Short communication

Listeria monocytogenes isolated from vegetable salads sold at supermarkets in Santiago, Chile: Prevalence and strain characterization

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1. Introduction

Large outbreaks of human listeriosis associated with contaminated foodstuffs have occurred in several countries since the early 80s and numerous studies have been performed on detection of Listeria monocytogenes in different types of food, including vegetables (Farber and Peterkin, 1991; Beuchat, 1996; Crépet et al., 2007). However, Listeria contamination of food in Chile was unknown until 1990 when a research on its presence in high consumption foods in Santiago, Chile was started. Initially, dairy, meat products and crustaceous shellfish were analysed and percentages of contamination in ready-to-eat products ranged from 0.8% to 6.7% [ice cream (3.5%), soft cheese (0.8%), processed meat products (2.1%) and cooked shellfish (6.7%) (Cordano and Rocourt, 2001)].

The growing interest in healthy diets, which entail increased consumption of vegetables, turned our interest to vegetable salads. Besides the rise in consumption, changes in life-style have meant a shortage of time for home cooking that has led many people to buy ready-to-eat food. Therefore, a study was undertaken, over a six-year period, on the presence of L. monocytogenes in vegetable salads sold in supermarkets in Santiago, with the aim to ascertain the risk of these products for consumers and to provide the health authorities with the relevant information. Three main supermarket chains were chosen for the study. Samples were purchased as any customer would do, considering all brands and type of salads offered for sale. This paper shows the results obtained from the analysis of the three types of vegetable salads available to customers at supermarkets: frozen, cooked or raw ready-to-eat salads industrially prepared. Enumeration of L. monocytogenes was done by plate count for 20 positive frozen samples, randomly chosen. Most of them (90%) had <10 cfu/g. MPN technique was performed for 34 another positive samples; 12 had ≥1100/g, five ranged between 240 and 93, eight between 23 and three and nine had <3.0. No L. monocytogenes was recovered after cooking 12 contaminated frozen samples. Isolation of strains was done using three selective agars. Sixty-two L. monocytogenes were isolated from lithium chloride phenylethanol moxalactam agar, 95 from Listeria selective agar Oxford formulation, and 103 from polymixin acriflavine lithium chloride ceftazidime aesculin mannitol agar. Fifty isolates (45.5%) belong to PCR group IIb (including strains serovar 1/2b), 41 (37.3%) to PCR group IVb (including strains serovar 4b), 17 (15.5%) to PCR group IIa (including strains serovar 1/2a), and 2 (1.8%) to PCR group IIc. With the use of DNA macrorestriction patterns analysis, 17 different clusters were detected among 71 isolates, with P10, the most frequent with 25 isolates (35.2%) of PCR group IIb.

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vegetable salads freshly prepared at three supermarkets, sold loose and dispensed by staff in plastic disposable food containers and (iii) 154 packaged samples of two industrial brands of minimally processed raw ready-to-eat salads with a ten-day shelf life. All three types of salads were made from single or mixed vegetables.

(i) Frozen salads were either mixed vegetables (63 samples) or a single product such as sweet corn (39), green beans (37), broad beans (33), green peas (33), broccoli (21), fresh kidney beans (20), cauliflower (17), carrot (17), mushrooms (14), spinach (13), onion (12), asparagus (12), artichoke (8), and Brussels sprouts (8). Twelve positive frozen samples were boiled according to the manufacturers' instructions and tested afterwards for L. monocytogenes. Boiling time ranged from 5 to 10 min depending on the product.

(ii) Ready-to-eat vegetable salads freshly prepared at the supermarkets were: mixed vegetables (87), broad beans (20), sweet corn (15), raw celery (13), beetroot (10), raw tomato (10), raw carrot (6), mushrooms (6), seaweed (6), green peas (6), fresh kidney beans (6), cauliflower (6), broccoli (5), bean sprouts (5), raw cabbage (5), green beans (5) and raw lettuce (5).

