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Survey of *Listeria monocytogenes* and Other *Listeria* sp. Contamination in Different Common Ready-to-Eat Food Products in Jordan

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Abstract: Incidence and contamination levels of different *Listeria monocytogenes* serovars and different *Listeria* sp. in 360 samples of common ready-to-eat food products in Jordan were investigated. The presence of *L. monocytogenes* was determined using EN ISO protocol and confirmed using PCR technique. Five *Listeria* sp.: *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. welshimeri* and *L. seeligeri* were isolated. *L. monocytogenes* was isolated from 19 samples (5.3%), from 6 RTE dairy samples; 6 RTE vegetables samples and 4 traditional dishes samples and 3 miscellaneous samples. *L. innocua* and *L. ivanovii* were the most and least frequently isolated species, 24 and 3 samples, respectively. *L. welshimeri* was isolated from 8 samples and *L. seeligeri* from 7 samples. The contamination levels of *L. monocytogenes* were found to be ≤ 100 CFU g⁻¹ in 84.2% (16 samples) of the positive samples. Only 15.8% (3 samples; 1 vegetable, 1 traditional dish and 1 miscellaneous samples) of the positive samples were found with counts >100 CFU g⁻¹. *L. monocytogenes* strains isolated fell into 2 serotype, 1 and 4 and to 5 different serovars, 1/2a, 1/2b, 1/2c, 4a, 4c.

Key words: *Listeria monocytogenes*, *Listeria* serovars, ready-to-eat-food-products, Jordan, *L. monocytogenes* chromogenic agar

INTRODUCTION

Listeria monocytogenes is the causal agent of listeriosis illness which is one of the most virulent foodborne diseases that regulatory agencies throughout the world have been trying to keep contained (Schlech, 2000). Listeriosis is characterized by a range of symptom manifestations including mild flu-like symptoms such as fever, fatigue, nausea, vomiting and diarrhea and severe symptoms such as septicemia, meningitis and death (European Food Safety Authority, 2007; Farber and Peterkin, 1991). While the incidence of human listeriosis is low (2-15 per million habitants), the case fatality rate is reported to be approximately 20-40%, that increases up to 75% in high risk groups, such as pregnant women, neonates, elderly (>65 years) and immunocompromised adults (Rocourt *et al.*, 2003). *L. monocytogenes* is widely distributed in nature (soil, irrigation water, vegetables etc.) and food processing environments (surfaces, machines, floors etc.) (Farber and Peterkin, 1991).

As a result to the change in life style and consumer food habits, consumption rates and demands of Ready-to-Eat (RTE) food products have greatly increased in Jordan and around the world. In response to this increased demands, food processing companies have developed new products. Part of the appeal of these products is their

convenience, once it does not need cooking prior to serving (Norrung, 2000).

Along with the increased consumption of RTE foods has been the rise in incidence of illness resulting from contaminated RTE food products (Awaisheh and Ibrahim, 2009; Guerra *et al.*, 2001; Schlech, 2000). *Escherichia coli* O157:H7, *Salmonella* sp., *Campylobacter* sp. and *Staphylococcus aureus* are among the most common health risk foodborne pathogens associated with RTE food products, but recently, several outbreaks have occurred involving *L. monocytogenes* (Norrung, 2000). The incidence of *L. monocytogenes* in different RTE food products has been variable ranging from 2.7 to 20%, whereas, prevalence of *Listeria* sp. ranging from 1.8 to 54.0% (Center of Food Safety and Applied Nutrition, 2000; Meloni *et al.*, 2008; Vitas and Garcia-Jalon, 2004). During the last 15-20 years there has been an increasing concern world-wide about *L. monocytogenes* and its implications for food safety. Several large well documented foodborne outbreaks and sporadic cases have been described and *L. monocytogenes* has been isolated from a wide range of raw and ready-to-eat meats, poultry, dairy products, sea foods and vegetables and from various food processing environments (Norrung, 2000; Schlech, 2000).

