

Prevalence of *Listeria monocytogenes* and Related Species in Minimally Processed Vegetables

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Abstract *Listeria* spp. were isolated from a total of 402 naturally contaminated domestic ready-to-eat (RTE) vegetable samples by the conventional Food and Drug Administration protocol and confirmed by API-*Listeria* kit. Also, the susceptibility to 12 antibiotics, polymerase chain reaction (PCR) assay for virulence gene of pathogenic *Listeria monocytogenes* isolates, and *in vitro* virulence assay using myeloma and hybridoma cells from murine and human sources were tested. Among the samples, 17 samples (4.2%) were found to be contaminated with *Listeria* species. Among the 17 strains of *Listeria* spp. isolates, only 2 strains (11.8%) of *L. monocytogenes* and 15 strains (88.2%) of *L. innocua* were identified. Antibiotic susceptibility test showed that the *Listeria* spp. isolates were very susceptible to the antibiotics tested, except for nalidixic acid. Among 17 strains of *Listeria* spp., PCR analysis showed that 2 strains of *L. monocytogenes* isolates proved to have a virulence *hly* gene, but none of *L. innocua* had the *hly* gene. Also, hybridoma Ped-2E9 cells assay showed that only *L. monocytogenes* isolates killed approximately 95–99% hybridoma cells after 6 h, but *L. innocua* isolates had about 0–5% lethal effect. These results indicate that PCR assay with *hly* primer or hybridoma Ped-2E9 cells assay could be used as a good monitoring tool or *in vitro* virulence test for *L. monocytogenes*.

Key words: *Listeria monocytogenes*, incidence, hybridoma cells, antibiotic susceptibility, PCR

Listeria monocytogenes is widespread in the environment and has been isolated from soil, animals, seafoods, raw vegetables, fresh produce, dairy products, sea foods, poultry, and meats [10, 26, 27, 30, 34, 35]. *L. monocytogenes* is one of the food pathogens that has a very wide growth temperature range (1–44°C), therefore, contamination of refrigerated foods could represent a significant health hazard

[19, 32]. This organism has emerged as one of the most serious pathogens due to its ability to cause foodborne listeriosis [11]. The pathogen can cause meningitis, septicemia, and abortion among susceptible individuals. Those at high risk include the elderly, the immunocompromised, pregnant women, fetus, and newly born infants [1].

Epidemics of foodborne listeriosis with high fatality rates (30%) have caused concern about the incidence and control of *L. monocytogenes* in the food supply and the environment [3, 7, 16]. Fresh vegetables are of particular interest. An epidemic in 1981 was linked to raw cabbage, lettuce, celery, and tomatoes which were suspected vehicles of transmittal in another cluster of cases [13, 29]. These facts caused great concern about the incidence and control of *L. monocytogenes* in the food supply and the environment.

Although a number of outbreaks of foodborne listeriosis have been reported, most of them were from developed countries such as USA, Canada, England, France, Australia, and The Netherlands [20, 23, 24, 26, 34]. In Korea, there has been little information until now on the occurrence of *L. monocytogenes* in raw or minimally processed vegetables, even though a few scientific articles describing the presence of *L. monocytogenes* in other types of processed foods have been reported [2, 9]. Baek *et al.* [2] first reported about the incidence and characterization of *L. monocytogenes* from domestic and imported foods in Korea, which showed 122 samples (7.9%) contaminated with *L. monocytogenes* out of a total of 1,537 samples, including shellfish, ice cream, frozen food, beef, pork, chicken, and milk. Very recently, Choi *et al.* [9] reported that about 8 samples (2%) of beef, chicken, pork, frozen foods, and sausage from a total of 410 samples were contaminated with *L. monocytogenes*. The purpose of this study was to determine the prevalence of *L. monocytogenes* in ready-to-eat (RTE) vegetables in Korea and to characterize the isolates in terms of biochemical tests, antibiotic susceptibility test, polymerase chain reaction assay, and virulence test using hybridoma Ped-2E9 cells assay.

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MATERIALS AND METHODS

Sampling

A total of 402 vegetable samples were collected at random from five supermarkets located at Chunchon city in Korea from September 2001 to May 2002. The tested RTE vegetables were already trimmed, peeled, washed, and kept at refrigeration temperature at the supermarkets. Collected samples included bean sprouts, celery, spinach, Chinese cabbage, lettuce, mushroom, perilla leaf, welsh onion, chrysanthemum, and chicory. Among them, most samples were air-packaged or wrapped, except for the Chinese cabbage and the Welsh onion which were non-packaged. All samples were transferred in an ice cooler to the laboratory for analyses on the same day.

