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Recovery of Different *Listeria* Ribotypes from Naturally Contaminated, Raw Refrigerated Meat and Poultry Products with Two Primary Enrichment Media

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Isolation rates for *Listeria monocytogenes* and the other *Listeria* spp. typically improve when samples are enriched in more than one primary enrichment medium. This study evaluated the abilities of two primary enrichment media, University of Vermont-modified *Listeria* enrichment broth (UVM) and *Listeria* repair broth (LRB), to recover different ribotypes of *Listeria* spp. from raw meat and poultry samples. Forty-five paired 25-g retail samples of ground beef, pork sausage, ground turkey, and chicken (160 samples) underwent primary enrichment in UVM and LRB (30°C for 24 h) followed by secondary enrichment in Fraser broth (35°C for 24 and 40 h) and plating on modified Oxford agar. After 24 h of incubation at 35°C, 608 *Listeria* colonies from selected positive samples were biochemically confirmed as *L. monocytogenes* (245 isolates), *L. innocua* (276 isolates), and *L. welshimeri* (89 isolates) and then ribotyped with the automated Riboprinter microbial characterization system (E. I. du Pont de Nemours & Co., Inc.). Thirty-six different *Listeria* strains comprising 16 *L. monocytogenes* (including four known clinical ribotypes), 12 *L. innocua*, and 8 *L. welshimeri* ribotypes were identified from selected positive samples (15 samples of each product type; two UVM and two LRB isolates per sample). Twenty-six of 36 (13 *L. monocytogenes*) ribotypes were detected with both UVM and LRB, whereas 3 of 36 (1 *L. monocytogenes*) and 7 of 36 (3 *L. monocytogenes*) *Listeria* ribotypes were observed with only UVM or LRB, respectively. Ground beef, pork sausage, ground turkey, and chicken yielded 22 (8 *L. monocytogenes*), 21 (12 *L. monocytogenes*), 20 (9 *L. monocytogenes*), and 19 (11 *L. monocytogenes*) different *Listeria* ribotypes, respectively, with some *Listeria* ribotypes confined to a particular product. More importantly, major differences in both the number and distribution of *Listeria* ribotypes, including previously recognized clinical and nonclinical ribotypes of *L. monocytogenes*, were observed when 10 UVM and 10 LRB isolates from five samples of each product were ribotyped. When a third set of six samples per product type was examined from which two *Listeria* isolates were obtained by using only one of the two primary enrichment media, UVM and LRB failed to detect *L. monocytogenes* (both clinical and nonclinical ribotypes) in two and four samples, respectively. These findings stress the importance of using more than one primary enrichment medium and picking a sufficient number of colonies per sample when attempting to isolate specific *L. monocytogenes* strains during investigations of food-borne listeriosis.

Emergence of *Listeria monocytogenes* as a serious food-borne pathogen dates back to the 1980s, with several well-documented outbreaks of listeriosis directly linked to consumption of contaminated coleslaw (46), Mexican-style cheese (33), and Vacherin Mont d'Or soft ripened cheese (11, 45). Following a massive 1985 outbreak of listeriosis in California traced to Mexican-style cheese, additional concerns were raised regarding the possible role of meat and poultry products as vehicles of listeric infection. One retrospective case-control study suggested that approximately 20% of the 1,600 annual listeriosis cases in the United States likely resulted from consuming uncooked hot dogs and undercooked chicken (48). Hard evidence of large-scale involvement of commonly consumed meat and poultry products in human listeriosis is still lacking, with only a few sporadic cases reported worldwide directly linked to consumption of pork sausage (13, 18, 45), chicken (30, 31, 45), and turkey frankfurters (4, 45). However, between 1988 and 1993 two specialty meat products, namely, paté (22, 28, 38, 45) and jellied pork tongue (1, 2, 22, 28), were traced to widely publicized listeriosis outbreaks in England and France. Given *L. monocytogenes* contamination rates as high as 52, 80, 20, and 85% for ground beef, ground pork, ground turkey, and chicken, respectively (45), the lack of additional listeric infections linked to consumption of these products is surprising.

