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Evaluation of Surface Contamination and the Presence of *Listeria monocytogenes* in Fish Processing Factories

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ABSTRACT

The main objective of this study was to determine the level of surface contamination in fish processing factories and the presence of *Listeria* in the factory environment and products. Another objective was evaluation of the different hygiene-monitoring methods. Total aerobic heterotrophic and enterobacteria, yeast and mold samples were collected and ATP levels measured in 28 factories. The number of well or adequately washed and disinfected factories was small (2 of 28), in terms of total aerobic heterotrophic bacterial counts on the surfaces. Most surfaces contaminated with bacteria were heavily contaminated. Results of the ATP and the total bacteria contact agar slide methods were poorly correlated ($r = 0.21$) although 68% of the samples were categorized as good to moderate or unacceptable with both methods. The *Listeria*-positive surface samples usually contained increased numbers of total bacteria (70.9%). The contamination of products and raw fish together with *Listeria* spp. was 45% and with *Listeria monocytogenes* 12%. Cold smoked fish was the most contaminated, with 75% *Listeria* spp. and cold salted fish with 20% *L. monocytogenes*. *Listeria innocua* was found in the samples more than twice as often as *L. monocytogenes*.

The hygiene of the process and processing environment is a significant factor in the production of microbiologically safe and good quality products in the fish industry. Both quantity and the specific type of microbial flora are important factors for evaluating the hygiene of the processing environment.

While there are very few publications concerning hygiene in the fish industry *(1),* surface hygiene monitoring has been conducted in slaughterhouses and in the meat industry *(5, 12, 21, 23),* the dairy industry *(2),* breweries *(19),* and in retail food premises *(8, 25).*

Methods for monitoring surface hygiene have been developed in recent decades *(7, 10, 17, 24, 26),* and some of them have also been commercialized. The most widely used method for monitoring hygiene for research purposes is swabbing either with swabs or sponges, rinsing, and cultivating the collected bacteria. ATP measurement and contact agar methods are easier to carry out, especially in routine hygiene monitoring.

Hygienic production is essential, especially for products that are eaten uncooked such as cold smoked and cold salted fish and roe. Microbial growth may reach high levels in nutrient-rich fish products if they are stored incorrectly. Vacuum packaging, cold storage, and high salt concentration may favor growth of pathogenic *Listeria monocytogenes* compared with competing microbial flora. Cold smoked fish is usually vacuum packed and has a long shelf life of 5 weeks *(15)* to 12 weeks *(3),* although it has been recommended that the time be limited to 3 weeks *(14, 15).*

The objective of this study was to determine the level of surface hygiene in fish processing factories with different hygiene-monitoring methods. Total aerobic heterotrophic bacteria, enterobacteria, yeast and mold, and ATP levels were measured in samples from the processing environment of 28 factories. The presence of *Listeria* on the factory surfaces and products was determined.

MATERIALS AND METHODS

Surface samples. Samples (866) were taken from 28 factories from 1996 through 1998. Surface samples were taken after cleaning and just before processing began. Surfaces in direct contact with the products (e.g., knives, chopping boards, and conveyor belts) or indirect contact (e.g., control panels, door handles) were sampled (35 to 45) at each factory. *Listeria* samples (943) were taken from 23 factories also including surfaces like floors and drains.

Microbiological analyses. ATP measurements were performed by swabbing the surfaces (approximately 100 cm^2) with ATP-free swabs moistened with 0.9% saline. Within 4 h, the swabs were agitated in cuvettes containing 500 μl of releasing reagent from a surface monitoring kit (Bio-Orbit, Turku, Finland). After 2 min, 500 μl of monitoring reagent (Bio-Orbit) was added. The bioluminescence was measured in a luminometer, 1253-Luminometri (Bio-Orbit), as relative light units (RLU).

