Animal Health Research Institute, 
Assiut Regional Laboratory.

INCIDENCE OF AEROMONAS HYDROPHILA IN FRESH WATER FISH (TILAPIA NILOTICUS) AND READY TO EAT FRIED FISH IN ASSIUT CITY 
(With 4 Tables)

By
H.H. ESSA; A.M. MANAA and N.H. MAKAR
(Received at 14/10/2009)

SUMMARY

This study was carried on 80 random samples 50 of fresh water fish (Tilapia niloticus) and 30 samples of ready to eat fried fish these samples were obtained from different shops; represented different localities of different sanitation levels of Assiut city. All samples were examined for the presence of Aeromonas hydrophila group; using enrichment
procedure and surface spread plate technique. The results obtained pointed out that 48% and 10% of the examined Tilapia niloticus fish and ready to eat fried fish samples were positive for the presence of *Aeromonas hydrophila* organism with an average counts of $3.7 \times 10^3$ and $1.9 \times 10^2$ /g fish respectively. In this study 30(60%) *Aeromonas* strains were isolated from fresh water fish (Tilapia niloticus) and were characterized according to species level as follow; 16 (53.33%) *Aeromonas hydrophila*; 9(30%) *Aeromonas caviae* and 5(16.67%) *Aeromonas sabria*. On the other hand 16(53.33%) strains were isolated from ready to eat fried fish and were characterized according to species level as follows: 7 (43.75%) *Aeromonas caviae*, 5 (31.25%) *Aeromonas sabria* and 4 (25%) *Aeromonas hydrophila*. All strains were examined for their ability to produce haemolysin as a virulence factor. The hygienic and public health importance as well as some recommended measures for improving the quality of such products were discussed.

**Key words:** Fish, fresh water fish, fried fish, *Aeromonas* spp.

**INTRODUCTION**

*Aeromonas hydrophila* (A. hydrophila), a gram negative bacteria, is widely distributed in aquatic environment (Nakano, *et al.*, 1990; Fiorentini, *et al.*, 1998; Legnani, *et al.*, 1998). A. hydrophila has received a particular attention because of its association with infections in a wide variety of hosts including, human, reptiles fish and invertebrates (Kodjo *et al.*, 1997; Pearson *et al.*, 2000; Roux *et al.*, 2000). More ever the bacterium is considered as one of the newly emerging water and food borne pathogens (Merino *et al.*, 1995; Gugnani, 1999). In fish A. hydrophila typically causes an exploded haemorrhagic septicemia and has been implicated in different outbreaks associated with heavy losies (Qian *et al.*, 1995; Son *et al.*, 1997).

Species of *Aeromonas* are short, gram negative, faculatively anaerobic, non spore forming, motile bacilli with a single flagellum, and can ferment glucose with or without the production of gas (Andrade, *et al.*, 2006). The most important three motile species associated with human illness are *Aeromonas hydrophila*, *A. caviae* and *A. sobria* (Brooks *et al.*, 1995). Isolation of these bacteria (*Aeromonas hydrophila* group), have been reported from a variety of food including fishes (Adithepchaikram *et al.*, 2008)

In addition to gastro-enteritis *Aeromonas hydrophila* group infects human causing infections such as septicaemia, acute diarrhea of
short duration, urinary tract infection and ear infection (Koneman et al., 1994). The *Aeromonas hydrophila* group produces a number of potential virulence factors, including enterotoxins, haemolysins, cytotoxins and proteases (Ljungh and Wadstrom, 1983). The Aeromonas microorganisms are normal inhabitant of the intestinal tract of *O. niloticus* (Akelah, 1978). However, *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas caviae* has been implicated in some cases of diarrheal disease. *Aeromonas sobria* and *Aeromonas hydrophila* are the primary enteropathogenic species, however *Aeromonas caviae* has been implicated in some cases of diarrheal disease (Topic et al., 2000).

In addition, Beta haemolytic strains of *Aeromonas* are assigned to *Aeromonas hydrophlia* and *Aeromonas sobria*, although haemolytic strains of *Aeromonas caviae* have been also found (Deodhar et al., 1991). Burke et al. (1981) mentioned that the haemolytic activity is strongly associated with enterotoxin production in members of *Aeromonas* genus.

