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Listeria spp. and *L. monocytogenes* Prevalence in Shrimps from Seafood Industry in Albania

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Abstract

Seafood industry in Albania is growing fast in the last decade. With the increased demand for shrimps and prawns consumption, the public health concern also has increased. Member of *Listeria* spp. especially *Listeria monocytogenes* has a significant role as a human pathogen. The presence of these bacteria in the seafood-production plants is strongly related to its presence in marine environment, cross-contamination, resistance and survival at low temperature condition, making this pathogen a serious threat to this industry.The aim of this study is to determine the overall prevalence of *Listeria* spp. and more *specifically L. monocytogenes* in frozen and processed shrimps from Albania seafood industry, as well as environment contamination rate of processing plants. 333 shrimps samples (processed and frozen), and 25 swabs samples from processing environment were collected during May 2021- December 2022 period. Samples of shrimps taken before and after processing, and samples of swabs were analyzed for the presence of *Listeria* spp. and *L. monocytogenes* using ISO standard microbiological methods. 30 (8.3%) out of 358 samples examined, were found positive for *Listeria* spp. The average prevalence of *L. monocytogenes* in shrimps resulted 1.2%, meanwhile in swabs is 8%. The prevalence is higher in frozen shrimps (4.7%) compared to processed shrimps (0.37%). The results clearly indicate that the *Listeria* spp. and *Listeria monocytogenes* are present in the shrimps from food processing industry in Albania, but contamination rate in these categories is low and represents a low risk for public health. The higher prevalence of *Listeria* spp. and *Listeria monocytogenes* in frozen shrimps samples compare to processed shrimps indicate that the effective processing procedures are in place.

Keywords: *Listeria* spp., *Listeria monocytogenes*, shrimps, swab, prevalence, Albania

1. Introduction

Albania has natural water potentials, which are suitable for growing seafood. Fishing is an important sector of the Albanian economy. Fishing data is collected by the Ministry of Agriculture and Rural Development based on the methodology of GFCM (General Fisheries Commission for the Mediterranean) for data collection based on segments of the fishing fleet, collection of logbooks from ships, interviews with aquaculture operators etc. Across 2019 alone, the demand for Albania shrimps and prawns (seafood category) has increased.

The country has developed also seafood industry recently which contributes in economy. The fish processing industry is concentrated around the main ports of Shengjin, Durres, Vlore, and Sarande. In 2020 there were 28 fish processing plants in Albania, and all of them were certified to supply not only the domestic market, but also to export to the EU.

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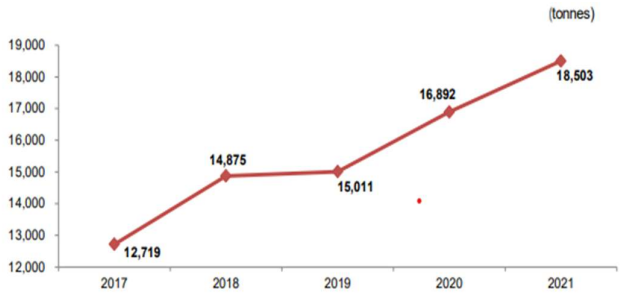

Figure 1. Total fish catches by INSTAT 2021 [6]

The production for export was over 6 855 thousand tonnes worth over 45 million euros. Major species processed are anchovy, sardine, and other small pelagic species for export primarily to the EU. Fishing water categories are: marine, brackish waters, lagoons, inland waters, aquaculture and mollusks.

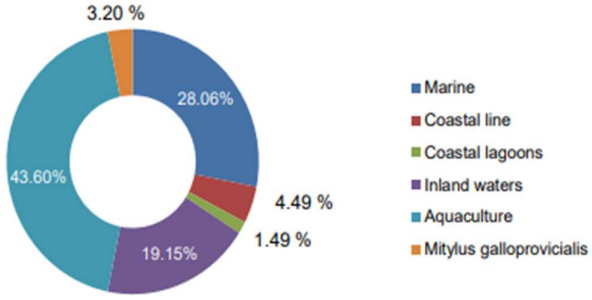

Figure 2. Catches structure by water categories (INSTAT 2021)

The main categories which represent the biggest percentage of fish catches are respectively "Aquaculture" with 43.60 % and “Marine” fishing with 28.06 % followed by “Inland waters” with 19.15 % of the total catches.

