Determination of *Listeria Monocytogenes* In Fresh Shrimps Using New FDA *Listeria* Method

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Abstract

This paper describes a study to determine whether the new FDA *Listeria* method could detect *Listeria monocytogenes* in fresh shrimp (*Penaeus monodon*) grown in Java, Indonesia.

It was found to be applicable; selective medium MMA was however more sensitive than LPM agar.

Introduction

Outbreaks of *Listeria monocytogenes* infections caused by the consumption of contaminated cole slaw, pasteurized milk and cheese made with pasteurized milk have been reported (Lee and McClain, 1986). Until recently, there has been no report that in Indonesia, outbreaks of *L. monocytogenes* infection have been caused by the consumption of shrimps. Shrimps exported to the USA must be certified free of *L. monocytogenes*.

Indonesia's export of shrimps was slightly more than 60,000 mt in 1989 and has increased yearly. Most Indonesian shrimps are exported to Japan, with other shipments to USA and Europe. Shrimps exported to these countries are accompanied by a Certificate of Health from the Provincial Laboratories of Fishery Quality Control.

L. monocytogenes is a food-borne pathogen which can cause a variety of symptons, such as meningoencephalitis, flu - like low - grade *septimia in gravida*, septicemia in the prenatal period, pneumonia, endocarditis, urethritis and abortion (Gray and Killinger, 1966).

The aim of this work was to establish a standard method of *L. monocytogenes* analysis, which could then be adopted for use by the Provincial Laboratory of Fishery Quality Control in Indonesia, thus increasing confidence in the quality of the product.

Materials And Method

Fresh shrimps (*Penaeus monodon*) were collected from a shrimp factory in Jakarta, Indonesia. The cultured shrimps were from brackish-water ponds along the north Java coast.

Listeria monocytogenes were transported in an insulated box to the laboratory of the National Center for Fishery Quality Control and Processing Development. To maintain their freshness, crushed ice was added. In the laboratory, the shrimps were separated into two groups: Group I, which would undergo irradiation, and Group II with no irradiation. Each group contained two lots as replications. A 25 g sample of each lot was added to 225 cm^3 of *Listeria* enrichment broth (LEB). Each homogenate of group A was then inoculated with 10^3 *L. monocytogenes* and gently shaken.

The methodology for isolation and identification of these bacteria consisted of four basic steps:

Note: This paper was presented at the Seminar by Mr Santoso.

1. Enrichment

The initial step, in which the food sample was enriched in a non-selective nutrient medium.

2. Isolation

In this step selective media plates were used to restrict the growth of bacteria other than *L. monocytogenes*. Suspect colonies were examined using beamed white light powerful enough to illuminate the plate well, and to streak the plate bottom at a 45-degree angle. When examined in this oblique transmitted light from an eye positioned directly above the plate, *Listeria* colonies appear blue-grey to blue. Media used were Modified McBride agar (MMA) and lithium chloride phenylethanol mexalactam (LPM) agar. Typical colonies from these two media were picked and streaked onto trypticase soy agar with 0.6% yeast extract (TSA-YE).

3. Identification
   1. Examination of TSA-YE plates for typical colonies was performed using the oblique illumination system.
   2. Examination of typical colonies by wet mount, using 0.85% saline for suspending medium.
   3. Catalase test.
   4. Gram stain.
   5. Growth of typical colonies in trypticase soy broth with 0.6% yeast extract (TSB-YE) for biochemical tests.
   6. Haemolysis test using blood agar plate.
   7. Biochemical test:
      urease, nitrate, MR, VP, xylase, mannitol, esculine, maltose, glucose and motility of the bacteria in SIM medium; H_2S formation, acid/base on TSI (butt and slant).

4. Camp Test.

Results And Discussion

Results of determination of *L. monocytogenes* in shrimps (*P. monodon*) are shown on Tables 1, 2 and 3.