Regulatory protocols for isolating listeriae from food samples, such as the Food and Drug Administration and U.S. Department of Agriculture (USDA) Food Safety and Inspection Service methods, use one primary enrichment broth, and in the case of the USDA Food Safety and Inspection Service method, one secondary enrichment broth, followed by plating on *Listeria*-selective media. Although used as standard methods in the United States and elsewhere, these *Listeria* isolation procedures are not entirely effective, with about 30% of all *L. monocytogenes*-positive food samples escaping detection (25). However, when results from the USDA Food Safety and Inspection Service and Food and Drug Administration methods are combined, approximately 90% of all *Listeria*-positive samples are typically identified. These findings clearly demonstrate the importance of using more than one enrichment broth when attempting to recover *L. monocytogenes* from raw meat and poultry products. Possible reasons why some *Listeria*-positive

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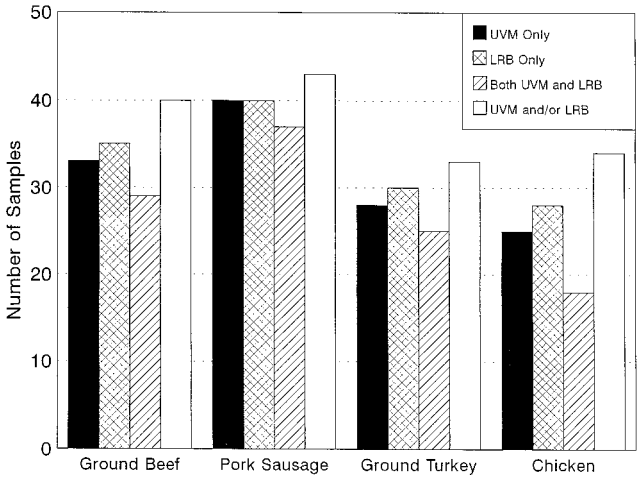

FIG. 1. Numbers of meat and poultry samples positive for *Listeria* spp. with UVM and LRB for primary enrichment.

samples go undetected include overgrowth by other *Listeria* spp. and/or natural background flora during enrichment and differing abilities of *Listeria* strains to grow competitively. To answer such strain-related questions, the diversity of specific *Listeria* strains in the sample must first be determined. While many strain-specific genotypic typing methods, including multilocus enzyme electrophoresis (5), restriction fragment length polymorphism analysis (23, 27), restriction enzyme analysis (40), and pulsed-field gel electrophoresis (8), can characterize listeriae beyond the species level, a newly developed automated ribotyping technique which defines bacterial strains in accordance with their rRNA patterns was our method of choice for reasons of sensitivity, specificity, rapid throughput, ease of operation, and reproducibility of results (10, 26).

In this study, naturally contaminated, raw refrigerated samples of ground beef, pork sausage, ground turkey, and chicken were analyzed in parallel for listeriae by using two different primary enrichment broths to (i) determine the overall incidence of *Listeria* spp. in the products tested, (ii) identify the incidence of multiple *Listeria* ribotypes (RTs) in selected *Listeria*-positive samples, (iii) examine the diversity of *Listeria* RTs recovered, and (iv) assess the ability of the two primary enrichment broths to recover different *Listeria* RTs, with particular emphasis on *L. monocytogenes* RTs linked to previous cases of food-borne listeriosis.

MATERIALS AND METHODS

Products tested. Forty-five retail size packages each of raw ground beef, pork sausage, ground turkey, and chicken were purchased from five area stores over a 5-week period (nine packages of each product per week). All meat and poultry products were stored overnight at 4°C and tested before the printed "sell by" date on the package.

Media. The following two primary enrichment broths were used to examine all meat and poultry samples: the University of Vermont-modified *Listeria* enrichment broth (UVM; Difco Laboratories, Detroit, Mich.) recommended by the USDA Food Safety and Inspection Service (36) and the *Listeria* repair broth (LRB) developed by Busch and Donnelly (12) and used very successfully by others (19, 43), with slight modification. LRB was prepared by completely dissolving 0.30 g of ferrous sulfate (Sigma Chemical Company, St. Louis, Mo.) in 1 liter of deionized, distilled water. Thereafter, 30.0 g of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), 5.0 g of glucose (Sigma), 6.0 g of yeast extract (Difco), 8.5 g of 3-*N*-morpholinepropanesulfonic acid (MOPS)-free acid (Sigma), and 13.7 g of MOPS sodium salt (Sigma) were added, completely dissolved, and followed by 2.46 g of magnesium sulfate (Sigma). The medium was brought to a boil and removed from the heat source, and 10.0 g of Na pyruvate (Sigma) was slowly added. LRB was dispensed into bottles in 225-ml aliquots and autoclaved at 121°C for 15 min. After inoculation of LRB, an initial 3-h period of nonselective incubation at 30°C (19) rather than 37°C (12) was used for repair of any potentially stressed or injured *Listeria* cells. Thereafter, the following filter-sterilized selective agents (Sigma) were added: 0.675 ml of 0.5% acriflavine (aqueous), 1.125 ml of 1% cycloheximide (ethanol-water ratio, 40:60), and 1.80 ml of 0.5% nalidixic acid (aqueous). All samples were then reincubated at 30°C as recommended by the USDA for meat and poultry products. Fraser broth (Difco) was used for secondary enrichment. Selective and nonselective plating media for isolating and purifying listeriae included modified Oxford agar (Difco) and brain heart infusion agar (Difco), respectively.

