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Comparison of Conventional Culture Method and Fluorescent In Situ Hybridization Technique for Detection of *Listeria* spp. in Ground Beef, Turkey, and Chicken Breast Fillets in İzmir, Turkey

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ABSTRACT

The occurrence of *Listeria* species in refrigerated fresh chicken breast fillet, turkey breast fillet, and ground beef was evaluated, comparing the conventional culture method and fluorescent in situ hybridization (FISH). FISH uses hybridization of a nucleic acid sequence target of a microorganism with a specific DNA probe labeled with a fluorochrome and imaging by a fluorescence microscope. First, *Listeria* was inoculated in chicken breast fillet, turkey breast fillet, or ground beef, and the applicability of the FISH method was evaluated. Second, *Listeria* was detected in fresh chicken breast fillet, turkey breast fillet, and ground beef by culture and FISH methods. *Listeria* was isolated from 27 (37.4%) of 216 samples by the standard culture method, whereas FISH detected 25 (24.7%) preenriched samples. Of these isolates, 17 (63%) were *L. innocua,* 6 (22%) *L. welshimeri,* and 4 (14.8%) *L. seeligeri.* Overall, the prevalences of *Listeria* spp. found with the conventional culture method in chicken breast fillet, turkey breast fillet, and ground beef were 9.7, 6.9, and 20.8%, whereas with the FISH technique these values were 11.1, 6.9, and 16.7%, respectively. The molecular FISH technique appears to be a cheap, sensitive, and time-efficient procedure that could be used for routine detection of *Listeria* spp. in meat. This study showed that retail raw meats are potentially contaminated with *Listeria* spp. and are, thus, vehicles for transmitting diseases caused by foodborne pathogens, underlining the need for increased precautions, such as implementation of hazard analysis and critical control points and consumer food safety education.

Listeria species are bacteria with high environmental adaptability, ubiquitous in animals and in vegetables. The genus *Listeria* comprises six species: *L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. ivanovii,* and *L. grayi (42). L. monocytogenes* is commonly associated with human listeriosis. The universal occurrence of *L. monocytogenes* in food and the risk of contracting foodborne *L. monocytogenes* listeriosis have been thoroughly reviewed *(53).* There has been a significant increase in human listeriosis cases in Europe since 2004 *(11, 13).* The incidence of listeriosis in the European Union in 2006 was three cases per million inhabitants per year *(18).* In Europe, the incidence is increasing, primarily *(27)* due to the increasing numbers of the elderly and immunocompromised (from chemotherapy or other therapies that inhibit the immune system) *(11).* Although dairy products have been implicated as sources of listeriosis outbreaks, *L. monocytogenes* has been isolated from animal and vegetable foodstuffs, both raw and recontaminated *(18).* The main sources and routes of contamination have not been fully described; however, this microorganism is a concern for the food industry because of its ability to colonize food contact surfaces and to survive or grow at low temperatures. The incidence of *L. monocytogenes* in processed poultry products varies between 20% *(28)* and 60% *(52).* Also, *L. monocytogenes* contamination at a wide range of levels has been observed in retail ground beef products. *L. monocytogenes* was present in 52% of 100 raw ground beef samples from retail markets in Canada *(9)* and in 3.5% of 512 ground beef samples from retail outlets in the state of Washington *(44).* Fantelli and Stephan *(20)* and Gudbjornsdottir et al. *(28)* investigated the prevalence of *L. monocytogenes* in minced beef and detected *L. monocytogenes* in 10.75, 15.6, and 12.2% of minced beef samples. In a farm to fork approach, it is necessary to assess the incidence of *L. monocytogenes* along the entire production chain and particularly at the primary production step in meat production systems, which could be a source of introduction of this pathogen into food plants *(11).*

Conventional detection methods for food pathogens involve culturing the organisms in selective media and identifying isolates according to their morphological, biochemical, and immunological characteristics, a process that is laborious and time-consuming. The food industry needs more rapid methods to detect the possible presence of pathogens in raw materials and finished products, to control the manufacturing process, and to monitor cleaning and

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TABLE 1. *Bacterial strains used in this study*[a]

Bacterial species	Strain designation	Origin
Non-*Listeria*		
Escherichia coli	ATCC 25922	Clinical isolate
E. coli	NCTC12900	Serotype O157:H7, nontoxigenic
Staphylococcus aureus	RSSK01009	Clinical isolate
Salmonella Typhimurium	CCM 5445	
Salmonella Enteritidis[b]	ATCC 13076	
Salmonella Typhimurium[b]	ATCC 51812	Human blood
Listeria spp.		
L. innocua Seeliger	NRRL-B 33314	Turkey/ham/cheese deli sticks (CA)
L. monocytogenes[b]	ATCC 19111	Poultry meat (UK), serotype 1
L. monocytogenes[b]	ATCC 19118	Chicken (UK)

[a] Strains were obtained from the culture collection of the Food Engineering Department, Izmir Institute of Technology, except as noted.
[b] Strains were purchased from MicroBioLogics, Inc.

hygiene practices. The fluorescent in situ hybridization (FISH) method, using a fluorescent labeled oligonucleotide probe designed from specific DNA and RNA sequences, has proven to be a useful tool to detect a specific microorganism in environmental *(41)* and clinical samples *(34),* and its application in food microbiology has been investigated *(8, 15, 16, 19, 23, 38, 54)*. In these studies, *Enterobacteriaceae,* lactic acid bacteria, *Listeria,* and *Salmonella* were detected by the FISH method in such foods as wine, cheese, pork, and water. FISH usually has four steps: sample fixation and permeabilization, hybridization of the target sequence and the fluorescent probe, stringency washes, and detection of the hybridized cells.

