Vol 5 (1), January 2014; 30-35

Bacteriological Analysis of Nile Tilapia Fish (*Oreochoromis niloticus*)

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ABSTRACT

Fish is a major food in great demand throughout the world. Fish protein is better and safer than meat (animal protein) because it contains lower cholesterol. A research was conducted to analyze the bacterial habitat and content of Tilapia fish. Total seven fishes were taken. The samples were examined in microbiology laboratory by serial dilution method. Sections of the skin, gills and intestine of fishes were aseptically removed by means of a sterile scalpel and pair of sterile scissors. A serial dilution was prepared and from last dilution 0.1ml was plated on nutrient agar plates. Total eighteen different species of bacteria were isolated and identified. Gram negative include *Pseudomonas sp.* **and** *Enterobacter sp***., while gram positive species include** *Staphylococcus aureus* **and** *Streptococcus sp***. Some of these pathogens have tendancy to transmit to man (who eat fish meat or deal with fish and fish products). Streptococcus infection was detected in high prevalence among cultured fresh water fishes, especially during summer seasons. The most common signs of Streptococcosis in fish were septicemia, skin ulcers, hemorrhages of the eye, in some cases changed cloudy and destructed (pop –eye) and hemorrhages on the skin especially in the base of fins and tail. Hence it is considered that a variety of bacterial species can be associated with fresh Tilapia fish related pathogen to humans.**

Keywords: Cultural Fish, Gills, Intestine, Nile Tilapia

INTRODUCTION

Fish has been one of the main source of foods for humans for many centuries and still constitute an important part of the diet in many countries (Leisner, *et al*. 2001). As a result, there is a considerable increase in the demand for fish being the cheapest source of animal protein (Ladipo, *et al*. 1981). The advantage of fish is its easy digestibility and high nutritional value. These important attributes makes the commodity readily susceptible to microbial attack particularly bacteria. Fish flesh naturally contains very low levels of bacteria (Adam & Tobaias, 1999). Disease out breaks in fish tank spreads very quickly and you have to first identify the type of disease before you can take action. The bacteria are transmitted by fish that have made contact with other diseased fish. Bacterial fish disease and infections are very common and are one of the most difficult health problems to deal with. Bacteria can enter the fish body through the gills or skin or it can stay on the surface of the body (Douglas, 2007). Shell fish such as Tilapia have a particular large pool of nitrogenous extractives and are even more prone to rapid spoilage, a factor which accounts for the common practice of keeping them alive until immediately prior to consumption.

The speed with which a product spoils is also related to the initial microbial load on the product. The natural flora of the environment may be contaminated with organisms associated with man such as members

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of the Enterobacteriaceae and Staphylococcus aureus which can grow well at 30-37ºC (Micheal, *et al*. 2007) Fish is among the most important sources of protein to human consumption, thus the study of the signs and lesions, induced by fish diseases, helps the protection in our national economy. Infectious diseases of cultured fish are among the most notable constraints on the expansion of aquaculture and the realization of its full potential (Plumb, 1994). The organisms from the environment around the fish may become closely associated with the external surfaces of the fish. There may be accumulation of the organisms at sites of damage, such as missing scales or abrasions. The organisms may enter the mouth with water or food and pass through and/or colonies the digestive tract (Austin & Austin, 1987). Scrutiny of the available literature suggests that fish have only low bacterial populations on the skin. Bacterial populations of skin is 10^2 to ~10^4 cm-2. Muscle has been considered by some to be sterile (Apun, *et al*. 1999), whereas other investigators have reported the presence of bacteria. Also, some workers have found bacteria in kidney and liver of healthy fish (Evelyn and McDermott, 1961). Gill tissue has been found to harbour high bacterial populations, e.g., up to 106. Enterobacteria, Gram-positive cocci, Pseudomonads, and Vibrios have been recovered from the gills of healthy juvenile trout (Nieto, *et al*. 1984).

