Bacteriological evaluation of raw Catfish (Clarias gariepinus)

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ABSTRACT

Fish is a worldwide inexpensive source of protein especially in the developing countries like Egypt. This study aimed to bacteriologically evaluate fifty random samples of catfish collected from retail and supermarkets in Banha city, Qalubiya governorate, Egypt. Results revealed that the mean values of aerobic plate count (APC), total enterobacteriaceae, and total coliforms counts were 9.74×10^3, 3.25×10^3 and 2.35×10^3 CFU/g, respectively. Escherichia coli, Salmonellae, and Yersenia enterocolitica were detected in 18, 6, and 10% of examined samples, respectively, which went in further serological identification. This study indicated that the hygienic status of raw catfish is strongly related to source of collection, conditions of storage and handling, and recommended following proper hygienic properties of rearing, transportation, storage and handling.

1. INTRODUCTION

African catfish (C. gariepinus) is generally considered to be one of the most important tropical catfish species for aquaculture in Africa (Clay, 1979), it is widely distributed throughout Africa, inhabiting tropical swamps, lakes, and rivers, some of which are subjected to seasonal drying (Olufemi et al., 1991).

Fish is a highly perishable food, which needs proper handling and preservation if it is to have a long shelf life and retain a desirable quality and nutritional value. The most obvious method for preserving the quality of fish is to keep them alive until they are ready for cooking and eating. So, farm fish is better way to have fresh, healthy, non-preserved fish, and higher nutritional value (Brenner, 2003). On the other hand, retailed fish must be handled very carefully so they can be delivered to the next part of the marketing chain in a fresh and undamaged condition, and to allow consumer to have all the nutritional value of fish (Ananou et al., 2007).

Fish is subjected to many risks of contamination from different sources either during their aquatic environment, sewage pollution of harvesting areas and/or after being harvesting by workers, utensils and equipment during transportation, distribution and food preparation (National Academy of Science, 1985; El-Leboudi, 2002).

Although fish flesh is generally thought to be sterile immediately after catching (Kasing et al., 1999), the hygienic quality of fish is often more difficult to control due to variations in species, sex, age, habitats and action of autolytic enzymes as well hydrolytic enzymes of microorganisms on the fish muscle (Venugopal, 2002), which may become contaminated with different microorganisms during subsequent handling (Sumner and Rose, 2002) as these microorganisms can penetrate from skin and the gut to the flesh (Samaha et al., 2004).

Contamination of fish with organisms of public health significant remains primarily a problem of handling and processing (WHO, 1999). Enterobacteriaceae group has an epidemiological importance as some of its members are pathogenic and may cause serious infections and food poisoning to human. High prevalence of Enterobacteriaceae indicates unsatisfactory hygienic measures during catching and distribution of the fish (Pogorelova et al., 1993; Valdivia et al., 1997).

Foodborne diseases are still one of the major public health problems worldwide and account for considerably high cases of illness. Many reports indicated that Salmonella species and pathogenic E. coli were considered the most frequent pathogens (White et al., 2002). In addition, Aziz and Dapgh (2005), and Pao et al. (2008) considered Salmonellae spp. and E. coli as Enterobacteriaceae members of a potential public health hazard; where it causes food poisoning associated with severe diarrhea and gastroenteritis in infants and adults as well.

Therefore, the aim of the present study was investigation and evaluation to the hygienic status of raw catfish sold in Benha city.

2. MATERIAL AND METHODS

2.1. Collection of samples

A total of 50 samples of African catfish were collected from different retails and markets located in Benha city, Qalubiya governorate, Egypt. Samples were transported to the laboratory under complete aseptic conditions in an ice box within one hour and examined for bacteriological detection of hygienic quality.

2.2. Preparation of sample according to APHA (2013)

Twenty-five grams of muscle sample were mixed with 225 ml sterile 0.1% peptone water. The contents were
homogenized at Stomacher (M A 106402, France, 450 to 640 strokes per minute) for 2 minutes and 1 ml of the mixture was transferred into separate tube each containing 9 ml sterile 0.1% peptone water, from which tenth-fold serial dilutions were prepared. The prepared samples were subjected to the following bacteriological examination:

2.3. Determination of Aerobic plate count (APC) according to ISO 4832-1: 2013.

2.4. Identification and enumeration of Enterobacteriaceae
Identification of family Enterobacteriaceae was conducted according to Cowan and Steel (1974) performed by Gram’s stain, Biochemical tests, and motility tests.

