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Original Article

Listeria monocytogenes: An emerging food-borne pathogen and its public health implications

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Abstract

Introduction: *Listeria monocytogenes* is considered one of the most important food-borne pathogens transmitted to humans via contaminated food. The aim of the present study was to demonstrate the importance of *L. monocytogenes* as a food-borne pathogen.

Methodology: A total of 340 samples were collected from different localities in El Giza Governorate, Egypt, to check the occurrence of *L. monocytogenes* in that area. The collected samples comprised 250 food samples, 40 swabs from food refrigerators, and 50 stool specimens from diarrheic children. *L. monocytogenes* was isolated from the examined samples according to the International Organization for Standardization. The isolates were tested biochemically using *Listeria* Microbact 12L and confirmed by polymerase chain reaction.

Results: The isolation rates of *L. monocytogenes* were 8% in beef burger, 4% in minced meat, 4% in luncheon meat, while sausage samples were all negative. Eight percent of raw milk samples were positive for *L. monocytogenes*, whereas cheese samples and refrigerator swabs were negative. Only *Listeria grayi* was isolated from human stools (2.5%).

Conclusion: The high isolation rates of *L. monocytogenes* among the examined food stuffs highlight the crucial role of food as an important vehicle for this pathogen. More efforts should be made to ensure safe handling and processing of these foods to reduce the transmission of *L. monocytogenes* to humans.

Key words: *Listeria monocytogenes;* food-borne; meat; milk; Egypt.

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Introduction

Listeriosis is one of the most important bacterial infections worldwide that arises mainly from the consumption of contaminated food [1,2]. The disease is caused by *Listeria monocytogenes*, which is considered an opportunistic pathogen that affects mainly those with underlying immune conditions, such as pregnant women, neonates, and elders, resulting in septicemia, meningitis, and/or meningoencephalitis [3]. Food-borne listeriosis is relatively rare but is a serious disease with high fatality rates (20%–30%) compared with other food-borne microbial pathogens [4].

Severe *L. monocytogenes* infections are responsible for high hospitalization rates (91%) among the most common food-borne pathogens [5], may cause sporadic cases or large outbreaks, and can persist in food-processing environments and multiply at refrigeration temperatures, making *L. monocytogenes* a significant public health concern.

Ready-to-eat (RTE) meat products represent high risk to the consumers because they are usually cooked during manufacturing and are consumed without further heating, so cross-contamination with food-borne pathogens during the processing cannot be overcome [6].

The occurrence of *L. monocytogenes* in foods is usually overlooked due to the low count of the pathogen, the high population of competitive bacteria, and the inhibitory effect of some food additives [7]. Thus, the aim of the present study was to investigate contamination of foods with *L. monocytogenes*, and investigate its carriage in children with symptoms of fever and diarrhea.

Methodology

A total of 340 samples were collected from El Giza Governorate over the period of October 2013 to September 2014. These samples included 250 food samples (25 minced meat, 25 luncheon meat, 50 sausage, 50 beef burger, 50 raw milk, and 50 cottage cheese samples), and 40 swabs from food refrigerators collected randomly from retail markets, groceries, and

restaurants. Additionally, stool specimens (n = 50) were collected from diarrheic and feverish children between one and seven years of age admitted to Embaba Hospital for Tropical Diseases in Egypt.

Isolation and identification of L. monocytogenes

Listeria monocytogenes was isolated from the examined food samples according to the International Organization for Standardization procedure [8]. Briefly,25 grams of samples (or 25 mL of milk samples) were added to 225 mL half-Fraser broth (Oxoid, Basingstoke, UK) in a 500 mL flask and mixed well by shaking. The enrichment broth was incubated at 30°C for 24 hours. Then, 0.1 mL from the half-Fraser broth was transferred into 10 mL of Fraser broth (Oxoid) and incubated at 37°C for 48 hours. From the culture obtained in Fraser broth, a loopful of the culture was streaked onto chromogenic *Listeria* agar plates (Oxoid) and incubated at 37°C for 24 to 48 hours. *L. monocytogenes* appear as blue-green regular round colonies due to β-glucosidase using a specific chromogenic substrate and show an opaque halo, which helps to easily differentiate them from other species of *Listeria.* The halo is due to the activity of a phospholipase involved in the infection process of pathogenic species.

