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# Assessment of *Listeria monocytogenes* in salad vegetables through kitchen simulation study

(Penilaian *Listeria monocytogenes* dalam sayuran ulam melalui kajian simulasi dapur)

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**Abstract**

This study was to investigate the occurrence of cross-contamination and decontamination in the kitchen via *Listeria monocytogenes* contaminated vegetables during salad preparation. In this study, naturally contaminated produce were used to provide realistic quantitative data as opposed to information obtained through artificial inoculation. The study was designed to simulate the real preparation of salad in kitchens in Malaysia which simply involved washing the vegetables in tap water and cutting them on a chopping board prior to serving. It was found that the mean percentage of transfer rates for *L. monocytogenes* from vegetables to wash water was 32.4–60.2%; from wash water to cucumber 24.9–66.3%; from vegetables to chopping board 18.9–32.2%; from chopping board to cucumber 5.4–75.3%. Washing of the vegetables in tap water caused a 0.3-log reduction of *L. monocytogenes* attached to the vegetables.

## Introduction

The demand for fresh, minimally processed vegetables has increased significantly through the years due to health concerns and diet trends of consumers. Unfortunately, this has resulted in a higher frequency of foodborne illnesses associated with raw produce (Gorny 2006; Meldrum et al. 2009). The *Listeria monocytogenes* bacterium is of particular concern since it is a robust gram-positive pathogen capable of causing life-threatening diseases including listeriosis, meningitis, septicaemia and cervical or intra-uterine infections which results in spontaneous abortion or stillbirth (Cossart 2007).

*Literia monocytogenes* has been detected in a large variety of foods (Pini and Gilbert 1988; Heisick et al. 1989; Little et al. 2007). In Malaysia, a few studies have been done over the years in which *L. monocytogenes* has been detected in a variety of foods including poultry, vegetables and seafood (Arumugaswamy et al. 1994; Endang et al. 2003; Tan et al. 2007; Ponniah et al. 2010). The presence

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of *L. monocytogenes* in vegetables is a cause for concern since vegetables are often consumed in the minimally processed form in local dishes such as *ulam*, a kind of fresh salad and as an accompaniment to popular dishes such as *nasi lemak*, which is a traditional rice dish that is consumed for breakfast.

There is no published data on the dissemination of *L. monocytogenes* from naturally contaminated vegetables to kitchen surfaces and to other ready-to-eat foods from kitchens in tropical countries where the ambient temperature and air humidity is higher although many studies have been carried out on artificially contaminated products (Adams et al. 1989; Nguyen-the and Carlin 1994) in temperate countries. Steinbruegge et al. (1988) suggested that a warm humid environment may allow *L. monocytogenes* to grow to detectable levels in vegetables and therefore data on pathogen transmission in this environment would be very useful in obtaining realistic transfer data that can confidently be used in quantitative microbial risk assessment to be carried out in this region.

Accurate quantitative data on cross-contamination and decontamination rates that target the food handling practices of the specific population would undoubtedly ensure the reliability of the risk estimate, and selection of the most effective intervention. Availability of relevant data would also reduce the number of assumptions that need to be made and assist in making the risk assessment more acceptable to the local population.

The aim of this study was to investigate how *L. monocytogenes* was transferred from naturally contaminated vegetables to other surfaces during handling of fresh vegetables in kitchens in tropical countries with high ambient temperature and air humidity. To demonstrate the real situation in the kitchen, naturally contaminated vegetables were used in this study and these vegetables were merely washed in tap water prior to cutting as per common practice to ensure the data obtained would give accurate contamination and decontamination rates.

## Materials and methods

### *Sampling*

Three vegetables that are commonly consumed as *ulam* were selected for this study, i.e. *Vigna unguiculata* (yardlong bean), *Cosmos caudatus* (ulam raja) and *Oenanther stolonifera* (Japanese parsley). These vegetables were found to be contaminated with *L. monocytogenes* in an earlier prevalence study (Ponniah et al. 2010). Fifteen samples of each of the three vegetables were purchased from a wet market in Selangor, Malaysia. All raw vegetables were packed individually and transported to the laboratory immediately.

### *Kitchen simulation*

The handling of the raw vegetables for *ulam* preparation was simulated including washing and cutting. The study was designed according to common vegetable handling practices in the kitchen by local consumers in Malaysia. Raw vegetables of 100 g weight were first washed in a bucket of water (1 litre) for about 10 min. Approximately 50 g of peeled cucumber was soaked together with the vegetables at the same time to simulate cross-contamination from washing water to another vegetable. After washing, half of the vegetables were cut on a plastic chopping board. Without washing the chopping board, a clean cucumber was sliced into 2 mm thick pieces to simulate cross-contamination from the chopping board to another vegetable.