Total aerobic heterotrophic bacterial counts from the surfaces were examined by pressing both sides of the Hygicult TPC contact agar slide (PC agar, 9.6 cm^2; Orion Diagnostica, Espoo, Finland) firmly against the surface. Slides were incubated for 2 days at 30°C. Enterobacteria were sampled with Hygicult E contact agar slides (modified violet red-bile agar containing glucose in addition to lactose, 9.6 cm^2; Orion Diagnostica), as described for the total

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TABLE 1. *Surface hygiene guidelines for total aerobic heterotrophic bacteria, enterobacteria, yeasts, and molds on food processing surfaces (CFU/cm²) investigated using contact agar slides*

Microbial group	Good	Moderate	Unacceptable
Total aerobic heterotrophic bacteria	1.8	1.8–5	>5
Yeasts	<1	1–5	>5
Molds	<0.6	0.6–1.6	>1.6
Enterobacteria	<0.1	0.1–1.0	>1

bacteria samples. Slides were incubated for 2 days at 37°C. Yeasts and molds were sampled with Hygicult Y&F contact agar slides (malt agar, antibiotics, and low pH 5.5 to inhibit the growth of bacteria, 9.6 cm²; Orion Diagnostica), as described for the total and enterobacteria samples. Slides were incubated for 5 days at 25°C. All the Hygicult contact agar slides were interpreted according to the manufacturer's chart models, except for small counts that were calculated. Both sides of the slides were examined, and the mean was presented as the final result.

Listeria from the surfaces was sampled with contact plates (25.5 cm²) of Oxford-agar (Oxoid Ltd., Basingstoke, Hampshire, UK). The contact plates were incubated for 2 days at 37°C. Each suspected *Listeria* colony was isolated and tested by Gram stain, β-hemolysis on blood agar (Orion Diagnostica), and API-Listeria test (bioMérieux, Marcy l'Etoile, France).

Duplicate samples from raw fish and processed products were examined for *Listeria* with modified Nordic Committee on Food Analysis method number 136 using a one-step enrichment. Samples (25 g) were homogenized for 30 s in 225 ml EB broth (Oxoid Ltd.). Homogenized samples were incubated for 48 ± 2 h at 30°C, then cultivated on Oxford agar and incubated for 48 ± 2 h at 37°C. Suspected colonies were further examined as described earlier.

Microbiological guidelines. Results of total aerobic heterotrophic bacterial counts were evaluated according to Houhala et al. *(13).* Yeast, mold, and enterobacterial counts were evaluated according to the manufacturer's instructions. The guidelines are presented in Table 1. The limit value for ATP measurement was set to 1 RLU. When the surface is properly cleaned the ATP result is below 1 RLU.

Statistical analysis. Correlations between different surface hygiene methods were calculated with equation 1.

$$\sigma_{x,y} = Cov(X, Y)/\sigma_x\sigma_y \quad (1)$$

where Cov = covariance of two (X, Y) data sets; σ_x = standard deviation of data set (X); and σ_y = standard deviation of data set (Y).

RESULTS

Total aerobic heterotrophic bacterial counts on the surfaces. Based on levels of total aerobic heterotrophic bacteria, about half (52.2%) of the cleaned surfaces were clean or only slightly contaminated (<1.8 CFU/cm²), 21.8% moderately contaminated (1.8–5 CFU/cm²), and 26.0% unacceptable (>5 CFU/cm²). Most (79.6%) of the unacceptable samples had counts >20 CFU/cm².

The total aerobic heterotrophic bacterial results of each factory categorized into three contamination classes are presented in Figure 1. In only one factory were over 90% of the samples clean (<1.8 CFU/cm²). More factories (4 to 10) had 60 to 70% clean samples under this limit. When the microbiological limit was increased to moderate cleanliness (5 CFU/cm²) or further (10 CFU/cm²) only two factories had more than 90% of their samples within the limit.

