

Listeria spp. Found on Fresh Market Produce

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From October 1987 to August 1988, 1,000 tests were conducted on 10 types of fresh produce from two Minneapolis area supermarkets to detect *Listeria* spp. The produce included broccoli, cabbage, carrots, cauliflower, cucumbers, lettuce, mushrooms, potatoes, radishes, and tomatoes. The vegetables were tested by the Food and Drug Administration method for isolation of *Listeria* spp., with the addition of LiCl-phenylethanol-moxalactam agar in the last 280 tests; 8.6 and 11.4% of these tests were positive by modified McBride and LiCl-phenylethanol-moxalactam agars, respectively. *Listeria monocytogenes* was isolated from cabbage, cucumbers, potatoes, and radishes; *L. innocua* was isolated from cucumbers, lettuce, mushrooms, potatoes, and radishes; *L. seeligeri* was isolated from cabbage and radishes; and *L. welshimeri* was isolated from cucumbers, potatoes, and radishes. The isolates were of various serotypes; however, the *L. monocytogenes* isolates were predominantly serotype 1 (82%). Only potatoes (25.8% positive) and radishes (30.3% positive) showed significant amounts of *L. monocytogenes* contamination.

Epidemics of food-borne listeriosis with high (about 30%) fatality rates (1, 3, 12) have resulted in concern about the incidence and control of *Listeria monocytogenes* in the food supply and the environment. Fresh vegetables are of particular interest. An epidemic in 1981 was linked to raw cabbage (12), and lettuce, celery, and tomatoes were suspected vehicles of transmittal in another cluster of cases (6). This study attempted to determine *Listeria* contamination of 10 types of fresh produce obtained from two Minneapolis area supermarkets from October 1987 through August 1988.

MATERIALS AND METHODS

A total of 1,000 test samples of produce were examined by the Food and Drug Administration method for isolation of *Listeria* spp. (9, 10), with LiCl-phenylethanol-moxalactam (LPM) agar (7) added in the last 280 tests. The produce included broccoli, cabbage, carrots, cauliflower, cucumbers, lettuce, mushrooms, potatoes, radishes, and tomatoes.

All produce was tested as obtained from the supermarket, without any further rinsing or cleaning. The vegetables were cut, and 25-g portions of each were spooned into 225 ml of enrichment broth and incubated at 30°C. At 24 h and at 7 days, the broth was streaked on modified McBride agar (MMA). The MMA was incubated for 48 h and examined by reflected light (9, 10). LPM agar (7) was streaked along with the MMA in the last 280 tests (28 tests of each type of produce). The isolates were tested for beta-hemolysis and identified by traditional biochemical tests as described previously (9, 10) as well as by the computerized Vitek Identification System (McDonnell Douglas Co., Hazelwood, Mo.). The Vitek system uses photo-optic sensors to read biochemical reactions in a plastic card. Results were obtained in less than 24 h, with 100% correlation to the traditional tests.

For serological identification, *Listeria* spp. were isolated by washing live cells from a tryptose agar slant with approximately 2 ml of 0.85% saline. This cell suspension was then tested against polyvalent types 1 and 4 *Listeria* antisera (Difco Laboratories, Detroit, Mich.). An organism showing positive agglutination in type 1 antiserum was further tested

against type 1a, factor 1 antiserum and type 1a, factors 1 and 2 antiserum. An organism testing positive in type 4 antiserum was further tested against the following antisera: type 4a, factors 7 and 9; type 4b, factors 5 and 6; type 4b, factor 5; and type 4d, factor 8.

RESULTS AND DISCUSSION

Listeria spp. were isolated from 6 of the 10 types of produce tested (Table 1). The proportion of radishes and potatoes positive for *Listeria* spp. was significantly higher than proportions of other vegetables (5). When the 95% confidence limits (11) are considered, the 25.8% contamination of potatoes in this study indicates that a true incidence lies between the limits of 18 and 33%, and the 30.3% contamination for radishes, indicates a true incidence between 22 and 38%. No *Listeria* spp. were isolated from broccoli, carrots, cauliflower, or tomatoes. However, this does not mean that there was no *L. monocytogenes* contamination in the lots from which these units were drawn. In fact, there is 1 chance in 20 that *Listeria* contamination exceeds 3% when 92 independent units are observed to be negative. It can be speculated that broccoli, cauliflower, and tomatoes show less contamination because they have less contact with the soil in which they are grown than do root crops such as carrots. A study in progress suggests that some component of carrots may be toxic to *Listeria* spp. (unpublished observations). When shredded carrots were dipped in a suspension of *L. monocytogenes*, the bacterial population was immediately reduced. This decrease was not observed with other types of vegetables (L. R. Beuchat, University of Georgia, personal communication).