Refrigerator samples

Swabs from food refrigerators were incubated in 225 mL half-Fraser broth at 30°C for 24 hours for primary enrichment, then secondary enrichment with Fraser broth at 37°C for 48 hours (as described in food samples). A loopful from the culture obtained in Fraser broth was streaked onto chromogenic *Listeria* agar plates.

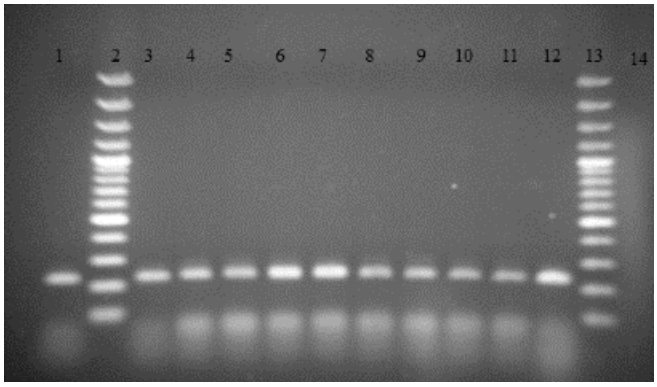
Human stool samples

Stool samples were streaked directly on chromogenic *Listeria* agar plates as previously described [9].

Biochemical identification

Colonies suspected to be *Listeria* spp. were transferred to tryptic soya agar plates with 0.6% yeast extract (TSA-YE) for further biochemical identification using *Listeria* Microbact 12L (Oxoid).

Figure 1. Electrophoretic profile of polymerase chain reaction for *hylA*gene in *L. monocytogenes* isolates



Lanes 2 & 13: DNA ladder (100 bp); lane 1: positive control (*L. monocytogenes* ATCC 35152); lane 14: negative control; lanes 3–12: positive *L. monocytogenes* isolates showing specific bands at 234 bp

Molecular identification

Extraction of DNA from *L. monocytogenes* isolates was done using DNA extraction kits (GF-1, Vivantis, Selangor, Malaysia) according to the manufacturer's instructions.

The amplification of the *hlyA* gene was carried out using the following primers (Table 1).

Polymerase chain reaction (PCR) amplification conditions were: 5 minutes at 94°C, 35 cycles of 30 seconds at 94°C, 45 seconds at 55°C, 45 seconds at 72°C, and a final extension of 5 minutes at72°C. The PCR products were analyzed using 1% agarose gel electrophoresis and examined using a UV transilluminator. The gel was photographed in order to obtain a permanent record using a digital camera (Figure 1).

Results

Of the 340 examined samples, ten *L. monocytogenes* isolates (all from food samples) were confirmed by PCR, while six isolates of other *Listeria* spp. were identified by *Listeria* Microbact 12L – two isolates from each of *L. ivanovii*, *L. seeligeri*, and *L. grayi* (Table 2).

Table 1. Primers sequence, annealing temperature, size of amplified fragment and targeted gene used in polymerase chain reaction PCR for confirmation of *L. monocytogenes*

Primer sequences orientation 5´ to 3´	Anneal temp. (°C)	PCR product (bp)	Targeted gene	Reference
LMA: CGGAGGTTCCGCAAAAGATG LMB: CCTCCAGAGTGATCGATGTT	55	234	hlyA (α-hemolysin, listeriolysin O)	[10]

