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# Detection and isolation of *Listeria* spp. and *Listeria monocytogenes* in ready-to-eat foods with various selective culture media

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ABSTRACT

The objectives of this study were to determine the prevalence of *Listeria* spp., specifically *Listeria monocytogenes* in ready-to-eat (RTE) foods and ascertain the efficiency of detecting *L. monocytogenes* with different selective culture media. A total of 396 RTE food samples were purchased from hypermarkets and streetside hawker stalls to examine the presence of *Listeria* spp. and *L. monocytogenes*. The presumptive isolates were characterized biochemically and were further confirmed by Polymerase Chain Reaction (PCR). Out of 396 samples, *Listeria* spp. was detected in 71 (17.9%) samples in which 45 (11.4%) were positive for *L. monocytogenes*. Among the studied RTE foods, salads and vegetables had the highest prevalence (14.7%) of *L. monocytogenes*, followed by chicken and chicken products (13.2%), beverages (10%), eggs and egg products (9.5%), beef and beef products (6.7%), lunch boxes (6.7%) and seafood and seafood products (6.7%). Both *Listeria* selective agar and PALCAM agar displayed a low sensitivity and specificity in *L. monocytogenes* detection compared to CHROMagar™ *Listeria* which demonstrated 96.9% of sensitivity and 99.1% of specificity in *L. monocytogenes* detection in naturally-contaminated foods. In conclusion, this work revealed consumption of RTE foods as a potential risk of listeriosis in this region. The high contamination rate of *L. monocytogenes* in salads and vegetables from hypermarkets and streetside hawker stalls was of great concern due to emerging fresh produce-borne *L. monocytogenes* globally. The scenario warrants further surveillance and action by the local authority to control the incidence of *L. monocytogenes* contamination in RTE foods.



## 1. Introduction

*Listeria monocytogenes* is a Gram positive, non-spore forming, aerobic to facultative anaerobic psychrotrophic bacteria with low C + G content (Monk, Gahan, & Hill, 2008) and high mortility rate (Mead et al., 1999). It has the ability to cause severe diseases in humans and animals. *L. monocytogenes* is ubiquitous and can be found in foods, water, soil, vegetables as well as animals and humans (Cocolin et al., 2005; Liu, 2008).

*L. monocytogenes* is the causative agent of listeriosis and is transmitted to susceptible individuals via consumption of contaminated foods (Wesley, 1999). The major population group at risk for invasive listeriosis are the immunocompromised such as pregnant women, new born babies, elderly people and AIDS patients (Kuhn, Scortti, & Vázquez-Boland, 2008). Recently, in the USA, human listeriosis attributed to consumption of contaminated cantaloupe was reported (CDC, 2011). Also, smoked fish, cooked marinated products, meat products, and vegetables were found to be contaminated with *L. monocytogenes* (Meloni et al., 2009).

Numerous food surveys conducted in Malaysia had reported on the detection of *L. monocytogenes* in various types of foods, including raw and RTE foods (Marian et al., 2012), raw salad vegetables (Ponniah et al., 2010), burger patties (Wong et al., 2011) and vegetarian burger patties (Wong et al., 2012). However, the actual incidence of foodborne listeriosis cases in Malaysia is not known. There is no official data on food poisoning/infection caused by *L. monocytogenes* in Malaysia because *L. monocytogenes* is rarely tested in the food poisoning/infection cases. Nonetheless, the recent outbreaks of foodborne listeriosis in USA and other countries and the high prevalence of *L. monocytogenes* in local foods have drawn the attention of local authorities on the possible widespread of *L. monocytogenes* in the country.

