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Incidence and Characterization of *Listeria monocytogenes* in Foods Available in Taiwan

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A variety of foods were examined for the incidence of *Listeria monocytogenes*, and the bacterial isolates were further characterized. *L. monocytogenes* was selected on LiCl-phenylethanol-moxalactam agar after enrichments and identified by several biochemical, mobility, and CAMP tests. *L. monocytogenes* was isolated from 58.8% of pork samples, 50% of chicken carcasses, 38% of turkey parts, 34% of frozen semiready foods, 24% of beef steaks, 12.2% of vegetables, 10.5% of seafoods, and 4.4% of frozen dim sum but was not found in the Chinese pickles and fermented milks. Isolates from seafoods, turkey parts, and beef samples had higher hemolytic activity than those from other samples. The isolates were highly susceptible to ampicillin, cephalothin, chloramphenicol, erythromycin, gentamicin, kanamycin, neomycin, novobiocin, penicillin, and streptomycin. About 14.5% of the isolates were resistant to methicillin, and 14.5% were resistant to tetracycline. The majority of the isolates from turkey parts and beef steaks were serotype 1, and those from chicken and pork samples were serotype 4 and others. Hemolytic activity, methicillin susceptibility, and serotype distribution of the isolates from domestic and imported food samples were significantly different. The results suggest the presence of food- or geography-specific *L. monocytogenes* strains.

Listeria monocytogenes is a psychrotropic, gram-positive, rod-shaped bacterium causing listeriosis in humans and animals (13). It has been implicated as the causative agent in several large outbreaks of food-borne disease in North America and Europe. The fatality rate of food-borne listeriosis is usually high (about 30%). The suspected vehicles in these cases were various vegetables, dairy products, or seafoods (13). Therefore, there have been great concerns about the incidence of this bacterium in the food supply.

Occurrence of *L. monocytogenes* in fresh produce (10), seafoods (7), meats (1, 2, 5), raw milk (6, 12), and manufacturing plants (8) has been reported. However, only a few reports concerned the characterization of the *L. monocytogenes* isolated from various types of foods (1, 10, 12).

Outbreaks of food-borne listeriosis have been reported only in the industrialized countries. No cases from the developing countries have been reported, and also, little is known about the distribution of this pathogen in the food supply in these countries. In this study, we examined the incidence of *L. monocytogenes* in various types of foods in this developing country and characterized the isolates obtained in terms of hemolysis, antibiotic susceptibility, and serotype distribution. Characteristics of these isolates from different food were also analyzed.

MATERIALS AND METHODS

Bacterial strains. *L. monocytogenes* V7, RmI, RmII, Scott A, V37CE, and ATCC 7644 were kindly furnished by W. H. Lee, U.S. Department of Agriculture, and used as reference cultures in these studies. Bacteria were cultured in tryptic soy agar (Difco Laboratories, Detroit, Mich.) slants at 37°C and stored at 4°C.

Isolation and identification of *L. monocytogenes*. Samples (25 g) were blended in 5 to 10 ml of sterile distilled water by a stomacher and added to enrichment media (11). *L. monocytogenes* was isolated with or without alkaline treatment on LiCl-phenylethanol-moxalactam agar and identified by biochemical, motility, and CAMP tests (11). The suspected isolates were also identified by the API 20 Strep test (API System, Montalieu Vercieu, France).

Assay of hemolytic activity. The hemolytic activity of different isolates was determined by both the blood agar plate and the microplate methods (15). Washed sheep erythrocytes (3%) were incorporated into tryptic soy agar supplemented with 0.6% yeast extract. The isolates were spotted on this blood agar plate and incubated at 37°C overnight. Results were registered as follows: −, nondetectable; +, weak hemolysis; ++, moderate hemolysis; +++, strong hemolysis (15). For the microplate method, 100 μl of 3% washed sheep erythrocytes was added to 50 μl of serial dilutions of overnight brain heart infusion (Difco) cultures in microtiter plates and the mixture was incubated at 37°C for 10 to 12 h. Minimum hemolysis units are the reciprocal of the highest dilution at which hemolysis was detected (15).

Antibiotic susceptibility. Antibiotic susceptibility was determined by the disk agar diffusion method in accordance with the instructions of the antibiotic disk supplier (BBL Microbiology Systems, Cockeysville, Md.).

Serotyping. A colony-blot double-stain method (14) was used to determine the serotypes of the *L. monocytogenes* isolates. Serotype 1 and 4 antisera were purchased from Difco. Protein A-horseradish peroxidase conjugate was purchased from Sigma Chemical Co. (St. Louis, Mo.).

Beta-hemolysin sequence homology. DNA colony hybridization was used to detect sequence homology in the isolates. Methods for probe preparation and colony hybridization followed the procedures of Datta et al. (3). The DNA probe was an internal *Hin*dIII-*Hin*cII region of about 500 base pairs of a beta-hemolysin gene of *L. monocytogenes* obtained from A. R. Datta.