In meat products, *L. monocytogenes* isolates were found in four of the examined beef burger samples (8%), in addition to which one *L. ivanovii* isolate (2%) and one *L. seeligeri* isolate were also identified. For both minced meat and luncheon meat, only one *L. monocytogenes* isolate was recovered from each (4%). No sausage samples yielded *L. monocytogenes*, but both *L. ivanovii* and *L. seeligeri* were isolated in 2%of each. Moreover, 4/50 examined milk samples were positive for *L. monocytogenes* (8%), but all examined cheese samples were negative (Table 3). On the other hand, neither refrigerator swabs nor children's stool specimens were positive for *L. monocytogenes*. *L. grayi* was isolated from one refrigerator swab and one diarrheic child (Table 2).

Discussion

L. monocytogenes has been recognized as one of the most serious emerging bacterial diseases during the last two decades that is transmitted through the consumption of contaminated foods [11-13]. The results of the current study revealed that minced meat samples were found to be contaminated with *L. monocytogenes* (4%).The contamination of raw meat with *Listeria* spp. could be due to either fecal contamination during evisceration or due to the practices of food handlers [14]. This result was in close agreement with that obtained by Akpolat *et al*. [15] and Yücel *et al*. [14], who isolated *L. monocytogenes* from minced beef samples at rates of5% and 4.7%, respectively. However, a lower rate of contamination was detected by Molla *et al*. [16] in Ethiopia (1.6%). Additionally, one luncheon meat sample yielded *L. monocytogenes* (4%); luncheon products undergo extensive processing and handling during their production, and this may have a higher associated risk of *L. monocytogenes* contamination [17,18]. Also RTE cooked meats are frequently contaminated with *L. monocytogenes* during post-processing steps [19]. The contamination of RTE cooked meat by *L. monocytogenes* is an important safety concern because RTE cooked meats may have longer shelflife and are consumed without further heating; moreover, *L. monocytogenes* can proliferate and exceed minimum infectious dose levels during refrigerated storage [20]. A higher prevalence of *L. monocytogenes* (12%) was detected by El-Shenawy *et al*. [21] in street-vended RTE luncheon meat sandwiches in Egypt, while a lower prevalence was found by Gombas *et al*. [22] (0.89%).

Surprisingly, *L. monocytogenes* was not isolated from sausage samples, but *L. ivanovii* and *L. seeligeri* werere covered at a rate of 2% each, highlighting the occurrence of pathogenic *Listeria* spp. rather than *L. monocytogenes* in this product. *L. ivanovii* was reported to infect ruminants only [23], but it has been isolated, although rarely, from infected humans, indicating its pathogenic potential for humans [24]. *L. seeligeri* may also carry a virulence gene cluster similar to that of *L. monocytogenes* and *L. ivanovii* [25].

Interestingly, beef burger samples showed higher isolation rates for *L. monocytogenes*, *L. ivanovii*, and *L. seeligeri*. It is noteworthy that the high isolation rate of *L. monocytogenes* from beef burger underscored the potential role that may be played by this product to

Table 2. Occurrence of *L. monocytogenes* and other *Listeria spp.* in the examined samples.

Type of samples	Number of examined samples	*L. monocytogenes*		*L. ivanovii*		*L. seeligeri*		*L. grayi*	
		No. positive	(%)	No. positive	(%)	No. positive	(%)	No. positive	(%)
Food samples	250	10	4%	2	0.8%	2	0.8%	0	0%
Refrigerator swabs	40	0	0%	0	0%	0	0%	1	2.5%
Human stool	50	0	0%	0	0%	0	0%	1	2%