For enumeration to the Enterobacteriaceae 0.1 ml from each of the previously prepared dilution was spread on Violet Red Bile Glucose agar (VRBG) and incubated at 37 °C for 24 hours. All purple colonies were then counted, and the average number of colonies was determined (ICMSF, 1996).

2.5. Determination of coliform count
1 ml from each of the previously prepared dilution was cultured in Violet Red Bile agar (VRBA) by pour-plate technique and incubated at 37 °C for 24 hours. All purple colonies were then counted, and the average number of colonies was determined (ISO 4832:2006).

2.6. Isolation and identification of E. coli:

2.6.1. Isolation of E. coli.
1 ml from each of the previously prepared dilution was cultured in Tryptone-Bile-Glucuronic Agar (TBX) by pour-plate technique and incubated at 44 °C for 24 hours. All bluish-green colonies were then counted, and the average number of colonies was determined (ISO 16649-2:2001).

2.6.2. Identification of E. coli.
Gram’s Stain according to (Cruickshank et al., 1975), and Biological tests (MacFaddin, 2000).

2.6.3. Serological Identification of E. coli according to (Kok et al., 1996).

2.7. Isolation and identification of Salmonellae

2.7.1. Isolation of salmonellae: According to (ISO 6579:2017), Pre-enrichment in non-selective buffered peptone water broth, which then incubated at 37±1 °C for 18 ± 2 hours.

Enrichment in Rappaport Vassiliidis broth (RV broth), then the tube was incubated at 43 °C for 24 hours.

Selective Plating on Xylose lysine Desoxy cholate (XLD) agar and Brilliant Green agar. The plates were incubated at 37 °C for 24 hours. Plates were examined for suspected Salmonella colonies which appeared as red with black centers on XLD agar and pink on Brilliant Green agar.

2.7.2. Identification of salmonellae
Gram’s Stain according to Cruickshank et al. (1975), biochemical identification Krieg and Holt (1984), and Serological identification (Confirmatory test) according to Kaufman (1974).


2.9. Statistical analysis
The obtained results were statistically analyzed according to Feldman et al. (2003).

3. RESULTS AND DISCUSSION

The microbial quality of fish meat is a reflection of the hygienic status of the rearing environment, handling and storage of caught fish. Gram and Huss (2000) reported that high coliforms counts in the examined samples an indicative for massive contamination with deteriorative bacteria, which mostly lead to flavor deterioration in the fish.

Results demonstrated in table (1) showed the incidences and counts of APC, Enterobacteriaceae, and coliform which were 100, 96, and 94%; with mean counts 9.74×10^3, 3.25×10^3, and 2.35×10^3 CFU/g, respectively, which indicating a high prevalence of Enterobacteriaceae and coliforms in the examined samples. The obtained results somewhat agreed with those reported by Mahmoud (2001), El-Shabasy (2009) and Mhango et al. (2010), who recorded that the counts of TEC, APC, and TCC were 2.3×10^3, 1.4×10^3, and 4.2×10^3 CFU/g, respectively. While, results were lower than those recorded by Mahmoud (2001), who recorded that the mean APC was 3.5×10^3, and El-Shabasy, (2009), who recorded that the mean TEC, and TCC in her examined samples were 1.48×10^4, and 6.4×10^3 CFU/g, respectively. Moreover, results were higher than those recorded by Danha et al. (2014), and Budiati et al. (2015) (2.24×10^3, and 2.8×10^2 CFU/g for APC and TCC, respectively).