Recently, European Commission Regulations (EC No. 1441/2007) considered all foods which could be associated with transmission of listeriosis to be mostly

RTE foods that support growth of *L. monocytogenes*. Products with pH >4.4 or $a_w > 0.92$, products with pH >5.0 and $a_w > 0.94$, products with a shelf-life of more than 5 days shall be automatically considered to belong to this category. Accordingly, most of the RTE food products traded around the world and in Jordan fall in the category of products supports the growth of *L. monocytogenes* and classified as high risk foods.

There are a number of regulations and standards concerning the microbiological criteria of *L. monocytogenes* in foods. In a number of countries including the United States, the microbiological criterion is 0 CFU of *L. monocytogenes* per 25 g sample of RTE-foods (Center of Food Safety and Applied Nutrition, 2000). However, some European countries have microbiological criteria of 100 CFU g⁻¹ of *L. monocytogenes* at the point of consumption (European Food Safety Authority, 2007).

In Jordan, the microbiological criteria of *L. monocytogenes* are not specified in the official standards for many types of foods, specifically RTE foods. As a result, there is a little information concerning incidence and distribution of *L. monocytogenes* in foods, particularly high risk RTE foods. Due to the potential and virulent health threat of this pathogen there is a need to develop a database of information concerning the incidence and distribution of *L. monocytogenes* in different Jordanian foods, so that possible outbreaks can be avoided. To meet this need the aims of the study were to: Survey incidence of *L. monocytogenes* and other *Listeria* sp. and contamination levels of *L. monocytogenes* in high risk RTE food products in Jordan and Survey the serotype groups of *L. monocytogenes* isolates.

MATERIALS AND METHODS

Food samples: A total of 360 samples of different common RTE food products in Jordan were randomly collected during the period of May, 2007 to July, 2008 from different street vendors, markets and processing outlets in Amman, Jordan. The analyzed products included dairy, vegetables (fresh and processed), traditional foods, salads, dressing and miscellaneous products (Table 1). Samples were kept refrigerated until analyzed. Analysis was performed within 24 h of collection.

Chemical-physical analysis: For all samples, the pH and water activity (a_w), were determined. The potentiometric measurement of pH was carried out by inserting the pin electrode of a pH-meter GLP 21 (Crison, Carpi, MO, Italy)

directly into each sample. Water activity (a_w) was determined using an Aqualab CX3 (Decagon, Pullman, WA, USA).

***L. monocytogenes* enrichment and isolation using EN ISO 11290-1:1997/Amd 1: 2004:** In order to recover the maximum number of stressed cells of *Listeria* sp. two-step selective enrichment protocols were used (EN ISO, 1997/Amd 1:2004). Half-Fraser Broth (HFB) was used as primary enrichment and Fraser Broth (FB) as a secondary enrichment. HFB was prepared using Fraser Broth Base (Oxoid, UK), supplemented with a half of the Fraser Selective Supplement. Twenty five grams of each sample was added to 225 mL primary enrichment broth. The mixture was then homogenized for 60 sec in a Stomacher Lab-Blender 400 (Seward Medical, England). Inoculated broth was incubated for 24 h at 30°C and then 0.1 mL samples were transferred to tube containing 10 mL of the secondary selective Fraser enrichment. After 24 and 48 h of incubation at 30°C, a loopfull was collected from secondary enrichment broth preparations and streaked onto samples of *Listeria* Chromogenic Agar (LCA) (Oxoid, UK).

Purification and identification: After incubation at 37°C for 48 h, five characteristic colonies were selected from LCA agar and streaked onto tryptone soya yeast glucose agar (TSYGA) plates for purification (EN ISO, 1997/Amd 1:2004). On LCA, *L. monocytogenes* colonies typically appear as blue-turquoise colonies with a white precipitation zone around the colony; whereas other non pathogenic *Listeria* sp. colonies appear blue-turquoise without precipitation zones (Reissbrodt, 2004). Accordingly, all isolates from LCA plates with typical characteristic colony appearance were selected and presumptively considered *L. monocytogenes*. Isolated colonies were tested for Gram reaction and plated onto sheep Blood Agar (BA), (Blood Agar Base No. 2; Oxoid, overlayers with the same medium containing 7% (v/v) Sheep Blood). All the isolates that showed haemolysis on BA were tested for motility and catalase activity and biochemical identification (API-*Listeria*, bioMerieux).