With the increased demand for this seafood category consumption, the public health concern also has increased. One of the major problems faced by the shrimp industry, besides insufficient production and disease outbreaks, is shrimp product safety. This concern has contributed to the enforcement of standards and regulatory procedures for shrimp products.

One of the main concerns related to shrimps consumption, is the bacterial pathogen *L. monocytogens*. Since this bacteriais a major human pathogen, there is widespread agreement that the goal should be to exclude this organism from the food chain wherever possible, and to maintain conditions that will inhibit its multiplication in foods in which this bacterium can grow [14].

The genus *Listeria* comprises six species: *L. monocytogens, L. innocua, L. ivanovii, L. welshimeri, L. seeligeri,* and *L. grayi*. In humans, *L. monocytogenes* is the major pathogen, although very rare cases of infection due to *L. ivanovii* and *L. seeligeri* have been described [11].

This pathogen is ubiquitous in the environment and can grow in vacuum and gas-packaged products at refrigeration temperatures. Thus, ready to-eat (RTE) food products which are stored at refrigeration temperature for more than 10–15 days and are consumed without sufficient heating to kill living bacterial cells belong to the risk foodstuffs causing listeriosis. It has also been reported that L. monocytogenes has shorter generation times in seafood (pH 6.1– 7.6) than in meat and meat products (pH 5.1–6.2). This is partly due to the effect of pH on the growth of *L. monocytogenes*, which has higher growth rates at near neutral pH.

Listeria monocytogenes is the causal agent of listeriosis, a disease that can be serious and is often fatal in susceptible individuals. Listeriosis is a severe and often fatal illness with clinical manifestations resembling sepsis or meningitis in immunocompromised patients and neonatal babies, and flu-like illness or abortion during pregnancy in women [2]. An estimated 1,600 people get listeriosis

each year, and about 260 die [1]. Invasive listeriosis is characterized by severe symptoms and a high mortality rate (20–30%) [16]. Major outbreaks of listeriosis have been associated with the consumption of foods of animal origin [14], especially sea foods such as shrimp, mussels and undercooked fish [12]. This organism has been isolated frequently from fish and fish products from different parts of the world [12].

The aim of this study is to determine the overall prevalence of *Listeria* spp. and more specifically *L. monocytogenes* in frozen and processed shrimps from Albania seafood industry, as well as environment contamination rate of processing plants.

2. Material and Methods

A total of 333 shrimps' (raw and semi-processed) samples, belonging to the species *Pandalus borealis* were obtained from some seafood processing plants, located in Albania, during May 2021- December 2022 period. The processing plants were selected based on their facilities and capacity as representative plants for the region.

Shrimps' samples were collected from the plants randomly at monthly intervals (Figure 3). Samples of shrimps were taken before and after processing.

Number of samples were distributed not proportionally in each month and were immediately placed in ice in insulated boxes with a drain valve and brought to the laboratory within an hour for analysis, to determine the presence of *Listeria* spp. It was recorded also the samples information, which included the name and location of the processing plant, pack type, date and time of sampling, temperature, sanitary status of raw material, place and date of lot production.

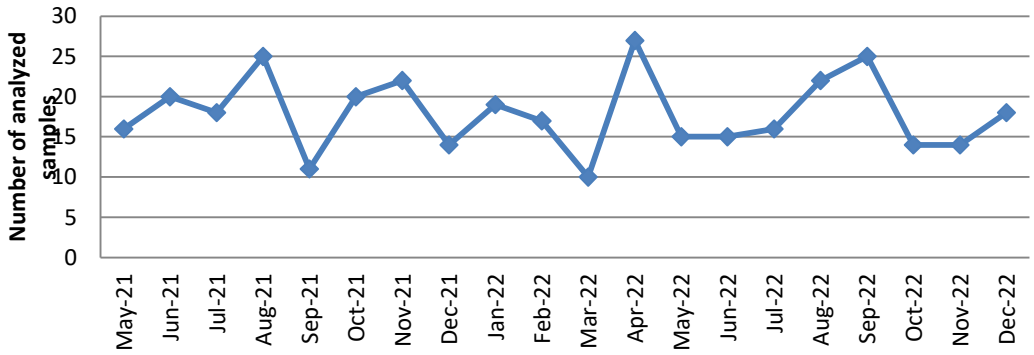

Figure 3. Number of analyzed samples per month

25 swabs samples from plant contact surfaces were collected also. Seafood contact surfaces examined from sampling site under this study belong to production environment (floors, walls, doors, etc.), and the surrounding environment. The samples were taken by swabbing surfaces in each plant. Swab samples were collected based on SSH EN ISO 6887-1:2017. Contact area (25 cm2) was swabbed using sterile cotton swabs moistened with sterile saline and diluted in a total volume of 25 ml of sterile physiological saline.