### *Data collection*

Freshly purchased vegetables were examined for initial *L. monocytogenes* concentration using the Food Drug Administration-Bacteriological Analytical Manual Standard for detection of *Listeria* (Hitchins 2003). About 10 g of vegetable and cucumber were sampled after washing and cutting to quantify *L. monocytogenes* using the procedure stated above.

Ten ml of wash water was collected and mixed with Bufferred Listeria Enrichment Broth (Merck, Darmstadt, Germany) and incubated for 30 min at room temperature before proceeding with the analysis. The chopping board was rinsed thoroughly into a sterile stomacher bag filled with 100 ml of Bufferred Listeria Enrichment Broth (Merck) and incubated for 30 min at room temperature before 10 ml was taken for analysis. The mixture was treated as one sample and was assumed to contain all the *L. monocytogenes* organisms on the chopping board.

***Data analysis***

All quantitative data collected were in Most Probable Number/g (MPN/g) or MPN/ml units. Before calculating the transfer rate (percentage) and log reduction, the actual number of *L. monocytogenes* organisms (MPN) involved in the cross-contamination and decontamination events were obtained by taking the total weight or volume (for wash water) of the sample used in the simulation into account. The appropriate transfer rates (percentages) and log reduction were calculated as follows:

$$\text{Transfer rate} = \left(\frac{\text{MPN on recipient}}{\text{MPN on source}}\right) \times 100$$

Log reduction = log MPN/g on source – log MPN/g after washing

The range and means were determined for cross-contamination rates from contaminated vegetable to wash water and subsequently to cucumber and from washed vegetables to the chopping board and consequently to the cucumber. Decontamination rates were determined for washing practices.

Data were statistically analysed to determine if there were significant differences at 0.05 level of confidence in the transfer rates among the three types of vegetables as well as among the processing steps using Analysis of Variance (ANOVA). Decontamination rates were also analysed to determine if there were significant differences before and after washing using paired t-test. The data were also analysed to determine if there were significant differences in decontamination rates among the three types of vegetables using Analysis of Variance (ANOVA). Statistical analysis was carried out using SPSS software (version 6.0).

**Results and discussion**

The cross-contamination and decontamination of *L. monocytogenes* during handling of raw vegetables in the kitchen was observed using naturally contaminated vegetables purchased from the wet market. The wet market was chosen as the sampling location as it is the place that is favoured by consumers from both the rural and urban population. The wet market was also found to provide more choices as there are a greater number of stalls that offer the same product. It is also more reliable in terms of product availability, since it caters for the needs of consumers who come especially to purchase the exotic salad vegetables that are generally more difficult to get in supermarkets and grocery shops.

The study attempted to imitate as closely as possible the real events occurring in the kitchen to give realistic quantitative data on how *L. monocytogenes* is disseminated from contaminated raw vegetables to other contact surfaces such as the chopping board, wash water and other ready-to-eat foods that are prepared at the same time in the kitchen. Naturally contaminated vegetables were used throughout the entire study as it has been proven that inoculated bacteria are different from bacteria naturally harboured in foods (Whyte et al. 2003; Purnell et al. 2004).

There is also a tendency to use higher microbial loads when carrying out artificial inoculation although vegetables have generally been found to carry a much lower bacterial concentration (Petran et al.1988; Norrung et al.1999). Carlin and Nguyen-the (1994) had found that *L. monocytogenes*

grew faster when initially present at lower (10–1000 CFU/g) than at higher ($10^5$ CFU/g) concentrations. Therefore, the use of naturally contaminated raw vegetables would reflect a more realistic potential exposure of *L.monocytogenes* to the consumer.

Numerous studies have shown that microorganisms on food processing surfaces are important sources of food contamination and can lead to food spoilage and transmission of diseases (Jessen and Lamnert 2003; Reij and Den Aantrekker 2004). Vegetables that are to be consumed in the minimally processed state carry a greater risk as these products do not undergo any heat treatment prior to serving and thus retain much of their indigenous microflora. *Listeria monocytogenes* is widely diffused in the environment and this fact can cause the contamination of vegetables during growing, harvesting, postharvesting, handling or distribution ( Farber and Addison 1994).

