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Prevalence of *Listeria* spp. over Commercial Frozen and Refrigerated Foods at the Supermarket Level

In-Ho Cha†, Sung-Hyun Jin, Eun-Hee Park, Sung-Ah Park, Seon-Bong Kim*, Hyeon-Cheol Cho, Young-Sook Lee and Young-Guen Lee**

Pusan Institute of Health and Environment, Pusan 613-104, Korea
**Dept. of Food Science and Technology, Pukyong National University, Pusan 608-373, Korea*
***Dept. of Food Science, Miryang National University, Miryang 627-130, Korea*

Abstract

As a part of investigation for listeriosis, we attempted isolation of *Listeria* spp. from commercial frozen and refrigerated foods at the supermarket level. Seven strains of *Listeria* spp. were isolated from 6 samples (7.1%) among 84 samples of frozen foods, and ten strains of *Listeria* spp. were also isolated from 8 samples (7.6%) among 105 samples of refrigerated foods at the supermarkets in Pusan area. From a total of 189 commercial foods, the number of isolated *Listeria* spp. and ratio were 6 strains (3.2%) of *L. grayi*, one strain (0.5%) of *L. welshimeri*, 6 strains (3.2%) of *L. innocua* and 4 strains (2.1%) of *L. monocytogenes*. *Listeria* spp. isolates except *L. monocytogenes* did not show β-hemolysis on blood agar and positive reaction in CAMP test with *Staphylocccccus aureus*. In the antibiotic susceptibility, most isolates of *Listeria* spp. were susceptible to 12 antibiotics such as ampicillin, cephalothin, penicillin, amikacin, gentamicin, erythromycin, kanamycin, vancomycin, tobramycin, carbenicillin, tetracycline and trimethoprim/sulfamethoxazole. Four strains of *L. monocytogenes* were susceptible to antibiotics used in this study except nitrofurantoin. The serotype of 3 strains and one strain of *L. monocytogenes* were classified into *Listeria* O serotype 1 and 4, respectively.

Key words: *Listeria*, serotype, susceptibility, frozen foods, refrigerated foods

INTRODUCTION

Food poisoning is a worldwide problem in developed and developing countries alike. Reports show that pathogenic organisms found in foods cause thousands of individual cases, hundreds of outbreaks, and several deaths each year in various areas (1). Many pathogenic bacteria can be found in various food sources, and can be transmitted to consumers and occupationally exposed persons (2-7).

Listeria monocytogenes, a gram-positive intracellular pathogen, is responsible for causing listeriosis in humans and animals (8). In recent years, outbreaks of listeriosis which have been associated with the consumption of contaminated food have been recognized (9-11). Human listeriosis is associated with meningitis, septicemia, and abortion, with mortality rates as high as 30 to 40%, particularly in immunocompromised individuals (12).

L. monocytogenes is widely distributed in soils, water, vegetation, animal silage, and other environments (8,13-16). Because of its food-borne mode of transmission by these contaminants, considerable efforts have been made in detecting and preventing listerial contamination (17-19). Although extensive research has been conducted to determine the incidence of *L. monocytogenes* on various samples, only a little study was reported the prevalence of *Listeria monocytogenes* on commercial frozen and refrigerated foods in spite of its ability to grow at low temperatures.

The objective of this study was to determine the distribution of *Listeria monocytogenes* as well as other *Listeria* spp. for commercial foods maintaining at low temperatures, and characteristics of isolates such as biochemical properties, serotype and antibiotic susceptibility.

MATERIALS AND METHODS

Materials and sampling

One hundred and eighty-nine samples of frozen and refrigerated foods were obtained at random from supermarkets in Pusan area, and classified according to odinary name and food source. The kind and collected number

†Corresponding author

of samples are shown in Table 1. The frozen and refrigerated foods were selected as fresh samples which could have been on the supermarket shelf of the freezed and refrigerated display case. The samples were immediately transforted using ice coller after sampling for isolation of *Listeria* spp.

Isolation and identification of *Listeria* spp.