(iii) Industrially minimally processed, ready-to-eat raw vegetable salads were either mixed (45) or single vegetables such as carrot (21), lettuce (21), celery (18), green cabbage (17), red cabbage (16) and spinach (16). These products have a ten-day shelf life, contain no additives or preservatives and should be kept refrigerated.

Samples were taken to the laboratory under refrigeration. Frozen samples were kept at −20 °C and all others at 4 °C until they were subjected to bacteriological analysis.

2.2. Bacteriological methods

2.2.1. Isolation and identification of L. monocytogenes

Bacteriological analysis of the samples was performed as recommended by the FDA Bacteriological Analytical Manual (Hitchins, 1995). Twenty-five grams out of 350–500 g of sample in 225 ml of enrichment broth plus yeast extract—both Difco, Detroit, MI (catalogue nos. 0370-17-3 and 0127-17-9) plus selective agents, were homogenized for one minute in a Stomacher Lab Blender 400 (Seward Medical, London, England), incubated 48 h at 30 °C and plated simultaneously on three different isolation media: lithium chloride phenylethanol moxalactam agar (LPM) prepared with phenylethanol (Difco catalogue No. 0504-17-2) as base agar and the formula was completed with the relevant ingredients including esculin and ferric ammonium citrate (Merck, Darmstadt, Germany); polymyxin acriflavine lithium chloride ceftazidime aesculin mannitol agar (Palcam), and Listeria selective agar Oxford formulation (Oxford), Oxford and Palcam were Oxoid (Basingstoke, UK catalogue nos. CM856 and CM877, Selective Supplements catalogue nos. SR140E and SR150E, respectively). Sodium pyruvate (Merck, 106619) was added to the enrichment broth for frozen salads.

Four suspected Listeria spp. colonies from each isolation media were purified on tryptic soy agar (Difco, O369–17) plus yeast extract and identified by morphological, cultural and biochemical characteristics. Tests performed were: Gram staining, catalase and nitratase tests, mobility, β–haemolysis, and acid production from dextrose, esculin, maltose, mannitol, rhizomannose, and xylose (Hitchins, 1995).

2.2.2. Enumeration

Plate count was performed on 20 positive frozen samples using the ISO quantitative method (ISO, 1998). Samples were randomly chosen. One millilitre of each of the decimal dilutions of each sample was seeded on four Oxford agar plates (0.25 ml each) and incubated 48 h at 35 °C. Test was performed on duplicate. MPN method was applied on another 34 positive frozen samples (Hitchins, 2003). Decimal dilutions of each sample were seeded in series of three tubes of single-strength Enrichment broth with yeast extract and incubated 48 h at 30 °C. A loopful of culture from each tube was streaked on Oxford agar and incubated 48 h at 35 °C. In both cases, suspected colonies were identified as described before.

2.2.3. Serotyping

Out of the 110 L. monocytogenes strains isolated, 38 (isolated from 2000 to 2002) were serotyped according to Seeliger and Höhne’s (1979) scheme and 72 (isolated from 2003 to 2005) were typed using a PCR technique (Doumith et al., 2004, 2005). Serotyping was done by Laboratoire des Listeria (Institut Pasteur, Paris, France).

2.2.4. DNA macrorestriction patterns analysis

Seventy-one L. monocytogenes isolates were characterized by DNA macrorestriction patterns obtained after treatment with the restriction enzymes Ascl (BioLabs, Massachusetts, Beverly, USA) and Apal (MBI Fermentas, Burlington, Ontario, Canada) and separation of the generated fragments with a previously described protocol (Graves and Swaminathan, 2001). This was done by Laboratoire des Listeria (Institut Pasteur, Paris, France).

Out of the 71 randomly chosen isolates, 40 were isolated from frozen samples of four brands: brand C (9 strains), E (8), D (11) and H (12), 16 from frozen samples sold loose (from supermarkets No. 1 and No. 2) and 15 from fresh supermarket prepared salads (from supermarket No. 1 and No. 3). Simpson’s index of diversity (ID) was calculated as described elsewhere (Hunter and Gaston, 1988). Two profiles were considered as different if they differed by one band.