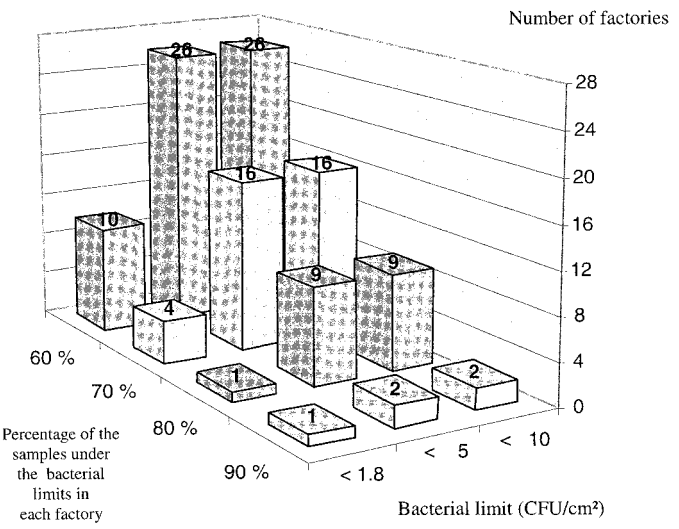

FIGURE 1. *Number of factories (total 28) at which total aerobic heterotropic bacteria results from surfaces were below the bacterial contamination limits (1.8 to 10 CFU/cm²) in at least 60 to 90% of the samples in each factory (<1.8 CFU/cm² = good; 1.8 to 5 CFU/cm² = moderate; >5 CFU/cm² = unacceptable).*

The mean counts of total aerobic heterotrophic bacteria on all the fish processing surfaces studied were well above levels considered to be clean (Table 2). High standard deviation values indicate that results differed considerably between factories and corresponded to visual observations of cleanliness. Contamination was greatest on scale, brine basin, chopping board, and shovel surfaces from 13 common fish factory surfaces. Additionally many conveyor belts, tables, roe grids, and slicing and skinning machines remained heavily contaminated after cleaning.

ATP activity on the surfaces. ATP activity exceeded the microbiological limit of 1 RLU in 43.3% of all the samples. The mean ATP results from 13 common fish factory surfaces are presented in Table 2. ATP levels of 10 to 30 times the guideline were found from brine basins, doors, door handles, aprons, and scales.

Enterobacteria, yeast, and mold counts on the surfaces. The level of enterobacteria exceeded the guideline value of 0.1 CFU/cm² for good hygiene in 19.7% of all the samples, and 9.2% of the samples exceeded the unacceptable limit (1.0 CFU/cm²). A total of 35.2% of the surfaces were at least moderately (>0.6 CFU/cm²) contaminated with molds. The percentage of unacceptable surfaces (>1.6 CFU/cm²) was 12.2%. In the case of yeasts, 24.0% of the surfaces were moderately contaminated (1 CFU/cm²). The percentage of unacceptable (>5 CFU/cm²) surfaces was 6.2%. The mean values of enterobacteria, molds, and yeasts from 13 fish factory surfaces are presented in Table 2. Some chopping boards in particular were heavily contaminated with enterobacteria. Most of the molds were found from

TABLE 2. *Mean total aerobic heterotrophic bacteria, enterobacteria, mold, and yeast counts and ATP results from the fish factory surfaces analyzed with contact agar slides and ATP method from fish factory surfaces*

