

## Research

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# Prevalence of *Listeria* species in Fresh Salad Vegetables and Ready-to-Eat Foods Containing Fresh Produce Marketed in Canterbury, New Zealand

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### ABSTRACT

Fresh produce, the most common food, is source of high value nutrients in human diet. However, some pathogenic bacteria associated with fresh produce may cause illness. For example, *Listeria monocytogenes* is a microorganism that causes a disease of variable severity, such as mild gastroenteritis, severe infections of the blood stream and/or the central nervous system, and even leads to abortion in pregnant women. The aim of this study was to determine the prevalence of *Listeria* spp. in fresh produce. Four common fresh vegetables (lettuce, carrot, purple cabbage, green cabbage) were tested in the amount of *Listeria* spp. over a 5-week period, and eight individual samples of each fresh vegetable were collected from two fruit and vegetable markets each week. *Listeria* spp. were detected in all the purple cabbage samples collected from these two markets. *Listeria* spp. were also present in 60% and 100% of the green cabbage samples obtained from market 1 and market 2, respectively. *Listeria* spp. were found in the lettuce samples collected from both markets during week 1 to week 3. Carrot samples had the lowest percentage (20%) of *Listeria* spp. Generally *Listeria* spp. were present in all the four vegetables sampled. However, variations were recorded between vegetable types and sources. Selected ready-to-eat products containing fresh produce were also collected to test the presence of *Listeria* spp. and it turned out that two samples were *Listeria* spp. positive. Our data suggested that *Listeria* spp. were generally prevalent in fresh vegetables and Ready-to-Eat (RTE) food products containing fresh produce.

**KEYWORDS:** *Listeria monocytogenes*; fresh produce; enumeration.

### INTRODUCTION

Fresh produce have high nutritional qualities and several health benefits attributed to them. From a nutritional point of view, fresh produce are low energy-dense foods relatively rich in vitamins, minerals and other bioactive compounds as well as being good sources of fiber.<sup>1</sup> Consumption of fresh produce is encouraged by government health agencies, health groups and health professionals.<sup>2</sup> Generally, fresh fruits and vegetables are believed to protect against a range of illnesses such as cancers and cardiovascular diseases.<sup>3</sup> This campaign is helping to change dietary habits toward higher per capita consumption of fresh fruits and vegetables, particularly, in developed world. For instance the average intake of fruits and vegetables in New Zealand is nearly 400 gram per day.<sup>2</sup> Without doubt, fresh fruits and vegetables are important components of a healthy and balanced diet, however, several new food safety issues are being associated with their consumption.<sup>4</sup> One of the major issue is the emergence of pathogens that were not traditionally associated with raw produce. This situation has enhanced the potential for outbreaks associated with raw fruits and vegetables.

Bacterial pathogens such as *Salmonella*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Clostridium botulinum*, *Shigella*, *Staphylococcus*, *Vibrio cholera*, *Aeromonas*, *Campylobacter jejuni* and *L. monocytogenes* were frequently isolated from fresh produce.<sup>5</sup> Among all of these pathogens, *L. monocytogenes* is a kind of pathogenic bacterium that causes a serious disease called listeriosis. This infection has variable degree of health consequences, such as mild gastroenteritis, severe infections of the blood stream and/or the central nervous system, and even abortion for pregnant women.<sup>6</sup> This illness is a relatively rare but has high fatality rates (30%).<sup>7</sup> *L. monocytogenes* had previously been isolated from market produce i.e. cabbage, corn, lettuce, sprouts, potatoes, cucumbers, parsley, watercress and salad vegetable.<sup>8</sup>

Several outbreaks of *L. monocytogenes* infection associated with fresh produce have been reported in various parts of the world. Recently *L. monocytogenes* was responsible for the deaths of 10 people in food poisoning outbreaks of chopped celery in 2010 and 30 people due to contaminated melon in 2011.<sup>9,10</sup> The foodborne outbreaks caused by contaminated fresh produce are predicted to increase in coming years.<sup>9</sup> Therefore, this study was planned to investigate the status of *Listeria spp.* contamination in salad vegetables and selected Ready-to-Eat (RTE) food products containing fresh produce. To our knowledge this is the first investigation on *Listeria spp.* contamination levels in fresh produce marketed in Canterbury region, New Zealand.

