INCIDENCE OF *LISTERIA MONOCYTOGENES* IN FRESH TILAPIA NILOTICA FISH

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ABSTRACT

In the present study one hundred random samples of fresh Tilapia nilotica samples were purchased from different fish markets in Cairo, Kalyobia and Beheira governorates. The collected samples were bacteriologically examined for detection of *L. monocytogenes*. The obtained results revealed that 7 of *L. monocytogenes* were isolated from fresh Tilapia nilotica fish samples in Egyptian fish market identified by biochemical test. The 7 isolates were exposed to PCR technique and only 4 isolates were confirmed to be *L. monocytogenes*. The highest prevalence of *Listeria monocytogenes* (3%) was observed in fresh Tilapia nilotica from marketed fish while lower prevalence of *L. monocytogenes* (1%) was seen in fresh Tilapia nilotica from farm fish. Concerning the other *Listeria* species, 27 isolates, eight isolates (8%) were *L. ivanovii*, five isolates (5%) were *L. innocua*, five isolates (5%) were *L. seeligeri*, six (6%) isolates were *L. welshimeri*, and three (3%) isolates were *L. grayi*. The public health significance of the isolated microorganisms and the probable sources of Tilapia nilotica contamination as well as the suspected recommendations to prevent them to gain access to such food items were discussed.

KEY WORDS: *Listeria*, *Listeria monocytogenes*, Tilapia nilotica.

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1- INTRODUCTION

The shortage of human dietary protein can be provided by fish protein, particularly in developing countries, where the protein shortage is serious. One of the most important perishables types is the fish protein as Tilapia spp.

Listeria monocytogenes is a Gram-positive, facultative bacterium that is ubiquitous in nature. Fish and seafood harvested from natural environments have been identified as potential sources of *Listeria* in the human diet (Farber *et al.*, 1991).

There is possibility that *L. monocytogenes* could survive and / or grow in the foods as they move along the distribution chain. Three outbreaks of listeriosis that occurred in the 1990s were traced to fish products. These demonstrate that fish and fish products are suitable vehicles for the transmission of the pathogen to human (FAO, 1999).

Listeria monocytogenes is unlike most other food-borne pathogens in that it only rarely causes the typical symptoms of gastroenteritis. The illness in human can range from a mild flu-like symptom to severe manifestation. Severe manifestation usually takes the form of meningitis or septicemia. The elderly and those with weakened immune systems are especially vulnerable to infection, and this goes some way to explain the high mortality rate 30%. 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.*, 1997).

However, rarely, persons without these risk factors can also be affected. According to CDC (2002) there are about 1,600 cases of listeriosis annually in the United States and listeriosis is the third leading cause of

death among major pathogens transmitted commonly through food.

Therefore, the purpose of the present study is to throw a light on the incidence and initial levels of *Listeria monocytogenes* in fresh *Tilpia nilotica* sold at the Egyptian market level and study the effect of heat treatment on the survival of *L. monocytogenes.*

2. MATERIAL AND METHODS:

2.1. Collection of samples:

A total of 100 random samples of fresh *Tilapia nilotica* samples (body weight of each fish ranging from 500 ± 50) were purchased from different fish market in Egypt. Moreover, 40 samples were purchased from different fish markets in Shoubra and Abbasia, Cairo governorates. 30 samples were purchased from different fish markets in Benha, Kaliobeya governorates and 30 samples were purchased from farm fish in Beheira governorate. The collected samples were transferred directly to the laboratory in an ice box under complete aseptic conditions. The collected samples were subjected to bacteriological examination for detection of *Listeria monocytogenes*.

