Prevalence of *Listeria* in Smoked Fish

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**ABSTRACT**

Smoked fish samples (71) were surveyed from Newfoundland retail markets and tested for the prevalence of *Listeria*. *Staphylococcus aureus* and fecal coliforms were also detected in the samples. *Listeria* was present in 11.3% of the smoked seafood products; 4 smoked cod, 3 smoked mackerel, and 1 smoked caplin were found to harbor the bacterium. The Food and Drug Administration protocol was also analyzed with regards to testing smoked seafoods. The secondary enrichment broth showed a 68% false-positive rate, whereas all positive samples were detected after 24 h of primary enrichment.

*Listeria monocytogenes* has emerged as an important foodborne pathogen associated with several outbreaks and sporadic cases of listeriosis in many parts of the world (7,12,14,41,44,45,46,51). *Listeria* can proliferate at refrigeration temperatures and freezing has no detrimental effect on its viability and it is also fairly thermostable (16,19,26,32). *Listeria* is widely distributed in nature and has been shown to occur in a wide variety of foods. Several outbreaks have occurred since 1979 implicating vegetables (37), dairy products (10,20,29,38), and fish (37) as vehicles of infection.

In recent years, many surveys have been conducted to detect different species of *Listeria* in foods. *Listeria* species has been detected in foods such as milk (9,18,25,39); cheese (1,4,7,46) ice cream (2,3) vegetables (27,47); meats (8,33,40,54) and fish (1,6,21,23,41).

Smoked fish is a ready-to-eat food commodity. It is either kept frozen or held at refrigeration temperatures and is often consumed without further cooking or heating. Those from older generations that consume *Listeria*-contaminated smoked fish are put at a higher risk and become target population for *Listeria* infections. Since there is little information regarding *Listeria* in smoked seafoods, a survey was carried out to determine its prevalence in smoked seafoods and to analyze the application of the Food and Drug Administration (FDA) protocol for *Listeria* as it pertains to smoked fish.

**MATERIALS AND METHODS**

Smoked fish samples

Samples of smoked fish were obtained from local outlets and fish industries in various regions within Newfoundland.

Isolation of *Listeria*

The modified Canadian version of the FDA *Listeria* isolation protocol (55) was used to isolate *Listeria* in accordance with the scheme shown in Fig. 1. The following media were used in the protocol: *Listeria* Enrichment broth and UVM *Listeria* enrichment broth (Difco) in the primary and secondary enrichment steps, respectively, and Oxford *Listeria* Selective agar, lithium chloride-phenylethanol moxalactam medium (LPM) (Difco), and PALCAM were used as the selective agars.

Cultures

*Listeria* cultures were obtained from Department of Fisheries and Oceans, St. John’s, Newfoundland, and the remainder cultures were collected from stock cultures in the Biology Department, Memorial University of Newfoundland. The *Listeria* cultures were: *Listeria monocytogenes* 3a (HPB #59), *L. monocytogenes* 1/2b (HPB #395), *Listeria innocua* (HPB #8), *Listeria ivanovii* (HPB #28). Other bacterial cultures consisted of *Staphylococcus aureus* and *Escherichia coli*. All cultures were maintained on tryptic soy agar-yeast extract (Fisher Scientific Ltd.) and transferred monthly. They were kept at 4°C at all times.

Microbiological methods

Overall microbiological indicators of food quality were tested and included total aerobic counts, *S. aureus*, coliforms and *Listeria*.

Total aerobic counts

This was done by the standard pour plate method (11). Serial dilutions of each sample were prepared in sterile physiological saline and duplicate pour plates prepared. Total aerobic counts were obtained from trypticase soy agar plates incubated at 30°C for 48 h.

Coliforms

A 3-tube most probable number procedure (MPN) was used to determine coliform numbers (42), using EC medium. Eosine methylene Blue plates were also spread plated with diluted samples and incubated at 35°C for up to 48 h for confirming typical coliforms.

*Staphylococcus aureus*

One-tenth-ml volumes of appropriate dilutions were spread in duplicate on *Staphylococcus* medium 110 (Difco), the plates were incubated at 35°C for 48 h. Typical yellowish pigment producing colonies were counted and confirmed by coagulase plasma test (49) and microscopic examination.
Samples of smoked fish were collected and processed to detect *Listeria*, fecal coliforms, and *Staphylococci*. Analytical measurements included salt, pH, and water activity. For smoked fish samples, the pH ranged from 5.5-6.8, had a salt percent of 2.9 to 14.0%, and a water activity of 0.69 to 0.98 (Table 1). Smoked caplin has the highest salt percents (10-14%) and lowest water activities (0.68). The remainder of smoked fish had a water activity of >0.95 and a salt content of 2.93 to 4.88% (Table 1). *Listeria* can grow in up to 10% salt and will survive in up to 30% salt (26). The above parameters for the smoked fish, including caplin, will permit the growth of *Listeria* if present. *Listeria* has been found to survive in other low water activity products such as alpha tablets (17) and dry skim milk powder (35) indicating its ability to withstand dry conditions.