This study evaluated the use of the FISH method to detect *Listeria* spp., foodborne pathogens used as a food safety index in ground meat, in a food market in Izmir, Turkey. First, after the pathogen was inoculated in ground beef, turkey, and chicken breast fillets, the applicability of the FISH method was evaluated; second, the pathogen was detected in fresh ground beef, turkey, and chicken breast fillets sold in the market by standard culture methods and the FISH method.

MATERIALS AND METHODS

Reference bacterial strains. Bacterial strains used in this study are listed in Table 1. *Listeria* and non-*Listeria* reference bacterial strains (MicroBioLogics, Inc., St. Cloud, MN) were reconstituted and cultivated onto nonselective tryptic soy agar (TSA; Difco, BD, Sparks, MD) plates as recommended by the suppliers. After cultivation, selected colonies were isolated and subcultured before use in the experiments. These bacteria included *L. innocua* and *L. monocytogenes,* the *Listeria* species that occur most frequently in foodstuffs, stored at 2 to 8°C. Stock cultures were stored at −80°C (Thermo Forma -86 ULT freezer).

Meat samples. Samples of ground beef, turkey, and chicken breast (at least 200 g of ground beef and three fillets each of turkey and chicken) were purchased from local retail outlets in İzmir province. Samples were transported to the laboratory in a cold box filled with ice, and all samples were stored at 4°C after sampling until the analysis was conducted.

Experimentally contaminated samples. Meat samples (ground beef, turkey, and chicken breast fillets) that tested negative for *Listeria* spp. on preliminary analysis were experimentally contaminated with *Listeria* spp. Before artificial inoculation, preliminary analysis of the meat samples for *Listeria* detection (isolation and identification) was done according to the culture-based method. Samples were experimentally contaminated by spiking 25 g of product with 1 ml of the *Listeria* culture to obtain 10^4 to 10^8 cells per g of meat sample.

Naturally contaminated samples. From March 2009 to February 2010, 216 samples of the ground beef (72 samples from three markets [A, B, C] and three butchers [D, E, F]), turkey (72 samples from market A), and chicken breast fillets (72 samples from markets A, B, C) were purchased from local supermarkets and were analyzed using the standard culture and the FISH method. Eight visits were made to each market; on each visit, three chicken breast fillet samples were purchased. Samples were subjected to microbiological analysis within 2 h of collection.

Oligonucleotide probes. In the present study, 16S rRNA-targeted probes were applied for the in situ detection of all members of the genus *Listeria*. Probe sequences, as well as specificities that have been used in this study, are shown in Table 2. The probe EUB338, complementary to the region of 16S rRNA specific to the *Bacteria* domain, was used as a positive control to test the efficiency of hybridization. A nonsense probe, NonEub338 (which has a nucleotide sequence complementary to the nucleotide sequence of probe EUB338), was used as a control for nonspecific staining. Oligonucleotide probes used in this study were 5′-labeled with the fluorochromes fluorescein isothiocyanate. Cyanine (Cy3) was used to label the Lis-637 probe. The labeled probes were purified by high-performance liquid chromatography. All probes were obtained commercially (Thermo Fisher Scientific, Ulm, Germany).

Preparation of bacterial strains. Bacteria were grown in Luria-Bertoni broth at 30°C for 18 to 24 h. Broth cultures were washed three times by means of centrifugation (3,000 rpm for 15 min; Hettich Instruments) in 0.85% NaCl solution and were suspended in saline at a cell density of 10^9 cells per ml. Cells were further diluted in saline to achieve the desired cell density. Microbial culture densities (yielding from 10^3 to 10^8 CFU/ml) of suspensions were standardized using an eight-channel NanoDrop

TABLE 2. *16S rRNA–targeted oligonucleotide probes used for the FISH method in the study*

Probe	Full name[a]	Sequence (5′–3′)	Specificity	Position[b]	Reference
NONEUB338		ACTCCTACGGCAGGCAGC	—	—	*1*
UNIV1390	S-*-Univ-1390-a-A-18	GACGGGCGGTGTGTACAA	All known organisms	1407-1390	*36*
EUB338	S-D-Bact-0338-a-A-18	GCTGCCTCCCGTAGGAGT	*Eubacteria*	355-338	*1*
Lis-637		CACTCCAGTCTTCCAGTTTC	*Listeria* spp.[c]	658-637	*47*

[a] Details on oligonucleotide probes are available at oligonucleotide probe database probeBase *(36)*.
[b] Probe position according to the *E. coli* gene numbering. Second number, target sequence position of first base of each oligonucleotide.
[c] *Listeria* spp., except *L. grayi*.

(Thermo Fisher Scientific) spectrophotometer. At 550 nm, 0.5 McFarland Standard corresponds to 1.5×10^8 bacterial density. Final cell numbers of the culture suspensions were confirmed by determining viable cell counts on TSA (Difco, BD) plates. The bacterial suspensions prepared were used for the FISH technique and for the experimental contamination of the meat samples.

Inoculation of meat samples with *Listeria*. To activate the pathogens, they were streaked onto TSA medium; after 18 h at 37°C, organisms were collected by centrifugation at 5,000 rpm (Hettich Instruments) for 5 min at 4°C. Then, after the supernatant was discarded, the precipitate was resuspended in 10 ml of buffered peptone water, and the cell number was evaluated by determining viable cell counts using selective agar medium according to the methods described previously *(6)*. Each pathogen was further diluted in phosphate buffered saline (PBS; pH 7.0) to appropriate cell density. Then, the dilutions were used to prepare artificially inoculated ground beef, turkey, and chicken breast samples.

