Detection of *Listeria monocytogenes* in wild and cultured Nile Tilapia fish (*Oreochromis niloticus*)

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Abstract

In the present study one hundred random samples of wild and cultured (50 each) of fresh Nile Tilapia fish (*Oreochromis niloticus*) which were purchased from fish sale markets in Assiut City. These samples were subjected to bacteriological examination for determination their contamination by *Listeria* spp. The obtained microbiological results showed that the overall incidence of *Listeria* spp. were 22 % and 16 % in the examined wild and cultured Nile Tilapia fish samples, respectively. The identified *Listeria* spp. were *L. monocytogenes* (6 and 10 %). Concerning the other *Listeria* species, 11 isolates, nine isolates (19%) were *L. ivanovii*, one isolate (2%) were *L. seeligeri* and one (2%) isolate were *L. welshimeri*. In conclusion, the obtained results revealed that some wild and cultured Nile Tilapia fish was contaminated with *L. monocytogenes*; this pathogens could pose serious risk to public health. It is thus necessary to perform continuous surveillance for *L. monocytogenes* in aquatic fish in Assiut.

Keywords: wild tilapia, cultured tilapia, *Listeria monocytogenes*

1. Introduction

Fish plays an important role in the human diet, and there is an observed increase in the consumption of fish in Egypt. Nutritionists recommend seafood because of its high nutritional value. Seafood is an excellent source of high-quality protein and contains lipids with high levels of unsaturated fatty acids, which are claimed to reduce the risk of cardiovascular disease. In addition, seafood is tender, easily digested, and a good source of many important vitamins and minerals (Ghanbari et al., 2013)⁶.

Nile tilapia (*Oreochromis niloticus*) production is ranked on the top of aquaculture fish in Egypt (Omaima M. Ahmed, 2019)¹⁵. Tilapia is one of the most important economic freshwater fish in Egypt and considered one of an excellent quality fish characterized by poor fat and rich vitamins and good sensorial properties of fish making it more suitable and an appetizing fish to the consumers. The intensive farming of tilapia, *Oreochromis* spp. is rapidly expanding and tilapias are the second most widely farmed fish in the world with annual production exceeding 3 million tons in 2010 (FAO, 2012)³.

*Listeria monocytogenes* is unlike most other food borne pathogens in that it only rarely causes the typical symptoms of gastroenteritis and the illness in human can range from a mild flu-like symptom to severe manifestation which usually takes the form of meningitis or septicemia. The elderly and those with weakened immune systems are especially vulnerable to infection. The other group particularly at risk from listeriosis is pregnant women, who may suffer mild symptoms that lead to infection of the fetus and then to a miscarriage or stillbirth (Rocourt et al., 2001)¹⁸.

In Assiut, wild and cultured fresh Nile tilapia are the most perishable in fish markets as they are marketed without inspection or quality control as well as, in many of these markets the adopted hygienic measures during storage and sale of fish are neglected which may lead to economic losses or risk the public health. So, this study was designed to investigate the presence of *L. monocytogenes* in wild and cultured Nile Tilapia fish samples collected from selected fish markets in Assiut city, Egypt.

2. Materials and methods

2.1 Sampling: One hundred random samples of wild and cultured fresh Nile Tilapia fish samples (50 each) of different sizes were obtained from fresh fish retailers and fish markets in Assiut city. Fish samples were immediately transferred to individual sterile bags and buried in ice in an ice box. The collected fish samples were dispatched within 1 h to the laboratory with a minimum of delay. At the laboratory, the collected samples were subjected to bacteriological examination for detection of *Listeria monocytogenes*.

2.2 Microbial analysis

2.2.2 Preparation of samples: For bacteriological examination, the scales were removed by sterile instrument then the skins of tilapia fish were cleaned and disinfected using 70% ethanol and Povidone-iodine. By using a sterile scalpel blade the skin was removed from muscles and the musculature of the fishes were cut aseptically using sterile forceps and scalpels (Ondo-Azi et al., 2013)¹⁶. Samples were homogenized and inoculated in media specific for *Listeria monocytogenes*.