The samples were transferred to the Food Microbiology Laboratory at Bio-V Laboratory in insulated cold-boxes. Samples were analyzed on the day they were collected. The total number and type of samples collected and analyzed are given in Figure 4.

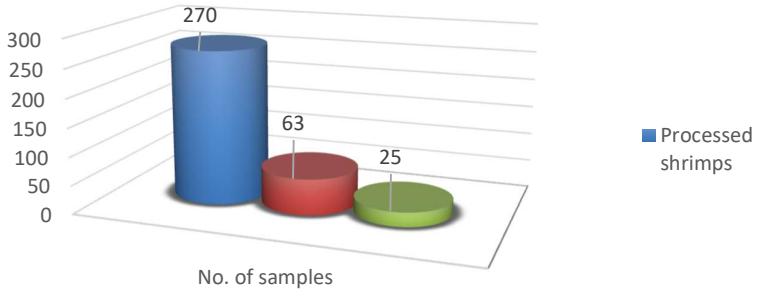

Figure 4. Numbers and types of analyzed samples

The samples were tested for the presence of *Listeria* spp. and *L. monocytogenes* using the selective enrichment and isolation protocol recommended by International Organization for Standardization (SSH EN ISO 11290:2 2017). After incubation 24 ± 2 h, were exanimated the Petri dishes for the presence of presumptive colonies of *L. monocytogenes* and *Listeria* spp. The blue-green colonies surrounded or not by an opaque halo (on ALOA agar) and black colonies with black sunken center (on Oxford agar) were considered as *Listeria* spp. At least five suspected colonies were sub cultured onto Tryptone Soy Agar supplemented with 0.6% of yeast extract (TSAYE) and incubated at 37°C for 18 h to 24 h. All isolates were subjected of identification by biochemical tests including hemolysis reaction and API Listeria

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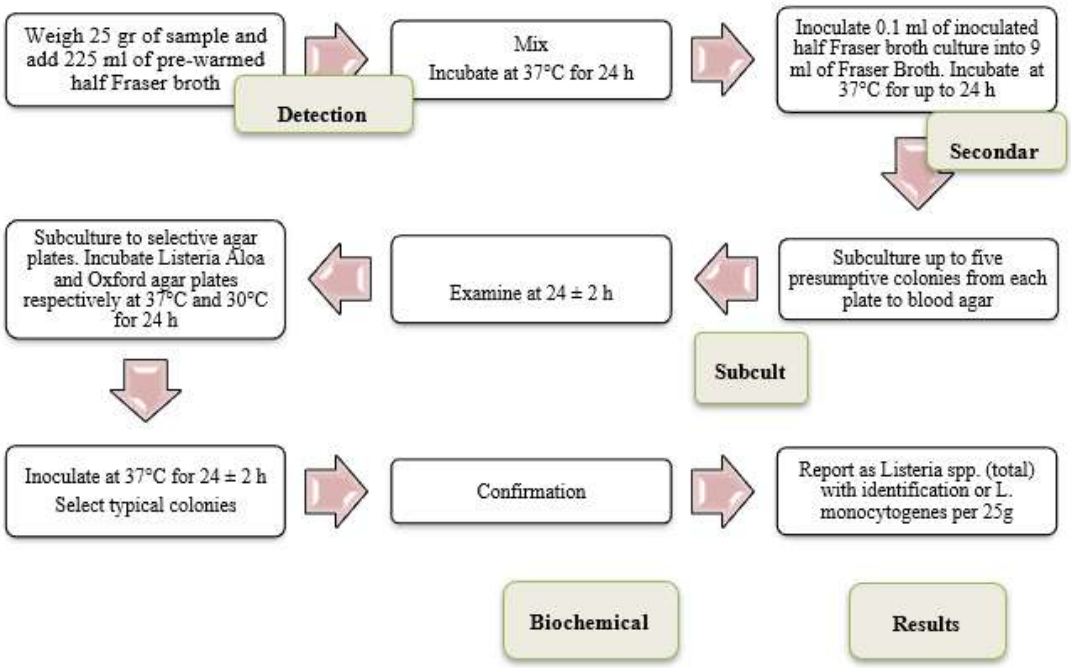