Isolation and Identification of *Listeria* Species

RTE vegetable samples were analyzed for the presence of *L. monocytogenes*, using the enrichment and isolation procedures recommended by the U.S. Food and Drug Administration method [15]. Twenty-five grams of samples were aseptically taken, blended for 2 min in 225 ml of *Listeria* enrichment broth (LEB; Difco), and incubated for 24 h at 30°C. A portion (1 ml or 0.1 ml) of the homogenate was inoculated into 9 or 9.9 ml of Fraser broth (Difco Laboratories, Detroit, U.S.A.) for a second enrichment and then incubated at 35°C for 24 h or 48 h. Positive Fraser broth was streaked to modified Oxford agar, and plates were examined for typical *Listeria* colonies after 48 h of incubation at 35°C. The suspected colonies were transferred to the trypticase soy agar supplemented with 0.6% yeast extract (TSBYE; Difco, U.S.A.) and incubated overnight at 35°C. Biochemical tests, including Gram staining, catalase, oxidase, motility, β -hemolysis, carbohydrate utilization, and the CAMP test, were carried out according to Bergey's Manual of Systematic Bacteriology [17]. Also, the API-*Listeria* (BioMereux, sa Marcy-1 Etoile, France) kit was used to differentiate the identity of *L. monocytogenes* from the other *Listeria* spp.

Antibiotic Susceptibility

Antibiotic susceptibility was determined by the disk agar diffusion method [4] in accordance with the instructions of the antibiotic disk supplier (BBL Microbiology Systems, Cockeysville, U.S.A.). Throughout the experiments, *Escherichia coli* ATCC 25922 was used as control.

In Vitro Virulence Test

The hybridoma cell (Ped-2E9) was kindly provided by Dr. Michael G. Johnson, Dept. of Food Science, Univ. of Arkansas, U.S.A. Ped-2E9 cells were maintained at the log phase in RPMI supplemented with 10% fetal bovine serum and were incubated at 37°C in 7% CO₂ under humidified

conditions. Each experimental procedure was carried out according to the methods described by Bhunia *et al.* [5].

Polymerase Chain Reaction Assay

A polymerase chain reaction (PCR) was applied to determine the virulence gene of *L. monocytogenes* isolates. Both *hly* 1 (TCCGCCTGCAAGTCCTAAGA) and *hly* 2 (GCGCTTGCAACTGCTCTTTA) primers, which were previously reported by Klein and Juneja [21], were used as virulence marker (58-kDa virulence factor listeriolysin D). The amplified product of each primer was 713 bp (1620–2333). DNA sample was prepared using the alkaline lysis method, modified as follows: addition of both lysozyme (2 mg/ml) and proteinase K (200 μ g/ml), then boiling the mixture for 15 min. The DNA samples were amplified in a 50 μ l reaction mixture composed of 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 100 μ M dNTP, 0.5 μ M each primer, 1.25 U of Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT, U.S.A.), and 10 μ l of chromosomal DNA. The mixture was overlaid with 100 μ l of mineral oil and subjected to 38 cycles of amplification in a Touch-Down Thermal Cycler (Hybaid, Teddington, U.K.). Parameters for the amplification cycles were denaturation for 1 min at 94°C, annealing of primers for 1 min at 56°C, and primer extension for 1.5 min at 72°C. After the last cycle, the PCR tubes were incubated for 5 min at 72°C. After PCR amplification, 10 μ l of PCR product was loaded on the horizontal submarine 1.5% agarose gels and subjected to electrophoresis in TAE buffer. The gels were stained with ethidium bromide and photographed under UV transillumination.

RESULTS AND DISCUSSION

A total of 402 samples in various RTE vegetables were examined for the presence of *Listeria* species. Of these, 17 samples (4.2%) were found to be contaminated with *Listeria* species (Table 1). Among the 17 strains of *Listeria* spp. isolates, 2 strains (11.8%) of *L. monocytogenes*, and 15 strains (88.2%) of *L. innocua* were identified, and no other *Listeria* spp. were detected.