***Listeria* enrichment and isolation.** Paired samples of ground beef, pork sausage, ground turkey, and chicken weighing 25 g were inoculated into 225 ml each of UVM and LRB, homogenized for 2 min in a Lab-Tek 400 Stomacher (Tekmar, Cincinnati, Ohio), and incubated at 30°C. After a total incubation period of 22 to 26 h at 30°C, 0.1 ml of each UVM and LRB primary enrichment was inoculated into a separate tube containing 10 ml of Fraser broth. Following 24 and 40 h of incubation at 35°C, all secondary enrichments, regardless of color change due to esculin hydrolysis, were streaked onto modified Oxford agar plates. All plates were incubated at 35°C for 24 h, after which two presumptive *Listeria* isolates per sample for each primary enrichment medium were streaked onto brain heart infusion agar plates for purification and incubated at 35°C for 24 h. An additional eight presumptive UVM and LRB *Listeria* isolates per primary enrichment medium from five samples of each of the four products were also streaked onto plates of brain heart infusion agar and similarly incubated.

Identification of *Listeria* spp. Presumptive *Listeria* isolates were confirmed and identified to the species level on the basis of Gram stain, catalase reaction, typical umbrella motility, and fermentation of mannitol, rhamnose, and xylose. A modified CAMP test (37) for enhanced hemolysis in the presence of *Staphylococcus aureus* alone also was performed on each isolate.

Strain selection for RT analysis. A total of 608 *Listeria* strains were selected from the entire pool of samples for ribotyping to obtain (i) two UVM and two LRB strains from 15 paired ground beef, pork sausage, ground turkey, and chicken samples when both UVM and LRB were positive for listeriae (240 strains); (ii) eight additional UVM and LRB strains from 5 of the previous 15 paired ground beef, pork sausage, ground turkey, and chicken samples when both UVM and LRB were positive for listeriae (320 strains); and (iii) two UVM or two LRB strains from 2 to 4 ground beef, pork sausage, ground turkey, and chicken samples when either UVM or LRB was positive for listeriae (48 strains). Within the confines of this selection scheme, emphasis was given to analyzing as many samples positive for *L. monocytogenes* as possible.

RT analysis. The aforementioned 608 *Listeria* strains were ribotyped by using the automated Riboprinter microbial characterization system, alpha version, developed by E. I. du Pont de Nemours & Co., Inc. (Wilmington, Del.) (10, 26). This six-stage, largely automated process used to identify selected *Listeria* strains to the species level and further characterize them is based on the simultaneous separation and transfer of *Eco*RI DNA restriction fragments followed by hybridization with a chemiluminescently labeled DNA probe from *Escherichia coli* encoding rRNA.

RESULTS

***Listeria* incidence.** Combined results obtained with UVM and/or LRB indicated that 40 (89%), 43 (96%), 33 (76%), and

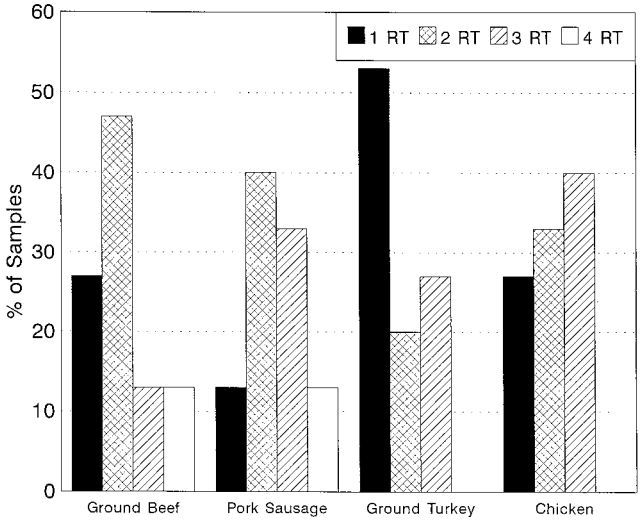

FIG. 2. Percentages of meat and poultry samples from which one, two, three, or four *Listeria* RTs were recovered.