RESULTS

Incidence of *L. monocytogenes* in foods. *L. monocytogenes* was isolated from 25.3% of the total food samples purchased

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TABLE 1. Incidence of *L. monocytogenes* in various foods

Item	No. of samples	Incidence (%)
Domestic		
Vegetables	49	12.2
Chinese pickles and fermented milk	12	0.0
Pork	34	58.8
Chicken carcass	16	50.0
Seafood[a]	57	10.5
Frozen semiready foods[b]	68	34.0
Frozen cooked foods[c]	45	4.4
Imported		
Turkey parts	50	38.0
Beef steak	25	24.0
Total	356	25.3

[a] Frozen and refrigerated fishes, squids, and crabs.
[b] Various types of dumplings (prepared by wrapping mixtures of vegetables and meats such as pork, fish, shrimp, or peanuts, etc., in a thin layer of bread dough), fish balls, and meat balls. All needed to be cooked before serving.
[c] Various types of frozen dim sum needing to be heated before serving.

from the local markets (Table 1). High incidences were found in the raw meat samples, such as pork, chicken carcasses, turkey parts, and beef steaks, while no samples of the Chinese pickles and fermented milk products were contaminated (Table 1). *L. monocytogenes* was isolated from 2 of 45 samples of frozen cooked foods (frozen dim sum). *L. monocytogenes* was isolated only from lettuce, Chinese cabbage, and green onion among a variety of fresh produce and only from fish and squid samples among the seafoods examined.

Hemolytic activity. A total of 173 different *L. monocytogenes* isolates were obtained from the contaminated samples. Hemolytic activities were determined by the blood agar plate and microplate methods, and the results were similar, so only the results from the microplate method are presented in Table 2. Isolates from seafoods, turkey parts, and beef samples showed higher hemolytic activity than those from other samples. A number of isolates showed nondetectable hemolytic activity in the blood agar plate or when assayed by the microplate method (Table 2). The colony hybridization experiment showed weak to strong signals corresponding to the hemolytic activity of the isolates as determined by the blood agar plate and microplate methods (data not shown). Also, all isolates showed weak to strong hemolysis in the CAMP test (data not shown).

Antibiotic susceptibility. The *L. monocytogenes* isolates tested were highly susceptible to ampicillin, cephalothin, chloramphenicol, erythromycin, gentamicin, kanamycin, neomycin, novobiocin, penicillin, and streptomycin (Table 3). About 14.5% of the isolates were resistant to methicillin, 14.5% were resistant to tetracycline, and 2.9% were resistant to streptomycin (Table 3).

TABLE 3. Antibiotic susceptibility of *L. monocytogenes* isolates from foods

Antibiotic	μg/disk	No. (%) that were:	
		Resistant	Susceptible
Ampicillin	10	1 (0.6)	172 (99.4)
Cephalothin	30	1 (0.6)	172 (99.4)
Chloramphenicol	30	2 (1.2)	171 (98.8)
Erythromycin	15	1 (0.6)	172 (99.4)
Gentamicin	10	1 (0.6)	172 (99.4)
Kanamycin	30	4 (2.3)	169 (97.7)
Methicillin	5	25 (14.5)	148 (85.7)
Neomycin	30	4 (2.3)	169 (97.7)
Novobiocin	5	1 (0.6)	172 (99.4)
Penicillin	10	3 (1.7)	170 (98.3)
Streptomycin	10	5 (2.9)	168 (97.1)
Tetracycline	30	25 (14.5)	148 (85.5)

Serotype distribution. All 10 isolates from vegetables were of serotypes other than types 1 and 4, while those from seafoods were all types 1 and 4. More than 90% of the isolates from the imported turkey and beef steaks were type 1 (Table 4).

Characteristics of isolates from domestic food versus imported foods. Characteristics of the isolates from imported foods (turkey parts and beef steaks) were compared with those of isolates from the domestic samples and analyzed by the chi-square test. Statistically significant ($P < 0.05$) characteristics are summarized in Table 5. Isolates from the imported meats had stronger hemolytic activity, were more susceptible to methicillin, and mostly were serotype 1, while isolates from domestic food samples were weaker in hemolytic activity, were more resistant to methicillin, and had a more even serotype distribution.

DISCUSSION

L. monocytogenes is commonly found in the environment, and it has been isolated from a variety of foods, even in processed meat (2, 19). Our findings indicate that the incidences of *L. monocytogenes* in different types of foods in this developing country are similar to other reports (1, 2, 5, 7, 10). Generally, high incidences were found in raw meats (2, 19). So, it is not extraordinary to find a high incidence in the fresh pork and chicken samples obtained from the local markets.

L. monocytogenes was not detected in the pickles and fermented milk samples. This may be due to the low contamination of the raw materials, good sanitation practices

TABLE 2. Hemolytic activities of *L. monocytogenes* isolates from various food sources

Hemolysis assay (MHU)[a]	% of isolates from:							
	Vegetables	Pork	Chicken carcass	Seafood	Frozen semi-ready food	Frozen cooked food	Imported turkey	Imported beef
0	90.0	94.4	92.3	0.0	68.8	28.6	2.8	10.0
6	0.0	0.0	0.0	0.0	3.1	0.0	0.0	0.0
12	10.0	0.0	7.7	15.8	0.0	14.3	33.3	10.0
24	0.0	0.0	0.0	21.1	12.5	28.6	41.7	10.0
48	0.0	5.6	0.0	47.4	15.6	28.6	19.4	35.0
96	0.0	0.0	0.0	15.8	0.0	0.0	2.8	5.0

[a] Hemolytic activity was assayed by the microplate method. MHU, Minimum hemolytic unit.