To interpret data collected, the new FDA *Listeria* Method was used. In this method, *L. monocytogenes* are characterized as gram positive, rods, positive catalase, hydrolize urea without producing H_2S, give an acid butt and acid slant on TSI medium, able to decompose rhamnose, esculine, maltose and glucose with acid production, but were not able to decompose mannitol and xylose. In MR-VP broth, these bacteria give +/+ reaction, but they are not able to reduce nitrate. In blood agar plates, they produce a slightly cleared zone around stab. On Camp Test, there is an interaction between *Staphylococcus aureus* and *L. monocytogenes* leading to production of clear haemolysis zone.

In the selection (isolation) step, both MMA and LPM produced typical colonies of *L. monocytogenes*. Colonies on LPM appeared to be sparkling blue, close to white, while colonies on MMA were blue grey. Trypticase soy agar, yeast extract plates (TSA-YE) were grown by typical colonies of *L. monocytogenes*.

All samples A,B,C and D on both selective media LPM and MMA contained typical colonies of these bacteria. Sample A on LPM did not contain a typical colony, but it did on MMA. On TSA-YE, only sample B was determined not to contain a typical colony. Biochemical and other tests confirmed that samples A and B did not contain *L.monocytogenes*, while samples C and D were confirmed to contain positive *L. monocytogenes*.

The second trial showed that sample B, on both LPM and MMA, did contain typical colony of *L. monocytogenes*, but sample A on the same media showed typical colonies and further isolation on TSA-YE gave positive results. On TSA-YE, samples C & D were found to contain typical colonies. Biochemical and further identification tests confirmed that colonies isolated from LPM & MMA representing sample C, and from MMA representing sample D, were *L. monocytogenes*. However, colonies isolated from LPM on sample D were confirmed not to contain these bacteria.

The third trial showed that *L. monocytogenes* were recovered from samples C & D. Although, typical colonies were found from MMA on sample

Table 1. Result of determination of *L. monocytogenes* on shrimp, first trial.

MEDIA		Code of sample							
		A		B		C		D	
1		2		3		4		5	
Selective agar		LPM	MMA	LPM	MMA	LPM	MMA	LPM	MMA
TSA-YE			+	+	-	+	+	+	+
TSB-YE	25		x	x		x	x	x	x
	35		+	+		+	+	+	+
Hemolysis Test	stab		o	+		o	+	+	+
	camp		-R/-S	-R/-S		-R/-S	+S	+S	+S
Gram stain			-	+		-	+	+	+
Catalase Test			-	+		+	+	+	+
Rhamnose			+	-		-	+	+	+
Xylose			+	+		-	-	-	-
Mannitol			-	-		+	-	-	-
Esculine			+	-		+	+	+	+
Maltose			+	+		+	+	+	+
Glucose			+	+		-	+	+	+
SIM			+	-		-	+	+	+
Urea			+	+		-	-	-	-
Nitrate			+	+		+	-	-	-
MR			-	-		+	+	+	+
VP			-	+		-	+	+	+
TSI	butt		a	a		k	a	a	a
	slant		k	k		k	a	a	a
	H_2S		+	+		-	-	-	-
	gas		+	+		+	-	-	-
Interpretation		neg.	neg.	neg.	neg.	neg.	pos.	pos.	pos.

Table 2. Result of determination of *L. monocytogenes* on shrimp, second trial.