Among the RTE vegetables tested, only celery (2.7%) and mushroom (2.2%) were contaminated with *L. monocytogenes*, whereas bean sprout (6.8%), celery (10.8%), lettuce (2.3%), mushroom (2.2%), Chinese cabbage (5.5%), and Welsh onion (5.7%) were contaminated with *L. innocua*. Of the 15 *Listeria* spp. isolates, *L. innocua* (88.2%) was the most predominant isolate identified in a wide variety of RTE vegetables. These results are in good agreement with those findings of Petran *et al.* [26] who did not find *L. monocytogenes* in any of the market samples of fresh and frozen vegetables, and with those results of Farber *et al.* [10] who found *L. ivanovii* only

Table 1. Incidence of *Listeria monocytogenes* and *Listeria* spp. from ready-to-use vegetables.

Type of sample	Number of sample	<i>Listeria</i> spp. (%)	
		<i>L. monocytogenes</i>	<i>L. innocua</i>
Bean sprouts	44		3 (6.8%)
Celery	37	1 (2.7%)	4 (10.8%)
Lettuce	43		1 (2.3%)
Mushroom	46	1 (2.2%)	1 (2.2%)
Spinach	41		
Chinese cabbage	73		4 (5.5%)
Perilla leaf	33		
Welsh onion	35		2 (5.7%)
Chrysanthemum	30		
Chicory	20		
Total	402	2 (0.5%)	15 (3.7%)

in one out of 110 samples. In contrast, in previously reported articles [30, 31], *L. monocytogenes* was found in 4 out of 60 prepacked salads and in about 9% of the lettuce heads purchased at retail stores. Steinbreugge *et al.* [31] suggested that a warm humid environment might be necessary for *L. monocytogenes* to grow to detectable levels on lettuce leaves. On the other hand, Kerr *et al.* [20] reported that *L. innocua* was isolated in 100% rate (7 out of 7) from the raw chicken, whereas *L. innocua* was identified in only one in 102 (0.9%) samples of cooked chill chicken. MacGowan *et al.* [23] also reported that *L. monocytogenes* and *L. innocua* were the main species isolated from foods and fecal specimens. The thermal resistance of *Listeria* spp. and *L. monocytogenes* has drawn much debate; however, it is a general consensus that proper heating process would kill viable *Listeria* cells [25]. Therefore, the occurrence of *Listeria* spp. in cooked foods

Table 2. Antibiotic susceptibility of *Listeria monocytogenes* and *Listeria innocua* isolates from ready-to-use vegetables.

Antibiotic	Conc (µg/disk)	No. of susceptible strains					
		<i>L. monocytogenes</i> (2)			<i>L. innocua</i> (15)		
		S ¹	I ²	R ³	S	I	R
Ampicillin	10	2			15		
Chloramphenicol	30	2			14	1	
Carbemicillin	100	2			14	1	
Cephalothin	30	2			15		
Ciprofloxacin	5	1	1		10	5	
Gentamicin	120	2			15		
Kanamycin	30	2			15		
Nalidixic acid	30			2			15
Streptomycin	300	2			14		
SXT ⁴	1.25/23.75	2			15		
Tetracycline	30	2			14		1
Tobramycin	10	2			15		

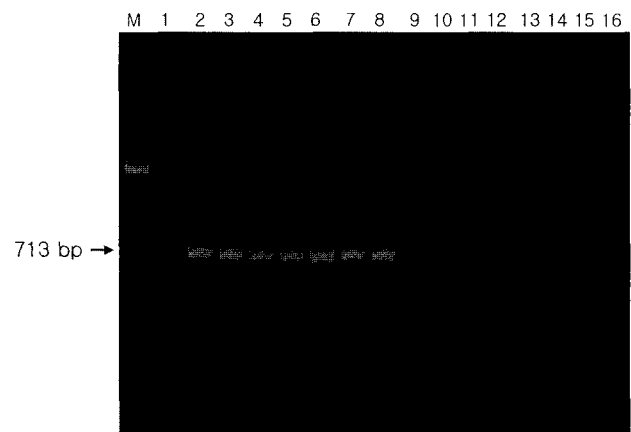
¹Susceptible, ²Intermediate, ³Resistant, ⁴Trimethoprim/Sulfamethoxazole.

must be considered as the consequence of inadequate heat treatment or post-heating re-contamination.