TABLE 4. Serotype distribution of *L. monocytogenes* isolates from foods

Item	No. (%[a]) that were:		
	Serotype 1	Serotype 4	Others
Domestic			
Vegetables	0 (0.0)	0 (0.0)	10 (100.0)
Pork	2 (5.6)	10 (27.8)	24 (66.7)
Chicken	1 (7.7)	5 (38.5)	7 (53.8)
Seafoods	14 (73.7)	5 (26.3)	0 (0.0)
Frozen semiready foods	10 (31.3)	9 (28.1)	13 (40.6)
Frozen cooked foods	5 (71.4)	1 (14.3)	1 (14.3)
Imported			
Turkey	35 (97.2)	0 (0.0)	1 (2.8)
Beef steak	18 (90.0)	1 (5.0)	1 (5.0)

[a] Percentage of total in row.

(8), and detrimental effects of the high-salt and high-acid environment (4). It is known that the incidence of *L. monocytogenes* in raw milk and other dairy products is usually low (5, 6), and its survival in milk is affected by the growth of different lactic acid bacteria (17, 18). This pathogen is completely inhibited at pHs below 4.75 in milk fermentation (16). The minimum pH in which this pathogen can grow is 4.39 at 30°C (9). The salt concentration of the Chinese pickles is usually more than 10%, and acidity is also high.

The frozen semiready foods examined were meat balls, fish balls, or mixtures of raw vegetables, raw meat, and bread dough. Frozen cooked foods were various types of cooked dim sum. These foods were mixtures of raw materials. Judging from the hemolytic activities and serotype distributions (Tables 2 and 4), isolates from these food samples seem to be a mixtures of isolates from individual raw materials such as vegetables, seafoods, pork, and chicken. Since these frozen semiready and cooked foods have to be cooked or reheated before serving, *L. monocytogenes* contamination may not cause a great health problem.

TABLE 5. Hemolysis, antibiotic susceptibility, and serotype distribution of *L. monocytogenes* isolated from domestic and imported foods

Characteristic[a]	No. of isolates (%[b])	
	Domestic foods[c]	Imported foods[d]
Hemolysis[e]		
−	81 (69.2)	2 (3.6)
+	13 (11.1)	18 (32.1)
++	13 (11.1)	25 (44.6)
+++	10 (8.5)	11 (19.6)
Methicillin		
Resistant	23 (19.7)	2 (3.6)
Susceptible	94 (80.3)	54 (96.4)
Serotype		
Type 1	32 (27.4)	53 (94.6)
Type 4	30 (25.6)	1 (1.8)
Others	55 (47.0)	2 (3.6)

[a] Analyzed by chi-square test. Only significant ($P < 0.05$) items are listed.
[b] Percentage of total in column.
[c] All food samples excluding imported turkey parts and beef steak.
[d] Imported turkey parts and beef steak.
[e] Hemolysis on blood agar plate: −, nondetectable; +, weak; ++, moderate; +++, strong.

Although there have been a number of surveys concerning a variety of foods, little is known about the characteristics of the *L. monocytogenes* isolated. In this study, characteristics of the isolates seem to be related to their food origins, e.g., isolates from turkey, beef, and seafoods had stronger hemolytic activity than others (Table 2) and the majority of the isolates from seafoods were serotype 1 (Table 4). Isolates from imported foods (turkey and beef) and other domestic foods differed in hemolytic activity, methicillin susceptibility, and serotype distribution (Table 5). *L. monocytogenes* from chickens in the southeastern United States is mainly serotype 1 (1), but the isolates from chicken in this report were mainly not serotype 1 (Table 4). These data suggest the presence of food- or geography-specific *L. monocytogenes* strains. More surveys and characterization should be done to draw any solid conclusion.

The turkey samples, mainly legs and wings, were imported from the United States. Beef steaks were imported from the United States or Australia. These meats were shipped in the frozen state and had already thawed when they were displayed in the market. The long freezing and thawing process may injure this pathogen and affect its recovery; nevertheless, comparably high incidences were still found in these imported products (Table 1). Our data suggest that serotypes of poultry isolates of *L. monocytogenes* may vary with geographical source, being mainly serotype 1 in the chicken survey of Bailey et al. (1) and mainly serotype 4 (38.5%) and non-1 or non-4 serotype (53.8%) in chickens from Taiwan (Table 4). Therefore, food-borne pathogens not only cause regional health problems, they may be problems in international trade. Recently, some local food suppliers have been requested by foreign firms and foreign governmental agencies to provide a monitoring program for *L. monocytogenes*.

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