In Malaysia, a wide variety of local foods sold by street hawkers is a major source of RTE foods for the locals. On the other hand,

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hypermarket is an emerging modern setting in the country for the locals to purchase foods. However, the hygiene status of street hawkers is questionable. Previous studies conducted locally (Modarressi & Thong, 2010; Ponniah et al., 2010) and in other countries (Díaz-López et al., 2011; Nyenje, Odjadjare, Tanih, Green, & Ndip, 2012) found high a prevalence of foodborne pathogens in street foods. The hygiene level of food courts in the hypermarket settings is generally assumed to be better. However, the foodborne pathogens and *L. monocytogenes* had been reported to be isolated frequently from raw and RTE foods sold in the hypermarkets in Malaysia (Ponniah et al., 2010). Therefore, the occurrence of this pathogen in various types of RTE foods, sold by hawkers and hypermarkets could pose a significant public health risk to the consumers.

Isolation and detection of *L. monocytogenes* in food is commonly carried out using methods approved by the International Standards Organization (ISO) (ISO 11290), US Food and Drug Administration (FDA) and US department of Agriculture (USDA). The choice of methods depends on the food matrix. Although various enrichment broths can be used in these methods, only limited selective/differential agars are available for the detection of *L. monocytogenes*. Hydrolysis of esculin is a common trait in all *Listeria* spp. and is therefore used to differentiate between *Listeria* species and other bacteria on *Listeria* selective agar (Encinas, Sanz, García-López, & Otero, 1999) and PALCAM agar (El Marrakchi, Boum'handi, & Hamama, 2005). However, differentiation of *L. monocytogenes* from other *Listeria* spp. is impossible with these media. Based on literature search, an alternative medium, CHROMagar™ *Listeria* is able to differentiate *L. monocytogenes* and *Listeria ivanovii* from other *Listeria* species through formation of phospholipase-C (El Marrakchi et al., 2005). Both *L. monocytogenes* and *L. ivanovii* grow as blue colonies with white-halo on CHROMagar™ *Listeria*; bacteria other than these two species could produce colonies of variable morphologies. Previous studies conducted by different researchers had found CHROMagar™ *Listeria* more superior than Oxford, modified Oxford agar and PALCAM agar (Aragon-Alegro et al., 2008; El Marrakchi et al., 2005; Pangloli, Ahmed, Stevens, Golden, & Draughon, 2007). In the reported works by Ritter, Kircher, Sturm, Warns, and Dick (2009) and Hedge, Leon-Velarde, Stam, Jaykus, and Odumeru (2007), the performance of CHROMagar™ *Listeria* in detection of *L. monocytogenes* in food samples were determined with both naturally- and artificially-contaminated samples. However, Ritter et al. (2009) used only naturally-contaminated raw food in the study; while Hedge et al. (2007) reported on the use of 50 different types of naturally-contaminated food samples, majority of the food samples tested were minimally processed RTE foods. In this study, the RTE foods tested included refrigerated RTE foods and high-heat processed foods. This will provide a test of the sensitivity and specificity of CHROMagar™ *Listeria* in correctly detecting *L. monocytogenes* from both minimally- and heat-processed RTE foods.

The aims of this study were: (i) to determine the prevalence of *Listeria* spp. and *L. monocytogenes* in RTE foods purchased from street hawkers and hypermarkets and; (ii) to determine the efficiency of various selective culture media in recovering *L. monocytogenes* from naturally contaminated foods.

## 2. Materials and methods

### 2.1. Samples

Between December 2006 and January 2012, 396 samples of RTE foods were sampled from street hawkers and hypermarkets in Selangor, Malaysia. In particular, 10 beverages (cold orange flavoured drink), 68 chicken and chicken products (fried chickens, chicken satay, chicken sausages, chicken burger, breaded chicken meatball, chicken gizzard, chicken heart, chicken liver and chicken fillet), 42 eggs and egg products (hard-boiled eggs shell removed, fried eggs and egg tarts), 30 beef and beef products (stir fried egg and beef burger), 45 seafood and seafood products (sushi, fried fish, fish roll, barbecue cuttlefish), 170 salads and vegetables (Malaysian salad with vinegar, potato salad with mayonnaise, fruit salads, raw bean sprout, blanched long bean, raw lettuce, raw cucumber, raw tomato and raw cabbage), 15 packed lunch, and 16 other types of RTE foods (noodles, steam rice, chicken soups, custard, etc.). All RTE foods included in this study, except for raw vegetables, salads and beverages, were subjected to high-temperature process (e.g. cooking, boiling, steaming, deep-frying, etc.). Normally, RTE foods sold by the street hawkers are fresh and without packaging. On the other hand, before display, RTE foods at the supermarkets are packed. The samples were transferred into sterile plastic bags and transported in icebox to the laboratory within two to 4 h of sampling.