Some taxa, such as Pseudomonas, have been implicated as causes of fish spoilage (Gillespie, 1981; Malle, 1994) by the production of histamines (Kim, *et al*. 2001) principally during storage of fish. Some of these pathogens that isolated from the skin and the internal organs of *O. nilotica* were *Aeromonas sp., Pseudomonas sp., Streptococcus sp.* (Abd El-Latif and Adawy, 2004; Laila, *et al*. 2004; El-Refaee, 2009; Attia, 2004). Some of these pathogens could transmit to man who eat fish meat or deal with fish and fish products (Goncalves, *et al*. 1992; Weinstein, *et al*. 1997; Zlotkin, *et al*. 2003). *Aeromonas sp*. and *Pseudomonas sp*. isolated from O. niloticus by 35.96% and 16.88% respectively (Abou El-Atta, 2003). Pseudomonas was widely distributed in ecosystem and was recognized as one of the primary cause of bacterial hemorrhagic septicemia in fish, pseudomonas septicemia, usually is associated with environmentally stressful conditions such as overcrowding, low temperature, injuries (Aly, 1994; Allen, *et al*. 1983). It may be a secondary invader of damaged fish tissue (Roberts and Home, 1978). *P. fluorescens* considered the causative agent of red spot disease attack all kinds of cultured fishes where the disease raised in running water ponds, stagnant water ponds as well as in cages (Angka and Lioe, 1982), the disease favored by stressor as low temperature, injuries and recorded that the incidence of pseudomonas septicemia was 11% (Eissa, *et al*. 1996). The infected fish showed dark body coloration, exophthalmia with corneal opacity and hemorrhage in the eyes, loss of balance, frayed and torn tail and fins, scale detachment and skin discoloration with scattered hemorrhages all over the body surface with slight ascites, petechial hemorrhages were seen on the ventral abdominal wall and the base of the fins (Badran & Eissa, 1991; El-Altar and Moustafa, 1996).

Tilapias are known to harbour bacterial flora in their guts (Sugita, *et al*. 1985; Al Harbi and Uddin, 2005) showed that bacteria species isolated from the intestine of a tilapia species are predominantly gram-negative rods (87%). The main aim of the present study is to analyze the bacterial load of Tilapia fish.

MATERIALS & METHODS

Sample collection: We took seven samples of tilapia fish. One fish was healthy and three were infected. Preserved in sterile polythene bag in fridge. Fish sample was cut from the gill, skin, and intestinal region with a sterile knife.

Preparation of stock cultures: The cut sample was crushed into small pieces in sterile mortar with about 10ml sterile water. Take 1ml crushed sample and 9ml distilled water to make up 1:10 dilution.

Isolation and identification of bacteria: Inoculate

0.1ml of the diluted solution in nutrient agar and streak with the help of wire loop. All plates were incubated at 34ºC for 24 hrs. The developed colonies on the plates were examined after the incubation period. These colonies were then isolated and identified according to their colonial, morphological and biochemical characterstics..

RESULTS & DISCUSSION

In the present study, isolates from seven specimens of Nile tilapia, (*Oreochromis niloticus*) have shown in Table I.

One specimen of Nile tilapia was fresh while six others were infected and used to isolate gram positive and gram negative bacteria. Both fresh and infected specimens were used in the study because sometimes apparently without any symptoms the fresh specimens may carry the pathogenic diseases. The findings of the study revealed the isolation of both gram – positive bacteria: *Streptococcus sp*. and *Staphylococcus aureus* and Gram – negative: *Enterobacter sp*. and *Pseudomonas aeruginosa* and occluded that heavy contamination of water affects the health of fishes and aquatic organisms. The study of bacteriological analysis from tilapia fish revealed the presence of possible pathogenic micro-organisms as well as high coliform bacterial counts from fish samples.

