Monitoring of some pathogenic bacteria in Nile fish

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ABSTRACT
The present study was designed to monitor the incidence of pathogenic bacteria in Nile fish. Ninety random samples of fresh Nile fish Oreoichromis niloticus (Nile tilapia), Clarias gariepinus (African catfish) and Cyprinus carpio (Common carp) (30 samples of each) were randomly and periodically collected from different markets in Gharbia governorate, Egypt. They were packaged and marked individually in polyethylene bags, and bacteriologically and serologically examined. Our result showed that the incidence of E. coli was 70% (n=21), 34% (n=10) and 73% (n=22) from Nile tilapia, catfish and carp, respectively. E. coli isolates from Nile tilapia (four), catfish (three) and carp (three) were serotyped. Serological identification revealed that the isolates from Nile tilapia were (O84, O26, O128 and O119), from catfish were (O84, O26 and O128) and from carp were (O17, O128 and O119). The incidence of S. aureus was 27% (n=8), 60% (n=18) and 67% (n=20) from Nile tilapia, catfish and carp, respectively. Salmonella was not detected in any of the examined fish samples. The incidence of Aeromonas spp was 80% (n=24), 100% (n=30) and 93% (n=28) from Nile tilapia, catfish and common carp, respectively. The incidence of Pseudomonas spp was 95% (n=28), 100% (n=30) and 53% (n=16) from Nile tilapia, Catfish and Common carp, respectively. It was concluded that Nile fish are contaminated with many food poisoning bacteria.

1. INTRODUCTION
Fish and fishery products are a very valued source of protein and essential micronutrients for balanced nutrition and good health (Arni, 2012). Nile tilapia (Oreoichromis niloticus) is the most commonly cultivated species among Tilapia in many countries around the world (Salem, 2015). Egypt is the second largest producer of farmed tilapia in the world (FAO, 2019). It has the largest aquaculture industry in Africa that provides about 75.46% of the country’s fish production (GAFRD, 2013). Catfish (Clarias gariepinus) is one of the widely spread fish species in tropical Africa which has become the most cultivated fish species. This is because of its high-quality flesh, high acceptance level of water characteristics, production and market values (Adeshina et al., 2016). Common Carp (Cyprinus carpio) is a freshwater fish that is widely cultivated. It has a rapid growth and high fertility, so it has a high importance to become a source of protein (FDA, 2012). Millions of bacteria are present on the surface slime, the gills and in the intestines of live fish, although the flesh itself is normally sterile. Bacterial growth and attack on the fish are barred by the body’s natural defense system during life but after death the defense system breaks down and the bacteria multiply and enter the flesh (Abolagba and Uwagbai, 2011). Human infections caused by pathogens transferred from fish are quite common. Enteric pathogenic bacteria insulated from fish that might be transferred to humans after the handling or consumption of fish which was E. coli and Salmonella typhi (Onyango et al., 2009). Staphyloccocus spp. causes nosocomial contamination in neonatal and urinary tract infections mostly in young women. Also, Staphylococcal food poisoning, toxic shock syndrome and scaled skin syndrome are caused by S. aureus (Goja et al., 2013). Aeromonas spp. causes bacteremia, pulmonary infections, meningitis, and wound infections. It may cause “summer-diarrhea”, which is a worldwide problem in children under five years old, the elderly, and travelers (Alvarez et al., 2006). In humans, P. aeruginosa is the cause of dermatitis, skin infections in severe burns, sepsis, meningitis and nosocomial infections of the urinary tract (Mitov et al., 2010). The aim of this work was to monitor the incidence of pathogenic bacteria in Nile fish (Nile tilapia, Catfish and Common carp).
2. MATERIAL AND METHODS

2.1. Samples:
A grand total of 90 random samples of fresh Nile fish Oreochromis niloticus (Nile tilapia), Clariasgariepinus (African catfish) and Cyprinus carpio (Common carp) (30 of each) was randomly and periodically collected from different markets in Gharbia governorate, Egypt. The samples were kept in separate plastic bags and transferred directly without undue delay to the laboratory in an insulating refrigerated container under complete aseptic conditions to avoid any changes in the quality of the sample.