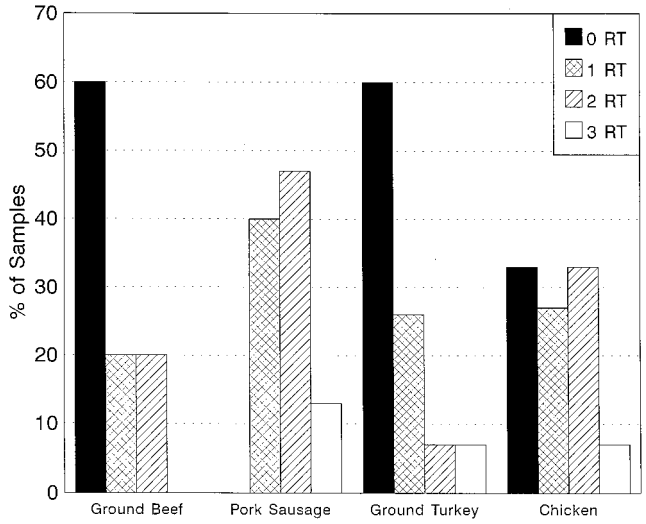

FIG. 3. Percentages of meat and poultry samples from which zero, one, two, or three *L. monocytogenes* RTs were recovered.

34 (73%) of 45 ground beef, pork sausage, ground turkey, and chicken samples, respectively, were positive for listeriae (Fig. 1). However, samples positive for *Listeria* with UVM were occasionally negative with LRB and vice versa, with consistently fewer samples yielding listeriae following both primary enrichment procedures. This observation was most evident for the 45 chicken samples with only half of the positive samples detected with both UVM and LRB. Overall, 125 (69.4%) of 180 UVM-enriched and 133 (73.8%) of 180 LRB-enriched samples yielded listeriae, with neither medium exhibiting statistical superiority ($P > 0.05$) with McNemar's chi-square test.

Incidence of multiple *Listeria* RTs. Analysis of two UVM and two LRB *Listeria* isolates from 15 samples of each product showed that raw meat and poultry frequently contained multiple strains of listeriae (Fig. 2) with 46 and 26% of pork sausage and ground beef samples, respectively, harboring three or four different *Listeria* RTs. In contrast, *Listeria* populations in ground turkey and chicken were somewhat less diverse, with neither product harboring four different RTs and over half of all ground turkey samples confined to a single RT. However, 40% of the chicken samples contained three different *Listeria* RTs—the highest percentage of any of the four products examined. While much of the RT diversity observed in all four products was attributable to multiple *Listeria* spp. and multiple *L. innocua* or *L. welshimeri* RTs in the same sample (data not shown), up to three different RTs of *L. monocytogenes* were detected in 15 samples of pork sausage, ground turkey, and chicken (when two UVM and two LRB isolates from each of 15 samples per product were tested) (Fig. 3), with 7 to 47% of all *Listeria*-positive samples harboring two different *L. monocytogenes* RTs. In addition, all 15 pork sausage samples selected for analysis yielded at least one RT of *L. monocytogenes*, whereas 60% of ground beef and turkey samples were negative for *L. monocytogenes.*

Diversity of specific *Listeria* sp. RTs. A total of 608 *Listeria* colonies from selected positive samples were biochemically confirmed as *L. monocytogenes* (245 isolates), *L. innocua* (276 isolates), and *L. welshimeri* (89 isolates) and then ribotyped. Thirty-six *Listeria* RTs comprising 16 of 47, 12 of 20, and 8 of 8 *L. monocytogenes*, *L. innocua*, and *L. welshimeri* strains recognized in the Riboprinter microbial characterization system database, respectively, were identified among the 21 samples of each product when two UVM and two LRB isolates were ribotyped per sample (Table 1). Pork sausage contained the highest number of *L. monocytogenes* RTs (12 RTs), followed by chicken (11 RTs), ground turkey (9 RTs), and ground beef (8 RTs). Four of the 16 *L. monocytogenes* RTs detected, namely, RTs 19071, 19231, 19161, and 19193, were clinical RTs previously associated with sporadic and/or epidemic cases of food-borne listeriosis (20). All four of these clinical RTs were detected in pork sausage, with ground beef, chicken, and ground turkey harboring 3, 3, and 2 clinical RTs, respectively. While 8 of 21 chicken samples contained RT 19161, the incidence and distribution of the two remaining clinical *L. monocytogenes* RTs in ground beef, pork sausage, ground turkey, and chicken were far lower and markedly scattered. Additional *Listeria* spp. detected in these meat and poultry products included *L. innocua* (12 RTs) and *L. welshimeri* (8 RTs). Among these RTs, *L. innocua* RT 19094 was particularly common and predominated in chicken, with 8 (38%) of 21 samples positive. Nine of 12 *L. innocua* and 6 of 8 *L. welshimeri* RTs were confined to three or fewer products.

Recovery of different *Listeria* RTs with UVM and LRB. Considerable variation between the numbers of *Listeria* RTs recovered with the two primary enrichment media was observed.