Toward the end of the study, LPM agar was used in addition to MMA because of its reported effectiveness (2, 4, 8, 13). More listeria-positive vegetables (Table 2) were detected with LPM agar than with MMA during the last 280 tests. The additional *Listeria* strains isolated from cucumbers (with LPM agar but not with MMA) were identified as *L. monocytogenes* (two strains), *L. innocua* (one strain), and *L. welshimeri* (one strain). In one instance, two species were isolated from the same cucumber portion. The additional strains isolated from radishes were identified as *L. welshimeri* (five strains). The overall recovery on the two agars did

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TABLE 1. Overall incidence of *Listeria* contamination on produce tested with MMA and LPM agar^a

Vegetable (no. tested)	No. (%) of test samples positive for <i>Listeria</i> spp.	95% Confidence limits
Lettuce (92)	1 (1.1)	0.0-3.2
Cabbage (92)	2 (2.2)	0.0-5.2
Cucumbers (92)	9 (10.9)	3.7-15.9
Mushrooms (92)	11 (12)	5.4-18.6
Potatoes (132)	34 (25.8)	18.3-33.3
Radishes (132)	40 (30.3)	22.5-38.1

^a No *Listeria* spp. were found in 92 tests each of broccoli, carrots, cauliflower, and tomatoes.

not differ significantly at the $\alpha = 0.05$ level; however, if the LPM agar had been used for the duration of the study, the difference might have been significant. The LPM agar showed marked selectivity, suppressing nearly all the background growth of the vegetable microflora.

Four species, *L. monocytogenes*, *L. innocua*, *L. seeligeri*, and *L. welshimeri*, were isolated from the various produce (Table 3): 1 isolate of *L. monocytogenes* was found in cabbage, 2 isolates were found in cucumbers, 28 isolates were found in potatoes, and 19 isolates were found in radishes. Overall, 48% of the isolates were *L. monocytogenes*. Radishes and potatoes were the only vegetables demonstrating a significant (5) amount of *L. monocytogenes* contamination. Contaminated radishes were found throughout the year, with the highest proportion found in February, possibly from a single contamination source. The contamination rate for potatoes was fairly consistent throughout the year.

The *Listeria* sp. found in all contaminated mushrooms (12%) was *L. innocua* (Table 3). Additional investigation would be needed to determine whether mushrooms, which are grown in a composted material, can be considered free of *L. monocytogenes* and, if so, whether *L. innocua* is the only *Listeria* sp. able to survive the composting procedure or whether something about the composting procedure changes the pathogenic hemolytic characteristic, resulting in the nonpathogenic *L. innocua* strain.

The *L. monocytogenes* isolates were identified as predominantly serotype 1a, factor 1 (Table 3). These isolates, which were found on vegetables, probably came from the soil (environment) in which the vegetables grew. Our experience with dairy products has shown that this serotype is also common in the environment of the production facility,

TABLE 2. Comparison of the number of positive *Listeria* spp. isolated on MMA and LPM agar^a

Vegetable (n = 28)	Test samples positive for <i>Listeria</i> spp.			
	No.		%	
	MMA	LPM	MMA	LPM
Lettuce	1	1	3.6	3.6
Cabbage	2	2	7.1	7.1
Cucumbers	6	9	21.4	32.1
Mushrooms	2	2	7.1	7.1
Potatoes	8	8	28.6	28.6
Radishes	5	10	17.9	35.7

^a The 280 test samples averaged 8.6% positive on MMA and 11.4% positive on LPM agar. These recoveries did not differ significantly at the $\alpha = 0.05$ level. No *Listeria* spp. were found in 28 tests each of broccoli, carrots, cauliflower, and tomatoes.

TABLE 3. Number of *Listeria* serotypes isolated from fresh produce^a

Vegetable	Species (% isolated) ^b	Serotype and factor(s)	No. of isolates
Potatoes	<i>L. monocytogenes</i> (21.2)	1a; 1	21
		4a; 7, 9	6
		4ab; 6, 7, 9	1
	<i>L. innocua</i> (4.5)	4a; 7, 9	4
		4b; 6	1
		4ab; 6, 7, 9	1
<i>L. welshimeri</i> (0.8)	4ab; 5, 7, 9	1	
Radishes	<i>L. monocytogenes</i> (14.4)	1a; 1	19
		4ab; 6, 7, 9	4
		4a; 7, 9	9
	<i>L. innocua</i> (3.0)	1a; 1	1
		4a; 7, 9	1
		4ab; 5, 7, 9	1
<i>L. seeligeri</i> (9.1)	4a; 7, 9	12	
Cabbage	<i>L. monocytogenes</i> (1.1)	1a; 1	1
		1a; 1	1
Cucumbers	<i>L. monocytogenes</i> (2.2)	4a; 7, 9	2
		4a; 7, 9	5
	<i>L. innocua</i> (5.4)	1a; 1	1
		4a; 7, 9	2
Mushrooms	<i>L. innocua</i> (12.0)	4a; 7, 9	10
		4ab; 6, 7, 9	1
Lettuce	<i>L. innocua</i> (1.1)	4a; 7, 9	1

^a A total of 132 tests each were conducted on potatoes and radishes; 92 tests each were conducted on all other vegetables. No *Listeria* spp. were isolated from broccoli, carrots, cauliflower, or tomatoes.

^b Two species of *L. welshimeri* were isolated from the same potato portion in one instance, two species of *L. welshimeri* were isolated from the same radish portion in six instances, and two species of *L. welshimeri* were isolated from the same cucumber portion in one instance.

whereas serotype 4 is more often isolated from the finished product (unpublished data). It is not known why this is so.

Estimates of sporadic cases of listeriosis have increased to more than 1,600 per year, with an estimated 400 deaths (C. V. Broome, Comprehensive Conf. *Listeria monocytogenes*, Soc. Ind. Microbiol., 1988, abstr. I-9, p.11). It is hoped that awareness of the distribution of *Listeria* spp. will help to prevent listeriosis. Although it is not known what, if any, degree of cleaning would eliminate contamination of fresh produce by *L. monocytogenes*, careful handling and thorough cleaning of all fresh produce are recommended.

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