Rugulska, A., et al. (1994) reported that the haemolytic activity of *Aeromonas hydrophila* and *Aeromonas sobria* act as marker of pathogenicity therefore, the initial purpose of this study was to determine the occurrence and level of *Aeromonas* organisms in fresh water fish (Tilapia niloticus) and ready to eat fried fish in Assiut city markets.

**MATERIALS and METHODS**

1 - Collection of samples:-

Atotal of 80 random samples of fresh water fish (*Tilapia niloticus*) (50 samples) and ready to eat fried fish (30 samples) were collected from fish markets, shops and restaurants of varied sanitary levels at Assuit city.

Each sample was put in a sterile plastic bag. The collected samples were transferred directly to the laboratory under aseptic conditions without any delay where they were organoleptically and bacteriologically examined.

2 - Organoleptic examination:-

Ready to eat fried fish were evaluated for their palatability and odour of the flesh, while the fresh water fish were examined for their skin condition, consistency, colour, scales, eyes and gills according to Anon, (1985).
3 - Bacteriological examination:-

The samples were analyzed by using enrichment method as recommended by Okrend et al. (1987), where 25 gram sample were aseptically transferred to 225 ml of trypticase soy broth containing 10 mg ampicillin / ml and blended for 2 min. The prepared samples were serially diluted up to $10^6$ in butterfieds phosphate dilutent, and the count was carried out on the aforementioned dilutions as recommended by Palumbo et al. (1985), using MacConky manitol ampicillin agar. The number of colonies which showed red colour in countable plates was enumerated as Aeromonas organisms.

a- Enrichment procedure:

This was done according to the technique adopted by Palumbo et al. (1989).

b- Isolation and identification techniques:-

The technique adopted was that used by Okrend et al. (1987), Ahmed et al. (1991), and Koneman et al. (1994).

c- Determination of the haemolytic activity of the isolated strains:

It was carried out using 5% sheep blood agar as recommended by Rogulska et al. (1994).

RESULTS

Table 1: Organoleptic examination of fresh and fried fish samples.

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>No. of Samples</th>
<th>Organoleptic examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fresh samples</td>
</tr>
<tr>
<td>Fresh water fish</td>
<td>50</td>
<td>50 (100%)</td>
</tr>
<tr>
<td>(Tilapia niloticus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ready to eat fried fish</td>
<td>30</td>
<td>30 (100%)</td>
</tr>
</tbody>
</table>

Table 2: Statistical analytical results of *Aeromonas* species count/g examined samples.

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>No. of Examined samples</th>
<th>Positive samples</th>
<th>Count/g of Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>Fresh water fish</td>
<td>50</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Ready-to-eat fried fish</td>
<td>30</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>
**Table 3:** Incidence of *Aeromonas* species isolated from the examined samples.

<table>
<thead>
<tr>
<th>Type of Samples</th>
<th>No. of Isolated Strains</th>
<th><em>Aeromonas hydrophila</em></th>
<th><em>Aeromonas caviae</em></th>
<th><em>Aeromonas sobria</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh water fish <em>(Tilapia niloticus)</em></td>
<td>30 (60%)</td>
<td>16</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>53.33%</td>
<td>30</td>
<td>16.76%</td>
</tr>
<tr>
<td>Ready-to-eat fried fish</td>
<td>16 (53.33%)</td>
<td>4</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25%</td>
<td>43.75%</td>
<td>31.25%</td>
</tr>
</tbody>
</table>

**Table 4:** Detection of haemolysin activity of *Aeromonas* species isolated from fresh water fish *(Tilapia niloticus)* and ready-to-eat fried fish.