Figure 5. Flowchart for the detection of *Listeria monocytogenes* and other *Listeria spp*., in shrimps

The API Listeria strip consists of 10 microtubes containing dehydrated substrates (DIM, ESC, αMAN, DARL, XYL, RHA, MDG, RIB, G1P, RAG) which enable the performance of enzymatic tests of sugar fermentation. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents.

The inoculum prepared from well isolated colonies, was distributed into each tube avoiding the formation of bubbles. After incubation for 18-24 hours at 36 ± 2°C in aerobic conditions, were read all the reaction within 3 minutes after adding ZYM B reagent to the DIM test. All the reactions were recorded whether they were positive or negative according to the table. Data were transferred to a Microsoft Excel spreadsheet for analysis.

3. Results and Discussion

3.1. Overall prevalence of Listeria spp. positive and L. monocytogenes positive samples

The overall prevalence of *Listeria spp*. in shrimps and swabs samples were 8.3%. Table 1 and Figure 6 give a breakdown of the types of samples tested and the types of positive samples. Out of 358 samples tested, 30 samples were positive for *Listeria spp*. meanwhile *L. monocytogenes* were identified in 5 samples with a prevalence 1.39%.

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Table 1. Prevalence of *Listeria spp*. and other species in shrimps and environmental samples. % expressed as the number of positive samples/ number of samples analyzed (x100).

Samples	No	*Listeria* spp.	*L. monocytogenes*	*L. innocua*	*L. welshimeri*	*L. ivanovii*
Semiprocessed shrimps	270	17 (6.2%)	1 (0.37%)	8 (2.96%)	4 (1.48%)	7 (1.48%)
Frozen shrimps	62	7 (11.1%)	3 (4.7%)	4 (6.3%)	2 (3.17%)	3 (4.7%)
Swabs	25	6 (24%)	2 (8%)	1 (4%)	1 (4%)	1 (4%)
TOTAL	**358**	**30 (8.3%)**	**5 (13.9%)**	**13 (36.3)**	**7 (19.5%)**	**11 (30.7%)**

The *Listeria spp*. and *L. monocytogenes* prevalence, obtained from our study is comparable to results reported from Japan [15], Spain [3] , Sweden [13] and India [4].

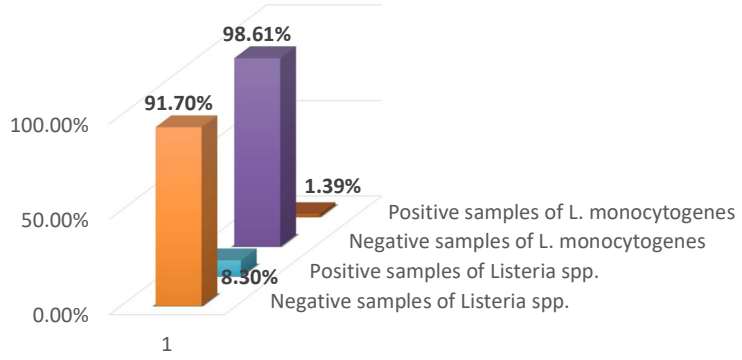

Figure 6. The total incidence of Listeria spp. and L. monocytogenes in shrimps and environments samples

Samples found positive included semi processed shrimp, frozen shrimp and swabs (Table 1). Listeria species were found in samples from different processing plants.

The remaining 25 isolates were identified as *L. innocua, L. welshimeriand L. ivanovii*. The Figure 7 presents the findings related to percentages of *Listeria spp*. strains isolated. The most common species identified were *L. innocua* with a total of 43.3%. The least common species were *L. monocytogenes* with a total of 16.6%. The two other non-pathogenic member of genus *Listeria, L. welshimeri and L. ivanovii*, were isolated in considerable percentage, respectively 23.3% and 33.3%.