Isolation and identification for *Listeria* spp. from commercial foods were followed by the method of Lovett (20). Sample (25g) was inserted aseptically into the sterile Stomacher bag containing 225 ml of sterile UVM modified Listeria enrichment broth (Difco), and incubated for 24 h at 30°C as first enrichment after homogenization by Stomacher (Pro-media SH-001, Japan). A sample was then inoculated onto Oxford-Listeria-selective agar (Oxford) for direct isolation and incubated for 48 h at 35°C. Simultaneously, first enriched culture (0.1 ml) was inoculated into 9.9 ml of Fraser broth (Difco) with 0.05% ferric ammonium citrate and incubated for 48 h at 35°C as secondary enrichment. Secondary enriched culture was then inoculated onto Oxford-Listeria-selective agar (Oxford) and incubated for 48 h at 35°C. Suspected colonies as *Listeria* spp. on this selective agar were inoculated onto Tryptic soy agar (Difco) containing 0.6% yeast extract, and incubated for 24 h at 35°C. *Listeria* sp. was identified by API Listeria kit (bioMerieux) and the following various tests: gram stain, β-hemolysis on blood agar, growth at 4°C, motility at 25°C, methyl red, Voges-Proskauer, indole, nitrate reduction, oxidase and CAMP for *Staphylococcus aureus*.

Serotyping

L. monocytogenes isolates were revived on Tryptic soy agar plates containing 0.6% yeast extract, and used for serotyping. Serotyping was performed by slide agglutination against *Listeria* O antiserum poly, O antiserum type 1 and 4 (20). A loopful of bacteria was mixed with 1 drop (50 μl) of each antiserum. The mixture was allowed to react for 30 to 45 sec, after which agglutination was read.

Table 1. The kind and number of sample collected from supermarkets

Class	Sub-class	No. of sample	Total
Refrigerated foods	Cheese	18	105
	Milk	17	
	Ham	22	
	Sausage	23	
	Processed fish meat	25	
Frozen foods	Ice cream	16	84
	Processed meat products	30	
	Mixed products of fish & meat	17	
	Frozen Mandoo	16	
	Frozen Pizza	5	
Total		189	189

Antibiotic susceptibility test

Antimicribial susceptibility test was conducted using the disk diffusion method described by Barry and Thornsberry (21), except that Mueller-Hinton agar (Difco) plus 10% sheep blood and growth for 48 h were used. The kind and concentration of antibiotic disks used for susceptibility test were as follows: ampicillin, 10 μg; cephalothin, 30 μg; penicillin, 10 unit; amikacin, 30 μg; gentamicin, 10 μg; kanamycin, 30 μg; erythromycin, 15 μg; vancomycin, 30 μg; nitrofurantoin, 300 μg; tobramycin, 10 μg; streptomycin, 10 μg; carbenicillin, 100 μg; tetracycline, 30 μg; and trimethoprim/sulfa-methoxazole, 1.25 μg/25.75 μg.

RESULTS AND DISCUSSION

Isolation of *Listeria* spp.

Seventeen strains of *Listeria* spp. were isolated from 14 samples (7.1%) among 189 commercial foods taken from randomly selected supermarkets in Pusan area. Of the 17 strains of *Listeria* spp. isolates, 6 strains (3.2%), one strain (0.5%), 6 strains (3.2%) and 4 strains (2.1%) were identified as *L. grayi*, *L. welshimeri*, *L. innocua* and *L. monocytogenes*, respectively (Table 2). Seven strains of *Listeria* spp. were isolated from 6 samples (7.1%) among 84 samples of frozen foods, and ten strains of *Listeria* spp. were also isolated from 8 samples (7.6%) among 105 samples of refrigerated foods. Especially, four strains of *L. monocytogenes* were isolated from four samples such as sausage and processed fish meat in refrigerated foods, and mixed products of fish & meat and frozen Pizza in frozen foods. Other species of *Listeria* spp., isolated in this study, were also isolated from mixed products of fish & meat, frozen Pizza and processed meat in frozen foods, and cheese, sausage and processed meat in refrigerated foods.

The epidemiological importance of listeriosis concerned with the carriage of *L. monocytogenes* by various foods and environmental sources has been indicated by

Table 2. Isolation ratio of *Listeria* spp. from frozen and refrigerated foods

Class of foods (No. of sample)	Sub-class of foods (No. of isolated sample)	No. of isolates (%)			
		L. grayi	*L. innocua*	*L. welshimeri*	*L. monocytogenes*
Frozen (84)	Mixed products[1](1)	-	1	-	1
	Frozen Pizza (3)	1	1	-	1
	Processed meat (2)	1	1	-	-
Subtotal	6 (7.1)	2 (2.4)	3 (3.6)	0	2 (2.4)
Refrigerated (105)	Cheese (2)	2	1	-	-
	Sausage (2)	-	1	-	1
	Processed fish meat (4)	2	1	1	1
Subtotal	8 (7.6)	4 (3.8)	3 (2.9)	1(1.0)	2 (1.9)
Total (189)	14 (7.4)	6 (3.2)	6 (3.2)	1(0.5)	4 (2.1)

