

PREVALENCE OF *LISTERIA* SPP AND *LISTERIA MONOCYTOGENES* IN MEAT AND FERMENTED FISH IN MALAYSIA

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Abstract. Fermented fish and meat samples were purchased from supermarket and wet market for microbiological analysis of *Listeria* species and *Listeria monocytogenes* contamination. *Listeria* species were isolated from 17 (73.9%) of 23 samples of imported frozen beef, 10 (43.5%) of the 23 samples of local beef and 14 (56%) of the 25 samples of fermented fish from wet market. *Listeria monocytogenes* occurred in 15 (75%) of the frozen beef samples, 6 (30.4%) of the 23 samples of local meat and 3 (12%) of the 25 samples from fermented fish. *Listeria* species was not isolated from any of the 23 samples of imported frozen beef from supermarket and from the 5 samples of buffalo meat examined. This highlights the possibility of *Listeria* spp or *L. monocytogenes* to persist in meat and fermented fish in wet market and raises the problem of illness due to the handling and consumption of *Listeria*-contaminated meat or fermented fish are likely as evidence by the high contamination rates of samples sold at the wet market.

INTRODUCTION

Listeria monocytogenes is an important food-borne pathogen having common occurrence in a variety of foods (Farber and Peterkin, 1991; Loncarevic *et al*, 1997; Brett *et al*, 1998; CDC, 1999). Listeriosis is a severe and often fatal illness with clinical manifestations like sepsis or meningitis in immunocompromised patients or neonatal babies and flu-like illness or abortion during pregnancy in women. The major outbreaks of listeriosis has been associated with consumption of foods of animals origin (Doyle, 1986; Iida *et al*, 1998) and fish (Noah *et al*, 1991). The case-fatality rate from listeriosis is generally about 20-30% (Farber and Peterkin, 1991). Fish and meat have been

reported as possible vectors of *L. monocytogenes* which has been linked to prenatal listeriosis (Noah *et al*, 1991; Ronda *et al*, 1992; Virendra *et al*, 1994). Listeriae can grow under various conditions. This microorganism can survive and grow under conditions found in refrigerated foods (Harrison *et al*, 1991). For example, it has been reported to grow in more than 10% NaCl (Monfort *et al*, 1998). However, contamination levels in foods in Malaysia remain uncertain and require special attention. The objectives of this study were to determine the incidence of *Listeria* species and *Listeria monocytogenes* isolated from frozen and fresh meat and fermented fish obtained from supermarket and wet market in Malaysia.

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MATERIALS AND METHODS

Sample collection

Sample of imported frozen beef or meat were purchased in wet and supermarket around

Kuala Lumpur, Subang, Serdang, Melaka and Kuala Terengganu (Table 1) and fermented fish (Pekasam) from Kedah. The samples were collected in sterile plastic bags and transported to the laboratory in boxes containing ice. The samples were kept at 4°C overnight before analysis.

Isolation procedure

Aseptically 25 g of samples were added to 225 ml *Listeria* enrichment broth (LEB) (Oxoid) in a stomacher bag and homogenized for two minutes in a stomacher (Coldworth Stomacher) at room temperature and incubated for 24 hours at 30°C. After incubation, three loopfulls from LEB were streaked onto the surface of Palcam Agar (Oxoid). The plates were incubated at 30°C for 24-48 hours. Five

presumptive *Listeria* species colonies on Palcam agar were streaked on Tryptone Soya Agar containing 0.5-0.6% yeast extract (TSAYE) for purification and confirmation of *Listeria* (Gregory and Michael, 1988; Donald, 1989; Andrews, 1992).

Confirmation

Listeria-like, bluish grey colonies and producing black zone of esculin hydrolysis, whether β-hemolytic or no blood agar, were identified by the following examination: gram-staining-positive, catalase-positive, oxidase-negative, motility at 20-25°C as umbrella-like growth on motility semi solid agar, urea-negative, TSI-produced acid but not gas, MR-VP, nitrate-positive or negative, hippurate hydrolysis positive or negative and *Listeria*

Table 1
Foods tested for isolation and identification of *Listeria* species.

