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Comparison of Lithium Chloride-Phenylethanol-Moxalactam and Modified Vogel Johnson Agars for Detection of *Listeria* spp. in Retail-Level Meats, Poultry, and Seafood

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The effectiveness of Modified Vogel Johnson agar and lithium chloride-phenylethanol-moxalactam agar for detection of *Listeria* spp. in foods was compared by using the media to analyze retail-level meat, poultry, and seafood both by direct plating and in conjunction with a three-tube most-probable-number enrichment. The most-probable-number protocol detected *Listeria* species, including *Listeria monocytogenes*, in a substantial portion of the fresh meat and seafood samples. In most instances the *Listeria* levels were less than 2 CFU/g, which precluded detection by direct plating. Modified Vogel Johnson agar performed as well as did lithium chloride-phenylethanol-moxalactam agar and was considerably easier to use because of its ability to differentiate *Listeria* spp. from other microorganisms.

Recent outbreaks of epidemic human listeriosis have emphasized the importance of food-borne transmission as a primary etiologic factor for this disease. The areas of concern, which were restricted initially to dairy and cabbage products, have been expanded to include a variety of meat, poultry, and seafood products. Although none of these products have been implicated directly in an outbreak of listeriosis, they are viewed as a potential source of *Listeria monocytogenes* because of the role of this microorganism as an animal pathogen, its apparent widespread distribution in the processing environment, and its ability to grow at refrigeration temperatures. Furthermore, a number of investigators have isolated *Listeria* spp., including *L. monocytogenes*, from meats (2, 4, 9, 13–15), poultry (6, 7, 10, 15), and fish or shellfish (2, 8). Although these investigations suggested that a variety of meat, poultry, or seafood products may harbor *L. monocytogenes*, essentially no quantitative data establishing the levels of this microorganism in these classes of food products have been reported.

Currently, the plating medium that seems to be the most commonly used for detection of *L. monocytogenes* in meats, poultry, and seafood is lithium chloride-phenylethanol-moxalactam agar (LPM) (11). Our laboratory recently developed a selective medium, modified vogel johnson agar (MVJ), that eliminates the need to view colonies under obliquely reflected light (3). Instead, MVJ relies on a tellurite-positive, mannitol-negative (Tel^+ Man^-) response by *Listeria* isolates, which results in black colonies on a red background. The objective of this study was to assess the comparative effectiveness of MVJ and LPM for detection of *Listeria* spp. in retail-level foods. The samples were examined with both media, using both direct plating and prior most-probable-number (MPN) enrichment to achieve a secondary objective: acquisition of quantitative data on the levels of *Listeria* spp. in food products.

MATERIALS AND METHODS

Food samples. All foods, except the samples of raw, naturally contaminated milk, were purchased at local supermarkets. All items were transported to our laboratory in their original containers and refrigerated until sampled. The elapsed time between purchase and sampling was generally under 60 min.

The contaminated milk samples were graciously provided by J. Bryner of the Agricultural Research Service National Animal Disease Center (Ames, Iowa). The milk was obtained from a herd of cows infected intramammarily with *L. monocytogenes* Scott A. The milk samples were shipped refrigerated by air freight and were received and analyzed within 12 h.

Media. MVJ was prepared as described by Buchanan et al. (3) except that the concentration of moxalactam was increased to 20 mg/liter. LPM was prepared as described by Lee and McClain (11). University of Vermont listeria enrichment broth (UVM) was prepared as described by Donnelly and Baigent (5), using an acriflavine level of 12 mg/liter. Tryptic soy agar (TSA; Difco Laboratories, Detroit, Mich.) was used for general enumeration of aerobic bacteria in milk samples.

Direct plating. A 10^{-1} dilution of each food sample was prepared by mixing 25 g with 225 ml of sterile 0.1% peptone water in a stomacher bag (Spiral Systems, Inc.). The sample was processed in the stomacher bag for 2 min. Three 0.5-ml portions of the diluted samples were plated onto triplicate prepoured plates of MVJ and LPM. All plates were incubated for 48 h at 37°C and then examined for *Listeria* spp. Milk samples were assayed in a similar manner except that duplicate plates of MVJ, LPM, and TSA were inoculated by using a Spiral Plater (Spiral Systems, Inc.).