The samples collected were hot (51%) or cold (49%) smoked. Herring, mackerel, and caplin were hot smoked, and salmon, charr, and cod were cold smoked, although some cod were hot smoked. The hot smoke process carried out by plants often deviated slightly from that outlined in the literature. A cold smoke involves a temperature range of ≤28°C for the entire smoke cycle. A hot smoke requires three stages: tempering at 30°C for 30-60 min; heating at 50°C for 60-90 min; and cooking at 80°C for 30-90 min. (13). The smoking plants surveyed do not follow the three stages. For the hot smoke, the product is smoked at a consistent temperature slightly greater than 28°C. As a result, it is likely that bacteria will be found on both hot and cold smoked fish.

*Staphylococci* contamination ranged between 33% for the caplin and 83% for mackerel, and the contamination of cod, kippers, and salmon was 50, 47, and 42%, respectively. Only 8% of salmon and 33% of the cod samples were found to carry coliforms at levels >100 CFU/ml. Other fish samples were free of these organisms. Table 2 indicates that *Listeria* contamination was 7, 27, and 50% for caplin, cod, and mackerel, respectively.

### Table 1. Specific parameters of the smoked fish products sampled from various supermarkets and industry.

<table>
<thead>
<tr>
<th>Product</th>
<th>$A_w^{a}$</th>
<th>Salt(%)</th>
<th>pH $^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kippers (15)$^b$</td>
<td>0.951 ± 0.017</td>
<td>4.88 ± 0.975</td>
<td>5.56 ± 0.432</td>
</tr>
<tr>
<td>Mackerel (6)</td>
<td>0.958 ± 0.009</td>
<td>4.12 ± 1.146</td>
<td>5.58 ± 0.185</td>
</tr>
<tr>
<td>Caplin (15)</td>
<td>0.697 ± 0.029</td>
<td>14.43 ± 3.49</td>
<td>5.95 ± 0.348</td>
</tr>
<tr>
<td>Cod (18)</td>
<td>0.980 ± 0.008</td>
<td>3.54 ± 1.251</td>
<td>6.83 ± 0.453</td>
</tr>
<tr>
<td>Charr (4)</td>
<td>0.982 ± 0.012</td>
<td>4.42 ± 3.120</td>
<td>5.49 ± 0.299</td>
</tr>
<tr>
<td>Eel (1)</td>
<td>0.966</td>
<td>2.93</td>
<td>5.55</td>
</tr>
<tr>
<td>Salmon (12)</td>
<td>0.971 ± 0.012</td>
<td>3.63 ± 1.377</td>
<td>5.96 ± 0.164</td>
</tr>
</tbody>
</table>

$^a$Mean ± standard deviation for water activity.

$^b$Mean ± standard deviation for percent salt.

$^c$Mean ± standard deviation for pH.

$^n$No. of samples.

#### RESULTS AND DISCUSSION

The secondary enrichment step with UVM *Listeria* enrichment broth produced false positives varying from 57 to 75% when plated out onto *Listeria* selective agars. From a total of 71 samples examined for *Listeria*, 25 were tested positive by the secondary enrichment method. However, further testing revealed that only 32% of these samples truly tested positive. In contrast, the primary enrichment detected *Listeria* in eight of these samples and were confirmed by further testing. The overall false-positive rate was 68% samples collected over a three-month period. All positives for *Listeria* were detected after 24 h primary enrichment, and no additional samples were found positive thereafter. These results coincide with those of Warburton et al. (56) who also found through an extensive analysis of the FDA and U.S. Department of Agriculture protocol that 92% of all positives were found after 24 h primary enrichment.

Data vary on the superiority of the three selective agars, LPM, PALCAM, and Oxford. *L. monocytogenes* 3a was shown to perform poorly on LPM (30). These scientists also showed Oxford as being superior to LPM. Curtis et al. (15) who formulated Oxford indicated that Oxford
### TABLE 2. Microbiological analysis of the smoked fish products sampled from various supermarkets and industry.