### 2.2. Isolation and detection

The ISO 11290 method was used for isolation and identification of *L. monocytogenes* in this study as described by Becker et al. (2006). Briefly, 25 g of samples were added to 225 ml of half Fraser broth (Oxoid, Basingstoke, UK) as the first enrichment culture in stomacher bag and were homogenized in a stomacher (Lab blender 400, Seward Medical, London, UK) and incubated for 24 h at 30 ± 1 °C. A loopful of first enriched broth culture was streaked on CHROMagar™ *Listeria* (CHROMagar, Paris, France) and incubated for another 24–48 h at 37 °C. On the other hand, 0.1 ml of half Fraser broth was added to 10 ml of Fraser broth as a second enrichment culture and incubated at 37 °C for 48 h. Then, a loopful of enriched Fraser broth-culture was streaked onto different selective agar, *Listeria* selective agar (Oxford Formulation) (Oxoid, Basingstoke, UK) and PALCAM agar (Oxoid, Basingstoke, UK) and incubated for 24–48 h at 37 °C. Three to 5 presumptive colonies from CHROMagar ™ *Listeria*, *Listeria* selective agar and PALCAM agar were re-streaked on tryptic soy agar (Oxoid, Basingstoke, UK) with 0.6% yeast extract (Oxoid, Basingstoke, UK) (TSAYE) as a non-selective medium and incubated at 37 °C for 24 h. The colonies from TSAYE were confirmed using biochemical tests (Gram staining, catalase, oxidase, urea, SIM, TSI, and MR-VP) and Polymerase Chain Reaction (PCR). The presence of *L. monocytogenes* in a particular sample was scored positive if any of the presumptive colonies obtained from any of the three media were confirmed as *L. monocytogenes*.

### 2.3. Confirmation of L. monocytogenes isolates by Polymerase Chain Reaction

Following biochemical testing, the presumptive isolates were subjected to PCR analysis to confirm whether they were *L. monocytogenes*. Crude DNA was prepared by direct boiling of a suspension of the cell lysates. In this study, a duplex PCR was performed to detect the simultaneous presence of *Listeria* spp. (LI1/U1) and *L. monocytogenes* (LM1/LM2) (Rossmanith, Krassnig, Wagner, & Hein, 2006). The LI1 primer sequence is complementary to a highly conserved 16S rRNA sequence present in all *Listeria* spp. The LM1 and LM2 primers were based on the sequence of listeriolysin O (LLO) gene to detect *L. monocytogenes*. The optimised PCR conditions consisted of an initial denaturation of 95 °C for 4 min and 30 cycles of 95 °C, 1 min, 52 °C, 45 s, 72 °C, 2 min and a final elongation 72 °C, 8 min. LI1/U1 primers amplify the 938 bp region in the 16S rRNA sequence which shows all *Listeria* spp. and

LM1/LM2 primers amplify the 701 bp region in the LLO gene that shows *L. monocytogenes*.

### 2.4. Determination of the performance of CHROMagar™ Listeria, PALCAM agar and Listeria selective agar

For each *L. monocytogenes* detection method using CHROMagar™ *Listeria*, PALCAM agar and *Listeria* selective agar, 3–5 presumptive colonies were selected for confirmation using biochemical assays and PCR. If *L. monocytogenes* was detected, isolated and confirmed using any of the media, the sample was scored positive for *L. monocytogenes*. If *L. monocytogenes* presumptive colonies were detected on the media, the sample is presumptively considered as "positive" based on the particular method/media, and *vice versa*. If none of the presumptive colonies isolated was confirmed to be *L. monocytogenes*, the sample was deemed "false positive". "False negative" was used to described the method/media that fail to detect *L. monocytogenes* in a *L. monocytogenes*-positive sample whether at the presumptive level (e.g. no presumptive colony grows on the media) or after the confirmation (e.g. presumptive colonies were detected on the media, but were confirmed to be non-*L. monocytogenes*).