Samples of turkey and chicken breast (25 g) or of ground beef (10 g) were weighed in a sterile plastic bag and used as the artificial contamination test samples. A sterile bent glass rod was used to spread 0.1 ml of pure bacterial culture (1.5×10^8 to 6.0×10^8 CFU/ml) of *Listeria* spp. across the turkey and chicken breast (25 g) fillet surface, and the ground beef was mixed to obtain the appropriate concentrations (from 10^4 to 10^8 CFU/ml each) of *Listeria* spp. The inoculated samples were allowed to rest 20 min for the attachment of bacterial cells, after which the samples were immediately processed for preenrichment in 225 ml of medium. Noninoculated meat samples mixed with pure Fraser broth were used as the blank controls; these were found to be free of *Listeria* spp. by FISH as well as standard culture methods.

Enrichment procedures. Experimentally and naturally contaminated samples were subjected to the enrichment protocol recommended by EN ISO 11290-1 *(2)*. Briefly, 25 g of each sample was aseptically removed and combined with 225 ml of half Fraser broth (bioMérieux, Marcy-L'Etoile, France) in sterile stomacher bags and was pummeled for 2 min in a stomacher (Interscience, St. Nom, France). The samples were incubated for 24 and 48 h without shaking at 30°C. After 24 and 48 h of incubation, an inoculation loop was streaked onto PALCAM (Difco, BD) and Oxford agar (Oxoid, Hampshire, UK), both with selective supplement. After the incubation period, 1 ml of the sample was withdrawn from the enriched sample and processed for *Listeria* detection by both conventional culture and FISH method.

***Listeria* spp. detection by standard culture method.** The detection of *Listeria* in meat samples was done according to the ISO 11290 method *(2)*. A 25-g meat sample was homogenized in 225 ml of half Fraser broth (bioMérieux) and incubated at 30°C. After 24 h, a loopful of the broth was streaked onto PALCAM agar (Difco, BD), including PALCAM antimicrobic supplement (Difco, BD), and onto Oxford agar (Oxoid), including Oxford *Listeria* selective supplement (Merck, Darmstadt, Germany), followed by incubation for 48 h at 37°C. Three typical single colonies were streaked onto TSA supplemented with yeast extract (Oxoid), incubated at 35°C for 24 h, and submitted to biochemical identification (motility, catalase, xylose, rhamnose, hemolysis, and CAMP) to *Listeria* spp. Colonies were identified as *Listeria* using API-*Listeria* identification strips (bioMérieux).

FISH probe check and optimization of stringency. Bacterial cell hybridization was performed at different temperatures (50 to 53°C), with increasing concentrations of formamide (0 to 50%, vol/vol), and with different probe concentrations (2, 5, and 8 ng μl^{-1}), to determine the optimal conditions for the designed probe. Conditions for in situ hybridization of the probes used in this study were optimized with reference strains by gradually increasing the formamide concentration of the hybridization buffer (Table 3). The optimal formamide concentration is the highest concentration that still yields good signals with the target cells but allows the discrimination of nontarget cells *(47)*.

Sensitivity and specificity of FISH method on pure bacterial cultures. From overnight cultures of *L. monocytogenes* (4.5×10^8 CFU/ml), FISH assays using the Lis-637 probes were carried out on 1-μl volumes of inocula from the dilution series (containing from 10^0 to 10^6 CFU) to determine the assay's limit of detection. The specificity of the probes was evaluated using cultures of a range of *Listeria* and non-*Listeria* bacterial species frequently found on raw meat products (data not shown). The applicability of the FISH technique was tested against *E. coli, L. monocytogenes,* and *L. innocua*. *Listeria* species-specific probe Lis-637 was used to detect *Listeria* spp.; the Lis-637 probe is considered suitable for all *Listeria* spp., excluding *L. grayi*. This study did not use *L. grayi* to test the specificity of the probe;

TABLE 3. *Optimum hybridization conditions for different probes used in the study*

Probe	Hybridization temp (°C)	Formamide (%)	Washing temp (°C)	NaCl in washing buffer (mM)	Reference
NonEub338	37	30	37	180	*1*
Univ 1390	37	20	37	180	*39*
Eub338	37	30	37	180	*1*
Lis-637					*47*

TABLE 4. *Comparison of* Listeria *detection results obtained for 216 fresh chicken or turkey breast fillet or ground beef samples analyzed by conventional culture (ISO) and FISH methods*

Sample	Detection results, no. (%)			
	Chicken breast fillet ($n = 72$)	Turkey breast fillet ($n = 72$)	Ground beef ($n = 72$)	Total ($n = 216$)
Positive agreement (ISO+, FISH+)	7 (9.72)	5 (6.94)	12 (16.67)	24 (11.1)
Negative agreement (ISO−, FISH−)	64 (88.89)	67 (93.06)	57 (79.17)	188 (87.0)
Positive deviation (ISO−, FISH+)	1 (1.39)	0	0	1 (0.5)
Negative deviation (ISO+, FISH−)	0	0	3 (4.17)	3 (1.4)

however, *L. grayi* was not isolated in any of the standard culture method analyses to detect *Listeria* in chicken breast fillet, turkey breast fillet, and minced meat samples.

