnosed as *Listeria* contamination; however molecular identification assay confirmed that two of them were *L. monocytogenes* (Figure 1).

Discussion

There is a growing interest regarding to the increase consumption of fast food in all over the world. As *L. monocytogenes* cause severe disease, the presence of this bacterium in food is related to customer's safety so this species would not be detected in foods origin (Jay et al., 2005). The bacterium can survive in the frozen meat even under the prolonged freezing situation (Foerster et al., 2015; Wong et al., 2011). One possible explanation for the presence of *L. monocytogenes* in the frozen burgers is

Table 1: The specific primer pairs used in this study

Primer	Target	Sequence 5'-3'	Size (bp)	Reference
actA-F	*actA*	GTGATAAAATCGACGAAAATCC	400	Vazquez-Boland et al., 2001
actA-R		CTTGTAAAACTAGAATCTAGCG		
MAR 1	*iap*	GGGCTTTATCCATAAAATA	453	Manzano et al., 1998
MAR 2		TTGGAAGAACCTTGATTA		

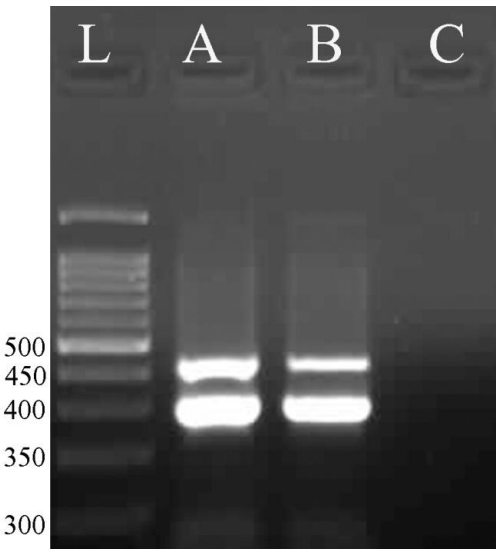

Figure 1: Agarose gel electrophoresis (1.5%) for assessing the PCR products. L: 100 bp DNA ladder; A: positive control of *L. monocytigenes* ATCC7644 (The PCR product length for *actA* gene was 400 bp and the one for *iap* gene was 453 bp); B: positive sample; C: negative control

related to the ability of this bacterium to multiply in freezing condition. Although a high prevalence of *L. monocytogenes* in many food products have been reported worldwide, the epidemiological data on the prevalence of this pathogenic bacterium in raw hamburger are limited. In a survey by Akpolat et al. (2004), 43 out of 830 samples of products of animal origin in Turkey were contaminated to *Listeria* among which one sample had contamination rate of more than 1000 colony forming unit/ml. According to another work in Malaysia carried out by Wong et al. (2012), it was found that the prevalence of *L. monocytogenes* in chicken patties (33.3%) was higher than that of beef (22.9%). In a similar study in Turkey, prevalence rate of *L. monocytogenes* in chicken burgers were identified 26.6% (Sireli et al., 2002). In all mentioned study, poor hygiene practice during food handling in domestic area was one of the important factors for the presence of this food-borne pathogen. In our study the prevalence of *L. monocytogenes* was only 2%. This low rate of infection comparing to the previous similar researches might be due to some factors such as small sample size or differences in applied detection methods. However, consumer should be aware about the heating temperature/time needed to completely inactive *L. monocytogenes* in burgers.

Conclusion

Although the prevalence rate of *L. monocytogenes* was not high in hamburger samples of Kerman, the risk of human listeriosis must not be underestimated or ignored. Further surveys are required in future for risk assessment of this bacterium in other food products distributed in the country.

Conflicts of interest

There are no conflicts of interest.

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