TABLE 1. Incidence of *Listeria* RTs in meat and poultry products[a]

Listeria sp.	RT	No. (%) of positive samples			
		Ground beef	Pork sausage	Ground turkey	Chicken
L. monocytogenes	19071[b]	4 (19)	10 (48)	1 (5)	2 (9)
	19231[b]	1 (5)	3 (14)	1 (5)	3 (14)
	19161[b]	1 (5)	1 (5)		8 (38)
	19193[b]		2 (9)		
	54132	1 (5)	3 (14)	1 (5)	1 (5)
	54081	4 (19)	4 (19)	1 (5)	1 (5)
	54183	1 (5)	2 (9)	1 (5)	1 (5)
	28623	1 (5)	1 (5)		1 (5)
	54184	1 (5)	1 (5)		
	28647		1 (5)		1 (5)
	19192			2 (9)	1 (5)
	19157			1 (5)	1 (5)
	54084			2 (9)	2 (9)
	19226		1 (5)		
	54135		1 (5)		
	19236			1 (5)	
L. innocua	19094	4 (19)	6 (29)	7 (33)	8 (38)
	19073	4 (19)	3 (14)	2 (9)	2 (9)
	19093	1 (5)	1 (5)	7 (33)	2 (9)
	19107	1 (5)		1 (5)	1 (5)
	19166	2 (9)		1 (5)	
	19224		1 (5)	2 (9)	
	54154			2 (9)	2 (9)
	19181	3 (14)			
	19196	2 (9)			
	54152	1 (5)			
	19188		1 (5)		
	54261				1 (5)
L. welshimeri	28643	3 (14)	3 (14)	1 (5)	1 (5)
	28622	3 (14)	3 (14)	1 (5)	1 (5)
	19098	1 (5)	1 (5)		
	19213	3 (14)		1 (5)	
	54185	4 (19)			
	19214	3 (14)			
	19226		2 (9)		
	19095			1 (5)	

[a] Twenty-one samples of each product type were tested.
[b] Clinical RT recognized in the World Health Organization typing study.

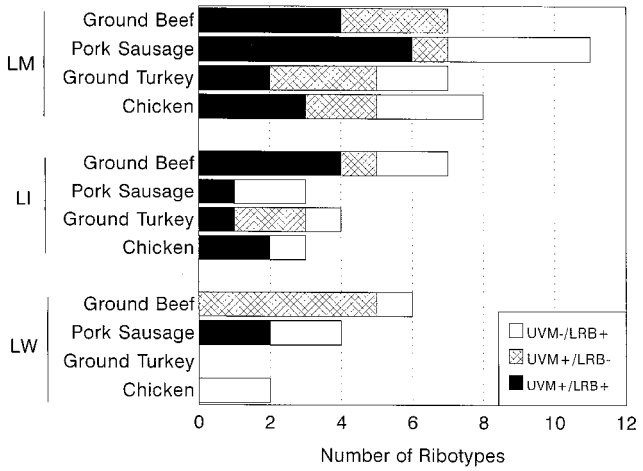

FIG. 4. Numbers of *L. monocytogenes* (LM), *L. innocua* (LI), and *L. welshimeri* (LW) RTs detected with UVM and LRB.

While 26 of 36 *Listeria* RTs (including 13 of 16 *L. monocytogenes* RTs) were detected with both UVM and LRB, 3 (including 1 *L. monocytogenes* RT) and 7 (including 3 *L. monocytogenes* RTs) of 36 *Listeria* RTs were observed only with UVM and LRB, respectively (results not shown). When two UVM and two LRB *Listeria* isolates from each of 15 ground beef samples were examined (Fig. 4), 3 additional *L. monocytogenes* RTs detected with UVM were not isolated with LRB and 7 and 10 non-*L. monocytogenes* RTs were recovered with LRB and UVM, respectively. On the basis of these findings, UVM would be preferred for testing of ground beef. UVM was also superior to LRB for ground turkey, with UVM detecting three more *L. monocytogenes* RTs (including one clinical RT) compared with two nonclinical RTs for LRB. Two additional *L. innocua* RTs also were recovered with UVM as the sole primary enrichment broth. In contrast, LRB was superior to UVM for recovering *L. monocytogenes* from both pork sausage and chicken. With LRB, four more *L. monocytogenes* RTs were detected in pork sausage compared with only one additional RT with UVM. Furthermore, none of the non-*L. monocytogenes* RTs were missed with LRB. For the 15 chicken samples examined, LRB detected three more *L. monocytogenes* RTs (including one clinical RT), compared with two additional nonclinical RTs for UVM. Three additional non-*L. monocytogenes* RTs (including the only two RTs of *L. welshimeri*) were also observed with LRB.