2.2. Bacteriological examination:
2.2.1. Preparation of sample was done according to ISO (2007) as follows:
Twenty-five grams of the examined sample was transferred to a sterile polyethylene bag. Then 225 ml of 0.1% sterile peptone water was aseptically added to the content and homogenized at 1400 rpm for 2.5 min to provide a homogenate.

2.2.2. Isolation and identification of E. coli was done according to ISO (2005) as follows:
Pre-enrichment: 0.1 ml of original homogenate was inoculated into MacConkey broth (Oxoid) with inverted Durham's tubes. Then tubes were incubated at 37 °C for 24-48 hrs.
Enrichment broth: a loopful from each positive pre-enrichment tube was inoculated into tube contain MacConkey broth and incubated at 44.5±0.5 °C for 48 hrs.
Plating media: a loopful from positive enrichment tube was separately streaked into (EMB) agar (Oxoid) and incubated at 37 °C for 24 hrs. Suspected colonies of green metallic color with dark purple center were picked and inoculated into nutrient slope tubes for further identification.

2.2.2.1. Morphological examination:
- Microscopical examination: by Gram staining G-ve, medium size stained evenly coccobacilli.
- Motility test: motile.

2.2.2.2. Biochemical Identification was done according to Mcfaddin (2000) as follows:
- Indole test, Methyl Red: positive reaction.
- Vogas Proskauertest, Citrate Utilization test: negative reaction.

2.2.2.3. Serological identification of E. coli:
The isolates were serologically identified according to Kok et al. (1996) by using rapid diagnostic E. coli antisera sets (Denka Seiken Co., Japan) for diagnosis of the Enteropathogenic types.

2.2.3. Isolation and identification of Staphylococcus spp. was done according to ISO (2003) as follows:
Accurately, 0.1 ml from nutrient broth was spread onto plats of Baird Parker (Oxoid). Plates were incubated at 37°C for 48 hrs. Staphylococcus appear as black shiny colonies with narrow white margins surrounded by a clear halo zone extending into the opaque medium.

2.2.3.1. Morphological identification:
- Microscopic identification: by Gram staining G+ve cocci grapes like clusters.
- Motility: non-motile.

2.2.3.2. Biochemical identification was done according to Mcfaddin, (2000) as follows:
- Catalase, Coagulase and Hemolysis-Detection: positive
- Mannitol test: showed yellow colonies surrounded by a halo zone.

2.2.4. Isolation and identification of Salmonella spp. was done according to ISO, (2002) as follows:
Sample pre-enriched: 25 g ranalytical portion of sample are simply stomached in 225 ml buffered peptone water, incubated for 18±3 hrs at 37 ±1°C.
Enrichment in selective liquid media: 0.1 ml of pre-enrichment broth culture added to 10 ml of RVS broth then incubated at 41.5°C for 24±3 hrs.
Plating out & identification: loopful of enrichment broth was streaked on XLD agar incubated at 37±1°C for 24±3 hrs. The suspected colonies subjected to biochemical testes (using API 20) and serological confirmation using (commercial antisera). Poly O and poly H slide agglutination testes.

2.2.4.1. Morphological identification:
- Microscopical examination: by Gram staining G - vecocco bacilli to medium size rods.
- Motility test: motile.

2.2.4.2. Biochemical Identification was done according to Quinn et al. (2002) as follows:
- Triple sugar iron media: Test with saline TSI agar. Inoculate the agar slope by stabbing to the bottom and streaking longitudinally along the slope incubates 37 °C for 24±3 hrs. Interpret the reactions.
- Citrate utilization, H2S, Methyl red test: Salmonella positive reaction.
- Vogas Proskauer, Indole production, gelatin hydrolysis and urease: negative reaction.

2.2.5. Isolation and identification of Aeromonas spp. was done according to Mcfaddin (2000) as follows:
0.1 ml of original homogenate was streaked onto tryptic soya broth and incubated at 37°C for 24hrs then streaked onto Aeromonas agar and incubated at37°C for 24hrs. Aeromonas appear as dark green colony.