[1]Mixed products of fish and meat

a number of recent reports (22,23). *L. monocytogenes* have been emphasized particularly on the contamination of foods stored in the low temperatures (24). *L. monocytogenes* have been isolated from 0~14% of raw milk and milk products (9,25,26), 47.0% of commercial chicken (27), 44.4% of ground beef (17) and 14.9% of commercial beef (28). In our study, *L. monocytogenes* was not isolated from 17 case of milk and 22 case of ham, 23 case of cheese, 16 case of ice cream, 16 case of Mandoo and 30 case of processed meat, but 2.1% of *L. monocytogenes* were isolated from frozen and refrigerated foods. This result indicats the serious problem in the aspect of public health, because of all samples used in this study were commercial foods at the supermarket, although detection ratio of *L. monocytogenes* was lower than those of other previous reports.

Characteristics of *Listeria* spp. isolates

Biochemical properties of 17 strains of *Listeria* spp. isolated from commercial frozen and refrigerated foods are shown in Table 3.

All strains showed positive reaction in utilization of carbohydrates such as glucose, esculin and D-arabitol, as well as α-mannosidase, α-methyl-D-glucoside, growth at 4°C, MR, VP, catalase and motility test at 25°C, but not in glucose-1-phosphate, indole, urease, nitrate reduction and oxidase test. Isolated the *Listeria* spp. did not show β-hemolysis on blood agar except 4 strains of *L. monocytogenes*. Seeliger (29) described that *L. monocytogenes* were differentiated from other species of *Listeria* spp. and other bacteria by motility test at 25°C, β-hemolysis on blood agar and CAMP test for *Staphylococcus aureus*. In our results, 4 strains of *L. monocytogenes* showed positive reaction in these tests (Fig. 1, 2 and 3).

Serotyping of *L. monocytogenes*

Serotype of 4 strains of *L. monocytogenes* isolated from commercial foods are shown in Table 4.

Three strains and one strain of *L. monocytogenes* were classified as serotype 1 and 4, respectively. Bille and Doyle (30) described that three serotypes (1/2a, 1/2b and

Table 3. Biochemical properties of *Listeria* spp. from commercial frozen and refrigerated foods

Characteristics	No. of positive reaction			
	L. grayi (n=6)	*L. innocua* (n=6)	*L. welshimeri* (n=1)	*L. monocytogenes* (n=4)
Glucose	6	6	1	4
Esculin	6	6	1	4
α-mannosidase	6	6	1	4
D-arabitol	6	6	1	4
Xylose	0	0	1	0
Rhamnose	5	4	1	4
α-Methyl-D-glucoside	4	6	1	4
Ribose	6	0	0	0
Glucose-1-phosphate	0	0	0	0
D-tagatose	0	0	1	0
Mannitol	5	0	0	0
Growth at 4°C	6	6	1	4
Methyl red (MR)	6	6	1	4
Voges-Proskauer (VP)	6	6	1	4
Indole	0	0	0	0
Urease	0	0	0	0
Nitrate reduction	0	0	0	0
Catalase	6	6	1	4
Oxidase	0	0	0	0
Motility[1]	6	6	1	4
CAMP for *S. aureus*	0	0	0	4
β-Hemolysis	0	0	0	4

[1]Motility test performed at 25°C

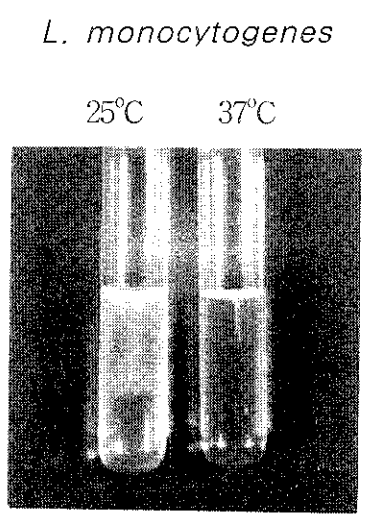

Fig. 1. Motility test of *L. monocytogenes* after 24 h at different temperatures.

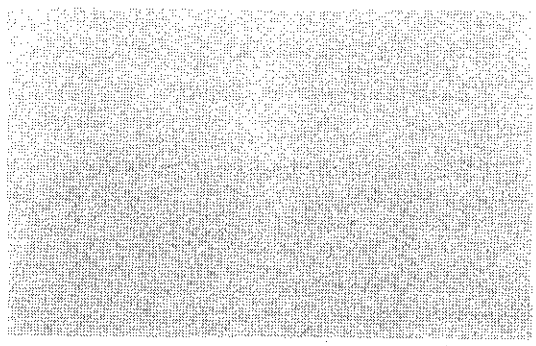

Fig. 2. β-Hemolysis property of *L. monocytogenes* on blood agar after 24 h at 35°C.