MEDIA		Code of sample							
		A		B		C		D	
1		2		3		4		5	
Selective agar		LPM	MMA	LPM	MMA	LPM	MMA	LPM	MMA
TSA-YE		+	+			+	+	+	+
TSB-YE	25	x	x			x	x	x	x
	35	+	+			+	+	+	+
Hemolysis Test	stab	o	o			+	+	o	+
	camp	-R/-S	-R/-S			+S	+S	-R/S	+S
Gram stain		-	-			+	+	-	+
Catalase Test		+	-			+	+	-	+
Rhamnose		-	+			+	+	-	+
Xylose		-	-			-	-	-	-
Mannitol		+	-			-	-	+	-
Esculine		-	-			+	+	+	+
Maltose		+	+			+	+	-	+
Glucose		-	+			+	+	+	+
SIM		-	-			+	+	-	+
Urea		+	+			-	-	+	-
Nitrate		+	+			-	-	-	-
MR		-	-			+	+	-	+
VP		-	+			+	+	-	+
TSI	butt	a	k			a	a	a	a
	slant	k	k			a	a	k	a
	H_2S	+	+			-	-	+	-
	gas	-	+			-	-	+	-
Interpretation		neg.	neg.	neg.	neg.	neg.	pos.	pos.	pos.

Table 3. Result of determination of *L. monocytogenes* on shrimp, third trial.

MEDIA		Code of sample							
		A		B		C		D	
1		2		3		4		5	
Selective agar		LPM	MMA	LPM	MMA	LPM	MMA	LPM	MMA
TSA-YE			+			+	+	+	+
TSB-YE	25		x			x	x	x	x
	35		+			+	+	+	+
Hemolysis Test	stab		o			+	+	+	+
	camp		-R/-S			+S	+S	+S	+S
Gram stain			-			+	+	+	+
Catalase Test			+			+	+	+	+
Rhamnose			+			+	+	+	+
Xylose			-			-	-	-	-
Mannitol			-			-	-	-	-
Esculine			+			+	+	+	+
Maltose			+			+	+	+	+
Glucose			+			+	+	+	+
SIM			-			+	+	+	+
Urea			+			-	-	-	-
Nitrate			-			-	-	-	-
MR			-			+	+	+	+
VP			+			+	+	+	+
TSI	butt		a			a	a	a	a
	slant		k			a	a	a	a
	H_2S		+			-	-	-	-
	gas		+			-	-	-	-
Interpretation		neg.	neg.	neg.	neg.	neg.	pos.	pos.	pos.

Note (for Tables 1,2 and 3):

+	:	positive reaction
-	:	negative reaction
o	:	no hemolysis
+	:	positive hemolysis
+ R	:	positive interaction with *Rhodococcus*
+ S	:	positive interaction with *S. aureus*
-R/-S	:	no interaction with both *Rhodococcus* and *S. aureus*
a	:	acid
k	:	alkaline/base
x	:	no test
A	:	Sample was not contamintated with the bacteria
B	:	Sample was not contaminated with the bacteria
C	:	Sample was contaminated with the bacteria
D	:	Sample was contaminated with the bacteria

A, they were confirmed in further biochemical tests not to be *L. monocytogenes.*

Conclusion

1. The new FDA *Listeria* Method was able to determinate the presence of *L. monocytogenes* in shrimp.
2. Selective medium MMA was more sensitive than LPM agar.

Collins - Thompson, D.L. and P.J. Slade. 1987. Two stage enrichment procedures for isolation *Listeria monocytogenes* from raw milk. Journal of Food Protection, 50(11): 904-908

Food and Drug Administration. 1988. Bacterioloical analytical manual, Chapter 29 - *Listeria* isolation, revised method of analysis, Notice, Part II.

Gray, M.L. and A.H. Killinger. 1966. *Listeria monocytogenes* and listeric infections. Bacteriol. Rev., 30: 309-382.

Lee, W.H. and D. McClain. 1986. Improved *Listeria monocytogenes* selective agar. Applied and Environmental Microbiology, 52(5): 1215-1217.

McNab, W.B. and R.B. Truscott. 1988. Comparison cf media and procedures for the isolation of *Listeria monocytogenes* from ground beef. Journal of Food Protection, 51(8): 626-628, 638.

Discussion

A comment was made about the determination of *Listeria monocytogenes* in food and the fact that one of the biochemical tests conducted, an SIM test, gave good results. Mr Santoso emphasized the fact that before 1987, there was no internationally-recognised method to test for this bacteria.