

# **MICROBIOLOGICAL ANALYSIS OF FARM FISHES IN ALEXANDRIA GOVERNORATE**

A Thesis

Presented to the Graduated School

Faculty of Veterinary Medicine, Alexandria University

In Partial fulfillment of the  
Requirement for the Degree

**Of**

**Ph.D. Of Veterinary Science**

**In**

**Milk Hygiene**

**By**

**Nahla Ahmed Ahmed El-Shabasy**

**2009**

# التحليل الميكروبيولوجي لأسماك المزارع فى محافظة الإسكندرية

رسالة علمية

مقدمة إلى الدراسات العليا بكلية الطب البيطري – جامعة الإسكندرية  
استيفاء للدراسات المقررة للحصول علي درجة

دكتور الفلسفة في العلوم الطبية البيطرية  
في

الرقابة الصحية على اللحوم والأسماك  
والمنتجات الحيوانية

مقدمة من

السيدة ط.ب./ نهلة أحمد أحمد الشباسى

2009

## **Advisors' Committe**

### **Prof. Dr. Ebeed Abd El-Atty Saleh**

Professor of Meat Hygiene  
Head of Food Hygiene Department,  
Faculty of Veterinary Medicine, Elbostan,  
Damanhour Branch, Alexandria University

### **Prof. Dr. Ahlam Amin El-Leboudy**

Professor of Milk Hygiene  
Head of Food Hygiene Department,  
Faculty of Veterinary Medicine,  
Alexandria University

## لجنة الإشراف

الأستاذ الدكتور/ عبيد عبد العاطى صالح  
أستاذ الرقابة الصحية على اللحوم  
ورئيس قسم الرقابة الصحية على الأغذية  
كلية الطب البيطرى بالبستان  
جامعة الإسكندرية- فرع دمنهور

الأستاذة الدكتورة/ أحلام أمين اللبـودى  
أستاذ الرقابة الصحية على الألبان  
ورئيس قسم الرقابة الصحية على الأغذية  
كلية الطب البيطرى بإدفينا  
جامعة الإسكندرية

## **1. INTRODUCTION**

In order to increase the level of the much needed protein of teeming population in Egypt, there has been increased interest of fish, which has traditionally been popular part of the diet in many parts of our country. Fish is considered one of the most nutritive and highly desirable food stuffs due to its contribution of high quality animal protein, its exceptional richness in calcium and phosphorous and generous supply of B-complex vitamins as well as its lower content of cholesterol.

In Egypt, the continuous increasing population renders the Egyptian government to embrace a program of intensive fishing from all available water sources. Thus, trials have been done during the last years by the concerned authorities to improve the fish quality and to preserve its high nutritive value.

The fish flesh, which is the main edible part, is generally sterile immediately after catching; however, it may become contaminated with different microorganisms during subsequent handling as these microorganisms can penetrate from skin and the gut to the flesh (*FAO, 1983; Brock et al., 1984 and Etzel et al., 1998*). Generally, fresh water fishes are subjected to many risks of contamination from various sources either during their presence in aquatic environment or after being harvested till they reach consumers (*Fattal et al., 1992*).

Accordingly, farm fish act as vehicle for certain types of bacteria and the chief sources of fish contamination are catching water, soil and handlers (*Austin, 1982 and Lamada, 1999*). Among these bacteria, Enterobacteriaceae group can be used as a useful indicator of faecal contamination and / or unsanitary handling of such fishes (*Nair and Nair, 1988*).

On the other side, the presence of Enterobacteria has an epidemiological interest as some of its members are pathogenic and may result in serious infections and food poisoning (*Varnam and Evans, 1991*). Examples of such serious pathogens include *E.coli*, *Salmonella*, *Vibrio parahaemolyticus*, *Staphylococcus aureus* and *Yersinia enterocolitica* organisms.

Enteropathogenic *E. coli* may constitute a public health hazard as it may give rise to severe diarrhoea in infants and young children as well as food poisoning and gastroenteritis among adult consumers (*Banwart, 1989*).

Salmonellosis is a worldwide problem responsible for food poisoning outbreaks but it is mainly difficult to determine whether the contamination of fishes occurred in their aqueous habitat or during their handling and marketing (*Etzel et al., 1998*).

*Vibrio parahaemolyticus* is widely distributed in water and recognized as the principal cause of gastroenteritis associated with consumption of fish (*West, 1989*).

*Yersinia enterocolitica* is considered the most psychrotrophic bacteria contaminating the fish and therefore illness results from eating of such fish although signs of spoilage may not be evident (*Varnam and Evans, 1991*). Moreover *Staphylococcus aureus* may represent a fatal public health hazard (*Bykawski, 1998*).

Fungal contamination of farm fishes is considered the main cause of spoilage which leads to off flavour and unpalatable taste and may constitute a public health hazard as well as many economic losses. Also, fungi were reported to be responsible for many fish diseases.