***Listeria* spp. detection by FISH method.** Bacterial cells from exponentially growing pure cultures or from samples before or after preenrichment (24 and 48 h) were harvested by centrifugation and resuspended in 0.5 ml of PBS (pH 7.0). Two aliquots (100 μl) of each cell suspension, adjusted to 10^4 to 10^6 cells cm^{-2}, were placed on 0.2-μm-pore-size polycarbonate filters (45-mm diameter; Isopore GTTP, Millipore, Germany) and gently vacuum filtered. Cells were fixed with an ethanol series of 70, 90, and 95% (vol/vol) (10 min at room temperature and air dried) or with paraformaldehyde. The hybridization was performed using the Cy3-labeled probe: 30 μl of hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl, pH 7, and 15% formamide) and 5 μl of probe (8 ng μl^{-1} final concentration) were added on each piece of filter. The hybridization was conducted in buffer (0.9 MNaCl, 0.01% sodium dodecyl sulfate, 0.01 M Tris-HCl, pH 7.2) for 1.5 h at 46°C, followed by a 10-min wash in prewarmed buffer (0.08 M NaCl, 0.01% sodium dodecyl sulfate, 0.01 M Tris-HCl, 0.05 M EDTA). The filters were kept at 50°C in the hybridization chamber overnight. After hybridization, the filters were transferred to a prewarmed vial containing 50 ml of washing buffer (0.9 M NaCl and 20 mM HCl-Tris, pH 7) and were incubated at 50°C for 20 min. The washing step was performed twice.

After hybridization, DAPI (4′,6′-diamidino-2-phenylindole) solution (1:10) was added to each filter section. After 30 min, they were washed twice with distilled water and, finally, air-dried. Autofluorescence of cells was also determined from the negative controls (20 sections of each sample). The preparations were examined by fluorescence microscope.

The FISH method was used to analyze samples of ground beef, turkey, and chicken breast fillets that had been experimentally contaminated with different concentrations of *Listeria* and also samples that had not been experimentally contaminated. The 16S rRNA FISH method using Lis-637 was used as a rapid method to detect *Listeria* in chicken or turkey breast fillet or ground beef samples; for comparison, analyses were performed in parallel following the standard culture method. To assess the matrix effect on the performance of the FISH technique, a calibration curve was constructed by adding *Listeria* suspensions (from 10^4 to 10^8 CFU/ml) to samples of ground beef, turkey, and chicken breast fillets having no endogenous *Listeria* spp. To assess the effect of the enrichment step applied before the FISH method, 25-g portions of experimentally and naturally contaminated ground beef, turkey, and chicken breast fillets were also aseptically removed, trimmed, and preenriched in Fraser broth. Aliquots (1 ml) from each of the overnight preenrichment samples were centrifuged and washed twice in 1 ml of sterile triple distilled water before being resuspended in 100 μl of water. Volumes (1 μl) of the washed cell suspensions were analyzed by FISH, using the method previously described.

Fluorescence microscopy. Samples in the wells of slides were examined by fluorescence microscope after addition of 5 μl of anti-fade mounting medium and a cover slide. To obtain the images, B, G, and UV excitation filters were used, depending on the wavelength of the probe. Images were obtained by using ProgRes (Jenoptik, Jena, Germany) digital camera and analyzed with Image-Pro Plus image processing and analysis software (version 6.0, Media Cybernetics, Bethesda, MD). In at least 20 randomly selected fields for each microorganism culture inoculated and for each purchased sample, cell counts were determined and average values were calculated.

Statistical analysis. The positive (false-positive results) and negative deviations (false-negative results) obtained by both methods were determined. Relative sensitivity and specificity of the FISH method was also determined (Table 4). The agreement between positive and negative results obtained by the methods and the relative accuracy, specificity, and sensitivity of the FISH method relative to the ISO standard culture method were determined by chi-square analysis (Table 5).

RESULTS

All *Listeria* cultures showed a positive hybridization signal with *Listeria*-specific Lis-637 probe (Figs. 1, 2A, 2C, 3B, and 3C). All strains were able to hybridize with Eub338 (Figs. 1, 2B, and 2D), and none hybridized with Non338. Gram-negative *E. coli* and *Salmonella,* members of phylum Proteobacteria and family *Enterobacteriaceae,* and gram-positive *S. aureus,* a member of phylum Firmicutes and family *Staphylococcaceae,* as representatives of different phyla and families were used in this study. Proteobacteria (which includes *Enterobacteriaceae*) and Firmicutes are dominating phyla in meat food products originating from the animal (intestinal and skin flora) microbiota living in a symbiotic relationship. Also, all members of the

TABLE 5. *Statistical evaluation results of FISH method for 216 fresh chicken or turkey breast fillet or ground beef samples according to conventional culture (ISO) method*[a]

Parameter	Results
No. of samples	216
No. of positive results	27
No. of negative results	189
Relative accuracy (%)	98.15
Relative specificity (%)	99.47
Relative sensitivity (%)	88.89
χ^2	1.733

[a] Tabular value for df = 2 is equal to 5.991 ($P < 0.05$).