2.2.5.1. Morphological identification:
- Microscopic examination: by Gram staining, G-ve straight rods with rounded ends.
- Motility test: motile.

2.2.5.2. Biochemical identification:
- Oxidase, Arginine hydrolysis, Indole, Gelatin liquefaction, H2S production, Nitrate reduction, catalase: positive.
- Methyl Red, Voges Proskauer, Citrate utilization: variable result.
- Urease: negative.
- Fermentation of sugars: ferment glucose, sucrose.
2.2.6. Isolation and identification of Pseudomonas spp was done according to Mcfaddin (2000) as follows: 1 ml of original homogenate was streaked onto tryptic soya broth and incubated at 37°C for 24 hrs then steak onto pseudomonas agar. Suspected colonies appeared as yellow colony.

2.2.6.1. Morphological identification:
- Microscopic examination: G- ve bacilli.
- Motility test: motile.

2.2.6.2. Biochemical identification:
- Catalase, Methyl red, Oxidation-Fermentation, Gelatin liquefaction and Citrate: positive.
- Indole production, Voges Proskauer, H₂S production: negative

3. RESULTS

As shown in table (1and2) the incidence of E. coli was 21(70%), 10(33.3%) and 22 (73.3%) from Nile tilapia, catfish and carp, respectively. Serological identification revealed that the isolates from Nile Tilapia (four) were belonging to (O84, O26, O128 and O111), from catfish (three) (O84, O26 and O128) and from carp (three) belonging to (O17, O128 and O119).

Table 1: Incidence of E.coli isolated from the examined fish samples (n=30).

<table>
<thead>
<tr>
<th>Fish type</th>
<th>Positive samples</th>
<th>Identified bacterium</th>
<th>Serodiagnosis</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oreochromis niloticus (Nile tilapia)</td>
<td>3 14.2%</td>
<td>E.coli O84: H21</td>
<td>EPEC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 28.5%</td>
<td>E.coli O26: H11</td>
<td>EPEC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 42.6%</td>
<td>E.coli O128: H2</td>
<td>EPEC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 14.2%</td>
<td>E.coli O119: H6</td>
<td>EPEC</td>
<td></td>
</tr>
<tr>
<td>Clarias gariepinus (African catfish)</td>
<td>5 50%</td>
<td>E.coli O128: H2</td>
<td>EPEC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 20%</td>
<td>E.coli O84: H21</td>
<td>EPEC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 30%</td>
<td>E.coli O26: H11</td>
<td>EHE</td>
<td></td>
</tr>
<tr>
<td>Cyprinus carpio (common carp)</td>
<td>7 31.81%</td>
<td>E.coli O119: H6</td>
<td>EPEC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 36.36%</td>
<td>E.coli O128: H2</td>
<td>ETEC</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Incidence of identified E. coli serotype isolated from the examined fish samples (n=30).

<table>
<thead>
<tr>
<th>Fish type</th>
<th>Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oreochromis niloticus (Nile tilapia)</td>
<td>21 70%</td>
</tr>
<tr>
<td>Clarias gariepinus (African catfish)</td>
<td>10 33.3%</td>
</tr>
<tr>
<td>Cyprinus carpio (common carp)</td>
<td>22 73.3%</td>
</tr>
</tbody>
</table>

Table 3: Suspected E. coli strains isolated from the examined fish samples (n=30).

<table>
<thead>
<tr>
<th>Fish type</th>
<th>No</th>
<th>%</th>
<th>Identified bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oreochromis niloticus (Nile tilapia)</td>
<td>5</td>
<td>55.5%</td>
<td>Mixed Culture</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>44.4%</td>
<td>Enterobacter</td>
</tr>
<tr>
<td>Clarias gariepinus (African catfish)</td>
<td>10</td>
<td>50%</td>
<td>Mixed Culture</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>50%</td>
<td>Enterobacter subaggregans</td>
</tr>
<tr>
<td>Cyprinus carpio (common carp)</td>
<td>8</td>
<td>100%</td>
<td>Enterobacter aerogenes</td>
</tr>
</tbody>
</table>

Table 4: Incidence of S. aureus isolated from the examined fish samples (n=30).