MPN assays. Three-tube MPN assays were performed by using 1.00-, 0.10-, and 0.01-g portions of the food samples in conjunction with sets of triplicate tubes containing 9 ml of UVM. The 1.00-g samples were added directly to triplicate tubes, whereas the 0.10- and 0.01-g portions were added by using the 10^{-1} sample dilution described above. After thorough mixing, all tubes were incubated for 48 h at 37°C. Portions (0.2 ml) of each tube were used to surface inoculate MVJ and LPM plates, which were incubated for 48 h at 37°C. Colonies were considered presumptively to be *Listeria* spp. if they grew on LPM or were Tel^+ Man^- on MVJ.

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TABLE 1. Comparison of MVJ and LPM direct plate counts of raw milk samples from cows infected with *L. monocytogenes* Scott A

Milk sample	Listeria (log CFU/ml)		Aerobic plate count[c] (log CFU/ml)
	MVJ[a]	LPM[b]	
A	3.55	3.58	3.73
B	3.51	3.84	4.10
C	3.60	3.75	3.74
D	3.42	3.60	3.93
E	2.80	4.02	4.44
F	3.01	3.56	3.67
G	3.02	3.33	3.47
H	3.13	3.70	4.04
Mean ± SEM	3.26 ± 0.11	3.67 ± 0.07	3.89 ± 0.11

[a] Only Tel^+/Man^- colonies were counted.
[b] All colonies were counted, although two or more colony types were evident on most plates.
[c] Determined on TSA.

Confirmation and identification of species. *Listeria* isolates were confirmed and identified as to species by techniques described by Lovett (12). Up to four colonies that were presumptively positive *Listeria* spp. from each positive LPM and MVJ plate were picked and examined microscopically. Colonies displaying the correct morphology were streaked onto TSA plates and incubated for 24 h at 37°C. These master plates were used to perform assays for Gram stain, motility (28°C; tested both by hanging drop, using phase-contrast microscopy, and by growth on motility test medium [Difco]), production of acid from glucose, esculin, catalase, and oxidase, and Voges-Proskauer reaction. Colonies from LPM plates were also restreaked onto MVJ plates to assess tellurite reduction. Colonies confirmed as members of the genus *Listeria* were then identified as to species by the CAMP test (28°C, 5% sheep blood agar) and acid production from mannitol, rhamnose, and xylose.

RESULTS

The effectiveness of MVJ was assessed initially by comparison against LPM for direct plating of milk samples from cows that had been infected intramammarily with *L. monocytogenes* (Table 1). Comparison of the *Listeria* counts (MVJ and LPM) versus total mesophiles (TSA) indicated that *Listeria* spp. were the major but not sole contaminants in the milk samples. Comparison of raw counts on LPM and MVJ indicated that the latter gave a slightly reduced (88.8%) recovery of *L. monocytogenes*. However, the evaluation was biased in favor of LPM in that all colonies growing on these plates were enumerated although more than one colony type was often evident. The decision to count all colonies on the plates reflected the lack of a quantitative means for directly differentiating *Listeria* spp. from cocontaminants on LPM. Conversely, since MVJ does incorporate a means for differentiation, only Tel^+ Man^- colonies were counted as *Listeria* isolates.

LPM and MVJ were compared further in conjunction with a quantitative survey of retail-level foods, mostly of animal origin. Samples were analyzed both by direct plating and by using a three-tube MPN whereby the samples were enriched for 48 h in UVM and then plated onto LPM and MVJ. Direct plating was almost totally ineffective because of the low levels of *Listeria* spp. in the retail samples. However, when an enrichment procedure was included, 16 and 13 of 62 food samples were *Listeria* positive with MVJ and LPM, respectively (Table 2). The greatest incidence rate was observed with fresh meat samples; 11 of 21 samples were positive for *Listeria* isolates. These results gave a combined isolation rate for both media of 52% (Table 3). When only *L. monocytogenes* was considered, the incidence rate was 43%. However, on the basis of the MPN values, only a few of the samples had more than 1.0 CFU/g, and none of the fresh meat samples had more than 5.0 CFU/g.