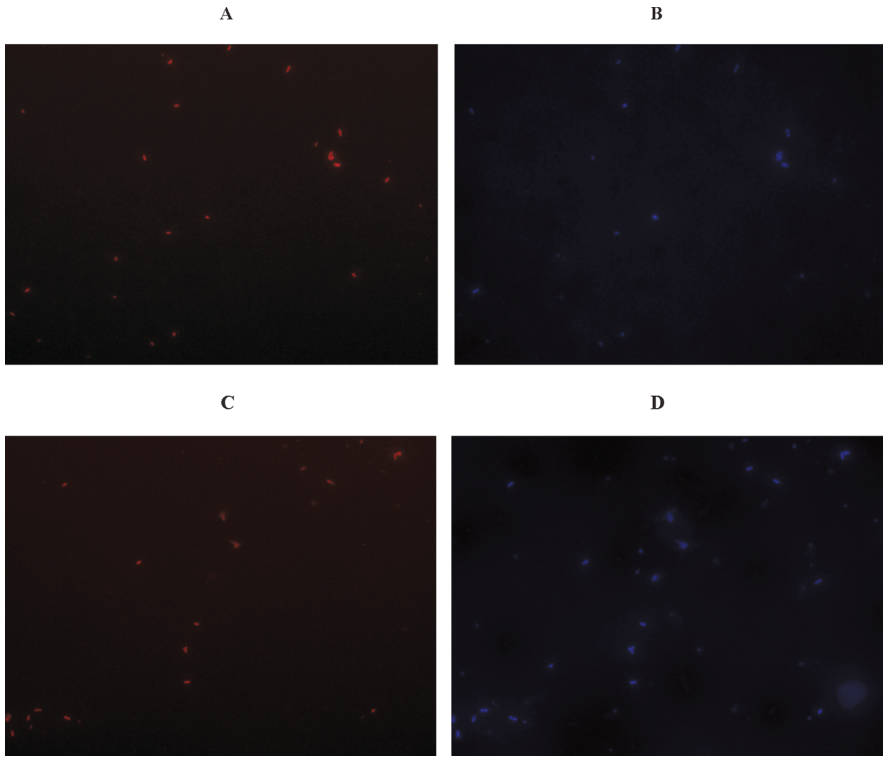

FIGURE 1. *Fluorescence microscope images of ethanol-fixed* L. innocua *([A] Cy3, [B] DAPI) and* L. monocytogenes *([C] Cy3, [D] DAPI) cells at* 10^4 *inoculum level.*

Proteobacteria phylum are gram negative, including *E. coli* and *Salmonella,* which is defined primarily in terms of ribosomal RNA (rRNA) sequences. Therefore, to show whether or not there would be an interaction with the rRNA probe used for the detection of *Listeria* spp., that is, for probe specificity in the study, *E. coli* and *Salmonella* were also used. No hybridization and no cross-reaction were observed for the non-*Listeria* bacterial strains used. FISH was performed for all fresh samples and preenriched samples, to evaluate the influence of this step in the FISH performance.

From the 216 analyzed samples, *Listeria* was isolated from 27 (37.4%) by the standard culture method, whereas FISH detected 25 (24.7%) preenriched samples (Fig. 4). Of these isolates, 17 (63%) were *L. innocua,* 6 (22%) *L. welshimeri,* and 4 (14.8%) *L. seeligeri.*

Overall, the conventional culture method found *Listeria* spp. prevalences of 9.7, 6.9, and 20.8% in chicken, turkey

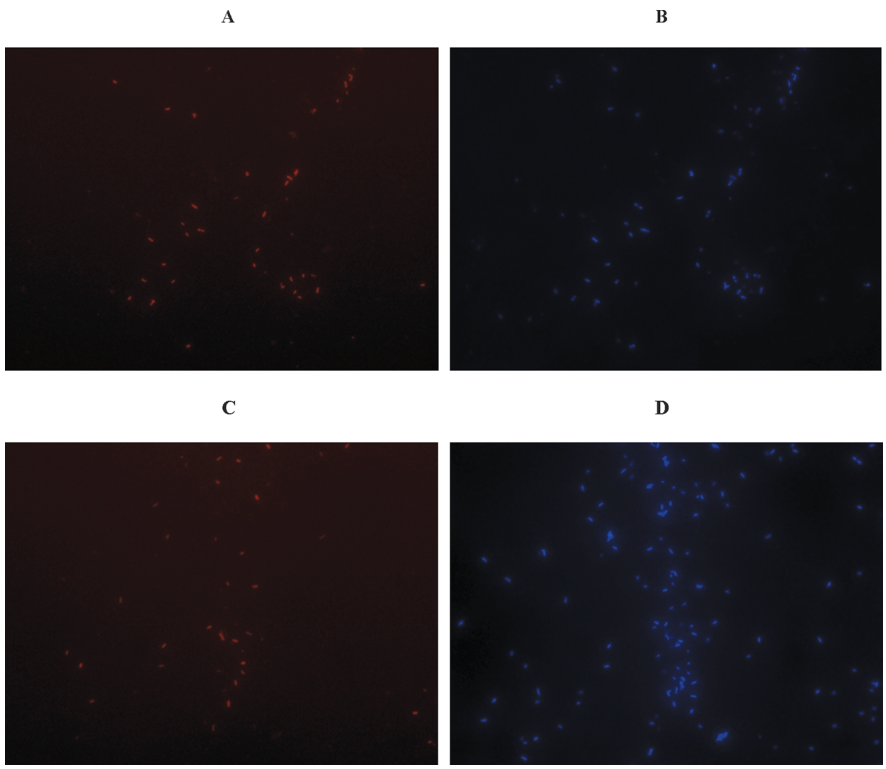

FIGURE 2. *Fluorescence microscope images of ethanol- and paraformaldehyde-fixed* L. innocua *([A] Cy3, [B] DAPI) and* L. monocytogenes *([C] Cy3, [D] DAPI) cells at* 10^8 *inoculum level.*