*Listeria monocytogenes* was detected in 17 out of 45 vegetable samples (*Table 1*) with 5 being found in *Vigna unguiculata* (yardlong bean), 5 in *Cosmos caudatus* (ulam raja) and 7 in *Oenanther stolonifera* (Japanese parsley). All initially negative samples remained negative throughout the entire simulation process and were not included in the statistical analysis to determine significant differences in contamination. *Table 1* summarizes the results of the cross-contamination experiments i.e. the transfer rates of *L. monocytogenes* originating from naturally contaminated vegetables to various recipients (wash water, chopping board and cucumber).

The role of cross-contamination in transmission of pathogens is of great concern to food safety. Chai et al. (2007) found that wash water too plays a role in the transmission of pathogens. In our study, it was found that vegetables contaminated with *L. monocytogenes* transferred the bacteria to the wash water at rates between 32.4–60.2 % (*Table 1*). The transfer rates from the raw vegetable to the wash water also differed among the three vegetables with *O. stolonifera* (Japanese parsley) having significantly higher transfer rates compared to the other two vegetables. There was also a significant difference in transfer rates from the chopping board to the cucumber among the three vegetables (5.4–75.3%) with *O. stolonifera* (Japanese parsley) showing a higher transfer rate. This was possibly due to the higher initial microbial load in this vegetable. Similarly, the transfer efficiencies of wash water to cucumber (24.9–66.3%) were highest from Japanese parsley while washed vegetable to cutting board (18.9–32.2%) were not significantly different among the three vegetables.

The data on decontamination of *L. monocytogenes* are summarized in *Table 2*. The mean of log reduction of *C. caudatus* was the highest (0.4) and was statistically significant ($p < 0.05$). Statistical analysis through paired t-test revealed that washing significantly reduced the number of *L. monocytogenes* on the vegetables ($p < 0.05$) (*Table 3*).

Decontamination of salad vegetables was achieved through washing and the mean of log reduction for *L. monocytogenes* from these vegetables was found to be 0.3 (*Table 2*). The value is quite similar to that of Chai et al. (2007) who reported a 0.4 log reduction in the *C. jejuni* when salad vegetables were washed in tap water. Since washing is obviously not very effective in reducing the microbial load, washing in antimicrobial solution should be considered.

Brackett (1987) reported that washing Brussel sprouts in a hypochlorite solution containing 200 mg/litre of chlorine decreased *L. monocytogenes* population approximately a hundred-fold. Zhang and Farber (1996) examined the effects of various disinfectants against *L. monocytogenes* on fresh-cut vegetables. For chlorine (200 ppm, 10 min), the maximum observed log reduction of *L. monocytogenes* at 4 °C and 22 °C were

Table 1. Most Probable Number/g (MPN/g) of *Listeria monocytogenes* in vegetable sources and recipients and calculated transfer rates

| Transfer source and recipient | Test vegetables | No. of vegetable samples positive with *L. monocytogenes* | Source | | Recipient | | Transfer rate (%) | |
|---|---|---|---|---|---|---|---|---|
| | | | Range | Mean | Range | Mean | Range | *Mean |
| Vegetables to wash water | *V. unguiculata* | 5 | 0.9–1.9 | 1.3 | 0.0–1.5 | 0.8 | 0–45.3 | 32.6b |
| | *C. caudatus* | 5 | 0.9–1.5 | 0.4 | 0.0–1.2 | 0.2 | 0–55.6 | 32.4b |
| | *O. stolonifera* | 7 | 1.3–2.2 | 1.8 | 1.2–2.0 | 0.7 | 30.0–71.4 | 60.2a |
| Wash water to cucumber | *V. unguiculata* | 5 | 0.0–1.5 | 0.8 | 0.0–1.3 | 0.5 | 0–72.4 | 24.9b |
| | *C. caudatus* | 5 | 0.0–1.2 | 0.2 | 0–1.0 | 0.2 | 0–76.5 | 43.1ab |
| | *O. stolonifera* | 7 | 1.2–2.0 | 0.7 | 1.0–1.9 | 1.3 | 55.0–93.5 | 66.3a |
| Vegetables to cutting board | *V. unguiculata* | 5 | 0.5–1.8 | 1 | 0.0–1.2 | 0.5 | 0–34.9 | 18.9a |
| | *C. caudatus* | 5 | 0.0–1.2 | 1 | 0.0–1.0 | 0.8 | 0–73.3 | 24.9a |
| | *O. stolonifera* | 7 | 1.0–2.1 | 1.7 | 1.0–1.6 | 1.2 | 0–68.7 | 32.2a |
| Cutting board to cucumber | *V. unguiculata* | 5 | 0.0–1.2 | 0.5 | 0.0–1.0 | 0.4 | 0–73.3 | 28.1b |
| | *C. caudatus* | 5 | 0.0–1.0 | 0.5 | 0.0–0.5 | 0.2 | 0–27.2 | 5.4c |
| | *O. stolonifera* | 7 | 1.0–1.6 | 1.2 | 0.9–1.2 | 1.1 | 65.4–88.4 | 75.3a |