Sample types	Location ^a	No. of samples tested	
Imported frozen beef	Kuala-Lumpur (S)	5	
	Kuala-Lumpur (W)	5	
	Kuala-Trengganu (S)	3	
	Kuala-Trengganu (W)	2	
	Subang (S)	5	
	Subang (W)	5	
	Serdang (S)	2	
	Serdang (W)	5	
	Kajang (S)	3	
	Kajang (W)	3	
	Melaka (S)	5	
	Melaka (W)	5	
	Local beef meat	Kuala-Lumpur (W)	5
Subang (W)		3	
Serdang (W)		5	
Kajang (W)		5	
Melaka (W)		5	
Buffalo meat	Melaka (W)	5	
Fermented fish (Pekasam):			
	Haruan (<i>Channa striatus</i>)	Kedah (W)	5
	Lampan (<i>Puntius javanicus</i>)	Kedah (W)	8
	Sepat (<i>Trichogaster pectoralis</i>)	Kedah (W)	12

^aS=supermarket; W=wet market.

latex slide agglutination test positive (Serobact, Medvet-Australia). Further identification were carried using Microbact 12L. *Listeria* identification system (Medvet-Australia) and conventional method of fermentation test of manitol, xylose, rhamnose, M-D-glucose, maltose, salicin and the CAMP test was performed with *Staphylococcus aureus* and *Rhodococcus equi* (Lovett and Hitchins, 1988; Donald *et al*, 1991; Andrews, 1992; Endang *et al*, 1998).

RESULTS AND DISCUSSION

Of the 101 samples tested, the results obtained pointed out to high positive rates for *Listeria* spp and *Listeria monocytogenes* (Table 2). *Listeria* spp were found in 41 (57.6%) out of 71 samples from the wet market. *Listeria* spp and *L. monocytogenes* were not isolated from any of the 23 samples of imported frozen beef from the supermarket or from the 5 samples of buffalo meat from the wet market.

Ninety-eight bacterial strains isolated from 17 samples of imported frozen beef from the wet market were identified as members of the genus *Listeria* (Table 2). Our data concur with those for *Listeria* spp reported by Donald *et al* (1991). Imported frozen beef examined harbored seven *Listeria* species: 15 *L. monocytogenes*, 18 *L. ivanovii*, 32 *L. innocua*, 2 *L. seeligeri*, 11 *L. grayi*, 7 *L. murrayi*, and 13 *L. welshimeri* isolates, respectively. The overall incidence was higher in comparison to that reported by Virendra *et al* (1994): they detected only two species: *L. monocytogenes* and *L. welshimeri* in retail food in the United Arab Emirates. De Simon *et al* (1992) reported only four species: *L. monocytogenes*, *L. welshimeri*, *L. innocua* and *L. seeligeri* in their raw foodstuff samples from Barcelona (Spain). In this study *L. monocytogenes* was detected in 75% from the wet market sample. However, in the study of Harvey and Gilmour (1993), 35% of food samples examined were positive for *L. monocytogenes*.

Fifty bacterial strains isolated from 10 of 23 (43.5%) samples of local meat from the

wet market were identified as members of the genus *Listeria*, as shown in Table 2. Local beef meat examined harbored seven *Listeria* species: 6 isolates of *L. monocytogenes*, 12 isolates of *L. ivanovii*, 14 isolates of *L. innocua*, a single isolate of *L. seeligeri*, 4 isolates of *L. grayi*, 4 isolates of *L. murrayi*, and 9 isolates of *L. welshimeri*. *L. monocytogenes* occurred in 6 (26.1%) of the samples. Ryu *et al* (1992) reported *Listeria* spp were isolated from 43 (56.6%) out of 76 samples of meat products and *L. monocytogenes* occurred in 26 (34%) of the samples. Similarly, Kamat and Nair (1993) showed the presence of *L. ivanovii*, *L. seeligeri* and *L. welshimeri* in meat samples, however, they were not able to detect incidence of *L. monocytogenes* in any of the samples tested. Retail minced beef in Japan was reported to have a 12.2% contamination rates with *L. monocytogenes* (Inoue *et al*, 2000).