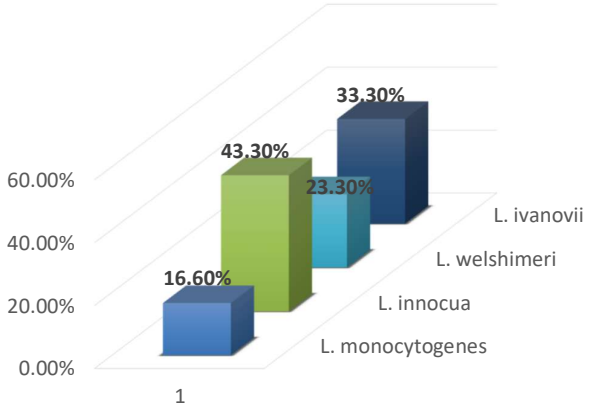

Figure 7. Incidence of *Listeria spp*. strains

These data are consistent with relative incidences reported from seafood's, shrimps and environmental samples. For example, the prevalence of *L. innocua* in shrimp in our study is comparable with other reports [7], [9]. As in other raw foods, fishery products more frequently contain *L. innocua* than *L. monocytogenes*. Since both species share ecological niches, the presence of *L. innocua* is considered an indicator of

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possible contamination with *L. monocytogene*s [8]. Higher incidence of *L. ivanovvi* were conveyed by Mashak et al. in 2021 [10]. They exhibited that the incidence of *L. ivanovii* in seafood sample was 20%.

3.2. Overall prevalence of Listeria spp. and L. monocytogenes positive samples in shrimps

The data given in Figure 8, characterizes the incidence of *Listeria spp*. and *L. monocytogenes* amongst two kinds of shrimp samples. From the total of 333 semi-processed and frozen shrimp samples tested, were found to be contaminated with *Listeria spp*. 24 samples (7.2%). Meantime *L.monocytogenes* was identified in 4 cases (1.2 %).

As the Figure 8 indicate, only 17 (6.2%) out of 270 samples of semi-processed shrimps examined for the incidence of *Listeria spp*. resulted positive. At the same time the prevalence of *L. monocytogenes* is very low, 1 out of 270 (0.37%).

Sixty-three frozen shrimp samples collected were analyzed also for the presence of *Listeria spp*. and *L. monocytogenes*. 7 (11.10%) out of the total 63 samples resulted positive for *Listeria spp*, from which three frozen shrimp samples (4.7%) showed the presence of *L. monocytogenes* (Tables 1, Figure 8).

If we discuss about the differences in prevalence of *Listeria spp* and *L. monocytogenes*, between semi processed and frozen samples, it is noticed that incidence of *L. monocytogenes* is very low in semi processed shrimps. The findings indicate the possible effect of processing. Many of the seafood products sampled in this study were semi-processed and would receive subsequent heat treatment prior to consumption. This would significantly reduce the potential of these foods as agents of food borne disease.

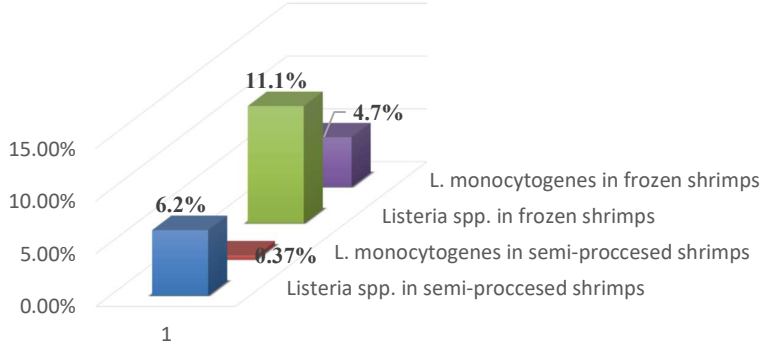

Figure 8. The total incidence of Listeria spp.and L. monocytogenes in semi-processed and frozen shrimps

The results in our study are in line with some previous studies. Mashak et al. 2021 [10], found higher incidence of *Listeria* spp. in seafood samples harbored (11.42%), meanwhile the incidence of *L. monocytogenes* amongst the fish shrimps, lobster and crab samples was 9%, 6%, 7% and 6%, respectively. Rahimi et al. 2012 [5], reported that the incidence of *L. monocytogenes* in frozen and fresh seafood samples were 1.9%. Zarei et al. 2012 [17] described the low incidence of *L. monocytogenes* in sea-food samples collected from Iran (1.4%).