The following values were calculated as described by De Boer and Beumer (1999) and Willinger and Manafi (1999): sensitivity (%) = true positives × 100/(true positives + false negatives), specificity (%) = true negatives × 100/(true negatives + false positives), and efficiency (%) = (true positives + true negatives) × 100/total.

### 2.5. Data analysis

The relationship between the true/false positive results and the food categories were analysed using Chi-square analysis. All statistical analysis was performed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA).

## 3. Results and discussion

Out of 396 RTE foods sampled, 71 were naturally contaminated with *Listeria* spp. The PCR result was used as final confirmation of the identity of presumptive colonies isolated in this current study (Fig. 1). A high prevalence of *Listeria* spp. (21.4%) (Table 1) and *L. monocytogenes* (14.1%) (Table 2) were detected in the RTE foods sampled from randomly selected street hawkers. It is common to assume that foods sold in hypermarkets are usually safer for consumption than street hawker foods. Therefore, in order to make

**Table 1**
Prevalence of *Listeria* spp. by sample types and settings.

| Food category | HM[a] samples | SF[b] samples | Total | *Listeria* (genus) | | |
|---|---|---|---|---|---|---|
| | | | | HM (%) | SF(%) | All (%) |
| *Beverage*[c] | 5 | 5 | 10 | ND | 1(20) | 1(10) |
| *Chicken and chicken products* | 34 | 34 | 68 | 7(20.6) | 9(26.5) | 16(23.5) |
| Fried chicken | 6 | 12 | 18 | 1(16.7) | 4(33.3) | 5(27.8) |
| Chicken satay | 0 | 12 | 12 | ND | 2(16.7) | 2(16.7) |
| Chicken sausage | 8 | 8 | 16 | 2(25) | 3(37.5) | 5(31.25) |
| Chicken burger | 2 | 2 | 4 | ND | ND | ND |
| Breaded chicken meatball | 4 | 0 | 4 | ND | ND | ND |
| Chicken gizzard | 5 | 0 | 5 | 1(20) | ND | 1(20) |
| Chicken heart | 2 | 0 | 2 | 1(50) | ND | 1(50) |
| Chicken liver | 3 | 0 | 3 | 1(33.3) | ND | 1(33.3) |
| Chicken fillet | 4 | 0 | 4 | 1(25) | ND | 1(25) |
| *Eggs and egg products* | 21 | 21 | 42 | 2(9.5) | 4(19) | 6(14.3) |
| Hard-boiled egg (shell removed) | 11 | 11 | 22 | ND | 3(27.3) | 3(13.6) |
| Fried egg | 5 | 5 | 10 | 2(40) | ND | 2(20) |
| Egg tarts | 5 | 5 | 10 | ND | 1(20) | 1(10) |
| *Beef and beef products* | 15 | 15 | 30 | 3(20) | 1(6.7) | 4(13.3) |
| Stir fried beef | 10 | 10 | 20 | 2(20) | 1(10) | 3(15) |
| Beef burger | 5 | 5 | 10 | 1(20) | ND | 1(10) |
| Packed lunch | 7 | 8 | 15 | 1(14.3) | 1(12.5) | 2(13.3) |
| *Salad and vegetables* | 60 | 110 | 170 | 6(10) | 28(25.5) | 34(20) |
| Malaysian salad with vinegar | 20 | 26 | 46 | 1(5) | 15(57.7) | 16(34.8) |
| Potato salad with mayonnaise | 10 | 10 | 20 | 1(10) | 2(20) | 3(15) |
| Fruit salad | 10 | 10 | 20 | 4(40) | 1(10) | 5(25) |
| Raw bean sprout | 0 | 10 | 10 | ND | 2(20) | 2(20) |
| Blanched long bean | 4 | 10 | 14 | ND | ND | ND |
| Raw lettuce | 4 | 14 | 18 | ND | 2(14.3) | 2(11.1) |
| Raw cucumber | 4 | 15 | 19 | ND | 2(13.3) | 2(10.5) |
| Raw tomato | 4 | 10 | 14 | ND | 1(10) | 1(7.1) |
| Raw cabbage | 4 | 15 | 19 | ND | 3(20) | 3(15.8) |
| *Seafood and seafood products* | 28 | 17 | 45 | 5(17.9) | 3(17.6) | 8(17.8) |
| Fried fish | 6 | 11 | 17 | 2(33.3) | 3(27.3) | 5(29.4) |
| Barbecue cuttlefish | 4 | 4 | 8 | 1(25) | ND | 1(12.5) |
| Sushi | 12 | 2 | 14 | 2(16.7) | ND | 2(14.3) |
| Fish roll | 6 | 0 | 6 | ND | ND | ND |
| *Others*[d] | 6 | 10 | 16 | ND | ND | ND |
| **Total** | 176 | 220 | 396 | 24(13.6) | 47(21.4) | 71(17.9) |