*Means of transfer rate with the same letter are not significantly different at $p \leq 0.05$

Table 2. Decontamination of *Listeria monocytogenes* in vegetable samples after preparation

| Test vegetables | No. of vegetable samples positive with *L. monocytogenes* | Log MPN of *L. monocytogenes* | | | | | |
|---|---|---|---|---|---|---|---|
| | | Before washing | | After washing | | Log reduction (log MPN/g) | |
| | | Range | Mean | Range | Mean | Range | *Mean |
| *V. unguiculata* | 5 | 0.9–1.9 | 1.3 | 0.5–1.8 | 1.0 | 0.1–0.4 | 0.2b |
| *C. caudatus* | 5 | 0.9–1.5 | 1.4 | 0–1.2 | 1.0 | 0.3–1.0 | 0.4a |
| *O. stolonifera* | 7 | 1.3–2.2 | 1.8 | 1.0–2.1 | 1.7 | 0.1–0.3 | 0.2b |
| Mean | | | 1.5 | | 1.2 | | 0.3 |

*Means of log reduction with same letters are not significantly different at $p \leq 0.05$

Table 3. Paired sample t-test showing significant difference in microbial load before and after washing

| Pair 1 | Log MPNRV – Log MPNWV (1.4826E0) – (1.1834E0) | | Paired differences |
|---|---|---|---|
| | Mean | | 0.2991974 |
| | Std. Deviation | | 0.2553333 |
| | Std. Error Mean | | 0.0619274 |
| | 95% Confidence Interval of the Difference | Lower | 0.1679171 |
| | | Upper | 0.4304777 |
| T | | | 4.8314198 |
| Df | | | 16 |
| Prob.> T | | | <.0001 |

MPNRV = MPN of vegetables before washing
MPNWV = MPN of vegetables after washing

1.3 and 1.7 for lettuce, and 0.9 and 1.2 for cabbage respectively. Brinez et al. (2006) found a 5 log reduction when peracetic acid in combination with hydrogen peroxide was used at concentrations of 0.1% and 10 min exposure.

## Conclusion

Vegetables that were contaminated with *L. monocytogenes* transferred the bacteria at rates between 5.4–75.3%. Among the three vegetables, *O. stolonifera* (Japanese parsley) had significantly higher transfer rates compared to the other two vegetables. Decontamination of salad vegetables can be achieved through washing and the mean of log reduction from these vegetables was found to be 0.3. The simulation of consumer's food handling practices in the kitchen provides a good model for cross-contamination and decontamination studies, taking into account local vegetable handling practices. The quantitative data obtained from this study can provide useful input for risk assessment studies by enabling appropriate incorporation of transfer data that are more suited to the local population.

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**Abstrak**

Kajian ini adalah untuk menyiasat proses kontaminasi-silang dan nyahkontaminasi yang berlaku pada sayur ulam yang tercemar dengan bakteria *Listeria monocytogenes* semasa penyediaan di dapur. Dalam kajian ini, sayur-sayuran yang tercemar secara semula jadi digunakan untuk memberi gambaran kuantitatif sebenar berbanding dengan sayur yang diinokulasi secara tiruan. Kajian dilaksanakan berdasarkan penyediaan sebenar ulam di dapur di Malaysia yang cuma melibatkan proses pencucian dengan air paip diikuti dengan pemotongan di atas papan pemotong sebelum sayur-sayuran sedia untuk dimakan. Didapati, purata peratus pemindahan *L. monocytogenes* dari sayur ke air bilasan ialah 32.4–60.2%; dari air bilasan ke timun 24.9–66.3%; dari sayur-sayuran ke papan pemotong 18.9–32.2%; dari papan pemotong ke timun 5.4–75.3%. Mencuci sayur-sayuran dengan air paip menyebabkan penurunan *L. monocytogenes* sebanyak log 0.3 berbanding dengan jumlah asal yang terdapat pada sayur-sayuran.

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