<table>
<thead>
<tr>
<th>Fish type</th>
<th>Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oreochromis niloticus (Nile tilapia)</td>
<td>8</td>
</tr>
<tr>
<td>Clarias gariepinus (African catfish)</td>
<td>18</td>
</tr>
<tr>
<td>Cyprinus carpio (common carp)</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 5: Incidence of Aeromonas isolated from the examined fish samples (n=30).

<table>
<thead>
<tr>
<th>Fish type</th>
<th>Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oreochromis niloticus (Nile tilapia)</td>
<td>24</td>
</tr>
<tr>
<td>Clarias gariepinus (African catfish)</td>
<td>30</td>
</tr>
<tr>
<td>Cyprinus carpio (common carp)</td>
<td>28</td>
</tr>
</tbody>
</table>

4. DISCUSSION

Bacterial diseases in fish usually do not develop just as a result of exposing a host to an infectious agent. In most occasions, disease occurs as the result of complex interactions between pathogen, fish and environmental stress, which affect the liability of the host to disease (Wedekind et al., 2010). E. coli was isolated from (70%) of the examined Nile Tilapia (Oreochromis niloticus) samples (Table 1). Nearly similar result were present in 73.9% of fry samples (17/23) and higher incidence 81.6% of adult fish’s samples (31/38) by Valenzuela-Armenta et al. (2018). Lower incidence by Hassan et al. (2012), 27%. E. coli was isolated from 33.3% of the examined Catfish (Clarias gariepinus) samples (Table 1). Nearly Similar results were obtained by Toyo et al. (2012) 23.2% and Egbebi et al. (2016) 24%. Lower incidence 17.5% by Akande and Onyedibe (2019). However, higher prevalence was reported from freshwater fish 72.7% (Jiang et al., 2012). E. coli was isolated from 73.3% of the examined Common carp (Cyprinus carpio) samples (Table 1). Nearly similar result were obtained from freshwater fish by Jiang et al. (2012); 72.7 %. Lower results were obtained by Sivakami et al. (2008); 50%; and Razavilar et al. (2013); 47.61%; in fish samples. The serotypes of E. coli isolates from the examined fish samples were O84, O26, O128, O17 and O119 (table 2). E. coli has been involved for a number of gastroenteric diseases such as diarrhea (traveler’s disease), dysentery, vomiting, fever, colitis, hemolytic uremic syndrome with renal failure (Egbere et al., 2010).

Staphylococcus spp are serious bacteria in public health due to the severity of some infections they cause. Even
when they were noticed at a very low frequency, their presence makes necessary to maintain microbiological quality investigation in tilapia culture and in general, in aquaculture (Allen et al., 2004). S. aureus was isolated from 26.6% of the examined Tilapia (O. niloticus) samples (Table 4). Nearly similar results were reported by (Hardi et al., 2018); 24.32%. Higher incidence (40%) was reported by Maysoon (2014). Lower incidence was by El-olemy et al. (2014); 4.5%. S. aureus was isolated from 60% of the examined Catfish (Clarias gariepinus) samples (Table 4). Lower results 23.21% by Danba et al. (2015) and 13.0% by Toyo et al. (2012). S. aureus was isolated from 66% of the examined Common carp (Cyprinus carpio) samples (Table 4). Higher incidence was obtained by Razavilar et al. (2013); 78.5%. Contamination of food by S. aureus may directly occur due to skin lesions of workers containing S. aureus or sneezing and coughing. Around 50% of human population carries S. aureus as commensals. Other contamination sources of S. aureus are soil, water, dust and air (Hanson et al., 2011).