ND: Not detected.
[a] HM: Hypermarket.
[b] SF: Street food.
[c] Beverage: Cold orange flavoured drink.
[d] Others: Noodles, steam rice, chicken soups, custard, etc.

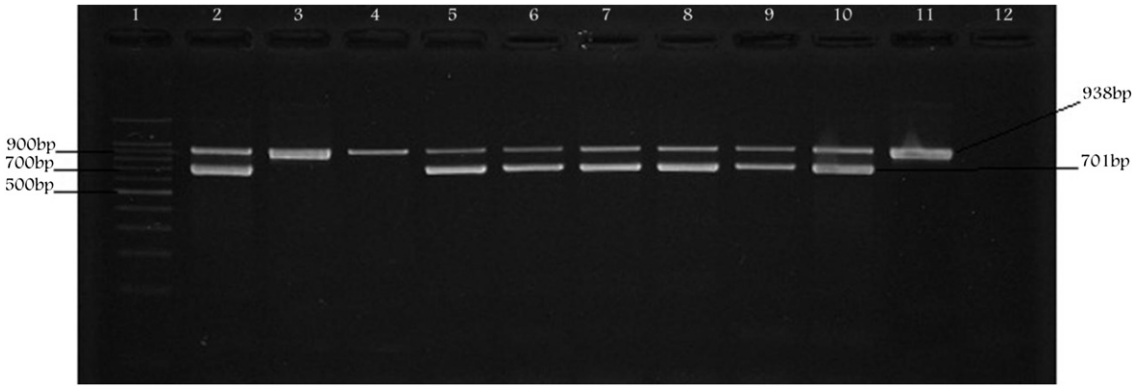


**Fig. 1.** A representative gel of PCR-amplified products of *Listeria* spp. *Listeria* spp. is indicated by a single band at 938 bp while *L. monocytogenes* is indicated by two bands, 938 bp and 701 bp. Lane 1, 100 bp molecular size marker; lane 2, positive control (*L. monocytogenes*, ATCC 19117); lanes 3,4, and 11: Non-*L. monocytogenes* from Malaysian salad with vinegar, hard-boiled egg (shell removed), fried chicken, and sushi; lanes 5–10, *L. monocytogenes* from beverage, chicken satay, fried egg, packed lunch, Malaysian salad with vinegar, and fruit salad; lane 12; negative control.