## MATERIAL AND METHODS

### Sampling plan and samples collection

Four most commonly consumed fresh vegetables, including green cabbage, purple cabbage, lettuce, and carrot, were selected in this study. These vegetables are consumed raw or in minimally processed form. Samples of these vegetables were collected from two different fruit and vegetable markets (reported as market 1 and market 2) located in Canterbury region of New Zealand over a 5-week period. All samples were bagged separately when purchased and brought to laboratory in a way normally consumers take home. Following the raw vegetables testing, samples of 12 different RTE products that contained fresh produce as ingredient were also tested to determine possible relationship between fresh produce and *Listeria spp.* detection. These RTE products were also collected from two different supermarkets (supermarket 1 and supermarket 2).

### Samples preparation

Briefly, 100 g of each raw vegetable sample was weighed into a stomacher bag. An appropriate amount of 0.1% peptone water was added before stomaching for 5 min. Afterwards, 1 mL of the liquid portion of stomacher contents was used to prepare serial dilutions in 0.1% peptone water. One mL of selected dilutions were transferred onto each *Listeria* selective agar plate (in duplicate) and incubated at 35 °C for 24 h.

### Media and enumeration of *Listeria spp.*

*Listeria* selective agar (CM0856, OXOID) was used in this study. Media plates were prepared according to manufacturer's instructions. Typical *Listeria spp.* colonies were brown color. Pure *Listeria monocytogenes* (strain V7) culture was used as a positive control (Figure 1) and 0.1% peptone water was the negative control. Confirmation of *L. monocytogenes* was not carried out in this study. The numbers of typical *Listeria spp.* colonies were counted after incubation and reported. Colony forming units (cfu/g) were calculated using the formula:

$$\text{cfu/g of sample} = \frac{A \times 10^n}{V_1} \times \frac{V_2}{m}$$

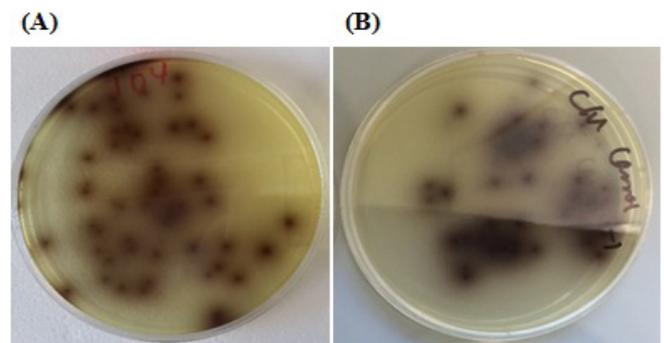
where A — number of colonies (average in two plates);

10<sup>n</sup> — level of dilution at which the counting was carried out;

V1 — volume of inoculum;

V2 — total volume of peptone water;

m — total sample weight



**Figure 1:** Typical *L. monocytogenes* colonies on *Listeria* selective agar (CM0856, OXOID). (A) *L. monocytogenes* (strain V7) pure culture colonies (B) Colonies of *L. monocytogenes* from a test product sample

## RESULTS

### *Listeria spp.* in selected salad vegetables

This study investigated the prevalence of *Listeria spp.* in four different vegetables, commonly used as salad, sampled from two fruit and vegetable markets in Canterbury region of New Zealand. All purple cabbage samples from both markets were consistently positive for *Listeria spp.* presence during the 5-weeks sampling period (Table 1). *Listeria spp.* were detected in 60% and 100% of green cabbage samples from market-1 and market-2, respectively. Lettuce samples collected from both markets during week 1 to week 3 were contaminated with *Listeria spp.*, while carrot samples had the lowest percentage (20%) of positive samples. Generally *Listeria spp.* was present in all vegetable types tested in this study. However, purple and green cabbage samples mostly carried *Listeria spp.* and the samples collected in market-2 had more number of positive samples for *Listeria spp.* compared to market-1. Carrots had the lowest

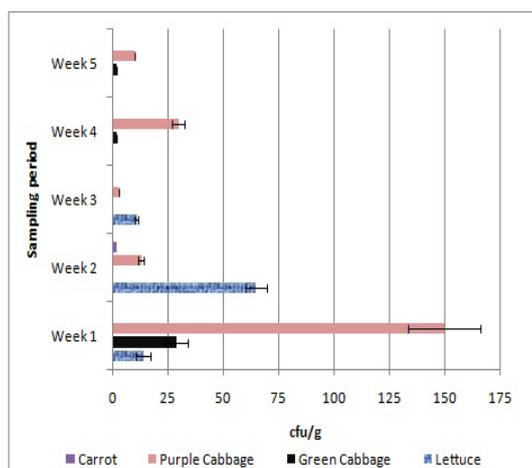
number of samples and frequency for *Listeria* spp. detection.