Ninety-four *Listeria* strains were collected from fermented fish (Pekasam) samples obtained in Kedah, Malaysia. 14 (56%) of the 25 fermented fish samples investigated were found positive for *Listeria* spp, out of the total of which 12% were positive from *L. monocytogenes*. The other species isolated were: 30 strains of *L. ivanovii*, 20 strains of *L. innocua*, 20 strains of *L. welshimeri*, 16 strains of *L. denitrificans* and 5 strains of *L. seeligeri*. Elsewhere, Ryu *et al* (1992) reported *L. monocytogenes* was isolated from 7 (6.1%) out of 114 samples of fish and fish products in Japan and Duarte *et al* (1999) reported a 31.3% isolation rates of *Listeria* spp from a smoked fish processing chain.

Overall, the discrepancies in results of prevalence of *Listeria* spp and *L. monocytogenes* can be partly explained by the fact that different sample material and different isolation protocols were used in the studies cited above and in the present one. As only 5 colonies per plate were picked for confirmation this could give rise to underestimation of the prevalence of *L. monocytogenes*. It has been reported that *L. innocua* grow faster than *L. monocytogenes* in enrichment broth media

Table 2
Results of incidence of *Listeria* species in food samples examined.

Sample ^a	Location ^b	<i>L. monocytogenes</i>	<i>L. ivanovii</i>	<i>L. innocua</i>	<i>L. seeligeri</i>	<i>L. grayi</i>	<i>L. murrayi</i>	<i>L. welshimeri</i>	<i>L. denitrificans</i>
Imported frozen beef	Serdang (24, 4/5)	3	3	5	1	4	2	6	-
	Subang (26, 4/5)	2	7	9	-	2	4	2	-
	KL (29, 5/5)	5	6	9	1	4	1	3	-
	Kajang (14, 3/3)	4	1	7	-	-	-	2	-
	KT (5, 1/5)	1	1	2	2	-	-	-	-
Local beef meat	Serdang (10, 2/5)	2	2	3	-	-	-	3	-
	Subang (5, 1/3)	-	3	2	-	-	-	-	-
	Kajang (15, 3/5)	2	2	3	1	2	1	4	-
	KL (10, 2/5)	1	2	4	-	1	1	1	-
	Melaka (10, 2/5)	1	3	2	-	1	2	1	-
Fermented Kedah fish	Haruan (15, 3/5)	-	2	5	3	-	-	3	2
	Lampuan (41, 5/8)	-	16	9	2	-	-	5	9
	Sepat (38, 6/12)	3	12	6	-	-	-	12	5

^aNone of the imported frozen beef from supermarket and the buffalo meat were positive for *Listeria* spp or *L. monocytogenes*.

^bKL=Kuala Lumpur; KT=Kuala Trengganu. Number in parenthesis indicates number of strains isolated and number of samples positive per number of samples analyzed.

(Curiale and Lewus, 1994) and that both species show identical reactions and colony morphology on plating media used. The influence of growth rates and possible underestimation of *L. monocytogenes* needs to be further investigated. The marked difference between the prevalence of *Listeria* spp and *L. monocytogenes* detected in meat sold in the supermarket and in the wet market confirmed the findings of Toh and Birchenough (2000) on the lack of good knowledge of good food handling and failure of hawkers to comply with health requirements in Malaysia. The wet markets where samples were purchased were observed to be crowded with limited access to basic sanitary facilities such as running water, garbage disposal and clean toilets. Thus we are tempted to speculate that the high rates of *Listeria* spp and *L. monocytogenes* isolation from wet market were more likely due to handling and cross-contamination. There are multiple approaches to prevention of contamination, but one of the most effective is amelioration of the critical societal determinants, that is educational activities to alert and inform those concerned about food safety.

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