The antibiotic susceptibility of *Listeria* species isolates from RTE vegetables are shown in Table 2. None of the 17 *Listeria* spp. isolates in the study were resistant to the 12 antibiotics tested, except in the presence of nalidixic acid. *L. monocytogenes* and *L. innocua* were the most resistant to nalidixic acid, which is used as antibiotic component of selective media for the *L. monocytogenes*. Also, *L. innocua* isolate was more resistant to tetracycline than that of *L. monocytogenes*, in agreement with other reports [8, 14]. In contrast, however, Rota *et al.* [28] reported that, of 144 *Listeria* spp. isolated from Spanish cheese and meat products, 41% were resistant to chloramphenicol, tetracycline, and erythromycin, and 25% to gentamicin. Recently, clinical strains or food isolates resistant to chloramphenicol, erythromycin, streptomycin, tetracycline, vancomycin, and trimethoprim have been described [6, 22]. These results suggest that *L. monocytogenes* or related species are slowly becoming antibiotic resistant by the acquisition of known antibiotic-resistance genes from the Gram-positive bacteria [12, 18].

PCR assay with specific primer, *hly* gene for the virulence gene of *Listeria* spp. isolates from RTE vegetables, and *in vitro* virulence assay on the *Listeria* spp. isolates, using myeloma and hybridoma cells from murine and human sources, were carried out.

Among the 17 *Listeria* spp. isolates, only 2 *L. monocytogenes* strains isolated from both celery and mushroom were shown to have a virulence *hly* gene and the remaining 15 *L. innocua* isolates did not show any band in the PCR assay (Fig. 1). PCR assay, using *hly*

**Fig. 1.** Agarose gel electrophoresis of *hly*-specific amplification by PCR for the *L. monocytogenes* and *L. innocua* isolates from ready-to-use vegetables.

M: Lambda DNA/*Hind*III markers; lane 1, negative control (no cell); lane 2, *L. monocytogenes* ATCC 19111; lane 3, *L. monocytogenes* ATCC 19113; lane 4, *L. monocytogenes* ATCC 19114; lane 5, *L. monocytogenes* ATCC 19114; lane 6, *L. monocytogenes* Scott A; lanes 7-8, *L. monocytogenes* isolates; lanes 9-16, *L. innocua* isolates.

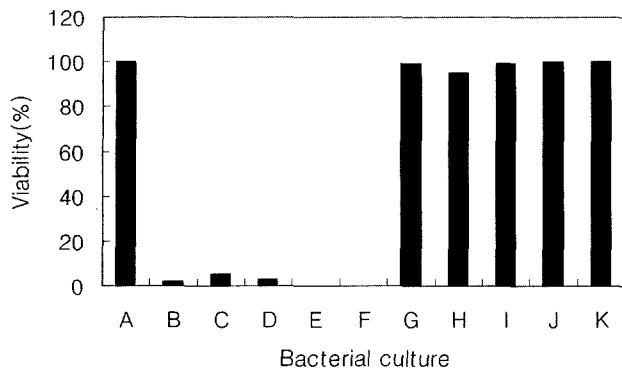


Fig. 2. Viability of hybridoma Ped-2E9 cells after 6 h of exposure to *L. monocytogenes* ATCC 19111 (B), ATCC 19113 (C), ATCC 19115 (D), *L. monocytogenes* isolates (E, F), and *L. innocua* isolates (G–K). The control (A) contained hybridoma cells only. Viability of hybridoma cells was determined by trypan blue staining.

primer for the determination of the virulence factor of the pathogenic *L. monocytogenes* isolates, can be utilized as an effective genetic marker for epidemiological investigation of the pathogenic spp. The viability of hybridoma Ped-2E9 cells after 6 h of exposure to the presence of various *Listeria* species are shown in Fig. 2. *L. monocytogenes* strains ATCC 19111 and *L. monocytogenes* isolates from both celery and mushroom killed approximately 95–99% of the hybridoma cells after 6 h of exposure, but little cytotoxic effect was observed in the *L. innocua* isolates, demonstrating that virulent *L. monocytogenes* isolates rapidly killed the hybridoma cells, while avirulent *L. innocua* isolates did not kill the cells. The hemolysin activity of *L. monocytogenes* might play an important role in killing of the hybridoma cells, because the *hly* negative mutant does not cause substantial hybridoma cell death [5]. Nevertheless, the pathogenicity mechanism has not yet been known. Based on these results, the hybridoma Ped-2E9 cells assay might be a good tool as a simple *in vitro* virulence test to confirm the virulence genes in *L. monocytogenes* and related *Listeria* species isolates in foods.