4b) represent more than 90% of *L. monocytogenes* isolates from human and animal sources. Also, Kang et al. (31) reported that serotypes of *L. monocytogenes* isolated from commercial and raw milk in Korea were classified as serotype 1 and 4. Since serotype 1 and 4 represent over 90% of *L. monocytogenes* isolates from human and animal sources, serotype of *L. monocytogenes* isolates from commercial foods in this study raises a serious concern in the epidemiological aspect.

Table 4. Serotype of *L. monocytogenes* isolates from commercial foods

Source	No. of isolates	Serotype	
		Type 1	Type 4
Frozen foods	2	2	-
Refrigerated foods	2	1	1
Total	4	3	1

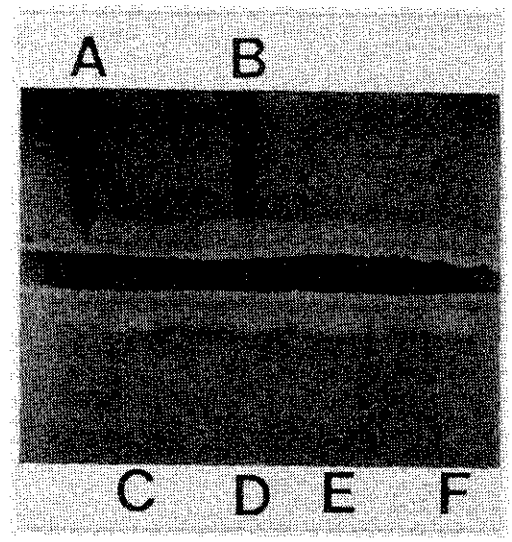

Fig. 3. CAMP test of *L. monocytogenes*. CAMP test done with *S. aureus* after 24 h at 35°C.
A, *L. grayi*; B, *L. innocua*; C, D, E and F, *L. monocytogenes*

Antibiotic susceptibility of *Listeria* spp. isolates

The antibitic susceptibility patterns of the isolates are shown in Table 5. The isolates were very susceptible to the antibiotics used in this study. *L. grayi*, *L. innocua* and *L. monocytogenes*, however, were resistant to nitrofurantoin. The antibiotic susceptibility patterns of *L. monocytogenes* were not definitely different from those of *L. grayi* and *L. innocua*. One strain of *L. welshimeri* was susceptible to the antibiotics used in this study except streptomycin.

Table 5. Antibiotic susceptibility patterns of *Listeria* spp. strains

Antibiotics	Conc. (μg/disk)	No. of susceptible strains			
		L. grayi (n=6)	*L. innocua* (n=6)	*L. welshimeri* (n=1)	*L. monocytogenes* (n=4)
Ampicillin	10	6	6	1	4
Cephalothin	30	6	6	1	4
Penicillin	10 units	6	6	1	4
Amikacin	30	6	6	1	4
Gentamicin	10	6	6	1	4
Kanamycin	30	6	6	1	4
Erythromycin	15	6	6	1	4
Vancomycin	30	6	6	1	4
Nitrifurantoin	300	0	0	1	0
Tobramycin	10	5	6	1	4
Streptomycin	10	2	3	0	4
Carbenicillin	100	6	6	1	4
Tetracycline	30	6	6	1	4
SXT[1)]	1.25/23.75	6	6	1	4

[1)]Trimethoprim/sulfamethoxazole

CONCLUSION

This study was performed to determine the distribution of *Listeria* spp. from frozen and refrigerated foods at the supermarkets in Pusan area. From 189 samples, the isolation ratio of *Listeria* spp. were 3.2% for *L. grayi*, 0.5% for *L. welshimeri*, 3.2% for *L. innocua* and 2.1% for *L. monocytogenes*. Among these isolates, we were able to differentiate 4 strains of *L. monocytogenes* from the other species of *Listeria* by the test of β-hemolysis on blood agar and CAMP test for *Staphylococcus aureus*. Serotypes of 4 isolates of *L. monocytogenes* were classified into *Listeria* O serotype 1 (3 strains) and 4 (one strain). In the antibiotic susceptibility, most isolates of *Listeria* spp. were susceptible to 12 antibiotics used in this study, and there were no differences in susceptibility pattern between *Listeria* spp. Our results clearly indicated that commercial frozen and refrigerated foods should not be ignored as far as listeriosis is concerned.

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