Enumeration of *L. monocytogenes* using EN ISO 11290-2:1998/Amd 1:2005: Positive samples for *L. monocytogenes* were tested by direct plating following EN ISO 11290-2:1998/Amd 1:2005. Twenty five grams of each sample were placed in a sterile plastic bag with 225 mL of Buffered Peptone Water (Oxoid, UK) and this initial blend was stored for 1 h ±5 min at 20±2°C in order to recover stressed microorganisms. Decimal dilutions

were prepared and plated in duplicate on LCA agar (Oxoid, UK). In order to increase the sensibility of the technique, 1 mL of the first decimal dilution was also plated (using one 140 mm plates or three 90 mm Petri dishes). The plates were incubated for 24 or 48±2 h at 37±1°C and typical colonies were counted and isolated in TSAY for further confirmation, as described in the previous section.

PCR identification of presumptive positive *L. monocytogenes*: All presumptive positive colonies identified by plating on selective agars and biochemical kits, were subjected to a Polymerase Chain Reaction (PCR) procedure which amplified a specific DNA sequence of the 730 bp listeriolysin (*hlyA*) gene. The gene identified using following primer sequences; 5'-CATTAGTGAAAGATGGAATG-3' (primer A) and 5'GTATCCTCCAGAGTGATCGA-3' (primer B) (Oligos-Midland, TX, USA). The primers are known to be highly specific for *L. monocytogenes* and do not amplify DNA present in any other *Listeria* sp. or non-*Listeria* organisms (Blais *et al.*, 2002). *L. monocytogenes* ATCC 7644 and *E. coli* ATCC 25922 have been used as positive and negative controls, respectively.

DNA extraction: The extraction procedure was based on a protocol previously described by Gouws and Liedemann (2005) for the detection of *L. monocytogenes* in food products. *L. monocytogenes* strains were stored for long term use in brain heart infusion medium with 10% sterile glycerol at -80°C. The strains were recovered by streaking samples of them on brain heart infusion agar plates and grown overnight at 37°C. Cells were then scraped and resuspended in 50 µL of 1X PCR buffer in a 2 mL microcentrifuge tube with an interlocking cap. A solution of 2% Triton X (50 µL) was then added to this cell suspension and thoroughly mixed. This mixture was heated at 100°C for 10 min and then allowed to cool to room temperature. For PCR amplification, 5 µL of this crude cell lysate were used.

PCR amplification: Amplification reaction mixtures were prepared using the primers at concentrations of 50 pmol µL⁻¹, 1 U of *Taq* polymerase, 1X reaction buffer (Fisher Scientific, Pittsburgh, Pa.), 0.2 mM each deoxynucleoside triphosphate (Oligos, Midland, TX, USA), 2.5 mM MgCl₂ and 2 µL of template DNA in a 25 µL reaction volume. PCR cycling conditions were as follows: Amplification conditions were optimized to the thermal cycler and were as follows: Amplification conditions were optimized to the thermal cycler and were as follows: 80°C

for 10 min, an initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec, then a final extension at 72°C for 2 min (Primus 96 Plus Thermal Cycler, MWG Biotech, High Point, NC, USA). The amplified DNA was analyzed by gel electrophoresis on a 1.2% agarose gel stained with ethidium bromide (3 µL/100 mL). A 100 bp ladder (Promega, WI, USA) was used as a reference marker. Tris-borate EDTA (0.5') was used as the running buffer and the gel was viewed using UV transillumination at a wavelength of 254 nm (Gouws and Liedemann, 2005).

Survey of *L. monocytogenes* serotypes: In order to survey the serotype groups of isolated *L. monocytogenes*, serotyping was performed following the scheme established by Seeliger and Hohne (1979). Strains were serotyped using antisera against somatic (O) and flagellar (H) antigens according to manufacturer instructions (Denka Seiken, Tokyo, Japan).