2.2. Isolation of L. monocytogenes:

The technique recommended by Hitchins (2003) was adopted. 5g of each section was pounded with a sterile mortar and pestle. Homogenization was carried out to obtain a uniform distribution of cells through stock culture. Five gram of each sample were blended with a Moulinex-type blender with 45 ml *Listeria* enrichment broth, and then incubated at 30ºC for 48 hours. A loopful from *Listeria* enrichment broth was streaked onto a PALCAM agar plate media, and then incubated at 37ºC for 24-48 hrs. Up to 34 typical colonies (bluish grey to black colonies with a black halo and a sunken center) were picked up and streaked onto a Trypticase soy broth, supplemented with 0.6 yeast extract (TSA-YE) and incubated at 30ºC for 24 hrs till obtaining satisfactory pure separate colonies, then submitted to biochemical identification to *Listeria* species (Van Netten *et al.,* 1989).

2.3. Identification of Listeria monocytogenes:

2.3.1 Microscopical examination:

Films were prepared from the pure culture of the organism and stained with Gram's stain and examined microscopically. Gram positive short rods, occurring in short chains of three to five organisms with a typical diphtheroid arrangement (Anneschuchat *et al.,* 1991) and Donnelly, 1994).

2.3.2 Biochemical identification:

Identification of *Listeria monocytogenes* was carried out according the outlines recommended by McClain *et al.* (1998).

2.3.3. DNA extraction, primers and PCR:

In order to confirm the genus of *Listeria monocytogenes*, a 702 bp of hyl gene specific for *L. monocytogenes* was amplified using polymerase chain reaction (PCR). DNA extraction was performed using boiling method described by Bansal (1996). In this study one set of primer was used, hyl gene specific for confirmation of *L. monocytogenes* and not any other type of *Listeria*. The sequence of hyl gene:
Sequence 5\- 3\ LM1 CCT-AAG -ACG-CCA-AT C-GAA
LM2 CCT-AAG -ACG-CCA-AT C-GAA
Amplification was performed in Mastercycler with an initial denaturation step at 95ºC for 5 min; 30 cycle of 95ºC for 15 sec; 57 ºC for 2 sec and72 ºC for 30 sec. Final extention at 72 ºC for 5 min. The amplicone size was 702 bp (Mengaud *et al.,* 1988). Five microliters of the reaction mixture was mixed with 2 µl of loading buffer and separated on a 1.3% agarose gel in 1x TBE using Gene Ruler 100 bp plus DNA Ladder. The PCR product was visualized by ethidium bromide staining.

3. RESULTS:

The obtained results revealed that 7 of *L. monocytogenes* were isolated from fresh Tilapia nilotica fish samples in Egyptian fish market identified by biochemical test. The 7 isolates were exposed to PCR technique and only 4 isolates were confirmed to be *L. monocytogenes*. Concerning the other *Listeria* species, 27 isolates, eight isolates (8%) were *L. ivanovii*, five isolates (5%) were *L. innocua*, five isolates (5%) were *L. seeligeri*, six (6%) isolates were *L. welshimeri*, and three (3%) isolates were *L. grayi*.

4. DISCUSSION:

Listeria monocytogenes is ubiquitous in nature; fish and seafood harvested from natural environments have been identified as potential sources of listeria in the human diet.

The results recorded in table (1) revealed that seven of *L. monocytogenes* isolated from fresh *Tilapia nilotica* fish samples in Egyptian fish market identified by biochemical test. The seven isolates which recorded by biochemical test was exposed to PCR technique (figure 1) and only four were confirmed to be *L. monocytogenes*, with higher percentage (5%) in fresh *Tilapia nilotica* collected from fish markets in Cairo than fresh *Tilapia nilotica* collected from fish markets in Benha, Kaliobeya (3.3%) and fresh *Tilapia nilotica* collected from farm fish in Beheira (3.3 %).

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), cross contamination (fish manipulation, using contaminated equipments and inappropriate transport) (Gudbjornsdottir *et al.,* 2004).

These results were nearly lower than those obtained by Ebrahim Rahimi *et al.* (2012), while similar results (4.6%) was recorded by Parihar *et al.* (2007) who examined fresh fish for *Listeria monocytogenes* in the seafood markets in Goa, India. On the other hand, higher results were reported by Krzysztof Kwiatek *et al.* (2004).