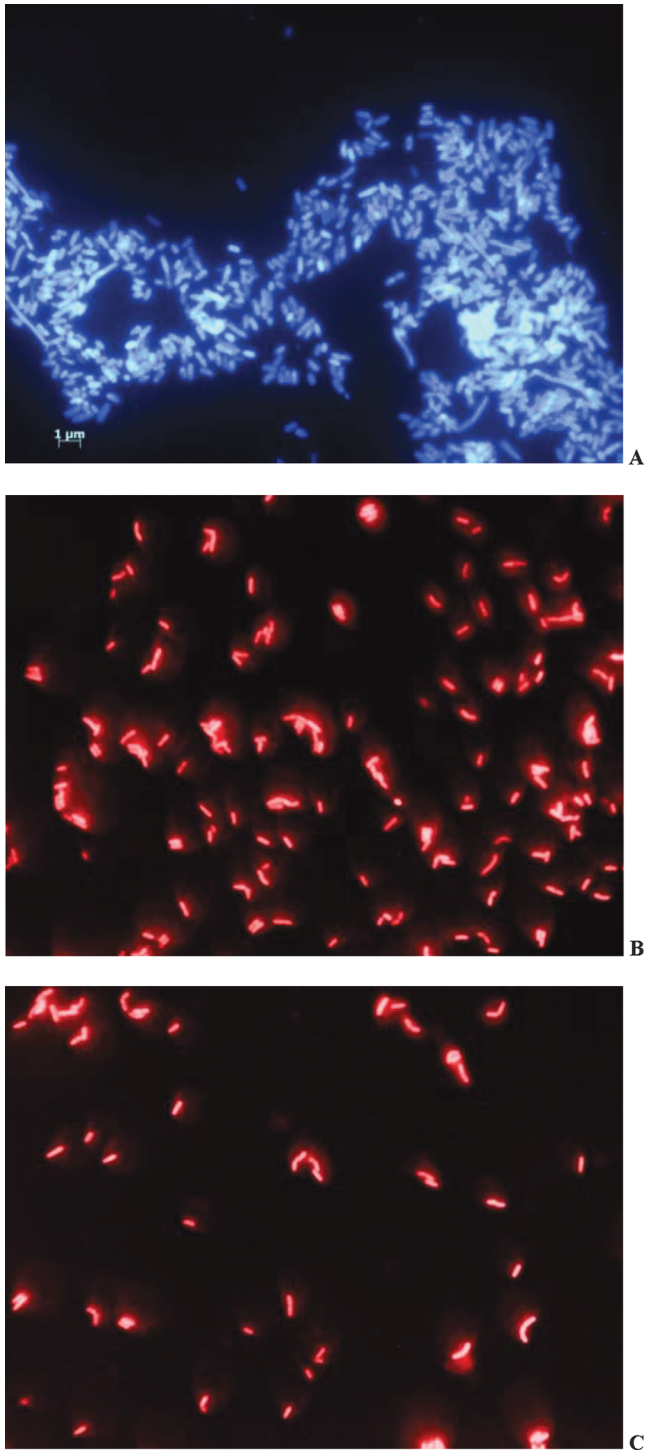

FIGURE 3. *Fluorescence microscope images of the total bacteria ([A] DAPI, blue) and* Listeria *([B]), [C] Cy3, red) detected by the FISH method using specific probes.*

breast fillet, and ground beef, respectively, whereas the FISH technique found prevalences of 11.1, 6.9, and 16.7%, respectively ($P < 0.05$). Detection of *Listeria* cells by the FISH method was possible after 1 or 2 days of sample enrichment.

Table 4 compares the conventional culture and FISH methods for the detection of *Listeria* species in a total of 216 samples of ground beef, chicken, and turkey breast fillets. This study has shown that the detection of *Listeria* in turkey

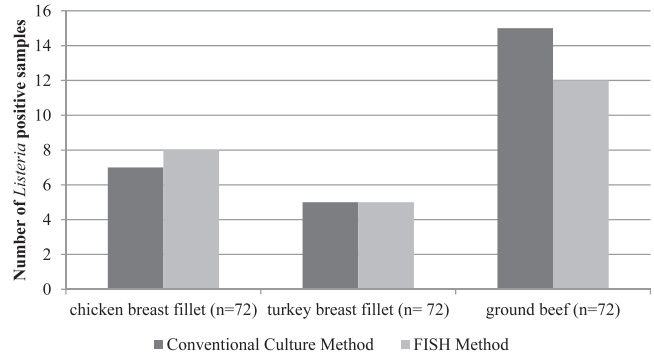

FIGURE 4. *Comparison of the conventional culture method and the FISH method for the detection of* Listeria *species in chicken and turkey breast fillets and ground beef.*

breast fillets and ground beef by the FISH method was highly specific (100%) because the false-positive results are zero (Table 4). Similarly, *Listeria* detection in chicken breast samples by the FISH method was found to be highly specific (98.61%) ($P < 0.05$). *Listeria* detection in turkey and chicken breast fillets by the FISH method was found to be highly sensitive (100%) because false-negative results are zero (Table 4). However, the FISH method had lower sensitivity (95.83%) for *Listeria* detection in ground beef, with three false-negative (4.17%) results (Table 4).

The FISH method used to test 216 fresh chicken or turkey breast fillets and ground beef resulted in a relative sensitivity estimate of 88.89% and a relative specificity estimate of 99.47% (Table 5). The chi-square value for FISH compared with the culture method for all samples was lower than the tabular value, showing that there is not a significant difference ($P > 0.05$) between the two methods.