Seafood had the second-highest incidence rate for *Listeria* spp. (28%) (Table 3), including isolations from both shellfish and finfish. When only *L. monocytogenes* was considered, the incidence rate was 11%, with positive isolations being restricted to two finfish samples (flounder and monkfish). The levels of *Listeria* spp. detected in the positive seafood samples encompassed a substantially greater range (0.36 to >110 CFU/g) than did the levels in fresh meats.

Positive isolations among cured meat samples were limited to the detection of low levels (0.36 CFU/g) of *Listeria innocua* in a single salami sample tested with MVJ. This result was equivalent to an observed incidence rate of 8% for all *Listeria* species and a 0% rate for *L. monocytogenes* specifically. *Listeria* spp. were not isolated from any of the poultry or potato salad samples.

Only three *Listeria* species, *L. monocytogenes*, *L. innocua*, and *Listeria welshimeri*, were identified among the isolates. Single species were isolated from the seafood samples and the one cured meat sample. In fresh meats, multiple species were detected in 45% of the positive samples. *L. monocytogenes* predominated, being present in 82% of the positive samples. *L. innocua* and *L. welshimeri* were detected in 55 and 18%, respectively, of the positive fresh meat samples.

DISCUSSION

Direct plating trials (Table 1) suggested that this technique can be effective if relatively high levels of *Listeria* spp. are present in the samples and interfering background microflora is minimal. The two media gave roughly equivalent recoveries. Although the counts were higher on LPM than on MVJ, the former tended to give inflated values because of the growth of cocontaminating streptococci (particularly enterococci) and staphylococci. These organisms grew on both media; however, they could usually be differentiated on MVJ. Attempts to observe the LPM plates with obliquely reflected light to quantitatively distinguish *Listeria* isolates were consistently unsuccessful and were discontinued as a means of detecting the microorganisms.

The two media were again roughly equivalent when used as part of an MPN protocol that included a single-stage, 48-h enrichment. The MPN values for positive samples detected on both media were equivalent statistically. However, a greater number of positive samples (16 of 62 versus 13 of 62) was detected with MVJ, largely because of the increased ease of differentiating *Listeria* colonies from the other microorganisms growing on the plates. Streptococci and staphylococci were again the major groups of non-*Listeria* organisms growing on both media. Occasionally, overgrowth with resistant streptococci or staphylococci, both of which are strongly mannitol positive, interfered with observation of the mannitol-negative reaction of *Listeria* isolates. However, differences in tellurite reactions and colony morphology still allowed presumptive identification of *Listeria* isolates. In these instances, representative colonies could be streaked onto MVJ to achieve adequate differentiation. Restreaking

TABLE 2. Comparison of MVJ and LPM when the media are used as part of an MPN[a] survey of fresh retail foods for incidence of *Listeria* species