RESULTS

Results of the chemical-physical analysis of the tested RTE food products are shown in Table 1: (a) Dairy products: The mean pH values were 4.2-6.8 and the mean *a_w* values were 0.84-0.98. The lowest mean pH values were in yogurt (4.2±0.2) and the highest were in milk and ice cream (6.8±0.1). The lowest mean *a_w* values were in boiled cheeses (0.84±0.02), followed by labaneh (0.92±0.03) and the highest were in milk, liquid jameed and shanineh (0.98±0.01); (b) Vegetable products: The mean pH values were 5.5-5.8 and the mean *a_w* values were 0.95-0.98; (c) Traditional dishes: The mean pH values were 5.0-5.6 and the mean *a_w* values were 0.94-0.96. The lowest mean pH values were in Hummus and mutable (5.0±0.2) and the highest were in soups and stews (5.6±0.2); (d) Miscellaneous: The mean pH values were 3.9-4.2 and the mean *a_w* values were 0.65-0.95. The lowest mean pH values were in ketchup (3.9±0.2), followed by mayonnaise and dried fruits and vegetables (4.2±0.2). Dried fruits and vegetables *a_w* were the lowest (0.65±0.1), followed by mayonnaise and ketchup (0.95±0.01).

The incidence of *L. monocytogenes* and other *Listeria* sp. in the samples tested is shown in Table 2. The 5.28% (19 samples) of the samples were positive for *L. monocytogenes*. The highest *L. monocytogenes* incidence occurred in dairy and vegetables samples, 5% (6 samples each). In traditional and miscellaneous samples 6.67 and 5.0% (4 and 3 samples) of the samples were *L. monocytogenes* positive, respectively.

Table 1: pH and *a_w* values (Mean±SD) of different groups of different ready-to-eat (RTE) food products tested for *L. monocytogenes*

| RTE food groups | Types of samples | No. of samples | <i>a_w</i> | pH |
|--------------------------|-------------------------------------|----------------|----------------------|---------|
| RTE dairy products | Milk | 20 | 0.98±0.01 | 6.8±0.2 |
| | Boiled cheeses | 20 | 0.84±0.02 | 6.2±0.3 |
| | Soft cheeses | 20 | 0.95±0.02 | 6.2±0.3 |
| | Yogurt | 15 | 0.97±0.02 | 4.2±0.2 |
| | Labaneh | 10 | 0.92±0.03 | 4.4±0.2 |
| | Liquid jameed | 10 | 0.98±0.01 | 4.5±0.2 |
| | Shanineh | 10 | 0.98±0.01 | 4.5±0.2 |
| | Ice cream | 15 | 0.97±0.01 | 6.8±0.1 |
| | Subtotal | | 120 | |
| RTE vegetable products | Fresh vegetables salad and tabooleh | 30 | 0.98±0.01 | 5.8±0.3 |
| | Coleslaw | 30 | 0.97±0.02 | 5.6±0.2 |
| | Sweet corn with mayonnaise | 30 | 0.95±0.02 | 5.6±0.2 |
| | Smashed vegetables with mayonnaise | 30 | 0.95±0.02 | 5.5±0.2 |
| Subtotal | | 120 | | |
| RTE traditional products | Hummus | 15 | 0.95±0.02 | 5.0±0.2 |
| | Mutable | 15 | 0.94±0.02 | 5.0±0.2 |
| | Taheeneh | 15 | 0.96±0.02 | 5.5±0.2 |
| | Soups and stews | 15 | 0.95±0.02 | 5.6±0.2 |
| Subtotal | | 60 | | |
| Miscellaneous | Dried fruits and vegetables | 20 | 0.65±0.10 | 4.2±0.2 |
| | Mayonnaise | 20 | 0.95±0.01 | 4.1±0.1 |
| | Ketchup | 20 | 0.95±0.01 | 3.9±0.2 |
| Subtotal | | 60 | | |
| Total | | 360 | | |