DISCUSSION

FISH is a cheap and quick method; it does not require costly technical equipment such as real-time PCR, genetic fingerprinting techniques (e.g., RFLP, DGGE), or microarray and radioisotope techniques. FISH has a low risk of contamination because it lacks a nucleic acid amplification step. Also, it enables in situ phylogenetic species-level identification and enumeration of individual microbial cells. Moreover, it can be combined with flow cytometer, mass, or Raman spectrometry for a high resolution advanced imaging technique and automated analysis of mixed microbial populations. Cell walls are the main obstacles to the entrance of rRNA-targeted nucleic acid probes into cells that have undergone fixation. The cell walls of gram-positive cells such as *Listeria* are thicker than those of gram-negative cells and have larger amounts of teichoic acid (up to 50% of the cell wall) *(26)*. This property delays entrance of DNA probes and also the passage of negatively charged polymers from cell wall. For this reason, an aldehyde-free fixation alternative fixation or the use of fixation protocols that include heating and alcohol-based methods is recommended *(37)*. Wagner et al. *(55)* also used ethanol fixation in FISH studies employing Lis-1255 DNA probe.

However, researchers have indicated that *L. monocytogenes* grown in brain heart infusion broth for 9 h could not

be detected by ethanol-based fixation alone; to allow passage of the probe, that is, to make the cell permeable, lysozyme and proteinase K application is necessary. In contrast to this finding, in our study Lis-637 probe was used and an effective hybridization was provided by using ethanol fixation for the cells at the stationary phase (18 to 24 h) without the need for an additional permeabilization stage (Figs. 1 and 2).

To ensure microbial quality and safety of food products, it is necessary to determine the kinds and number of viable microorganisms that occur in them. The FISH method has been used extensively to count and identify specific microorganisms found in environmental and clinical samples. The FISH method relies on the fact that an oligonucleotide probe generally binds specifically to a region of the target rRNA.

Sensitivity (the ability of the method to correctly detect which samples contain *Listeria*) and specificity (the ability of the method to correctly detect which samples do not contain *Listeria*) are related inversely to the false-negative and false-positive rates, respectively. That is, an increase in the false-negative rate indicates that the method has low sensitivity, whereas an increase in the false-positive rate indicates that the method has low specificity. The FISH method had a higher false-negative rate for ground beef than for chicken and turkey breast fillet samples (Table 4). However, *Listeria* detection by the FISH method yielded low total false-positive results (0.5%) and false-negative (1.4%) results and is, thus, proper, sensitive, and specific (Table 4). Therefore, the FISH method is appropriate for routine analysis because it has the sensitivity to reliably detect a positive sample with high relative accuracy and false-positive rates (0 to 1.39%) below 5%. A lower calculated chi-square value than tabular value for the FISH method compared with the culture method was confirmed for all samples, with good agreement overall (Table 5). The regions in the cells where significant amounts of copies of rRNA were found are very well preserved. Probes used in this method are rarely hybridized with dead cells in food samples. However, the FISH method may have false-negative results, depending on the concealment of target cells by the particles in food. Also this study obtained a very low false-negative rate (4.17%) in the detection of *Listeria* in ground beef. An increase in the false-negative rate is an indication of the lower sensitivity of the method.

Similarly, Schmid et al. *(47)* suggested that, in using the FISH method to detect *Listeria* spp. in raw milk, it is necessary to harvest bacterial cells. This is one of the restrictions that prevent the practical use of the FISH method in the food industry. To eliminate this restriction, Ootsubo et al. *(38)* proposed the use of a method referred to as FISH filter cultivation, which includes culturing cells on the surface of a membrane filter and then hybridization of the viable microcolonies that develop on the filter surface with an oligonucleotide probe. Wagner et al. *(55)* and Schmid et al. *(47)* recommended, respectively, the use of Lis-1225 and Lis-637 probes for 16S rRNA sequences of all *Listeria* species-specific hybridization. Although Lis-1255 is highly specific and has no mismatch within the 16S rRNA binding region of all *Listeria* spp., it completely matches the binding region of the 16S rRNA molecules of *Brochothrix* spp. (*B. thermosphacta* and *B. campestris*). On the other hand, Lis-637 perfectly matches the target region of all members of the genus *Listeria,* except *L. grayi*. Moreover, it did not react with members of the genus *Brochothrix (47).*

Finally, Brehm-Stecher et al. *(10)* designed peptide nucleic acid probe LisUn-11 for all of the 16S rRNA sequences of *Listeria* spp., but this probe is too expensive to be used in daily routine analysis. In the study conducted by Fuchizawa et al. *(23),* Lis-1400 probe designed from the 23S rRNA region of the genus *Listeria* was bound specifically with all *Listeria* spp. Culture counting or detecting methods (ISO 11290-1 and U.S. Food and Drug Administration methods) require 5 to 7 days to obtain a result, compared with 2 days for the FISH method.

Even though milk and milk products have been implicated as sources of human listeriosis cases and outbreaks, studies have shown that more meat and poultry products are contaminated with *Listeria* spp. *(22, 48).* The presence and growth of *Listeria* spp. in meat and meat products depend on the type of product, natural microflora, pH, and the level of contamination. Whereas optimum growth occurs at pH 6 and above, at pH 5 or below there would be very little or no growth.

Meat and meat products are exposed to significant contamination during processing, transport, and storage *(31).* Although commercial sterilization and cold storage of products are reliable deterrents to *Listeria* contamination, ready-to-eat products may be contaminated at later stages before reaching the consumer's kitchen *(31).* Indeed, the slaughterhouse and its environment were reported to be the first source of contamination for meat products *(33).* Studies have been carried out, worldwide and in Turkey, to determine the microbiological quality of meat and meat products offered for sale and have found the presence of a significant level of pathogenic microorganisms in meat products. Therefore, these foods are important in public health issues and economic losses resulting from foodborne infections and intoxications.