2.2.3 Isolation of *Listeria* spp. (Hitchins and Jinneman, 2011)⁸: Ten gram of sample was inoculated in 90 ml of Listeria selective enrichment broth (LSEB) (Oxoid, CM0862) and incubated at 30 °C for 24–48 h. Then spread 0.1 ml of the enrichment broth culture onto Oxford agar (Biolife, BC1801) plates. Plates were incubated at 35 °C. Bacterial growths were examined after 24 and 48 h (black to brown colonies). Serological identification of *Listeria* spp. was done by Listeria Latex Agglutination Kit.
3. Results

Table 1: Incidence of *L. monocytogenes* and other *Listeria* spp. in wild and cultured fresh Nile Tilapia fish (*Oreochromis niloticus*) samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>No.</th>
<th><em>Listeria</em> spp. +ve</th>
<th>%</th>
<th><em>L. monocytogenes</em> +ve</th>
<th>%</th>
<th><em>L. ivanovii</em> +ve</th>
<th>%</th>
<th><em>L. welshimeri</em> +ve</th>
<th>%</th>
<th><em>L. seeligeri</em> +ve</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild fish</td>
<td>50</td>
<td>11</td>
<td>22</td>
<td>7</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cultured fish</td>
<td>50</td>
<td>8</td>
<td>16</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>19</td>
<td>38</td>
<td>17</td>
<td>18</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Discussion

The obtained results of the current study which showed in Table 1 revealed that *Listeria* spp. (22% and 16%) in the examined freshwater and farmed tilapia, respectively. The identified *Listeria* spp. in the current study were *L. monocytogenes* (6 and 10%), *L. ivanovii* (14 and 2%), *L. welshimeri* (0 and 2%) and *L. seeligeri* (2 and 0%) in the examined freshwater and farmed fish, respectively (Table 1).

There are two sources of fish contamination with *Listeria*, which includes; the attack of *Listeria* from intestinal contents to other fish tissues like muscles especially when the time from fish death till removing viscera is more than a few hours (Ertas et al., 2005) [4], cross contamination (fish manipulation, using contaminated equipments and inappropriate transport) (Gudbjornsdtottir et al., 2004) [7].

Near similar results were recorded by other researchers as Eltholth et al., (2018) [3] who found the contamination of cultured (farmed) tilapia with *L. monocytogenes* (7.7%) in Egyptian fresh fish markets in Kafrelsheik governorate, Egypt. Also, Hussein et al. (2015) [9] who could isolate *Listeria* spp. from fish samples in Assiut City, with incidence of 7%, 33%, 3%, 8%, 5%, 1% for *L. monocytogenes*, *L. innocua*, *L. ivanovii*, *L. welshimeri*, *L. seeligeri*, *L. grayi*, respectively.

This obtained result was higher than that reported by Rahimi et al. (2012) [17] who isolated *L. monocytogenes* from raw fish with incidence of 2.5% and Mahmoud- Heba Allah (2016) [13] who recorded the presence of *L. monocytogenes* with percentage 2.7 and 0% in the examined farmed and fresh tilapia fish collected from commercial fish markets in Assiut city, Egypt. Also, lower incidence (4.3%) of *L. monocytogenes* was recorded by Terentjeva et al. (2015) [19] in freshwater fish in Latvia. On the other hand, higher percentages were obtained by Mena et al. (2004) [14] who could detect this pathogen with rate of 12% in raw fish in Portugal; Mohammed-Ghada (2012) [12] with incidence of 26.7% in raw fish sold in Assiut city and Ammar (2014) [14] with rate of 14% in freshwater fish in Assiut city, Egypt.

Further, many researchers recorded isolation of *Listeria* spp. from raw fish with variant percentages as Jallawar et al. (2000) [10] who could detect 39 (20%) isolates of *Listeria* spp. in India. Among them, 26 (67%), 8 (21%), 3 (8%) and 2 (5%) were classified as *L. monocytogenes*, *L. seeligeri*, *L. grayi* and *L. welshimeri*, respectively.

Compared to Listeriosis outbreaks associated with other foods, a low number of cases have been linked to seafood, potentially due to low numbers of *L. monocytogenes* generally present in seafood as well as reduced consumption of raw fish (EFSA and ECDC, 2013) [2]. Furthermore, the rate of contamination of raw fish with *L. monocytogenes* might vary among different geographical areas and processing plants (Jami et al., 2014) [15].

5. Conclusion

This study concluded that *L. monocytogenes* was isolated from the examined wild and cultured fresh Nile Tilapia with incidence 6 and 10%, respectively. Contamination of wild and cultured fresh Nile Tilapia with this pathogen indicated unsanitary condition under which they produced resulting in public health hazards. This study emphasizes the need for continuous surveillance for *L. monocytogenes* in aquatic fish in Assiut city, Egypt.

6. References

2. EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The European Union summary report on trends and sources of zoonosis, zoonotic agents and food-borne outbreaks in 2011. EFSA J. 2013.
5. FAO (Food and Agriculture Organization). The state of world fisheries and aquaculture, 2012, p209.