Table 2: Prevalence of *L. monocytogenes* and other *Listeria* sp. in different RTE food products (% of positive samples)

| RTE food groups | No. of samples | <i>L. monocytogenes</i> | <i>L. innocua</i> | <i>L. ivanovii</i> | <i>L. seeligeri</i> | <i>L. welshimeri</i> |
|--------------------|----------------|-------------------------|-------------------|--------------------|---------------------|----------------------|
| Dairy products | 120 | 6 (5.00) | 7 (5.83) | 1 (0.83) | 2 (1.67) | 2 (1.67) |
| Vegetable products | 120 | 6 (5.00) | 9 (7.50) | 2 (1.67) | 3 (2.50) | 3 (2.50) |
| Traditional dishes | 60 | 4 (6.67) | 4 (6.67) | - | 1 (1.67) | 1 (1.67) |
| Miscellaneous | 60 | 3 (5.00) | 4 (6.67) | - | 1 (1.67) | 2 (3.33) |
| Total | 360 | 19 (5.28) | 24 (6.66) | 3 (0.83) | 7 (1.95) | 8 (2.22) |

Table 3: Contamination levels of *L. monocytogenes* in ready-to-eat food products¹

| Ready-to-eat meat products | No. of positive samples | No. (%) of positive samples by colony (CFU g ⁻¹) | | |
|----------------------------|-------------------------|--|----------|-----------|
| | | <10 | >10-100 | >100-1000 |
| Dairy products | 6 | 5 (83.3) | 1 (16.7) | - |
| Vegetable products | 6 | 3 (50.0) | 2 (33.3) | 1 (16.7) |
| Traditional dishes | 4 | 2 (50.0) | 1 (25.0) | 1 (25.0) |
| Miscellaneous | 3 | 1 (33.3) | 1 (33.3) | 1 (33.3) |
| Total | 19 (100) | 11 (57.9) | 5 (26.3) | 3 (15.8) |

¹Contamination level was determined by using EN ISO 11290-2: 1998/Amd1:2004 with detection limits of 10-100 CFU g⁻¹ sample

L. innocua was the most extensively isolated *Listeria* sp., 6.66% (24 samples). It was isolated from 5.83% (7 samples) of the dairy samples, 7.50% (9 samples) of the vegetable samples and 6.67% (4 samples) of each of the traditional and miscellaneous samples. *L. welshimeri* was isolated from 2.22% (8 samples) of the samples. Highest *L. welshimeri* incidence was in vegetables samples, 2.50% (3 samples) and the lowest incidence was in traditional foods samples, 1.67% (1 sample). Both *L. seeligeri* and *L. ivanovii* were the least frequently isolated species, 1.95 and 0.83% (7 and 3 samples), respectively. Both species highest incidence was in vegetables samples, 2.50 and 1.67% (3 and 2 samples), respectively. *L. ivanovii* was isolated from 1 sample of the dairy samples and was not isolated from the traditional and miscellaneous samples. Whereas, *L. seeligeri* was isolated from 0.83% (2 samples) of the dairy samples and 1.67% of the traditional and miscellaneous

samples (1 sample each). It is worth mentioning that co-contamination with different species of *Listeria* was detected in several foods, especially in dairy and vegetable samples with *L. monocytogenes* plus *L. innocua* was the most frequent combination.

The level of *L. monocytogenes* in the positive samples was <10 CFU g⁻¹ in 57.9% (11 samples) of cases and was >10 and <100 CFU g⁻¹ in 26.3% (5 samples) of cases, in conformity with the food safety criteria provided for the RTE foods able to support the growth of *L. monocytogenes* (Commission Regulation EC N° 1441/2007). Only 3 samples (15.8%), coleslaw vegetable product, mutable traditional dish and mayonnaise, were found with counts higher than 100 CFU g⁻¹ (430, 350, 210 CFU g⁻¹, respectively) (Table 3).