Detection of the various *Listeria* RTs present in any sample depends on both the method of analysis, as shown by our data, and the number of isolates examined. In response to the latter, eight additional UVM and LRB *Listeria* isolates from five samples of each product were ribotyped, giving a total of 10 UVM and 10 LRB isolates from each of five samples for the four products tested. In ground beef (Table 2), UVM detected three different *L. monocytogenes* RTs in sample B, compared with a single *L. monocytogenes* RT (RT 54184) with LRB, with *L. monocytogenes* clinical RT 19071 going undetected in sample D with UVM. While comparable numbers of *L. monocytogenes* RTs were recovered from most pork sausage samples with UVM and LRB (Table 3), only one isolate of *L. monocytogenes* clinical RT 19071 was detected in sample A with UVM. Furthermore, *L. monocytogenes* clinical RT 19231 was missed in sample B by LRB and in sample D by UVM, with *L. monocytogenes* RTs 54081 and 54135 also going undetected in samples C and D with UVM and LRB, respectively. No appreciable difference in the distribution of *L. monocytogenes* RTs from ground turkey was observed when either UVM or LRB was used for primary enrichment (Table 4). However, sample A was only positive for *L. monocytogenes* with UVM (two different RTs were detected), whereas one additional *L. monocytogenes* RT not found in sample D with UVM was detected with LRB. Thirty and 21 *L. monocytogenes* isolates were recovered from raw chicken with UVM and LRB, respectively (Table 5). *L. monocytogenes* RT 19192 was not detected in chicken sample A with UVM, and *L. monocytogenes* RT 54132 was not recovered from sample C with LRB. Additionally, *L. welshimeri* was only detected with LRB.

Two *Listeria* isolates from each of six samples of the four products tested in which either UVM or LRB was negative for listeriae also were ribotyped (Table 6). LRB missed *L. monocytogenes* in one of three ground beef samples containing clinical RT 19071 and all three chicken samples, two of which harbored *L. monocytogenes* clinical RT 19161. Interestingly, *L. innocua* RT 19094 was recovered primarily with UVM, whereas *L. innocua* RTs 19073 and 19093 were detected only with LRB. Similarly, samples containing *L. welshimeri* RT

TABLE 2. Distribution of *Listeria* RTs recovered from ground beef samples with UVM and LRB[a]

Listeria sp.	RT	No. of isolates identified in UVM/LRB from sample:				
		A	B	C	D	E
L. monocytogenes	54184		1/8			
	28623		4/0			
	54081		5/0			
	19071[b]				0/2	
L. innocua	19196	2/0				0/1
	19094				8/8	10/4
	19073				1/0	0/4
	19163					0/1
L. welshimeri	19214	3/0	0/1	1/0		
	54185	3/0		9/0	1/0	
	19098	1/0				
	19213	1/10				
	28622		0/1			
	28643			0/10		

[a] Ten isolates per sample were tested.
[b] Clinical RT recognized in the World Health Organization typing study.

TABLE 3. Distribution of *Listeria* RTs recovered from pork sausage samples with UVM and LRB[a]

Listeria sp.	RT	No. of isolates identified in UVM/LRB from sample:				
		A	B	C	D	E
L. monocytogenes	19071[b]	1/8	8/7	7/8	8/7	2/2
	19231[b]		1/0		0/1	
	19161[b]				1/2	
	54135[b]				1/0	
	54081	9/2		0/1		
	54132			3/1		
L. innocua	19073		0/2			
	19094					0/2
L. welshimeri	19226					7/5
	28643		1/1			0/1
	28642					1/0

[a] Ten isolates per sample were tested.
[b] Clinical RT recognized in the World Health Organization typing study.

TABLE 4. Distribution of *Listeria* RTs recovered from ground turkey samples with UVM and LRB[a]

Listeria sp.	RT	No. of isolates recovered in UVM/LRB from sample:				
		A	B	C	D	E
L. monocytogenes	19192	5/0				
	54081	1/0				
	54084				2/6	
	54132				0/1	
L. innocua	19094	1/10		1/0		
	19073	2/0				
	19166	1/0				
	19093		10/10	9/10		
	54154				8/3	10/10

[a] Ten isolates per sample were tested.

28643 were identified only with LRB, whereas *L. welshimeri* RTs 28622 and 19095 were detected only with UVM. Except for two *L. welshimeri* ground turkey isolates from sample 1, all remaining *Listeria* isolates recovered from the same sample belonged to the same RT. These findings reinforce the importance of using two different primary enrichment media and picking a sufficient number of colonies when attempting to isolate specific *Listeria* strains from naturally contaminated raw meat and poultry.