**Table 2**
Prevalence of *L. monocytogenes* by sample types and settings.

| Food category | HM[a] samples | SF[b] samples | Total | *Listeria monocytogenes* | | |
|---|---|---|---|---|---|---|
| | | | | HM(%) | SF(%) | All(%) |
| *Beverage*[c] | 5 | 5 | 10 | ND | 1(20) | 1(10) |
| *Chicken and chicken products* | 34 | 34 | 68 | 3(8.8) | 6(17.6) | 9(13.2) |
| Fried chicken | 6 | 12 | 18 | ND | 4(33.3) | 4(22.2) |
| Chicken satay | 0 | 12 | 12 | ND | 2(16.7) | 2(16.7) |
| Chicken sausage | 8 | 8 | 16 | ND | ND | ND |
| Chicken burger | 2 | 2 | 4 | ND | ND | ND |
| Breaded chicken meatball | 4 | 0 | 4 | ND | ND | ND |
| Chicken gizzard | 5 | 0 | 5 | 1(20) | ND | 1(20) |
| Chicken heart | 2 | 0 | 2 | ND | ND | ND |
| Chicken liver | 3 | 0 | 3 | 1(33.3) | ND | 1(33.3) |
| Chicken fillet | 4 | 0 | 4 | 1(25) | ND | 1(25) |
| *Eggs and egg products* | 21 | 21 | 42 | 2(9.5) | 2(9.5) | 4(9.5) |
| Hard-boiled egg (shell removed) | 11 | 11 | 22 | ND | 1(9.1) | 1(4.5) |
| Fried egg | 5 | 5 | 10 | 2(40) | ND | 2(20) |
| Egg tarts | 5 | 5 | 10 | ND | 1(20) | 1(10) |
| *Beef and beef products* | 15 | 15 | 30 | 2(13.3) | ND | 2(6.7) |
| Stir fried beef | 10 | 10 | 20 | 2(20) | ND | 2(10) |
| Beef burger | 5 | 5 | 10 | ND | ND | ND |
| *Packed lunch* | 7 | 8 | 15 | 1(14.3) | ND | 1(6.7) |
| *Salad and vegetables* | 60 | 110 | 170 | 4(6.7) | 21(19.1) | 25(14.7) |
| Malaysian salad with vinegar | 20 | 26 | 46 | 1(5) | 11(42.3) | 12(26.1) |
| Potato salad with mayonnaise | 10 | 10 | 20 | 1(10) | 2(20) | 3(15) |
| Fruit salad | 10 | 10 | 20 | 2(20) | 1(10) | 3(15) |
| Raw bean sprout | 0 | 10 | 10 | ND | 2(20) | 2(20) |
| Blanched long bean | 4 | 10 | 14 | ND | ND | ND |
| Raw lettuce | 4 | 14 | 18 | ND | 1(25) | 1(5.6) |
| Raw cucumber | 4 | 15 | 19 | ND | 2(13.3) | 2(10.5) |
| Raw tomato | 4 | 10 | 14 | ND | ND | ND |
| Raw cabbage | 4 | 15 | 19 | ND | 2(13.3) | 2(10.5) |
| *Seafood and seafood products* | 28 | 17 | 45 | 2(7.1) | 1(5.9) | 3(6.7) |
| Fried fish | 6 | 11 | 17 | ND | 1(9.1) | 1(5.9) |
| Barbecue cuttlefish | 4 | 4 | 8 | 1(25) | ND | 1(12.5) |
| Sushi | 12 | 2 | 15 | 1(8.3) | ND | 1(6.7) |
| Fish roll | 6 | 0 | 5 | ND | ND | ND |
| *Others*[d] | 6 | 10 | 16 | ND | ND | ND |
| **Total** | 176 | 220 | 396 | 14(8) | 31(14.1) | 45(11.4) |

ND: Not detected.
[a] HM: Hypermarket.
[b] SF: Street food.
[c] Beverage: Cold orange flavoured drink.
[d] Others: Noodles, steam rice, chicken soups, custard, etc.

this comparison, an additional of 176 samples of RTE foods were purchased from several hypermarkets located in the Selangor state. Hypermarket usually sells foods under conditions that seem more hygienic than hawker-street. Although the use of air-conditioners and refrigerators might seem beneficial in the food preservation, but the level of hygiene is still questionable since the packaging process is done before the foods are displayed. Surprisingly, 13.6% of the samples comprised of chicken and chicken products, beef and beef products, seafood and seafood products, lunch boxes, salads and vegetables and eggs and egg products, were contaminated with *Listeria* spp. (Table 1). About 8% of the contaminated samples were also positive for *L. monocytogenes* (Table 2). The findings indicate wide distribution of *Listeria* spp. and *L. monocytogenes* in RTE foods in Malaysia. Consumption of RTE foods, whether it is purchased from the street hawkers or from the hypermarket pose almost equal risk of listeriosis to the consumers.