2.3. Statistical analysis

Chi² or Fisher’s test exact were used for statistical analysis (EpiInfo software; version 6.04; CDC). All tests of significance were at the 5% 2-sided level.

3. Results and discussion

L. monocytogenes was isolated from 110 out of 717 (15.3%) vegetable salads samples tested. This is the first report on L. monocytogenes contamination of vegetable salads in Chile.

L. monocytogenes is widely diffused in the environment (Farber and Peterkin, 1991) and this fact can cause the contamination of vegetables during growing, harvesting, post-harvesting, handling or...
distribution. Fresh vegetables may then pose a significant risk as they
are consumed raw. Salads have an additional risk of contamination
through preparation, distribution and storage. While the fresh salads
prepared daily at supermarkets analysed in this survey are consumed
locally, frozen and minimally processed ones are commercial brands
sold in most parts of the country.

A total of 347 frozen vegetable salad samples were tested and 88
were found to be contaminated with L. monocytogenes (25.4%). Out of
these, 62 (26.2%) were found in 237 samples of frozen vegetable
salads of seven different brands and 26 (23.6%) were found in 110
samples of frozen vegetable salads sold loose at two different
supermarkets. Listeria was not isolated from 154 samples of raw
minimally processed salads industrially prepared (Table 1).

A wide range of prevalences of contaminated frozen salads have
been reported elsewhere. In northern Spain, 1.8% of 1750 frozen
vegetable samples from retail outlets was reported contaminated by
L. monocytogenes (Vitas et al., 2004), while in Poland prevalence
reached 46% of frozen vegetables in a processing plant during a four-
year survey (Pappelbaum et al., 2008). In our study, L. monocytogenes
was isolated from 11 types of frozen salads: green beans (18 samples
contaminated out of 37 analyzed, 48.6%), mixed salads (27/63, 42.9%),
green peas (12/33, 36.4%), fresh kidney beans (6/20, 30%), sweet corn
(10/39, 25.6%), cauliflower (4/17, 23.5%), broad beans (7/33, 21.2%),
broccoli (2/21, 9.5%), asparagus (1/12, 8.3%), spinach (1/13, 7.7%) and
mushroom (1/14, 7.1%). By contrast, L. monocytogenes was not isolated
from carrots, onion, artichokes or Brussels sprouts.

Frozen samples with different counts of L. monocytogenes were
boiled following the manufacturer’s instructions and no Listeria was
recovered in samples after boiling. This indicates that this is an
efficient home preparation procedure for reducing the risk of infection
for consumers and that the risk of frozen vegetable salads is to be
considered mainly due to manipulation and to cross-contamination.

Two-hundred and sixteen fresh ready-to-eat salads prepared daily at
three main supermarkets in Santiago were analysed and 22 of them were
found to be contaminated (10.2%). Seventy two samples were analysed
from each supermarket; 10 samples from Supermarket No. 1 (13.9%),
four from Supermarket No. 2 (5.6%) and eight from Supermarket No. 3
were found contaminated (11.1%) (Table 1). L. monocytogenes was
isolated from 10 different types of these freshly prepared salads: cooked
seaweed (three positive samples out of six analyzed, 50%), cooked green
beans (1/3, 33.3%), raw tomato (2/10, 20%), cooked broccoli (1/5, 20%),
cooked kidney beans (1/6, 16.7%), raw celery (2/13, 15.4%), mixed salads
(9/87, 10.3%), cooked beetroot (1/10, 10%), cooked sweet corn (1/15,
6.6%) and cooked broad beans (1/20, 5%). L. monocytogenes was not
isolated from salads prepared with carrot, mushrooms, green peas,
cauliflower, bean sprouts, cabbage and lettuce. It is remarkable that half
of the cooked seaweed was found contaminated with L. monocytogenes.
All six samples were purchased at supermarket No. 3 at different dates. In
total, nine out of the 22 isolates were recovered from cooked produce; as