L. monocytogenes has been of much concern, particularly due to the contamination of foods stored at low temperatures [19, 32, 33]. A significant finding of our present study concerns the existence of pathogenic *L. monocytogenes* with commercially available RTE vegetables obtained from the supermarkets or retail stores. Because of its ability to grow at refrigeration temperature, the presence of pathogenic *L. monocytogenes* in the RTE vegetables makes it a potential public health hazard in the minimally processing industry of both fruits and vegetables. To establish and maintain microbial safety of RTU vegetables, continual monitoring, development of detection and control systems, as well as education of all parties involved in the food chain, should be carried out.

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REFERENCES

1. Azadiann, B. S., G. T. Finnerty, and A. D. Pearson. 1989. Cheese-borne *Listeria meningitis* in immunocompetent patient. *Lancet* **1**: 322–323.
2. Baek, S. Y., S. Y. Lim, D. H. Lee, K. H. Min, and C. M. Kim. 2000. Incidence and characterization of *Listeria monocytogenes* from domestic and imported foods in Korea. *J. Food Prot.* **63**: 186–189.
3. Barnes, R., P. Archer, J. Strack, and G. R. Istre. 1989. Listeriosis associated with the consumption of turkey franks. *Morb. Mortal. Weekly Rep.* **38**: 267–268.
4. Barry, A. L. and C. Thornsberry. 1991. Susceptibility test: Diffusion test procedures, p. 1117. In A. Balows, W. J. Husler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (eds.). *Manual of Clinical Microbiology*, 5th Ed. American Society for Microbiology, Washington, D.C.
5. Bhunia, A. K., P. J. Steele, D. G. Westbrook, L. A. Bly, T. P. Maloney, and M. G. Johnson. 1994. A six-hour *in vitro* virulence assay for *Listeria monocytogenes* using myeloma and hybridoma cells from murine and human sources. *Microbiol. Pathogene.* **16**: 99–110.
6. Biavasco, F., E. Giovanetti, A. Miele, C. Vignaroli, B. Facinelli, and P. E. Varaldo. 1996. *In vitro* conjugative transfer of VanA vancomycin resistance between *Enterococci* and *Listeria* of different species. *Eur. J. Clin. Microbiol. Infect. Dis.* **15**: 5–59.
7. Centers for Disease Control. 1985. Listeriosis outbreak associated with Mexican-style cheese. *Morbidity Mortal. Weekly Rep.* **34**: 357–359.
8. Cha, I. H., S. H. Jin, E. H. Park, S. A. Park, S. B. Kim, H. C. Cho, Y. S. Lee, and Y. G. Lee. 1998. Prevalence of *Listeria* spp. over commercial frozen and refrigerated foods at the supermarket level. *J. Food Sci. Nutri.* **3**: 157–162.
9. Choi, Y. C., S. Y. Cho, B. K. Park, D. H. Chung, and D. H. Oh. 2001. Incidence and characterization of *Listeria* spp. from foods available in Korea. *J. Food Prot.* **64**: 554–558.
10. Farber, J. M., G. W. Sanders, and M. A. Johnson. 1989. A survey of various foods for the presence of *Listeria* species. *J. Food Prot.* **52**: 456–458.
11. Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N. Engl. J. Med.* **312**: 404–407.
12. Francois, B., M. Charles, and P. Courvalin. 1997. Conjugative transfer of tet(S) between strains of *Enterococcus faecalis* is associated with the exchange of large fragments of chromosomal DNA. *Microbiology* **143**: 2145–2154.