Incidence of *Listeria* species other than *Listeria monocytogenes* isolated from examined samples of *Tilapia nilotica* fish was shown in table (2 and 3). Accurately, eight isolates (8%) were *L. ivanovii*, five isolates (5%) were *L. innocua*, five isolates (5%) were *L. seeligeri*, six (6%) isolates were *L. welshimeri*, and three isolates (3%) isolate was *L. grayi*.

Nearly similar results were obtained by Bayleyegn Molla *et al.* (2004) who isolated *L. innocua* (5.6%), *L. seeligeri* (6.7%), *L. welshimeri* (6.8), *L. ivanovii* and *L. grayi* (each 3%).

Current microbiological culture methods rely on growth in culture media, followed by isolation, and biochemical and serological identification. However, the detection of this pathogen in food by these standard culture methods is made difficult by the sporadic or low levels of contamination (<100 cfu/ g) can't be relied incase of the presence of a high level of background micro-flora and competitor organisms that could mask the presence of *L. monocytogenes*, or even interference due to food matrix components (Norton *et al.,* 2001). Moreover, these methods are laborious and time consuming, requiring a minimum of five days to recognize *Listeria spp.* and about 10 days to identify *L. monocytogenes* by confirmatory tests (Anon, 1996) while immediate action should be taken in case of contamination since it is of fundamental importance to ensure the safety of food products, especially the case of those food matrices having short shelf-lives, such as fresh fish.

In the past years, advancements in biotechnology have resulted in the development of rapid methods that reduce analysis time and offer great sensitivity

and specificity in the detection of pathogens (Olsen *et al.,* 1995). The results recorded in table (4) confirmed that PCR has been increasingly used for the rapid, sensitive and specific detection of food borne pathogens as only four isolates from seven gave a characteristic band at 702 bp to hyl gene specific for *L. monocytogenes* and confirmed to be *L. monocytogenes*.

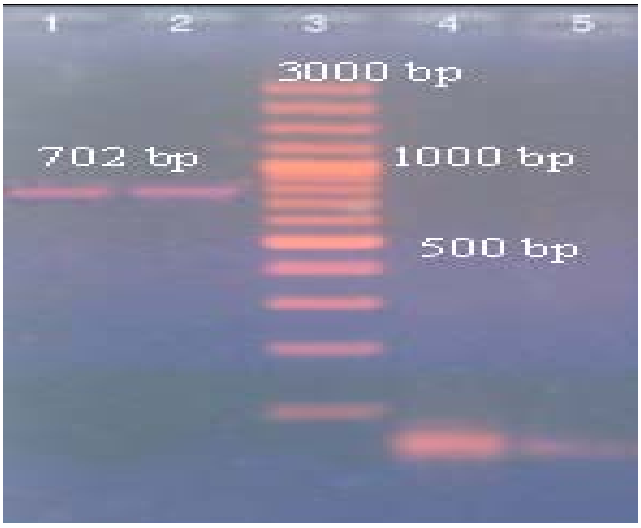
Listeria monocytogenes is unlike most other food borne pathogens in that it only rarely causes the typical symptoms of gastroenteritis. The illness in human can range from a mild flu-like symptom to severe manifestation. Severe manifestation usually takes the form of meningitis or septicemia. The elderly and those with weakened immune systems are especially vulnerable to infection, and this goes some way to explain the high mortality rate of around 30%. 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).

The present study showed that fresh *Tilapia nilotica* fish obtained from the Egyptian market contain different species of *Listeria* particularly *L. monocytogenes*, which regarded as an important human pathogen, which constitute a public health hazard.

Table (1): Incidence of *L. monocytogenes* in examined samples of *Tilapia nilotica*.