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<td><strong>Distribution of PFGE patterns and PCR groups for 71 strains of Listeria monocytogenes by type of salad and origin.</strong></td>
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<td><strong>PCR group</strong></td>
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<td><strong>Frozen salads</strong></td>
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<td><strong>Supermarket No. 2</strong></td>
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<td><strong>Supermarket No. 3</strong></td>
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| Total | 2 | 5 | 1 | 1 | 1 | 1 | 25 | 6 | 1 | 1 | 1 | 5 | 8 | 8 | 71 |

* Ready-to-eat daily prepared at supermarkets.
L. monocytogenes and for IVb 0.773. Index for diversity has different PCR groups was: for IIa 0.341, for IIb 0.840 for IIc 0.636 0.841 for restriction analysis of DNA and 0.636 for PCR groups. ID for two isolation media. This study has demonstrated, for the convenience of using simultaneously these media. No medium was able to detect all 110 positive samples of isolates recovered on Palcam were isolated only on the other two media as well. Eight out of the 103 isolates were obtained on Palcam, 95 on Oxford and 62 on LPM. The 71 isolates randomly selected for PFGE were statistically not different from the collection of 110 isolates from vegetable salads, according to PCR groups (P=6×10−1) or type of salas (P=1×10−4). Genetic characterization of 71 isolates by PFGE typing showed 17 profiles with Ascl and 17 profiles with Apol. With the combination of both enzymes, 17 different patterns were identified, P1–P17 (Table 2). The most frequent pattern was P10 with 21 of 25 isolates found in frozen products. Out of the 17 different PFGE patterns found, P10 appeared in 10 out of 12 isolates from brand H, six out of 11 from brand D and five out of nine brand-C isolates. This is likely to indicate contamination during processing. A similar situation can be observed for isolates from supermarket No. 3 fresh salads, for which out of eight isolates three clustered in P17, three in P15 and two in P5. These eight samples were collected at seven different dates. Out of the 11 isolates from frozen salads sold loose at supermarket No. 1, five clustered in P16 and four in P11. These nine isolates were found in six different frozen salad samples collected at only two dates of the same month. This suggests an original contamination at the factory, but cross-contamination could also have occurred at the supermarket as different frozen products are often dispensed using the same spoon. In contrast, all seven isolates from fresh salads prepared at supermarket No. 1 showed different patterns, a fact that may indicate different contamination sources (Table 2).

The Simpson's index of diversity (Hunter and Gaston, 1988) was 0.841 for restriction analysis of DNA and 0.636 for PCR groups. ID for the different PCR groups was: for Ila 0.341, for IIb 0.840 for Ilc 0.636 and for IVb 0.773. Index for diversity has confirmed to be more discriminating for DNA restriction than for PCR group. Nevertheless, ID for PCR group IIb has similar values by both methods.

Comparing the results obtained on the three isolation media used, 103 isolates were obtained on Palcam, 95 on Oxford and 62 on LPM. Out of the three isolation media used, Palcam proved to be more effective while LPM was not useful since all 62 isolates on LPM were obtained from the two other media as well. Eight out of the 103 isolates recovered on Palcam were not recovered on the other two media and three out of the 95 isolates on Oxford were isolated only on this medium. No medium was able to detect all 110 positive samples of this study; this proves the convenience of using simultaneously these two isolation media.

This study has demonstrated, for the first time, the presence of L. monocytogenes in frozen and in fresh ready-to-eat vegetable salads prepared on site and sold at supermarkets in Santiago. This provides for ascertaining the risk that the different types of vegetable salads represent as a source of L. monocytogenes contamination for consumers. At present, control of L. monocytogenes is not mandatory in Chile for any type of food; consequently, this study provides data to the Chilean health authorities for eventual future food regulations.

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**References**