Surface	Mean ± SD					Number of factories
	ATP (RLU/100 cm²)	Total bacteria (CFU/cm²)	Enterobacteria (CFU/cm²)	Molds (CFU/cm²)	Yeasts (CFU/cm²)	
Scale	10.0 ± 18.1	42.9 ± 36.0	0.9 ± 1.5	0.9 ± 0.7	2.6 ± 5.2	19–21
Brine basin	30.7 ± 53.9	33.7 ± 41.7	1.1 ± 2.1	0.5 ± 0.7	5.9 ± 12.5	12–13
Chopping board	3.4 ± 8.0	31.0 ± 37.8	2.5 ± 6.3	0.5 ± 0.6	6.7 ± 16.5	21–23
Shovel	2.3 ± 2.9	30.6 ± 39.7	0.9 ± 1.7	0.5 ± 0.6	6.8 ± 11.3	7–8
Packaging table	9.4 ± 23.4	23.8 ± 34.3	0.4 ± 1.4	1.0 ± 0.9	0.9 ± 1.4	10–12
Grindstone	5.2 ± 6.2	22.8 ± 38.7	0.2 ± 0.5	0.8 ± 0.7	1.8 ± 1.4	9–11
Knives	1.9 ± 4.0	17.2 ± 33.7	0.5 ± 1.2	0.3 ± 0.5	2.8 ± 6.4	15–19
Door and/or door handle	13.0 ± 23.7	15.0 ± 20.3	0.2 ± 0.6	0.3 ± 0.5	0.6 ± 1.1	22–24
Apron	10.1 ± 19.7	14.3 ± 20.6	0.2 ± 0.3	0.6 ± 0.5	5.3 ± 17.8	17–20
Faucet	7.8 ± 12.7	13.7 ± 16.0	0.0 ± 0.0	0.4 ± 0.7	0.3 ± 0.4	10–11
Vacuum packaging machine	4.3 ± 8.3	9.1 ± 21.4	0.0 ± 0.1	0.4 ± 0.6	3.4 ± 14.3	17–19
Ice basin/shovel	4.2 ± 6.5	7.7 ± 10.0	0.1 ± 0.2	0.7 ± 0.7	5.0 ± 10.1	11–13
Light/electric switch	0.9 ± 1.0	6.3 ± 9.5	0.0 ± 0.0	0.4 ± 0.3	0.2 ± 0.3	7–9

packaging tables and scales, and high counts of yeast were found from shovels and chopping boards.

Association of different microbiological methods. Correlation between the surface methods was slight (n = 812). The best correlation was between ATP measurement and enterobacterial counts (0.43). The total aerobic heterotrophic bacterial counts correlated moderately (0.33 to 0.21) with all the other methods. Hygiene monitoring with the contact agar slide method (total bacteria) compared with ATP measurement gave somewhat different results. The ATP method accepted 18.1% of the samples that the contact agar slide method found contaminated, and the ATP method discarded 13.6% of the samples that the contact agar slide method approved. Both methods agreed on 68.3% of the samples that were either under (38.4%) or over (29.9%) the microbiological limits used.

Figure 2 shows examples of how different microbes or ATP amounts can be the main hygiene problem in different factories. Molds were the main problem in factory A. In factory B the main problem was the high amount of ATP. Lots of yeasts were found in factory C compared with other factories. All methods gave clearly similar results in a well-cleaned factory, as can be seen in Figure 2, factory D.

***Listeria* results from surfaces.** *Listeria* spp. were found on 65% (15 of 23) of the factories sampled. In the factories that were *Listeria* positive, 7.2% of all the surfaces sampled were positive. One-third of the positive *Listeria* isolates were identified as *L. monocytogenes* and the rest were mainly *Listeria innocua*; *Listeria welshimeri* and *Listeria grayi* were also found. *Listeria* was found, e.g., on brine basin and machine, cold smoke skewer, grindstone, scale, apron, conveyor belt, skinning and slicing machine, door handle, light switch, packaging table and machine, and floors and drains.