The 19 *L. monocytogenes* strains isolated fell into 5 different serovars (Table 4): 1/2a, 1/2b, 1/2c, 4a and 4c. Seventy four percent of the strains belonged to serogroup

Table 4: Serotypes of *L. monocytogenes* strains isolated in ready-to-eat food products

| RTE food groups | No. of serogroup 1 (%) | No. of serogroup 4 (%) | No. of serotype positive (%) | | | | | Total |
|--------------------|------------------------|------------------------|------------------------------|----------|----------|----------|----------|---------|
| | | | 1/2a | 1/2b | 1/2c | 4a | 4c | |
| Dairy products | 4 (66.7) | 2 (33.3) | - | 3 (50.0) | 1 (16.7) | - | 2 (33.3) | 6 |
| Vegetable products | 4 (66.7) | 2 (33.3) | 2 (33.3) | 2 (33.3) | - | 2 (33.3) | - | 6 |
| Traditional dishes | 3 (75.0) | 1 (25.0) | 2 (50.0) | 1 (25.0) | - | 1 (25.0) | - | 4 |
| Miscellaneous | 3 (100.0) | - | 2 (66.7) | 1 (33.3) | - | - | - | 3 |
| Total | 14 (74.0) | 5 (26.0) | 6 (31.6) | 7 (36.8) | 1 (5.3) | 3 (15.8) | 2 (10.5) | 19(100) |

1 and 26% belonged to serogroup 4. Serotype 1/2a and 1/2b were the predominant serotypes (6 and 7 samples, 31.6 and 36.8%, respectively). Serotype 1/2a was equally isolated from vegetables, traditional and miscellaneous samples (2 samples each). Serotype 1/2a was not isolated from dairy samples. Serotype 1/2b was most frequently isolated from dairy samples (3 samples) and with less frequency in vegetables samples, traditional and miscellaneous samples (2, 1 and 1 samples, respectively). On the other hand, serotype 1/2c was only isolated from vegetables samples (1 sample). Within serogroup 4, serotypes 4a and 4c were the only prevalent serotypes. Serotype 4a was isolated from vegetables and traditional samples (2 and 1 samples, respectively). Whereas, serotype 4c was only isolated from dairy samples (2 samples).

DISCUSSION

Listeriosis is an emergent and virulent illness with a low incidence, but with a high fatality rate, especially in neonate, elderly, pregnant and immunocompromised individuals. Different kinds of foods have been reported to be associated with the transmission of *L. monocytogenes* (Mc-Lauchlin, 1996; Norrung, 2000), including vegetables (coleslaw and salads), dairy products (pasteurized milk and cheese) and meat products (RTE vacuum packaged meat products and Frankfurt sausages) (Norrung, 2000; Schlech, 2000).

Although, the minimal infective dose for human is unknown, different studies have implied that foods implicated in cases of listeriosis have contained elevated levels of the pathogen (Hitchins, 1996; Mc-Lauchlin, 1996). This is confirmed indirectly by the high distribution and prevalence of the microorganism in food as compared to the low incidence of listeriosis cases (Vitas and Garcia-Jalon, 2004). According to the listeriosis risk assessment report by USFDA, the consumption of RTE food products poses the greatest relative public health risk potential for listeriosis.

Most of the tested RTE products (except some samples of dairy products, i.e., boiled cheeses, yogurt and labaneh and dried fruits and vegetables), showed mean values of pH and a_w typical of products able to support

the growth of *L. monocytogenes* ($pH \geq 4.4$ or $a_w \geq 0.92$ and $pH \geq 5.0$ or $a_w \geq 0.94$, with shelf life more than 5 days) (Codex Alimentarius Commission, 2007; Meloni *et al.*, 2008).

In this study, 16.95% (61 samples) of 360 samples tested were *Listeria* positive, of which 5.28% (19 samples) were positive for *L. monocytogenes* and 11.67% (42 samples) were other *Listeria* sp. positive. The prevalence of *L. monocytogenes* and other *Listeria* sp. was relatively higher in the RTE dairy and vegetables samples than in traditional and miscellaneous samples. Dairy samples *L. monocytogenes* positive samples (3 samples, 5.0%) were 1 labaneh sample and 2 soft cheese samples. Incidence of *L. monocytogenes* and other *Listeria* sp. in dairy products is well documented in literature. Pintado *et al.* (2005) reported the incidence of *L. monocytogenes* (29%) and other *Listeria* sp. (*L. innocua* and *L. seeligeri*) (75%) in soft cheeses made from raw milk in Portugal. Vitas and Garcia-Jalon (2004) reported *L. monocytogenes* (6.8%) and other *Listeria* sp. (*L. innocua*, *L. ivanovvi*, *L. seeligeri* and *L. welshimeri*) contamination in raw cow and sheep milk, which could be upon improper heat treatment or post contamination transmitted to the finished products. However, listeriosis outbreaks linked to dairy products, such as pasteurized milk (Fleming *et al.*, 1985) or soft Mexican cheese (Linnan *et al.*, 1988) have caused several deaths.