3.3. Overall prevalence of Listeria spp. and L. monocytogenes positive samples in swabs

Out of a total 25 swab samples collected from the seafood processing plant, only six samples resulted positive for the presence of *Listeria spp*. The six positive strains belong to *L. monocytogenes* (8%), *L. innocua* (4%), *L. welshimeri* (4%) and *L. ivanovii* (4%). The overall incidence, 24% of *Listeria* spp. in swabs from environmental samples was high and indicates a possible route of contamination of shrimps during storage and processing. *L. monocytogenes* prevalence swabs is higher than in other species. The presence of the same species of *Listeria* (*L. monocytgenes, L. innocua, L. welshimeri, L. ivanovi*) both in swabs and shrimps, shows that the processing environment may be particularly important source of *Listeria spp*. contamination in food production chain. Similar results have been reported from the other studies in many countries.

Jeyasekaran et al. 2003 [7], have observed that 7.6% of environmental swab samples obtained from fishing harbour, fish landing centers, and seafood processing

plants, were positive for *L. monocytogenes*. Another study published in, reported that seafood processing plants had 4.1 % incidence of *L. monocytogenes.*

L. monocytogenes is a bacterial pathogen capable of adhering to many surfaces. It has been reported that humans may be carriers of *L. monocytogenes* and can contaminate the plants. Workers handling raw materials in food plants may carry Listeria on their hands and thus may contaminate surfaces, e.g. doors.This pathogen has the ability to persist for years in food industry environments, including seafood premises leading to product contamination. It has been reported that food production environmental areas can serve as source of *L. monocytogenes* contamination.

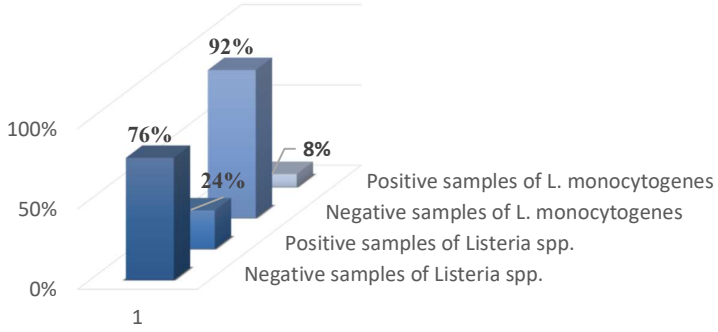

Figure 9. The total incidence of Listeria spp.and L. monocytogenes in swabs

The results clearly indicate that the *Listeria spp*. and *L. monocytogenes* are present in the shrimps from food processing industry in Albania, but contamination rate in these categories is low and represents a low risk for public health. The higher prevalence in frozen shrimps samples compare to processed shrimps indicate that the effective processing procedures are in place.

4. Conclusions

This study demonstrates that *Listeria spp*. and *L. monocytogenes* are present in seafood products. The total incidence (7.2%) indicates the potential public health hazard. The governmental institutions should take measures for monitoring and controlling during processing and in the market.

The incidence is highest in frozen shrimps (11.1% *Listeria spp*. and 4.7% *L. monocytogenes*).

Listeria spp. and *L. monocytogenes* were isolated in low percentage in processed shrimps (6.2% *Listeria spp*. and 0.37% *Listeria monocytogenes*). The results clearly indicate these pathogens are present in the shrimps, but contamination rate in these categories is low and presents a low risk for public health.

The higher prevalence of *Listeria spp*. and *L. monocytogenes* in frozen shrimps' samples compared to processed shrimps indicates that effective processing procedures are in place.

Listeria spp. and *L. monocytogenes* were present in processing environments in high percentage 24% and 8% respectively. The implementation of good hygiene practices, hazard analysis systems and critical point control (HACCP) are in place.

Our survey indicates that L. innocua (43% of *Listeria spp*.) were isolated more frequently than *L. monocytogenes* (16.6% of *Listeria spp*.). Since both species share ecological niches, the presence of *L. innocua* is considered an indicator of possible contamination with *L. monocytogenes*.

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