Of the *Listeria*-positive surfaces, 70.9% (22 of 31) were at least moderately (>1.8 CFU/cm²) contaminated, 67.7% were unacceptable (≥5 CFU/cm²) in terms of total aerobic heterotrophic bacterial counts, and 58.6% had an ATP result higher than 1 RLU. The ATP and/or total aerobic heterotrophic bacteria limit was exceeded in 77.4% of the *Listeria*-positive samples. Enterobacteria (≥0.1 CFU/cm²) were found in 55.6% of the *Listeria*-positive samples. Yeast and mold contaminations of the *Listeria*-positive surfaces were 44.4 and 42.9%, respectively. On the other hand, 8.8% of the all enterobacteria-contaminated surfaces were also *Listeria* contaminated. From the total aerobic hetero-

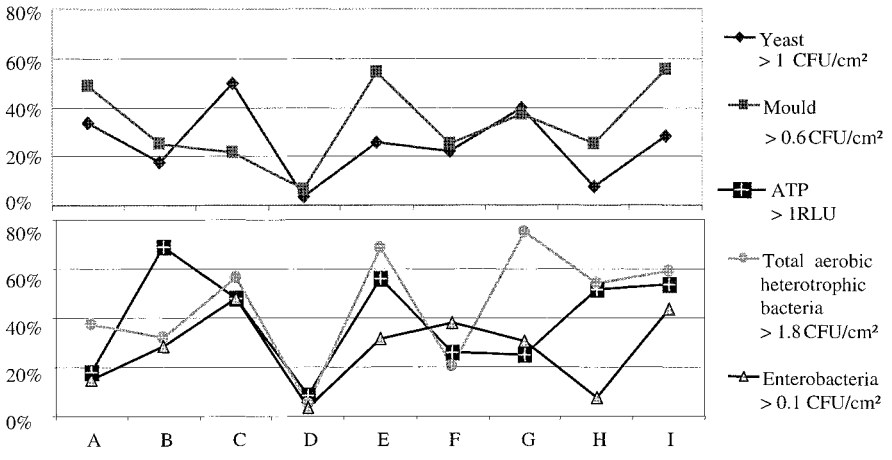

FIGURE 2. *Comparison of main hygiene problems and contamination levels caused by differenct microorganisms or ATP in different fish factories. Results of surface hygiene-monitoring methods from nine factories (A through I). Percentages of the samples that exceeded the microbiological limit of each method.*

TABLE 3. Listeria-*positive fish and fish products*

	No. of samples studied	No. (%) of samples positive for:			
		Listeria spp.	*L. monocytogenes*	*L. innocua*	Other *Listeria* spp.
Cold smoked	12	9 (75)	2 (17)	6 (50)	1 (8)[a]
Hot Smoked	13	3 (23)	1 (8)	1 (8)	1 (8)[b]
Cold salted	10	6 (60)	2 (20)[d]	4 (40)[d]	1 (10)[a]
Roe	5	2 (40)	0 (0)	1 (20)	1 (20)[c]
Raw fish	18	6 (33)	2 (11)[d]	4 (22)[d,e]	2 (11)[a,e]
Total	58	26 (45)	7 (12)	16 (28)	6 (10)

[a] *L. welshimeri.*
[b] Unidentified *Listeria* spp.
[c] *L. seeligeri.*
[d] Both *L. monocytogenes* and *L. innocua* in one sample.
[e] Both *L. innocua* and *L. grayi* in one sample.

trophic bacteria- and ATP-contaminated surfaces, *Listeria* was found in 7.5 and 4.8% of cases, respectively. Yeast- and mold-contaminated surfaces had *Listeria* in 5.8 and 3.9% of the samples, respectively.

***Listeria* results from raw fish and processed products.** The *Listeria* species and percentages of raw and processed fish samples positive for *Listeria* are presented in Table 3. *Listeria* were found on raw and processed fish in 69.6% (16 of 23) of the factories. *L. monocytogenes* was detected in samples from 7 factories (30.4%) and *L. innocua* in samples from 12 factories (52.2%). Other *Listeria* species were found in five factories. From five factories all the samples examined were contaminated with *Listeria.* In all factories where *L. monocytogenes* was found, *L. innocua* was also detected. *L. monocytogenes* was found in the brine at one factory, and its products were also contaminated with *L. monocytogenes.*

DISCUSSION

The number of well or adequately washed and disinfected factories was small (2 of 28) (Fig. 1) in terms of total aerobic heterotrophic bacterial counts on the surfaces. By comparison, in one Hungarian slaughterhouse over 95% of the sampled surfaces (n = 1,308) were found to be cleaner than 1 CFU/cm^2 *(12)* by the Ten Cate method *(26).* According to a fish industry study *(1),* the percentage of clean (<1.8 CFU/cm^2) surfaces was 61.2% with the contact agar method. In this study, the percentage of the samples cleaner than 1.8 CFU/cm^2 was 52.6%. Our results also indicated that the processing surfaces contaminated with bacteria were in fact heavily contaminated (Fig. 1).