Early outbreaks of listeriosis were epidemiologically associated with the consumption of raw vegetables. In addition, coleslaw was the cause of an outbreak in Canada in 1981 (Farber and Peterkin, 1991). Present results indicated contaminations patterns in vegetable products comparable to those reported in literature. Meloni *et al.* (2008) reported 2 and 24% contamination of *L. monocytogenes* and *Listeria* sp., respectively, in vegetables and vegetables products. Also, Vitas and Garcia-Jalon (2004) reported 1.8 and 10.4% contamination of *L. monocytogenes* and *Listeria* sp. (*L. innocua*, *L. seeligeri* and *Welshimeri*), respectively, in vegetables and vegetables products.

The low incidence of *L. monocytogenes* and *Listeria* sp. in traditional foods and miscellaneous products was shown in the present study. Samples with

L. monocytogenes contamination were of those products that handled and touched by human after processing and before serving. Handling of these products after processing could be the route through which *L. monocytogenes* is transmitted to food products.

In Jordan, food processors and handlers may have limited awareness about the high risks associated in not decontaminating the processing environment sufficiently to ensure complete eradication of foodborne pathogens like *L. monocytogenes*. This could explain the relatively high incidence of *L. monocytogenes* and other *Listeria* spp in Jordanian produced RTE food products. Also, despite the fact that the majority of RTE food products when produced and processed are stored at 0-5°C, many of the retail and domestic refrigerators in Jordan are too warm for the safe storage of food (reaching temperature $\geq 9^{\circ}\text{C}$), allowing the growth of *L. monocytogenes* and other spoilage organisms (Francois *et al.*, 2006). This also could partly account for the prevalence of *L. monocytogenes* and other *Listeria* spp in RTE food products sold and consumed in Jordan.

One of the objectives of this study was to quantify contamination levels of *L. monocytogenes* in different RTE food products in order to estimate actual consumer exposure to the organism. The analyzed samples were found to be able to support *L. monocytogenes* growth. Of the 19 positive samples only in 3 samples (15.8%) were in nonconformity with the food safety criteria provided for the RTE foods able to support the growth of *L. monocytogenes* (European Commission Regulations, 2007) with level of contamination $>100 \text{ CFU g}^{-1}$. The 84.2% of the positive samples with contamination level of $\leq 100 \text{ CFU g}^{-1}$ were in conformity with the Commission Regulation EC No 1441/2007. The most recent Codex document on microbiological criteria for *L. monocytogenes* in RTE foods able to support the growth of *L. monocytogenes* (Codex Alimentarius Commission, 2007) suggests a zero tolerance throughout the entire shelf life of the product.

With respect to the serological results, our results were similar to those of Garrido *et al.* (2009) and Vitas and Garcia-Jalon (2004). In our study, 2 serogroups were observed, serogroups 1 and 4. Serogroup 1 predominated by 74% and serogroup 4 by 26%, whereas Garrido *et al.* (2009) data prevailed 62 and 38% of serogroups 1 and 4, respectively in different RTE food products and Vitas and Garcia-Jalon (2004) data prevailed 75 and 25% of serogroups 1 and 4, respectively. The serotypes usually involved in cases of listeriosis are 1/2a, 1/2b and 4b, which were also the serotypes isolated in our study.

CONCLUSION

Even though the contamination levels of *L. monocytogenes* were limited but its high incidence poses a serious public health concern and risk and identifies the urgent need to raise awareness of Jordanian food processors and handlers to possible *Listeria* sp. contamination due to current production and processing practices. The findings from this study also suggest the need to increase their knowledge of the pattern and levels of incidence of *Listeria* sp. in both raw materials and finished products and to follow more strict hygienic procedures to avoid and prevent recontamination.

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