Considering all these hazards, farm fishes should not be subjected to unnecessary contamination and they should be free from such serious pathogens to ensure a maximum margin of consumer safety.

In Alexandria Governorate, farm fishes are the most purchasable fish in fish markets, but they are marketed without inspection or quality control measures. As well as, in many of these markets the adapted hygienic measures during fish sale and storage are neglected which may lead to risk of public health and economic loss.

Therefore, the present study was planned out to throw light on the hygienic quality of the most common farm fishes to determine their microbial contamination level to protect the health and welfare of

the consumers. Accordingly, the following examinations were carried out:

- 1- Determination of total bacterial count.
- 2- Determination of total Enterobacteriaceae count.
- 3- Determination of total coliform count. .
- 4- Identification of isolated Enterobacteriaceae.
- 5- Isolation and identification of enteropathogenic *E.coli*.
- 6- Isolation and identification of *Salmonellae*.
- 7- Isolation and identification of *Vibrio parahaemolyticus*.
- 8- Isolation and identification of *Yersinia enterocolitica*.
- 9- Determination of *Staphylococcus aureus* count.
- 10- Determination of total mould and yeast count.





## 2- REVIEW OF LITERATURE

### 2.1. Sources of fish contamination:

*Janssen and Mayers (1968)* revealed that fish may become actively infected with human pathogens by exposure to contaminated water and may constitute a hazard to public health.

*World Health Organization "WHO" (1968)* mentioned that fishes and shellfishes naturally do not carry a wide variety of pathogens, while contaminated ones are derived from polluted environment such as polluted water in which they live and improper handling.

*Lawsan (1970)* stated that fish do not normally suffer from food poisoning bacteria, however if they are harvested in polluted water, they may mechanically carry these organisms.

*Ghittino (1972)* reported that the bacterial flora of fish can reflect the hygienic condition of the water from which they were harvested. Fishes are considered to be indicators to the sanitary condition of water. He concluded that fish in polluted environment may be passive carriers of pathogenic bacteria, virus and fungi.

*Horsley (1973)* concluded that the fish flora on skin and gills may reflect the status of environmental pollution from which fishes were caught.

*Souter et al. (1976)* reported that the fish and fish products act as vehicles for all important bacteria causing food poisoning as



*Salmonella*, *Shigella*, *Staphylococci*, *Clostridium botulinum*, *Escherichia coli*, *Proteus*, *Streptococcus faecalis*, *Clostridium perfringens* and *Bacillus cereus*.

**Brown and Dorn (1977)** reported that fish retained in its digestive tract or in its integument human pathogens such as *E. coli*; *Salmonella* and *Shigella* species without becoming ill. Fish may be contaminated with polluted water during processing or by fishing vessel.

**Abo El-Naga (1978)** isolated species of *Acinetobacter* and *Micrococcus* from the skin of fresh water fish (*Burbus* species). He reported that a count ranged between  $16 \times 10^3$  to  $46 \times 10^6$  bacteria/cm<sup>2</sup> and from 0 to 6000 coliforms/cm<sup>2</sup> could be found on the skin of fish.

**Potter (1978)** mentioned that fish was a food usually involved in food poisoning due to food handlers.

**Bryan (1980)** discussed that the causes of bacterial food – borne illness transmitted by fish could be attributed to fish from polluted waters, improper sanitation procedures in fishing vessels, processing plants and storage facilities as well as diseased food handlers, improper cooking time and improper refrigeration facilities.

**International Commission on Microbiological Specifications for Foods "ICMSF" (1980)** reported that the handling of freshly caught fish would inevitably contaminate it with Gram – positive



bacteria, meanwhile coliforms can be imparted to such fish by continuous contact with nets, ropes, deck boards, human hands and clothing during catching and afterwards.

***El-Morshdy et al. (1981)*** stated that polluted water, hands of workers, and dirty instruments were considered the major sources of raw fish contamination with coliforms.

***Fawzy et al. (1981)*** found that the rate of contamination of fishes with enteric bacteria depends upon the time elapsed from netting till marketing.

***Kampelmacher (1981)*** mentioned that microbiological contamination of worker's hands was studied at 13 food and 3 non establishments the pathogenic organisms. Most frequently isolated at various food establishments were enterobacteriaceae (10-100% of workers) and faecal streptococci (7-100%). *Esherichia coli* was isdated from 4-100% *staphylococcus aureus* from 17-100% *clostridium perfringens* from (0-88%) and *Salmonella Spp.* He concluded that contamination of worker's hands by food especially that of animal origin is of more importance than the consequence of toilet use.

***Roberts (1981)*** stated that freshly caught fish were usually free from bacteria; however, they could become contaminated from humans during handling.



*Youssef et al. (1981)* reported that fish act as a vehicle for many types of microorganisms. The chief source of fish contamination is water, soil and fish handlers.