The contamination of *Listeria* spp. was observed in 17.9% of RTE foods purchased from hypermarkets and street hawkers. Among these contaminated samples, 63.4% harboured *L. monocytogenes*. A report from Brazil has recorded 65% of sliced cooked ham contaminated with *Listeria* spp., with 77% of them carried *L. monocytogenes* (Aragon-Alegro et al., 2008). Among 45 samples of seafood-based RTE as high as 17.8% were contaminated with *Listeria* spp. and 6.7% of the samples were positive for *L. monocytogenes*. El Marrakchi et al. (2005) had detected *Listeria* spp. in 4.5% of the raw mussel samples and 14.3% of the contaminated samples were *L. monocytogenes*. Furthermore, Salihu et al. (2008) reported the presence of *L. monocytogenes* in 25% of smoked fish. The high ratio of *Listeria* spp. to *L. monocytogenes* observed in the RTE foods pose a high public health risk to the consumer. However, the cause of this phenomenon that was observed in this study as well as in other studies (Aragon-Alegro et al., 2008) was unknown. It was speculated that *L. monocytogenes* was more resistant to harsh treatments during food preparation than other species of *Listeria*.

The contamination of RTE foods could be due to many factors. One of the possible factors is via cross-contamination after the foods were cooked. We could not rule out the possibility that the cooking process is not sufficient to inactive these tough bacteria. In a study done by Wong et al. (2011), *L. monocytogenes* was not detected after 6 min cooking of chicken burger patties but it was found when the cooking was done in 4 min. However, the high prevalence of *Listeria* spp. and *L. monocytogenes* in RTE foods was mainly distributed in salads and vegetables. Both salad and vegetables did not go through heat process. This explained why salads and vegetables were highly contaminated with *Listeria* spp. and *L. monocytogenes*. Furthermore, salad dressings are high in nutrients and this could encourage the growth of *Listeria* spp. that was present in the salads.

A total of 170 salads and vegetables were tested in this work. Salads and vegetables were the most contaminated with 20% carried *Listeria* spp. and 73.5% were *L. monocytogenes* positive. Ponniah et al. (2010) had reported a high prevalence rate of *L. monocytogenes* at 22.5% in raw vegetables purchased from wet markets and hypermarkets in Malaysia. Meanwhile, Pinto, Novello, Montemurro, Bonerba, and Tantillo (2010) reported a prevalence of 27% of *L. monocytogenes* in mayonnaise in deli salads sold in Italy.

The ISO 11290 method was used to detect and isolate *L. monocytogenes* from food samples in this study. Three plating media were compared for their detection efficiency for *L. monocytogenes* (Table 3). The process of detection and isolation using

**Table 3**
Sensitivity, specificity and efficiency of various selective culture media after 24 and 48 h incubation (based on 250 samples).

| Media (duration of incubation) | True positive | False positive | True negative | False negative | Sentivity[a] (%) | Specificity[b] (%) | Efficiency[c] (%) |
|---|---|---|---|---|---|---|---|
| CHROMagar™ *Listeria* (24 h) | 20 | 2 | 216 | 12 | 62.5 | 99.1 | 94.4 |
| CHROMagar™ *Listeria* (48 h) | 31 | 2 | 216 | 1 | 96.9 | 99.1 | 98.8 |
| *Listeria* selective agar (24 h) | 14 | 13 | 205 | 18 | 43.8 | 94.0 | 87.6 |
| *Listeria* selective agar (48 h) | 23 | 17 | 201 | 9 | 71.9 | 92.2 | 93.2 |
| PALCAM agar (24 h) | 12 | 8 | 210 | 20 | 37.5 | 96.3 | 88.8 |
| PALCAM agar (48 h) | 22 | 15 | 203 | 10 | 68.8 | 93.1 | 90.0 |

[a] Sensitivity (%) = true positives × 100/(true positives + false negatives).
[b] Specificity (%) = true negative × 100/(true negative + false positive).
[c] Efficiency (%) = (true positives + true negatives) × 100/total.