Fish	No. of examined Samples	Positive samples	
		No.	%
Cairo fish markets	40	4	10
Kalyobia Fish market	30	2	6.7
Farm Fish	30	1	3.3
Total	100	7	7

Figure (1): Electrophoretic pattern of Re-PCR products for examined *Tilapia nilotica* compared to the reference strain using hyl gene.



1- *L. monocytogenes* reference strain 2- *Tilapia niloticus* isolate 3- Marker (Fermentas)

Table (2): Incidence of *Listeria* species other than *L. monocytogenes* isolated from examined samples of *Tilapia nilotica*.

Fish	No. of examined Samples	*Listeria* species other than *L. monocytogenes*	
		No.	%
Cairo fish markets	40	14	35
Kalyobia fish market	30	6	20
Farm Fish	30	7	23
Total	100	27	27

Table (3): Incidence of *Listeria* species other than *L. monocytogenes* from examined fresh *Tilapia nilotica* samples.

Type of examined samples	No. of examined samples	Number and percentage of positive isolates									
		L. ivanovii		*L. innocua*		*L. seeligeri*		*L. grayi*		*L. welshimeri*	
		No.	%	No.	%	No.	%	No.	%	No.	%
Cairo fish markets	40	5	12.5	3	7.5	2	5	1	2.5	3	7.5
Kaliobeya fish market	30	1	3.3	1	3.3	2	6.6	1	3.3	1	3.3
Farm Fish	30	2	6.6	1	3.3	1	3.3	1	3.3	2	6.6
Total	100	8	8	5	5	5	5	3	3	6	6

Table (4): Incidence of *L. monocytogenes* in the examined samples of *Tilapia nilotica* confirmed by PCR.

Fish	No. of examined Samples	Confirmed *L. monocytogenes* isolated by PCR	
		No.	%
Cairo fish markets	40	2	5
Kaliobeya fish market	30	1	3.3
Farm Fish	30	1	3.3
Total	100	4%	4%

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مدى تواجد ميكروب الليستريا مونوسيتوجينز في السمك البلطى النيلى الطازج

ابو بكر مصطفي ادريس–أماني محمد سالم–مايكل عادل فؤاد

قسم مراقبة الأغذية –كلية الطب البيطري –جامعة بنها

الملخص العربي

تعتبر الأسماك أحد أهم مصادر البروتين الحيواني بالإضافة إلى احتوائها على نسبة عالية من الأملاح المعدنية وبعض الفيتامينات، إلا أنها قد تتعرض للتلوث بمختلف أنواع الميكروبات سواء المسببة للفساد أو الممرضة مثل ميكروبات الليستريا مما يشكل خطرا على الصحة العامة. لذلك قد تم جمع عدد (100) عينة من سمك البلطى النيلى الطازج وقد جمعت من أسواق السمك المختلفة فى محافظتى القاهرة والقليوبية وكذلك من مزارع سمكية فى محافظة البحيرة لتبيان مدى تلوثها بميكروب الليستريا مونوسيتوجينز. وقد دلت النتائج على أن نسب عزل ميكروبات الليستريا من عينات سمك البلطي النيلي هي 27%. كما أكدت نتائج الدراسة أن عينات سمك البلطى النيلى كانت ملوثة بميكروب الليستريا مونوسيتوجينز وقد تم التأكد من المعزولات بواسطة ال PCR (4%) فقط. وقد تم عزل أنواع أخرى من ميكروبات الليستريا من سمك البلطي النيلي محل الدراسة بنسب مختلفة مثل: *L. ivanovii, L. innocua , L. seeligeri, L. grayi and L. welshimeri* هذا وقد اهتمت الدراسة بتوضيح مصادر تلوث سمك البلطى النيلى بميكروبات الليستريا، مع بيان الأهمية الصحية لتلك الميكروبات المعزولة ووضع بعض التوصيات لتجنب تلوث هذه الأسماك بتلك الميكروبات الخطيرة من اجل حماية المستهلك.

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