<table>
<thead>
<tr>
<th>Product</th>
<th>Staphylococcus</th>
<th>E. coli</th>
<th>SPC</th>
<th>Listeria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kippers (15)</td>
<td>3.4 x 10³ (47%)</td>
<td>ND&amp;</td>
<td>2.8 x 10⁵ (53%)</td>
<td>ND</td>
</tr>
<tr>
<td>Mackerel (6)</td>
<td>2.4 x 10³ (83%)</td>
<td>ND</td>
<td>4.0 x 10⁵ (83%)</td>
<td>(50%)</td>
</tr>
<tr>
<td>Caplin (15)</td>
<td>1.1 x 10³ (33%)</td>
<td>ND</td>
<td>1.1 x 10⁵ (40%)</td>
<td>(6.7%)</td>
</tr>
<tr>
<td>Cod (18)</td>
<td>2.6 x 10⁵ (50%)</td>
<td>2.0 x 10⁴ (33%)</td>
<td>3.1 x 10⁵ (100%)</td>
<td>(27%)</td>
</tr>
<tr>
<td>Charr (4)</td>
<td>ND</td>
<td>Nil</td>
<td>4.0 x 10⁴ (25%)</td>
<td>ND</td>
</tr>
<tr>
<td>Eel (1)</td>
<td>ND</td>
<td>Nil</td>
<td>4.0 x 10⁴ (100%)</td>
<td>ND</td>
</tr>
<tr>
<td>Salmon (12)</td>
<td>9.1 x 10³ (42%)</td>
<td>3.2 x 10⁸ (8.3%)</td>
<td>1.2 x 10⁴ (58%)</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Standard plate count, average of the percent positives.

No. of samples analyzed.

Average of the percent positives.

ND - Not detected.

Fifty-one and 49% of the samples were hot and cold smoked, respectively.

was superior to McBride agar. However, Lee and McClain (36) proved their formulation, LPM, to also be better than McBride agar. Finally, van Netten et al. (52) who formulated PALCAM preferred their medium over LPM and Oxford. Another associate found LPM to be less inhibitory to Listeria isolates than PALCAM and Oxford (Powell, personal communications, 1990). The existing controversy relies heavily on the type of food product being analyzed, i.e., foods highly contaminated with bacteria have a better Listeria recovery with more selective plates, whereas for processed foods with fewer other competitors and possibly injured Listeria may need a less selective plate to recover the Listeria. Therefore, it seems best to use all three existing media to avoid any false negatives. Twenty-five out of 71 samples were directly plated onto Listeria selective agars, but no positives were found. It is evident that enrichment for Listeria is necessary. However, depending on the food item being analyzed, it may be possible to drop the secondary enrichment step from the method in many cases, and this will reduce the detection time.

Both hot and cold smoked samples contained Listeria indicating that hot smoking procedure used by the local smoking plants was not affecting the Listeria present. It is also possible that some samples could have been contaminated after smoking since some distributors buy smoked fish in bulk and package them at local outlets. No detailed information was available regarding the exact temperature conditions used by different smokers.

The confirmatory tests included catalase, Gram stain, wet mount, hemolysis, fermentation reactions, and motility. The positive samples indicated Listeria; however, it was difficult to distinguish between L. innocua and L. monocytogenes. The hemolysis test indicated L. innocua, but the controls indicated that the isolates could have been either, since no hemolysis was seen with the control L. monocytogenes 1/2b but was seen with L. monocytogenes 3a. Some blood, notably that of sheep, contains antibiotics against L. monocytogenes (48) and may account for this abnormality. However, in this work horse blood was used, and no hemolysis was detected with all the isolates tested. No information is available with respect to the presence of any antibiotics in the horse blood samples used. The only known property of L. monocytogenes which has been shown to be involved in virulence is a hemolysin, listeriolysin (34). However, there is evidence of nonhemolytic mutant of L. monocytogenes which is defined as nonvirulent (43). Hence, it leaves the possibility of mutants with virulence. Also, L. monocytogenes 1/2a was noted to have no indication of hemolysis on sheep blood (53). The carbohydrate tests indicated L. monocytogenes, but rhamnose is variable for L. innocua and therefore could have been either species. Both of these species of Listeria are the most commonly found in seafoods (21,22).

### CONCLUSIONS

Approximately five plants in Montreal, Vancouver, and Seattle have been shut down already because of Listeria contamination. Unless the problem can be controlled and solutions found, the entire industry may suffer from the consequences. Almost overnight Listeria has become the number one problem for the entire smoking industry that depends on a share of the U.S. market (24). The U.S. FDA has a zero tolerance for Listeria in all ready-to-eat foods entering the United States. The method used in this study is the Canadian version of the U.S. FDA. Jemmi (30,31) has detected L. monocytogenes in smoked fish products as well. The survey in this report verifies the prevalence of Listeria in the local smoked fish products.

This study also showed that about 10% of the samples tested showed greater than 100 CFU/g of smoked fish. No tests were carried out to determine the number of fecal coliforms present, although they were detected in the same 10% of the samples tested for coliforms.

It is necessary to further study the prevalence of Listeria in smoked fish samples in order to get a better understanding of the situation. Furthermore, all isolates will be verified, serotyped, and phage typed by a reference center in Ottawa. If L. monocytogenes is confirmed by the center, the validity of hemolysis test upon which great emphasis is placed will be questionable. Finally, the industry should be made aware of the problem, and ways of controlling should be implemented.

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REFERENCES

the isolation and enumeration of *Staphylococcus aureus*. In *Compendium of methods for the microbiological examination of foods*. American Public Health Association, Washington, DC.


**Jackson and Whiting. cont. from p. 861**