	Sampling period				
	Week 1	Week 2	Week 3	Week 4	Week 5
<b>Market-1</b>					
Lettuce	+	+	+	-	-
Green Cabbage	+	-	-	+	+
Purple Cabbage	+	+	+	+	+
Carrot	-	+	-	-	-
<b>Market-2</b>					
Lettuce	+	+	+	-	-
Green Cabbage	+	+	+	+	+
Purple Cabbage	+	+	+	+	+
Carrot	-	-	-	-	+

**Table 1:** Comparison of *Listeria* spp. presence in vegetable samples collected from two different markets

Figure 2 shows the number of colony forming units (cfu) detected in 1 g of fresh vegetable samples. Notably, in the first week, purple cabbage sample collected from market-1 had the highest number (i.e. 150 cfu/g) of *Listeria* spp. detected. Purple cabbage samples had more *Listeria* spp. counts than green cabbage and lettuce samples from market-1 in most of the cases. Lettuce samples from market-2 showed comparatively high level of *Listeria* spp. during the first three weeks sampling. Maximum number of *Listeria* spp. detected in green cabbage, lettuce and carrot were 33 cfu/g, 87 cfu/g and 2 cfu/g, respectively.

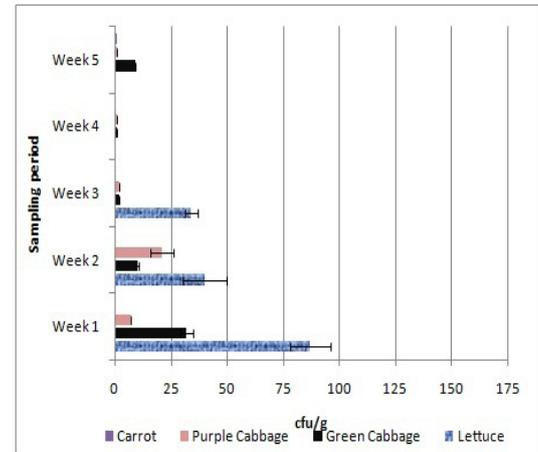
A)



### *Listeria* spp. in RTF containing fresh produce

Our curiosity to see whether *Listeria* spp. can be detected in RTE food products containing fresh produce led to

B)



**Figure 2:** Total number of *Listeria* spp. (cfu/g) detected in each of the vegetable sample from market-1 (A) and market-2 (B) over 5-week period. Error bars represent standard deviations of cfu detected each week.

collection of samples from two supermarkets (Table 2). Twelve samples were randomly collected once and two samples (Japanese style coleslaw and spinach and basil dip) were found in positive for *Listeria* spp. presence. This aspect of the study needs the detailed investigation. However, our results indicate there is potential risk associated with RTE products containing fresh produce as ingredient.

### DISCUSSION

Many research reports indicated the existence of *Listeria* spp., *L. monocytogenes* in particular, on the surface of fresh vegetables. *L. monocytogenes* is an important pathogen and was detected in 28% of fresh vegetables positive for *Listeria* spp. presence.<sup>11</sup> Althaus et al found that the samples of RTE lettuce were infected by *L. monocytogenes*.<sup>12</sup> In another report, Uzeh and Adepoju showed that *L. monocytogenes* was present on salad vegetables (sliced cabbage, lettuce and carrot).<sup>13</sup> The growth of *L. monocytogenes* was linked to the storage condition, especially temperature. Our results also strongly suggested that *L. monocytogenes* was prevalent in purple cabbage, green cabbage and lettuce. These observations are consistent with previous findings mentioned above.

According to guideline for the control of *L. monocytogenes* in RTE products, *Listeria* spp. level in fresh produce is expected to be as low as possible, and the maximal safety level limit should not exceed 100 cfu/g.<sup>14</sup> Obviously, purple cabbage from market-1 sampled in the first week had the level of *Listeria* spp. (150 cfu/g). The lettuce sample collected in week-1 from market-2 also found to have numbers close to maximum limits. All the other vegetable samples had detected numbers within the range of safety limits. However, we need to keep in mind that the guidelines of the food safety agencies are for regularity purpose and ambiguity of *Listeria* makes it more dangerous, thus *Listeria* guidelines need frequent review. In our opinion, it is not the number of pathogenic cells rather