Food sample	MPN (CFU/g)		Listeria species identified[b]
	MVJ	LPM	
Fresh meat			
Sausage			
1	0.03	0.03	M, In
2	ND[c]	ND	
3	ND	ND	
Pork sausage	0.03	ND	M
Hamburger			
1	ND	ND	
2	ND	ND	
3	0.74	0.92	M, In, W
4	ND	ND	
Veal, ground			
1	ND	ND	
2	0.74	0.30	In
3	0.62	0.30	M
4	0.30	0.62	M, In
Veal patties, ground	0.36	0.36	M
Veal sausage	0.36	ND	W
Liver, beef			
1	ND	ND	
2	ND	ND	
Liver, calf	ND	ND	
Lamb, ground			
1	0.74	4.27	M
2	2.31	1.47	M, In
3	0.64	2.10	M, In
Kidney, lamb	ND	ND	
Cured meat			
Corned beef	ND	ND	
Salami			
1	ND	ND	
2	0.36	ND	In
Bologna			
1	ND	ND	
2	ND	ND	
Ham and cheese loaf	ND	ND	
Luncheon meat	ND	ND	
Olive loaf	ND	ND	
Liverwurst	ND	ND	
Pepperoni			
1	ND	ND	
2	ND	ND	
Lebanon bologna	ND	ND	
Poultry products			
Chicken patties, frozen	ND	ND	
Chicken patties, refrig.	ND	ND	
Microwave chicken	ND	ND	
Chicken breast, sliced	ND	ND	
Liver, chicken	ND	ND	
Turkey breast, sliced	ND	ND	
Turkey roll, sliced	ND	ND	
Turkey, raw, ground			
1	ND	ND	
2	ND	ND	
Fish or shellfish			
Shrimp, uncooked, frozen	0.74	ND	In
Shrimp, uncooked, refrig.			
1	ND	ND	
2	ND	ND	
3	ND	ND	
Shrimp salad	ND	ND	
Seafood salad	ND	ND	
Crab meat, cooked, non-pasteurized			
1	>110	>110	W
2	ND	ND	
Crab meat, surimi	ND	ND	
Flounder, refrig.	0.92	0.36	M
Haddock, refrig.	ND	ND	
Scrod, refrig.	ND	ND	
Monkfish, refrig.	ND	0.36	M
Catfish, refrig.	23.98	23.98	W
Scallops, uncooked	ND	ND	
Oysters, stewing, uncooked	ND	ND	
Oysters, frying, uncooked	ND	ND	
Clams, uncooked, chopped	ND	ND	
Other			
Potato salad			
1	ND	ND	
2	ND	ND	

[a] Three-tube MPN using single 48-h enrichment in UVM.
[b] M, *L. monocytogenes*; In, *L. innocua*; W, *L. welshimeri*.
[c] ND, Less than 0.03 CFU/g.
[d] refrig., Refrigerated.

onto MVJ was also found to be very effective for identifying *Listeria* isolates from LPM plates containing resistant streptococci or staphylococci. *Kurthia* isolates were occasionally a problem with LPM. This microorganism shares numerous cultural and morphological characteristic with the genus *Listeria* and is differentiated primarily on the basis of lack of acid production from glucose. Our continued isolation of this microorganism emphasized the need to include glucose fermentation as an integral part of the confirmation protocol. The *Kurthia* isolates also grew on MVJ; however, their strong alkaline reaction permitted effective differentiation from *Listeria* spp. The ongoing problems associated with streptococci and staphylococci indicate that further enhancements in the specificity of the enrichment protocol would greatly facilitate the detection of low levels of *Listeria* spp. in food products of animal origin. The enrichment broth used in this study was chosen because it is buffered and relatively selective. However, the pattern of responses observed in a number of the MPN assays (i.e., skips at the lowest dilution) suggested that more accurate quantitation will require more effective enrichment formulations.

It is worth noting that there appears to be some variation in results achieved with MVJ among various laboratories. In some instances, growth has been slow and limited to pinpoint colonies. This result appears to be due to production lot differences in the activity of the bacitracin or moxalactam and can be overcome by adjusting the concentrations of the antibiotics such that control cultures of *Listeria* isolates grow adequately. Work is currently under way to better clarify the source of this variation.

This study provides some of the only quantitative data now available on the levels of *Listeria* spp. in retail foods of animal origin. Other studies have been restricted to single plus-minus samples. This is understandable considering the investment in time and effort that must be made to use an MPN protocol. Unless there is some overriding reason, the use of an MPN protocol does not seem warranted for the routine sampling of foods.

TABLE 3. Summary of data on isolation of *Listeria* spp. from retail-level food samples of animal origin

Food type (no. of samples)	No. positive (%)[a]					
	MVJ		LPM		Combined	
	All *Listeria* spp.	*L. monocytogenes*	All *Listeria* spp.	*L. monocytogenes*	All *Listeria* spp.	*L. monocytogenes*
Fresh meats (21)	11	9	9	8	11 (52)	9 (43)
Cured meats (12)	1	0	0	0	1 (8)	0 (0)
Fish or shellfish (18)	4	1	4	2	5 (28)	2 (11)

[a] No poultry product samples tested positive.