*El-Wakeel et al. (1982)* studied the relationship between the bacterial load of *Tilapia nilotica* and the degree of water pollution with *Salmonella typhimurium* and *Escherichia coli*. They recommended that the degree of water pollution affected the bacterial load on both slime and skin of live fish. Although no organism was detected in fish muscles, they stated that fish could act as a carrier for pathogenic bacteria if caught from polluted water and also the bacterial load of fish could reflect the degree of water pollution.

*Chen and Chai (1982)* reported that ice melt drainage in fishholds of fishing vessels contained bacteria at levels of  $10^7$  to  $10^9$ /ml. They concluded that the reuse of ice was neither a recommended nor an accepted procedure, since this ice was contaminated.

*Meadaw's and Snudden (1982)* examined 303 samples of water from Chippewa river in Wisconsin in USA, for presence of *Yersinia enterocolitica*. The organism was recovered from 28 samples (8.25%), 25 of the positive samples were from the rural areas and 3 from the urban areas.



*National Academy of Science "NAS" (1985)* stated that the fish might acquire pathogenic microorganisms from the natural aquatic environments, from sewage contaminating harvesting areas, and/or from contamination by workers and utensils during harvesting, distribution and food preparation.

*Ahmed et al. (1986)* examined bacteriologically 83 living fish of *Tilapia nilotica* caught from different localities of River Nile and El-Ebrahimia Canal at Assiut City. They isolated 108 isolates of Enterobacteriaceae, differentiated as, 12 *Escherichia coli*, 18 *Klebsiella*, 5 *Aerobacter*, 3 *Citrobacter* and other 70 isolates of different species. They recommended that the isolation of pathogenic and potentially pathogenic bacteria from fish proved that these cold – blooded animals shared with other reservoir animals the responsibility of transmitting these noxious agents to man and animals.

*De-Boer et al. (1986)* found that 40% of the examined surface water samples were positive for *Yersinia enterocolitica*.

*Mousa (1986)* stated that the presence of large number of viable count of coliforms in examined fish and shell fish samples could be attributed to polluted water of rivers, lakes or even sea which is now used for sewage disposal.

*Frazier (1988)* stated that the flora of living fishes depend upon the microbial content of the water in which they live. The author added that contamination of fish flesh with bacteria may be



originated from mud, polluted water and subsequently handling, as well as from exterior slim.

**Ward and Baaj (1988)** , mentioned that poor handling and absence or unsatisfactory hygienic adopted in method of catching, smoking, preservation, transportation, packaging, storage and marketing at retail market were the main parameters of fungal contamination of smoked fish and sea food.

**Sedik et al. (1989)** examined bacteriologically 40 fish of each of *Tilapia nilotica* and *Mugil cephalus* to illustrate the effect of handling and distribution in the microflora of fresh and brackish water fish. *Escherichia coli*, *Citrobacter freundii*, *Klebsiella* and *Enterobacter* species were isolated in varying extents, 2 isolates of *Salmonella* were recovered from market samples, one isolate from each fish species. The high bacterial counts recorded in market samples were attributed to careless handling and contact with dirty instruments and ice during transport and icing.

**Khalil et al. (1990)** examined bacteriologically 28 living *Tilapia nilotica* and *Bagrus bayad* as well as the water from which the fish were caught. *Aeromonas* and *Staphylococci* species were isolated form both *Tilapia nilotica* and water samples. They stated that water plays a major role in fish contamination.

**El-Leboudi (1991)** examined 90 marine water samples and 15 water samples from a lake for the presence of *Yersinia enterocolitica*. The author isolated 2 strains from lake water, and



failed to isolate any strain of *Yersinia enterocolitica* from any of marine water samples.

**Debashis and Gupta (1992)** performed a study on the concentration of *Escherichia coli* and *Salmonellae* in ponds and around Calcutta, India. They found a significant linear correlation between concentrations of these bacteria in pond water and their recovered from several tissues of the fish. The authors attributed the causes of most enteric and other infectious diseases, reported from hospitals, in this region to these bacteria.

**Fattal et al. (1992)** stated that fish inhabiting faecally - polluted water were often used for human consumption, therefore, such fish could be contaminated with enteric human pathogens, and might pose a potential risk to public health.

**Ogbondeminu (1993)** isolated 502 strains of enteric bacteria from 94 samples from each of freshwater fish intestines and culture waters. *Escherichia coli* organisms represented 18.39 and 11.51% of the total strains obtained from intestines and waters, respectively. *Citrobacter*, *Enterobacter*, *Proteus*, *Salmonella*, *Serratia*, and *Klebsiella* species were also representatives for enteric bacteria in both samples.

**Pogorelova et al. (1993)** studied bacterial contamination of river water and fish from the Volga Delta. Opportunist pathogens were detected in 51% of fish samples. Bacterial species in fish