Classification	Product name	Source	<i>Listeria</i> spp. detection
Cole Salad	Salad coleslaw	Supermarket 1	–
	Coleslaw salad	Supermarket 2	–
Slaw	Japanese style coleslaw	Supermarket 1	+–
	Herbslaw	Supermarket 1	–
	Broccoslaw	Supermarket 2	–
	Crispy salad	Supermarket 2	–
Dips	Spinach and basil dip	Supermarket 1	+–
	Spinach and feta dip	Supermarket 1	–
	Feta and spinach hummus	Supermarket 2	–
	Cucumber and mint yoghurt dip	Supermarket 2	–
<b>Burger/ Sand-wiches</b>	Meat lover roll (burger)	Supermarket 2	–
	Ham triple sandwiches	Supermarket 2	–

**Table 2:** *Listeria* spp. detection in RTE food products containing fresh produce sampled from two different supermarkets.

prevalence is more serious problem that allows this pathogen to reach to immune-compromised groups of the population (elderly, infant, pregnant women, etc).

Comparing the results of this study with Sant'Ana et al.<sup>15</sup> who reported  $7.67 \pm 0.09$  cfu/g of *L. monocytogenes* in cabbage samples, we have detected much higher number. However, carrot samples had  $6.15 \pm 0.02$  cfu/g, much more than our result (i.e. 2 cfu/g). Another investigation on the risk assessment of lettuce samples from home, restaurant and farm in Korea found that average final contamination level before consumption after washing treatment in restaurant or at home were  $-1.50$  log cfu/gor  $-0.146$  log cfu/g, respectively. Using food safety limit for *L. monocytogenes* population on fresh produce (2 log cfu/g) as threshold value, contamination levels on lettuce greater than this threshold value were observed as 12.86%, 15.03% and 31.04% for farm, restaurant and home, respectively.<sup>16</sup>

Microbiological analysis of raw vegetables was followed by *L. monocytogenes* testing in RTE products containing fresh produce. *L. monocytogenes* was not present in majority of its products (Table 2). Japanese style coleslaw and spinach-basil dip collected from supermarket-1 were positive in *L. monocytogenes*. However, numbers were less than threshold value for RTE products. Cordano and Jacquet<sup>17</sup> tested 154 samples of industrial minimally processed raw salads, and *L. monocytogenes* was not detected. However, Althaus et al<sup>12</sup> found 5 out of 223 samples of RTE lettuce contained *L. monocytogenes*. Number of *Listeria* spp. was less than 2 log cfu/g in each sample. Data from our study and several other reports strongly suggest that *Listeria* spp. could pose a serious threat to the safety of fresh produce and products that contain them as an ingredient. Therefore, it is important to use appropriate measures in order to reduce or minimize contamination well.

It could be assumed that most of the products sold

in supermarkets have gone through some treatments (heat or chemical) to eliminate or reduce pathogenic bacteria. Generally, the levels of *L. monocytogenes* could be reduced to the lowest level through different methods: 1) washing and cleaning before marketing (e.g.: making fresh produce in to salad); 2) applying a listericidal process (e.g.: pasteurization which in an effective way to reduce the presence of *Listeria* spp.); and 3) keeping the food in the environment which would inhibit the *Listeria* spp. growth.

Recently, several methods have been developed to reduce *L. monocytogenes* levels on the surface of fresh vegetables. For example, using balsamic vinegar from Moden approved more effective than washing in reducing pathogens including *L. monocytogenes*.<sup>18</sup> Authors also suggested that relative humidity and storage temperature also played a key role in keeping the low level of *L. monocytogenes*.<sup>19</sup> They showed that *L. monocytogenes* did not grow on lettuce or parsley at low temperature and a decline or stability in numbers was observed. High humidity caused *L. monocytogenes* to grow on cucumber epidermis and viable count increased from 4.0 to around 5.5 cfu/cm.<sup>2</sup> It suggests dry place is more fitful storage condition for fresh produce.

In conclusion, *Listeria* spp. have been frequently detected in market produce such as cabbage, corn, lettuce, sprouts, potatoes, cucumbers, parsley and watercress. In this study, all four fresh vegetables (lettuce, green cabbage, purple cabbage, carrot) were found in carrying the *Listeria* spp. more or less. These results indicate that food safety risks are associated with fresh produce consumption. However, RTE products (containing fresh produce) are relatively safe. Further, investigations are needed to establish level of risk associated with each product type and food safety threat to consumers.

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