In meat products, nonpathogenic *Listeria* spp. are present extensively. *L. innocua* has been isolated more frequently than *L. monocytogenes*. *L. seeligeri* and *L. welshimeri* are common; *L. grayi* and *L. murrayi* have also been identified. *Listeria* contamination in ground beef, in addition to its presence on the carcass or cuts of meat, may be introduced via knives, knife handles, and other materials and through improper handling by employees.

Schönberg et al. *(48),* detected *L. monocytogenes* in 85% of chicken meat analyzed, they also identified *L. innocua* and *L. welshimeri* in 8 and 1% of the samples analyzed, respectively. One study *(21)* identified *L. monocytogenes* in 56.3% of the chickens analyzed. Kwiatek et al. *(35)* isolated *L. monocytogenes* from 60% of raw chicken meat and isolated other *Listeria* species from 10% of the samples. Bailey *(3)* identified *L. monocytogenes* in 23% of chicken meat and other *Listeria* spp. in 38% of the samples. Sharif and Tunail *(49)* found *Listeria* spp. in 43% and *L. monocytogenes* in 38.6% of a total of 200 samples of a variety of meat products. Researchers reported that

Listeria isolates were identified as *L. monocytogenes* (23.49%), *L. innocua* (58.6%), *L. welshimeri* (14.88%), *L. grayi* (2.79%), and *L. ivanovii* (0.23%) *(49)*. In our country, Berktaş et al. *(5)* isolated *Listeria* spp. in 73 (73%) of 100 minced meats and in 37 (74%) of 50 meats.

Studies from different countries have reported broadly difference occurrence rates for *Listeria* spp. and *L. monocytogenes* in minced meat or ground beef, ranging from 38 to 93% and from 30 to 77.3%, respectively *(21, 48)*.

Many studies have looked at the microbiological load in ground beef or minced meat sold in butchers and markets in our country. Studies of the microbiological quality of ready ground beef or minced meat offered for sale in the provinces of İstanbul *(4),* Ankara *(17, 51),* Aydın, Afyon *(50),* Elazıg *(30),* Kayseri *(25),* Kars *(29),* and Van *(45)* show the risk that this food group poses to human health. The Centers for Disease Control and Prevention emphasizes that the most common food sources of fatal infections are meat and poultry, much of it due to *Listeria*. Together, beef, game, pork, and poultry accounted for fewer illnesses but for 29% of deaths. Poultry accounted for the most deaths (19%); many of those were caused by *Listeria* infections. This is partly due to three large *Listeria* outbreaks linked to sliced processed turkey that occurred in the last decade, although fewer have occurred in recent years *(40)*.

The literature shows that *L. monocytogenes* contaminates ground beef and many other meat products, at levels ranging from 1 to 77.3%, and that *Listeria* spp. have been detected in raw poultry meat *(7, 12, 20, 21, 24, 29, 30, 33, 43, 48, 56)*. Similarly, although *Listeria* spp. have been detected in ground beef samples in almost all of the research studies, to our knowledge, no study in the literature has found samples negative for *Listeria (3, 14, 21, 32, 44, 46, 48, 49).* The results in this study are similar to those from studies (worldwide and in Turkey) carried out on the microbiological load and detection of *Listeria* in poultry meat and minced meat from markets, butchers, and supermarkets.

To survey previous research on the subject in the scientific literature and do a quantitative comparison is beyond the scope of this study. However, compared to findings in studies performed in different cities in Turkey, the poultry meat analyzed was contaminated with the studied pathogens at lower rates. Note that these results were affected by such factors as use of different methods in detection, analysis sample and size, source of sample, variation in region and climate, hygienic condition of processing area, and personnel where the sampling was done. The effects of these and other factors are evaluated by performing the same studies to determine microbiological quality of poultry meat and ground beef on the market, using different microbiological methods at different laboratories simultaneously.

A number of studies of the microbiological load of the poultry and minced meat offered for sale in the market and at the butchers in our country not only provide information on the microbiological quality of these products but also show the significant risk to the health of consumers that these types of foods constitute.

To compare the results of this study with previous studies, various factors such as sampling time, origin of the samples, relative shelf life of the samples, sampling plan, sampling techniques, and the detection method applied should be considered. In chicken and turkey breast fillets, more positive *Listeria* results have been achieved with the FISH method than with the culture method, perhaps mainly because FISH has a higher sensitivity than the culture method in detecting a low number of cells or because FISH determines dead cells. Also, it is well known that background microflora can mask the presence of the pathogen in the samples analyzed by the culture method, and, in some circumstances, the target strains present in the sample are noncultivable. The probability of detection of dead cells by the FISH method can be eliminated by using the selective (secondary) enrichment stage or medium.

The food matrix can negatively impact detection by the FISH method because false-positive results can be matrix specific. In this study, only animal foods (turkey or chicken breast meat and ground beef) were used, and food matrix specific effects were not investigated; therefore, clear interpretation of the results obtained by both methods was not possible. It is important that the method used have low levels of false positives and false negatives. Using the FISH method, false negatives were obtained for the ground beef, and, for food safety, it was necessary to confirm the negative result. In other words, the implementation of both standard culture and FISH methods together will allow more accurate assessment of the results. FISH analysis gives results in less time than the culture method and has potential for use as a component of the food safety management systems of companies that process or produce poultry.

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