DISCUSSION

Widespread contamination of raw meat and poultry products with *L. monocytogenes* and other *Listeria* spp. has been extensively documented in the United States and elsewhere (6, 17, 21, 29, 35, 45, 47, 49) with 75 to 98% of our samples also harboring listeriae. However, very few *Listeria* isolates from raw meat and poultry products have been subtyped by serology or other means. In limited work by McLauchlin and Shah (39), 4 (31%) of 13 raw meat samples yielded multiple serotypes of *L. monocytogenes*, with up to three different serotypes detected in one sample. Raw meat harbored the widest range of *L. monocytogenes* serotypes when compared with 593 other samples of cooked or ready-to-eat meat, poultry, dairy, and confectionery products, and similar observations were made by Hayes et al. (25). Nonetheless, given the limited number of *Listeria* serotypes possible and the fact that most North American and European *L. monocytogenes* strains recovered from raw meat and poultry products belong to serotypes 1/2 and 4b, with the former predominating (3, 14, 18, 25, 29), serotyping lacks the inherent specificity required for full characterization of listeriae.

Complete characterization of all of the *Listeria* strains in a sample requires that (i) all healthy and injured *Listeria* strains grow to detectable numbers and (ii) all *Listeria* isolates be differentiated from each other by strain-specific typing techniques. According to Hayes et al. (25), analysis of 899 refrigerated foods by three different methods yielded 121 samples positive for *L. monocytogenes*, of which 42 samples were categorized as beef, pork, or poultry. The Food and Drug Administration, USDA, and Netherlands Government Food Inspection Service methods individually detected 26 (62%), 32 (76%), and 36 (86%) of the 42 positive meat and poultry samples, respectively. Although differences between these three methods were not statistically significant, combining results from the USDA Food and Drug Administration and USDA-Netherlands Government Food Inspection Service methods yielded significantly higher *L. monocytogenes* recovery rates, with 90 to 98% of all such samples being identified. The USDA and Netherlands Government Food Inspection Service methods also detected 8 and 9 of 10 positive ground beef samples (10 of 10 samples when combined), respectively, with multiple serotypes of *L. monocytogenes* observed in two samples. These findings and the results of our study strongly support the use of at least two primary enrichment media for near-optimal recovery of *L. monocytogenes* from naturally contaminated raw meat and poultry samples.

Many subtyping techniques, including ribotyping, restriction enzyme analysis, pulsed-field gel electrophoresis, multilocus enzyme electrophoresis, and phage typing, have been used to characterize listeriae. Compared with the aforementioned strain-typing strategies, the fully automated and user-friendly Riboprinter microbial characterization system offers 100% typeability and reproducibility and moderate discriminatory power, with *L. monocytogenes* and the genus *Listeria* presently divided into 47 and 90 unique RTs, respectively. However, as is true for the numerous *Listeria* isolation methods available, no single strain-specific typing method offers 100% typeability and reproducibility coupled with high discriminatory power, technical ease, and low cost, with a combination of genotypic and/or phenotypic typing methods invariably proving more effective.

RT analysis confirmed that raw meat and poultry products frequently harbor many *L. monocytogenes*, *L. innocua*, and/or *L. welshimeri* strains which cannot be easily differentiated without using strain-specific typing techniques. By using random amplification of polymorphic DNA, Lawrence and Gilmour (32) recently identified seven different types among 93 *L. monocytogenes* isolates from raw poultry products. By comparison, we identified 11 *L. monocytogenes* RTs in raw chicken and 9 RTs in ground turkey. These observations, along with the presence of up to four *L. monocytogenes* and five *Listeria* RTs in one sample (Tables 2 to 5), suggest multiple sources of contamination and/or heavily contaminated individual sites within the processing environment.

The ability of UVM and LRB to recover different *Listeria* RTs from the same sample when 10 isolates per sample were examined (Tables 2 to 5) was confirmed with automated RT analysis. Although Flanders et al. (19) reported similar *Listeria* recovery rates when naturally contaminated environmental samples were enriched at 30°C in both UVM and LRB, some UVM^- LRB^+ and UVM^+ LRB^- samples were observed. Thus, LRB likely allowed repair of potentially injured listeriae while greater selectivity of UVM increased discrimination between low levels of listeriae and the natural background flora. In our study, disparities between the number of isolates of any

TABLE 5. Distribution of *Listeria* RTs recovered from chicken samples with UVM and LRB[a]

Listeria sp.	RT	No. of isolates identified in UVM/LRB from sample:				
		A	B	C	D	E
L. monocytogenes	19161[b]	10/2				
	19192	0/1				
	54084		10/8	8/8		
	54132			2/0		
	19231			0/2		
L. innocua	19094	0/7	0/2		10/10	10/0
L. welshimeri	28622					0/9
	28623					0/1

[a] Ten isolates per sample were tested.
[b] Clinical RT recognized in the World Health Organization typing study.