**Table 4**
Comparison of the performance of chromogenic media for *L. monocytogenes* from various studies.

| | Sensitivity (%) | Specificity (%) | Efficiency (%) | Samples |
|---|---|---|---|---|
| Current study | 96.9 | 99.1 | 94.4 | NCFS[a] |
| Ritter et al. (2009) | 100.0 | 100.0 | NA | NCFS[a] and SFS[b] |
| Al-Wasify et al. (2011) | 93.7 | 90.0 | 92.8 | NCSW[c] |
| Hegde et al. (2007) | 99.0 | NA | NA | NCFS[a] and SFS[b] |

[a] NCFS: Naturally contaminated food samples.
[b] SFS: Spiked food samples.
[c] NCSW: Naturally contaminated surface water.

chromogenic media, CHROMagar™ *Listeria* took 48–72 h; while PALCAM agar and *Listeria* selective agar needed an extra enrichment step that prolonged the processing time to 96 h. Increase in incubation time for isolation on various plating media from 24 to 48 h increased the efficiency of *L. monocytogenes* detection by not more than 5.6%. In fact, the improvement of the detection sensitivity with the three plating media tested was almost two fold with an extra incubation time of 24 h. The detection of false negatives was decreased by 50% with 48 h of incubation in *Listeria* selective agar and PALCAM agar while it was decreased by about 91.7% in CHROMagar™ *Listeria*. There was no significant statistical relationship between the true/false positive results and the food categories ($P > 0.05$). All false positive samples contaminated by the presumptive colonies on *Listeria* selective agar and PALCAM agar were confirmed to be other types of *Listeria* spp. by PCR. However, both PALCAM agar and *Listeria* selective agar could not be compared to CHROMagar™ *Listeria* because the former two were generally used for detection of *Listeria* spp. while the latter enables direct detection of *L. monocytogenes*. The use of PALCAM agar and *Listeria* selective agar did not allow one-step detection of *L. monocytogenes* as further identification with biochemical test or PCR was needed to differentiate *L. monocytogenes* from other *Listeria* spp. The use of CHROMagar™ *Listeria* allowed direct detection and differentiation of *L. monocytogenes* in samples with high microbial background. Our result again agrees strongly with others on the performance of CHROMagar™ *Listeria* (Al-Wasify, El-Taweel, Kamel, & El-Laithy, 2011; Hegde et al., 2007; Ritter et al., 2009) (Table 4).

This study was designed to test as many as possible the available RTE foods sold by the street hawkers and hypermarkets. All the food samples tested were common foods taken by the locals and can be bought easily in Malaysia. During the sampling, the factors of different preparation methods of these RTE foods were not the main consideration as we sampled all types of RTE foods sold/available. However, we do intentionally included RTE foods prepared with different cooking methods to challenge the performance of CHROMagar™ *Listeria* in correctly detecting *L. monocytogenes* in different RTE foods. And we did not observe any indication that the performance was affected by food matrix and preparation methods.

In conclusion, the high prevalence of *L. monocytogenes* in RTE foods sold by hypermarkets and street hawkers pose a high risk to consumer since RTE foods are consumed directly after purchase with no further treatment applied to reduce *Listeria* in RTE foods. Therefore, the authorities have to look into the problem of sale and production of safe foods for the consumers. The chromogenic media performed better than *Listeria* selective agar and PALCAM agar in detection of *L. monocytogenes*. However, if one aims to detect *Listeria* spp. but not just *L. monocytogenes*, the combination use of two to three plating media are required to achieve higher efficiency of *Listeria* spp. detection.

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