Salmonella enterica, a principal cause of enteric diseases in human and animal with millions of sickness worldwide, whereas the non-typhoidal Salmonella species as a zoonotic agent is also principally associated with food borne infections (Van et al., 2012). Salmonella spp was failed to be isolated from the examined samples of Nile Tilapia (O. niloticus), Catfish (Clarias gariepinus) and Common carp (Cyprinus carpio). Similar results were recorded by Valenzuela-Armenta et al. (2018), who failed to detect Salmonella in adult or fry samples of tilapia. However, Omaima (2019) isolated Salmonella spp. (1/50, 2%). Higher incidence was reported by Maysoon (2014); 15%; and by Onyango (2009); 25.4%. In catfish (Clarias gariepinus) nearly similar results were obtained by Toyo et al. (2012); (7.5%). But higher incidence 32% was reported by Egbeb (2016). In common carp (Cyprinus carpio) similar results were obtained by Razavilar et al. (2013), who reported no salmonella in common carp. Salmonella is a type of bacteria with general occurrence in animals and the environment. The main sources of Salmonella are water, soil, animal feces, insects, surfaces of equipment, surfaces of utensils and food factories (Silva et al., 2007). It causes salmonellosis which in humans could result in severe typhoid fever (enteric fever) or salmonella fever (Egbere et al., 2010).

Gastrointestinal tract infection is the commonest cause of Aeromonads followed by wound infections. In immunosuppressed persons or those with hepatobiliary disease, aeromonads can cause otitis media, meningitis, endocarditis, peritonitis, cholecystitis, hemolytic uremic syndrome, septicemia and food poisoning (Guerra et al., 2007). Aeromonas spp was isolated from 80% of the examined Tilapia samples (Table 5). Nearly similar result was by Escarpulli et al. (2003) reporting incidences of A. salmoncindia and A. bestiarum 67.5% and 20.9%, respectively. Lower incidence was isolated 46.6% by Yagananth et al. (2009). Aeromonas spp was isolated from 100% of the examined catfish samples (Table 5). Nearly similar to result was reported by Rahayu et al. (2017) 95%. Lower result was 43.8% by Wamala et al. (2018). Aeromonas spp was isolated from 93.3% of the examined common carp samples (Table 5). Nearly similar result was found 99% by Kayis et al. (2018). Aeromonas is opportunistic bacteria also linked to several kinds of human infections, gastroenteritis, wound infections, septicemia, and respiratory infections (Parker and Shaw, 2011).

Pseudomonas spp. is widely extent in natural sources of water and accompanying with septicemia in aquatic animals. These bacteria are opportunistic pathogens, causing disease when the host exposed to stress (Magdy et al., 2014). Pseudomonas spp was isolated from 93.3% of the examined Tilapia samples (Table 6). This result was nearly similar to Maizhona et al. (2015), who observed 100% Pseudomonas spp in all cultured samples. Lower incidences were 30.83% Pseudomonas aeruginosa and 20.3% Pseudomonas fluorescens by Eissa et al. (2010). Pseudomonas spp. was isolated from 100% of the examined catfish samples (Table 6). Similar result 100% was found by Maizhona et al. (2015) in all cultured samples. Also, Kayis et al. (2018) 99%. Lower incidences were 27.5%, 19.62%, and 5% were recorded by Magdy et al. (2014), Danba et al. (2015) and Egbeb (2016), respectively. Pseudomonas spp was isolated from 53.3% of the examined common carp samples (Table 6). This result come in agree with El-Hady and Samy (2011) 55.3%. Lower incidence was 34.4% by Magdy et al. (2014). Higher incidence was 99% by Kayis et al. (2018). Contamination with enter toxigenic Pseudomonas has been testified from fish, food and drinking water resulting in diarrhea and skin infections in immune deficient persons (Wong et al., 2000).

5. CONCULSION

From the present study we concluded that Nile fish was contaminated by different microorganisms from polluted water or during handling and evisceration. These bacteria can be transported to human causing food borne illness. So, adequate cleaning and sanitization of utensils, effective training for workers on hygiene and safety, application of strict hygienic measures during handling of fish are required.

6. REFERENCES

knowledge exchange research and extension. FAO, Via Velluto Terme di Caracalla Vialedelle Terme di Caracalla 00153 Rome, Italy.