In the light of these major findings, the following recommendations are made; Public health authorities and food inspection authorities of the country should

Table I: Cultural & biochemical characteristics of isolated grampositive bacteria from fish samples

Samples	Colonies On N.A	Gram Staining	Mannitol Salt Agar	Blood Agar	Catalase Test	Coagulase Test	Isolated Organisms
Intestine	Transparent Small Colonies	Positive Cocci In Chain	N/A	No Hemolysis	Positive	Negative	Nonhemolytic Streptococci
Intestine	Transparent Small Colonies	Positive Cocci In Chain	N/A	No Hemolysis	Negative	Negative	Nonhemolytic Streptococci
Intestine	Transparent Small Colonies	Positive Cocci In Chain	N/A	No Hemolysis	Negative	Negative	Nonhemolytic Streptococci
Skin	Transparent Small Colonies	Positive Cocci In Chain	N/A	No Hemolysis	Negative	Negative	Nonhemolytic Streptococci
Skin	Transparent Small Colonies	Positive Cocci In Chain	N/A	No Hemolysis	Negative	Negative	Nonhemolytic Streptococci
Gills	Transparent Small Colonies	Positive Cocci In Chain	N/A	No Hemolysis	Negative	Negative	Nonhemolytic Streptococci
Eye	Off White Colonies	Positive Cocci Bunches	Yellow colonies	Beta Hemolysis	Positive	Positive	*S. aureus*
Intestine	Off White Colonies	Positive Cocci Bunches	Yellow colonies	Beta Hemolysis	Positive	Positive	*S. aureus*
Skin	Off White Colonies	Positive Cocci Bunches	Yellow colonies	Beta Hemolysis	Positive	Positive	*S. aureus*
Skin	Off White Colonies	Positive Cocci Bunches	Yellow colonies	Beta Hemolysis	Positive	Positive	*S. aureus*
Eye	Off White Colonies	Positive Cocci Bunches	Yellow colonies	Beta Hemolysis	Positive	Positive	*S. aureus*
Eye	Off White Colonies	Positive Cocci Bunches	Yellow colonies	Beta Hemolysis	Positive	Positive	*S. aureus*

Table II: Cultural & biochemical characteristics of isolated gram negative bacteria from fish samples

Sample	Gram Reaction	MacConkey Agar	EMB Agar	PIA Agar	TSI				Citrate	Organism
					Butt	Slant	gas	H2S		
Eye	Gram negative	Fermenting colonies	Purple colonies	N/A	Acidic	Acidic	++	-	Positive	Enterobacter
		Non fermenting colonies	N/A	White colonies	Alkaline	Alkaline	-	-	positive	Pseudomonas
Eye	Gram negative	Fermenting colonies	Purple colonies	N/A	Acidic	Acidic	++	-	Positive	Enterobacter
		Non fermenting colonies	N/A	White colonies	Alkaline	Alkaline	-	-	positive	Pseudomonas
Gills	Gram negative	Fermenting colonies	Purple colonies	N/A	Acidic	Acidic	++	-	Positive	Enterobacter
		Non fermenting colonies	N/A	White colonies	Alkaline	Alkaline	-	-	positive	Pseudomonas

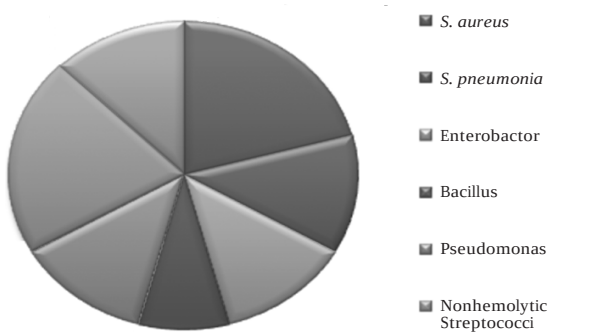

Figure 1: Prevalence of isolated bacteria from infected fish samples.

Figure 2: Infected Nile Tilapia Fish (*Oreochoromis niloticus*)

ensure adequate supervision and monitoring of food handling and sales especially ready to eat products like fried fish and cultured fish/fresh water fish. This research has brought to light those bacterial species associated with fresh Tilapia fish and has shown that they are potentially pathogenic to humans. Hence adequate measures should be taken in processing the fish before consumption.

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