### Table 1 Aerobic plate counts CFU/g in the examined samples of Catfish (n=50).

<table>
<thead>
<tr>
<th>Hygienic parameter</th>
<th>Positive samples No. (%)</th>
<th>Min.</th>
<th>Max.</th>
<th>Mean ± E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>50 (100.0)</td>
<td>4.9×10^3</td>
<td>1.8×10^4</td>
<td>9.74×10^3 ± 4.2×10^2</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>48 (96.0)</td>
<td>1.7×10^3</td>
<td>5.6×10^3</td>
<td>3.25×10^3 ± 1.4×10^2</td>
</tr>
<tr>
<td>Coliform</td>
<td>47 (94.0)</td>
<td>4.6×10^3</td>
<td>4.6×10^3</td>
<td>2.35×10^3 ± 1.5×10^3</td>
</tr>
</tbody>
</table>

Bacteriological and biochemical identification of Enterobacteriaceae members (Table 2) revealed the detection of Citrobacter diversus, Citrobacter freundii, Edwardsiella tarda, E. coli, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella pneumonia, Klebsiella oxytoca, Proteus mirabilis, Proteus vulgaris, Salmonella spp., Y. enterocolitica with incidences of 4, 4, 2, 18, 4, 6, 4, 8, 2, 2, 6, and 10%, respectively. Generally, 35 strains were isolated with total incidence of 70% from all examined samples. The results agreed with those recorded by Morshdy (1992), Mhango et al. (2010), and Hassan (2013), who detected Citrobacter diversus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella pneumoniae, Proteus mirabilis, and Proteus vulgaris in raw catfish samples.
The incidence of bacteriological detection of some pathogenic food poisoning bacteria and the incidence of hygienic acceptance of the examined samples were summarized in Table 3. *E. coli*, *Salmonellae*, and *Yersinia enterocolitica* were detected in 18.6, and 10.0%, respectively.

In addition, 41(82%), 47(94%), and 45(90%) of the examined samples were accepted in relation to the number and incidences of microbial detection following EEC, 2005 specifications. These results partially agreed with those recorded by Heinawy et al. (1989), Mikhail (2003) and Papadopoulou et al. (2007), who detected *Y. enterocolitica*, *Salmonella*, and *E. coli* in their examined samples at the incidence of 10.0, 6.2, and 14.0%, respectively. While, they were higher than those recorded by El-Adamy, (2002), who detected *E. coli*, *Y. enterocolitica*, in the examined samples by the incidence of 11.3, and 2.0%, respectively. While, Mahmoud (2001) could not detect *Salmonella* in the examined catfish samples. In addition, the present results were lower than those recorded by Ali (2017), who detected *E. coli*, *Salmonella*, and *Y. enterocolitica* at rate of 48.0, 32.0, and 16.0%, respectively, while they are totally disagreed with the results that recorded by Kasing et al. (1999), who could not detect any bacterial isolates in examined musculature samples.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Incidences</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrobacter diversus</td>
<td>2</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>2</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Edwardsiella tarda</td>
<td>1</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>E. coli</td>
<td>9</td>
<td>9</td>
<td>18.0</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>2</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>3</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>4</td>
<td>4</td>
<td>8.0</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>1</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>1</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>3</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>5</td>
<td>5</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>35</td>
<td>70.0</td>
</tr>
</tbody>
</table>

Table 2 Prevalence of Enterobacteriaceae species in examined samples of Catfish (n=50).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Incidences</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>O27:H2</td>
<td>4</td>
<td>8.0</td>
<td>EPEC</td>
</tr>
<tr>
<td>O163:H1</td>
<td>1</td>
<td>2.0</td>
<td>ETEC</td>
</tr>
<tr>
<td>O158:H4</td>
<td>3</td>
<td>6.0</td>
<td>ETEC</td>
</tr>
<tr>
<td>O159:H7</td>
<td>1</td>
<td>2.0</td>
<td>EHEC</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>18.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Serotyping of *E. coli* isolated from examined samples of Catfish (n=50).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Incidences</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Enteritidis</td>
<td>1</td>
<td>1.9</td>
<td>g,s,1,2</td>
</tr>
<tr>
<td>S. Saint Paul</td>
<td>1</td>
<td>1.4</td>
<td>4,5,12</td>
</tr>
<tr>
<td>S. Enteritidis</td>
<td>1</td>
<td>1.8</td>
<td>4,5,12</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>1</td>
<td>1.8</td>
<td>4,5,12</td>
</tr>
<tr>
<td>S. Haifa</td>
<td>1</td>
<td>1.8</td>
<td>4,5,12</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>18.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 5 Serotyping of *Salmonellae* isolated from examined samples of Catfish (n=50).

**ACKNOWLEDGMENT**

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**CONFLICT OF INTEREST**

The content of this report solely reflects the opinions of the authors, and we report no conflicts of interest.

**5. REFERENCES**