Table 3. Distribution of *L. monocytogenes* in different food samples

Type of food	Number of examined samples	Number of positive samples	(%)
Minced meat	25	1	4%
Luncheon	25	1	4%
Beef burger	50	4	8%
Sausage	50	0	0%
Cottage cheese	50	0	0%
Raw milk from small-scale farms outlets	30	4	13.3%
Raw milk from markets	20	0	0%
Total raw milk	50	4	8%

convey *L. monocytogenes* to the human gut. Similar results were obtained by Wong *et al*. [26], who detected *L. monocytogenes* in a higher ratio (22.9%) in beef burger in Malaysia and reported that spices added during the processing of burger patties and freezing during transportation and retailing are not sufficient to deactivate all *L. monocytogenes* that may present in the raw meat. Moreover, Wong *et al*. [27] found that *L. monocytogenes* was not detected after six minutes of cooking chicken burger patties, but it was detected after four minutes of cooking. Therefore, efficient cooking of burgers is very important to prevent food-borne illness from burgers that may be contaminated with *L. monocytogenes*. Raw milk samples were contaminated with *L. monocytogenes* (8%). All positive samples were collected from outlets of small-scale farms in rural areas; this high contamination may be due to lack of hygienic measures during the milking process and in transportation and milk storage tanks, which may be the source of contamination. This result agreed with that obtained by El Marnissi *et al*. [28], who detected *L. monocytogenes* in 8.33% of raw milk samples collected from traditional dairies in Morocco. The authors suggested that poor hygienic conditions during milking, transport, storage of milk, and management practices of cattle feed lead to contamination of raw milk with *L. monocytogenes*.AL-Ashmawy *et al*. [29] detected *L. monocytogenes* in 8% of bulk milk tank samples from dairy farms in Egypt, and Jamali *et al*. [30] found that the prevalence of *L. monocytogenes* in raw cow milk from dairy farm bulk milk tanks was 5.4% in Iran. Environmental contamination of milk during milking, storage, transportation; infected cows; and poor quality silage in addition to fecal contamination were reported as common sources of *L. monocytogenes* contamination of raw milk [31,32].

None of the examined cottage cheese samples yielded *Listeria* spp., a result comparable to that obtained by Ismaiel *et al*. [33], who did not isolate any *L. monocytogenes* from tested cheese samples from Egypt.AL-Ashmawy *et al*. [29] also failed to detect *L. monocytogenes* from cottage cheese samples using the colony PCR method. The low pH (approximately 4.2) and other antimicrobial compounds produced by lactic acid bacteria incorporated in cottage cheese may have a marked effect on the survival and growth of *L. monocytogenes*, which is usually inhibited at pH levels below 5.2. [34,35].

Furthermore*, L. monocytogenes* was not isolated from refrigerator swabs, but one sample swabbed from a refrigerator for meat storage in a restaurant was found to be positive for *L. grayi*.

All human stool samples were negative for *L. monocytogenes*, but *L. grayi* was isolated from a case of diarrhea in a five-year-old child. Grif *et al*. [36] attributed the lower isolation rate of *L. monocytogenes* from human stool samples to the secretion of gastric acid, which acts as an important protective factor against the passage of pathogenic organisms.

Rapose *et al*. [37] reported that infections with non-*monocytogenes Listeria* are rare, but they have the potential to cause human disease. The authors described a case of sepsis in a heart transplant recipient caused by *L. grayi*. Salimnia *et al*. [38] reported another case of *L. grayi* bacteremia in a stem cell transplant recipient.

The detection of *L. monocytogenes* by molecular methods is very specific [39], and the *hlyA* gene-based detection for *L. monocytogenes* has been frequently adopted by various investigators [40-42]. Also, introduction of chromogenic agars for isolation of *L. monocytogenes* takes 24 hours versus the three to four days it takes using Oxford and other conventional agars [43]. Most of these media have been tested on a wide range of different foods [44] and are now included in most protocols and standards [45,8].

Conclusions

High isolation rates of *L. monocytogenes* from beef burger and raw milk from farm outlets make both potential sources for *L. monocytogenes* food-borne illness. More efforts should be made to improve food safety in these chains for effective control of this infection.

In memoriam

Doctor Waffa Reda passed away on 27/11/2013. This work is dedicated to her memory and valuable work.

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