13. Greenwood, M. H., D. Roberts, and P. Burden. 1991. The occurrence of *Listeria* species in milk and dairy products: A national survey in England and Wales. *Int. J. Food Microbiol.* **12**: 197–206.
14. Heger, W., M. P. Dierich, and F. Allerberger. 1997. *In vitro* susceptibility of *Listeria monocytogenes*: Comparison of the E test with the agar dilution test. *Chemotherapy* **43**: 303–310.
15. Hitchins, A. D. 1999. *Listeria monocytogenes*. *Bacteriological Analytical Manual*. 8th Ed., Food and Drug Administration, pp. 10.01–10.13.
16. Ho, J. L., K. N. Shands, G. Friedland, P. Eckid, and D. W. Fraser. 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. *Arch. Intern. Med.* **146**: 520–524.
17. Holt, J. H., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams. 1994. *Bergey's Manual of Determinative Bacteriology*, 9th Ed. Williams & Wilkins, Baltimore, Maryland, U.S.A.
18. Jeon, S. U., H. J. Kim, K. B. Lee, J. W. Kim, and M. N. Kim. 2001. Antimicrobial effect of the surface-modified biomedical polyurethane against *Staphylococcus aureus* and *Staphylococcus epidermidis*. *J. Microbiol. Biotechnol.* **11**: 259–265.
19. Junttila, J. R., S. I. Niemala, and J. Hirn. 1988. Minimum growth temperature of *Listeria monocytogenes* and non-hemolytic *Listeria*. *J. Appl. Bacteriol.* **65**: 321–327.
20. Kerr, K. G., N. A. Rotowa, P. M. Hawkey, and R. W. Lacey. 1990. Incidence of *Listeria* spp. in precooked, chilled chicken products as determined by culture and enzyme-linked immunoassay (ELISA). *J. Food Prot.* **53**: 606–607.
21. Klein, P. G. and V. K. Juneja. 1997. Sensitive detection of viable *Listeria monocytogenes* by reverse transcription-PCR. *Appl. Environ. Microbiol.* **63**: 4441–4448.
22. McGowan, A. P., D. S. Reeves, and J. McLauchlin. 1990. Antibiotic resistance of *Listeria monocytogenes*. *Lancet* **336**: 513–514.
23. MacGowan, A. P., K. Bowker, J. McLauchlin, P. M. Bennett, and D. S. Reeves. 1994. The occurrence and seasonal changes in the isolation of *Listeria* spp. in shop bought food stuff, human faeces, sewage and soil from urban sources. *Int. J. Food Microbiol.* **21**: 325–334.
24. Marrakchi, A. E. L., A. Hamama, and F. E. L. Othmani. 1993. Occurrence of *Listeria monocytogenes* in milk and dairy products produced or imported into Morocco. *J. Food Prot.* **56**: 256–259.
25. Peel, M., W. Donachie, and A. Shaw. 1988. Temperature dependent expression of flagella of *Listeria monocytogenes* studied by electron microscopy, SDS-PAGE and western blotting. *J. Gen. Microbiol.* **143**: 2171–2178.
26. Petran, R. L., E. A. Zottola, and R. B. Gravani. 1988. Incidence of *Listeria monocytogenes* in market samples of fresh and frozen vegetables. *J. Food Sci.* **53**: 1238–1240.
27. Restaino, L., E. W. Frampton, R. M. Irbe, G. Schabert, and H. Spitz. 1999. Isolation and detection of *Listeria monocytogenes* using fluorogenic and chromogenic substrates for phosphate-dylinoditol-specific phospholipase C. *J. Food Prot.* **62**: 244–251.
28. Rota, C., J. Yanguela, D. Blanco, J. J. Carraminana, A. Arino, and A. Herrera. 1996. High prevalence of multiple resistance to antibiotics in 144 *Listeria* isolates from Spanish dairy and meat products. *J. Food Prot.* **59**: 938–943.
29. Schlech, W. P., P. M. Lavigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Brome. 1983. Epidemic listeriosis evidence for transmission by food. *N. Engl. J. Med.* **308**: 203–206.
30. Sizmur, K. and C. W. Walker. 1988. *Listeria* in prepacked salads. *Lancet* **1**: 1167.
31. Steinbreuge, E. G., R. B. Maxcy, and M. B. Liewen. 1988. Fate of *Listeria monocytogenes* on ready to serve lettuce. *J. Food Prot.* **51**: 596–599.
32. Walker, S. F., P. Archer, and J. G. Banks. 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. *J. Appl. Bacteriol.* **68**: 157–162.
33. Walker, S. J. and M. F. Stringer. 1987. Growth of *Listeria monocytogenes* and *Aeromonas hydrophila* at chill temperatures. *J. Appl. Bacteriol.* **63**: 20–23.
34. Weagant, S. D., P. N. Sado, K. G. Colburn, J. D. Torkelson, F. A. Stanley, M. H. Krane, S. C. Shields, and C. F. Thayer. 1988. The incidence of *Listeria* species in frozen seafood products. *J. Food Prot.* **51**: 655–657.
35. Wong, H. C., W. L. Chao, and S. J. Lee. 1990. Incidence and characterization of *Listeria monocytogenes* in foods available in Taiwan. *Appl. Environ. Microbiol.* **56**: 3101–3104.