TABLE 6. Variable recovery of *L. monocytogenes*, *L. innocua*, and *L. welshimeri* from meat and poultry products with UVM and LRB

Sample no.	Species[a] and RT							
	Ground beef		Pork sausage		Ground turkey		Chicken	
	UVM	LRB	UVM	LRB	UVM	LRB	UVM	LRB
1	LM 19071[b]		LI 19094		LW 19095		LM 19161[b]	
	LM 19071[b]		LI 19094		LW 28622		LM 19161[b]	
2	LI 19094		LI 19094		LI 19094		LM 19161[b]	
	LI 19094		LI 19094		LI 19094		LM 19161[b]	
3	LW 19213		LW 28622			LW 19213	LM 28623	
	LW 19213		LW 28622			LW 19213	LM 28623	
4		LW 28643		LI 19093		LI 19093		LI 19073
		LW 28643		LI 19093		LI 19093		LI 19073
5		LW 19213		LW 28643		LI 19094		LI 19093
		LW 19213		LW 28643		LI 19094		LI 19093
6		LI 19073		LM 54183		LW 28643		LM 19161[b]
		LI 19073		LM 54183		LW 28643		LM 19161[b]

[a] Abbreviations: LM, *L. monocytogenes*; LI, *L. innocua*; LW, *L. welshimeri.*
[b] Clinical RT recognized in the World Health Organization typing study.

particular RT identified in any one sample with UVM and LRB suggest possible differences in growth rates or ability of *L. monocytogenes* to compete with other *L. monocytogenes* RTs, other *Listeria* spp., and/or natural microflora (16). According to Petran and Swanson (41), *L. monocytogenes* and *L. innocua* attained similar populations in Trypticase soy broth with 0.6% yeast extract when grown competitively. However, when inoculated cheese sauce was enriched in both UVM and Fraser broth, *L. innocua* reportedly outgrew *L. monocytogenes* by 10- to 100-fold. Curiale and Lewus (15) analyzed samples of frozen beef broth and again found that *L. innocua* outgrew *L. monocytogenes* by a wide margin during enrichment in both UVM and Fraser broth. *L. monocytogenes* is also reportedly unable to compete with *L. innocua*, as well as *L. seeligeri* and *L. welshimeri*, in UVM (9). By using restriction enzyme analysis and pulsed-field gel electrophoresis, Loncarevic et al. (34) recently reported that direct plating was superior to the International Dairy Federation enrichment procedure for recovery of multiple *L. monocytogenes* strains from naturally contaminated sausage, salmon, and cheese. These findings reinforce the need for strain-specific typing of multiple *L. monocytogenes* isolates from the same sample during outbreak investigations.

All *L. monocytogenes* strains are pathogenic and a potential threat to human health; however, considerable heterogeneity in virulence levels has been reported for different serotypes and the two major groups of RTs (7). Human isolates recovered during listeriosis epidemics (44), as well as 40 to 85% of the strains responsible for sporadic cases (44), typically fall within serotype 4b, one major RT (24), and two major multilocus enzyme electrophoresis types (42). However, strains matching these characteristics are rarely isolated from meat and poultry (7). While our findings support this latter observation, with the *L. monocytogenes* RT responsible for major outbreaks in Nova Scotia, California, and Switzerland going undetected, four other clinical RTs included in the recent World Health Organization *Listeria* subtyping study (20) were identified in the meat and poultry products tested, including RT 19161, which was responsible for a large common-source outbreak involving paté (38). However, RT 19092, which was linked to one well-publicized case of listeriosis in Texas involving turkey frankfurters (4), was not observed.

Increasing evidence of the involvement of only a select few *L. monocytogenes* strains in major listeriosis outbreaks may eventually force a reevaluation of present *Listeria* isolation methods, with the eventual goal being the refinement of current isolation strategies to better detect these problem strains. Our findings suggest that the ability to recover specific *Listeria* RTs is at least partially dependent on the selectivity of the primary enrichment broth. Detection of certain *L. monocytogenes* clinical RTs that were apparently overgrown by other RTs of *L. monocytogenes* was sometimes only possible when 10 *L. monocytogenes* isolates were examined. Consequently, the current Centers for Disease Control and Prevention practice of subtyping a maximum of four *L. monocytogenes* isolates from the same food sample in outbreak investigations (24) may need to be reevaluated. Increased emphasis on strain-specific typing, coupled with additional surveys focusing on the incidence of the major epidemic strains of *L. monocytogenes*, is necessary to more accurately determine the relative public health risk of meat and poultry products in cases of human listeriosis.

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