<table>
<thead>
<tr>
<th><em>Aeromonas</em> Species</th>
<th>Haemolysin activity</th>
<th>Fresh water fish</th>
<th>Ready-to-eat fried fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>No. positive</td>
<td>% positive</td>
</tr>
<tr>
<td><em>A. hydrophila</em></td>
<td>16</td>
<td>11</td>
<td>68.75%</td>
</tr>
<tr>
<td><em>A. caviae</em></td>
<td>9</td>
<td>1</td>
<td>11%</td>
</tr>
<tr>
<td><em>A. sobria</em></td>
<td>5</td>
<td>2</td>
<td>40%</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This study was conducted to investigate the presence of *Aeromonas* species in fresh water fish *(Tilapia niloticus)* and ready to eat fried fish.

Although the organoleptic examination showed no abnormalities and all the examined samples were fresh and sound, yet *Aeromonas* organisms were recovered from fresh water fish *(Tilapia niloticus)* and ready to eat fried fish *(Table 1)*, therefore bacteriological examination must be associated with organoleptic examination to give the accurate judgment.

From *(Table 2)*, it is apparent that 24 (48%) and 3(10%) of fresh water fish *(Tilapia niloticus)* and ready to eat fried fish contained
Aeromonas species with an average count of \(3.7 \times 10^3\) and \(1.9 \times 10^2\)/gm respectively.

The obtained incidence are somewhat higher than that reported by Gobat and Jemmi (1992); Abdel. EL-Daym (1999) and Bastawrows and Mohamed (1999).

It was observed that the fresh water fish showed higher incidence and count than that from ready to eat fried fish as Aeromonas microorganisms are normal inhabitant of the intestinal tract of Tilapia niloticus (Akelah, 1978).

From Table (3), 30(60%) strains of Aeromonas organisms were isolated from the examined fresh water fish (Tilapia niloticus). Aeromonas hydrophlia was the most common species isolated (53.33%) followed by Aeromonas caviae (30%) strains and Aeromonas sobria (16.67%). On the other hand 16 (53.33%) strains were recovered from ready to eat fried fish where Aeromonas caviae was the most common species isolated (43.75%) followed by Aeromonas sobria (31.25%) and Aeromonas hydrophila (25%).

It is evident from the data presented in Table (4) that 11 (68.75%) of 16 Aeromonas hydrophila strains, 2(40%) of 5 Aeromonas sobria strains and only one (11%) of 9 Aeromonas caviae strains, while 3 (42.86%) of 7 Aeromonas caviae strains, 2 (40%) of 5 Aeromonas sobria and only one (25%) of 4 Aeromonas hydrophila strains from fresh water fish and ready to eat fried fish had the ability to produce haemolysin respectively.

Abyta et al. (1994) identified Aeromonas hydrophilia and Aeromonas sobria as the primary enterophogenic species, however Aeromonas caviae has been implicated in some cases of diarrheal disease (Topic et al., 2000).

In addition, Beta haemolytic strains of Aeromonas are assigned to Aeromonas hydrophila and Aeromonas sobria, although haemolytic strains of Aeromonas caviae have been also found (Deodhar et al., 1991).

Varnam, and Evans, (1991) reported that a number of phenotypic characters have been proposed as a markers of enteropathogenicity of Aeromonas species and they added that the most important of these markers was haemolysin production.

The present results disagree, with those reported by Okrend et al. (1987); Palumbo et al. (1989) and Freitas et al. (1992) since these authors pointed out that haemolysin was detected in 100% of Aeromonas hydrophila strains recovered from some varities of food.
On the other hand, Bastawrows and Mohammed (1999) found that more of the 12 strains of *Aeromonas caviae* recovered from fresh water fishes lysed the sheep erythrocytes.

In conclusion, the information given by the achieved results revealed that *Aeromonas* species existed in the examined fishes either fresh water fish or ready to eat fried fish and therefore these foods may play a significant role in the epidemiology of gastroenteritis, therefore, strict hygienic measures, good food handling practice at home and finally thoroughly and properly clean and sanitary equipments and contact surfaces should be recommended to avoid contamination with *Aeromonas* organisms.

**ACKNOWLEDGMENT**

I wish to express sincere thanks to Prof. Dr A. EL-Tamawy Professor of bacteriology, Faculty of Medicine, Assiut University for his help in Aeromonas serotyping.

**REFERENCES**