These results suggest that a substantial portion of retail fresh meat samples contain low levels of *Listeria* spp., including a substantial percentage with *L. monocytogenes*. Previous investigations have reported a range of incidence rates for meat products. Ternstrom and Molin (16) were unable to detect *Listeria* spp. on the surfaces of beef, pork, or poultry. Cottin et al. (4) were able to isolate *L. monocytogenes* and *L. innocua* from the organs of symptomatic and asymptomatic cattle at the time of slaughter but were unable to isolate the organism from the muscle tissue. Johnson et al. (9) also reported that the levels of *L. monocytogenes* in organ meats from cows experimentally infected with the microorganism were substantially higher than the levels found in the muscle meat. Our results most closely resemble those of Nicolas and Vidaud (13, 14) and Skovgaard and Morgen (15), who examined a variety of retail-level fresh and cured meat products, including delicatessen items. They detected *Listeria* spp. in a substantial portion of the retail-level products, with minced beefsteak having the highest incidence rate (40.9%). It is worth noting that Nicolas and Vidaud (13) also did not isolate *Listeria* spp. from the surfaces of meats at the time of slaughter, which suggests that the microorganisms isolated in the retail-level products were introduced after slaughter.

An interesting observation in our study was that a substantial portion of the positive fresh meat samples (72%) were from young animals (i.e., veal and lamb). At this time, it is unclear whether this represents a statistical anomaly (since the samples were not taken in a truly random fashion) or an actual hot spot for *Listeria* growth. It is possible that either some step associated with processing or the immature nature of the immune system in young animals may contribute to an increased incidence of *Listeria* spp. in these products.

Isolation of *Listeria* spp. from 28% of the seafood samples confirms and expands reports that the organism can be isolated from crustaceans and finfish (2). Although the incidence was less in seafood than in fresh meat samples, the higher levels noted suggest that this class of products may be at least as important as the former. The lack of positive isolations from poultry products was surprising, since other investigators have reported isolation rates of up to 60% in raw poultry (2, 6, 7, 10, 15). A probable explanation is that a majority of the poultry products sampled had received some form of thermal processing. This is also the probable reason for the lower incidence of *Listeria* spp. in cured meat products than in fresh meats.

The isolation of only three *Listeria* species, *L. monocytogenes*, *L. innocua*, and *L. welshimeri*, is in general agreement with results of previous investigations. Cottin et al. (4) isolated only *L. monocytogenes* and *L. innocua* from organ meat derived from infected cattle. Nicolas and Vidaud (14) isolated these species and *L. welshimeri* from various meat products. Johnson et al. (9) isolated a xylose-fermenting *Listeria* spp. that was presumably *L. welshimeri* from control meat samples used for the production of salami. Andre and Genicot (1) reported that *L. welshimeri* is primarily associated with environmental sources and only rarely associated with animal sources. However, the results cited above, including those of our study, indicate that *L. welshimeri* is a relatively common contaminant in meat and seafood. Additional insight into the sources and significance of *Listeria* species other than *L. monocytogenes* is needed, particularly if the genus in general is used as an indicator of *L. monocytogenes*. For example, does the relatively high level of *L. welshimeri* detected in samples of catfish and crabmeat represent an increased risk of the presence of *L. monocytogenes*? Additional information on the relative incidences of the various species in a variety of product classes is needed if this question is to be assessed effectively.

In summary, *Listeria* spp. were isolated from a substantial portion of retail-level fresh meats and seafoods, although the levels were generally low. LPM and MVJ were approximately equivalent with regard to recoveries with both direct plating and MPN protocols. However, because of its ability to directly differentiate *Listeria* spp. from cocontaminants, MVJ was substantially easier to use with meat, poultry, and seafood products that contained large numbers of other microorganisms.

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