Both the ATP measurement and the contact agar method have their strong and weak features. The ATP method measures the total amount of ATP in the samples, which is useful especially in hygiene monitoring because it is more rapid than culturing microorganisms. However, the minimal amount of ATP in stressed cells and spores may be below the limit of detection, although the cells can be detected by a cultivation method. Detergents and disinfectants also may reduce the activity of the luciferase enzyme *(9, 16).* Disinfectants may be similar to ATP-releasing reagents, and therefore residues found in the food industry can have additive effects on ATP measurements *(16).*

Enterobacteriaceae and fungi have also been recommended as indicator organisms for the presence of pathogenic bacteria on surfaces *(24).* Enterobacteria and total bacteria-contaminated surfaces were contaminated 2 and 1.5 times more often with *Listeria* than ATP, yeast, and mold samples. Nevertheless all the methods are important when evaluating hygiene problems and differences of individual factories as seen in Figure 2. The results were influenced by differences in buildings, processing conditions, equipment and machines, working habits, raw materials, and products, in addition to cleaning and washing procedures.

The contact plate surface detection method for *Listeria* was simple and rapid but not reliable. The sampled area was small (25.5 cm^2) and attachment of bacteria to the contact plate is limited *(13).* Selective agents in the contact agar may have also inhibited the recovery of detergent-stressed *Listeria* cells from the cleaned surfaces. Background flora, also complicated the isolation and identification of *Listeria* colonies. Nevertheless, *Listeria* was found in two-thirds of the factories sampled, at least sporadically. Many important process surfaces were found to be contaminated with *Listeria.*

The connection between the results from the hygiene-monitoring methods and the presence of *L. monocytogenes* in products cannot be evaluated with the small amount of products analyzed from each fish factory. The overall contamination of products and raw fish with *Listeria* spp. was 45% and with *L. monocytogenes* 12% (Table 3) compared with the other studies showing 27, 11 *(11),* 15, and 11%, respectively *(18).* Cold smoked and cold salted products were the most contaminated with *Listeria* spp. (75 and 60%) and with *L. monocytogenes* (17 and 20%).

As in many other studies *(4, 11, 22), L. innocua* was the most common (57%) *Listeria* species in the fish samples and also in the surface samples. Some researchers have reported almost similar incidences of *L. monocytogenes* and *L. innocua (6, 18). L. innocua* was found more than twice as often as *L. monocytogenes* in this study. *L. innocua* was

also found from all the factories or products where *L. monocytogenes* was present. The detection and recovery of *L. monocytogenes* may be influenced by *L. innocua* when enrichment culture technique is used *(20, 27).* In fact, many *L. innocua* strains have an inhibitory activity against strains of *L. monocytogenes (27).* Consequently *L. monocytogenes* may not be observed if *L. innocua* is present in the sample. For this reason, *L. innocua* is an important indicator of increased risk of *L. monocytogenes* in the sample.

The importance of good manufacturing practice and hygiene in the fish processing industry for product quality and safety cannot be underestimated, especially considering pathogens such as *L. monocytogenes.* After the evaluation of the hygiene results obtained in this study, it was possible to focus cleaning and disinfection on the most important surfaces and machines and to enhance the hygienic level and safety in the factories considerably. It is essential to assess the efficiency of the cleaning operations regularly with effective and appropriate hygiene-monitoring methods.

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