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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 *Oreochromis 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* was70% (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 tilapiawere (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* were 27% (n=8), 60% (n=18) and 67% (n=20) from Nile tilapia, catfish and carp, respectively. *Salmonella* was failed to be detected in any of the examined fish samples. The incidence of *Aeromonas* spp were 80% (n=24), 100% (n=30) and 93% (n=28) from Nile tilapia, catfish and common carp, respectively. The incidence of *Pseudomonas* spp were93%(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 (*Oreochromis 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 *(Clariasgariepinus*) 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 great 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).

Staphylococcus 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 problematic 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).

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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 withoutundue 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 wasaseptically 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 ℃ 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 ℃ for 48 hrs.
Plating media: a loopful from positive enrichment tube was separately streaked into (EMB) agar (Oxoid) and incubated at 37 ℃ 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 stainingG-ve, medium size stained evenly coccobacilli.
- Motility test: motile.

2.2.2.2. Biochemical Identification was done according to Macfaddin (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 toISO (2003) as follows**:**
Accurately, 0.1 ml from nutrient broth was spread onto plats of Baird Parker (Oxoid). Plates were incubated at 37ºC 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 gmanalytical 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 thenincubated at 41.5°Cfor 24±3 hrs.
Plating out & identification: loopful of enrichment broth was streaked on XLD agar incubatedat 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 stainingG - vecocco bacilli to medium size rods.
- Motility test:motile.

2.2.4.2. Biochemical Identification was done according toQuinn *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 ℃ 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 at37°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 at37°C for 24hrs 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, H_2S 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 O119), 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).

Fish type	Positive samples		Identified bacterium	Serodiagnosis	Strain
	No.	%			
Oreochromis niloticus (Nile tilapia)	3	14.2%	E .coli	O84: H21	EPEC
	6	28.5%	E .coli	O26: H11	EPEC
	9	42.8%	E .coli	O128: H2	ETEC
	3	14.2%	E .coli	O119: H6	EPEC
Clariasgariepinus (African catfish)	5	50%	E .coli	O128: H2	ETEC
	2	20%	E .coli	O84: H21	EPEC
	3	30%	E .coli	O26: H11	EHEC
Cyprinus carpio (common carp)	7	31.81%	E .coli	O119: H6	EPEC
	7	31.81%	E .coli	O 17: H18	EPEC
	8	36.36%	E .coli	O128: H2	ETEC

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

Fish type	Positive samples	
	No.	%
Oreochromis niloticus (Nile tilapia)	21	70%
Clarias gariepinus (African catfish)	10	33.3%
Cyprinus carpio (Common carp)	22	73.3%

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

Fish type	No	%	Identified bacterium
Oreochromis niloticus (Nile tilapia)	5	55.5%	Mixed Culture
	4	44.4%	*Enterobacter*
Clarias gariepinus (Africancatfish)	10	50%	Mixed Culture
	10	50%	*Enterobacte ragglomerans*
Cyprinus carpio (common carp)	8	100%	*Enterobacter aerogenes*

Table (4) showed that the incidence of *S. aureus* was 8(26.6%), 18(60%) and 20(66.6%) from Nile tilapia, catfish and carp, respectively. *Salmonella* was failed to be detected in any of the examined fish samples.

Table (5) showedthatthe incidence of Aeromonas spp were 24(80%), 30(100%) and 28(93.3%) from Nile tilapia, catfishand common carp, respectively.
Table (6) showed that the incidence of Pseudomonas spp were 28(93.6%), 30(100%) and 16(53.3%) from Nile tilapia, catfish and common carp, respectively.

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

Fish type	Positive samples	
	No.	%
Oreochromis niloticus (Nile tilapia)	8	26.6%
Clarias gariepinus (African catfish)	18	60%
Cyprinus carpio (common carp)	20	66.6%

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

Fish type	Positive samples	
	No.	%
Oreochromis niloticus (Nile tilapia)	24	80%
Clarias gariepinus (African catfish)	30	100%
Cyprinus carpio (common carp)	28	93.3%

Table 6 Incidence of *pseudomonas* isolated from the examined fish samples (n=30).

Fish type	Positive samples	
	No.	%
Oreochromis niloticus (Nile tilapia)	28	93.3%
Clarias gariepinus (African catfish)	30	100%
Cyprinus carpio (common carp)	16	53.3%

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 (Table1).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(Table1). 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 from73.3% of the examined Common carp*(Cyprinus carpio)*samples (Table1). 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 (Table4). 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 (Table4).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 (Table4).Higher incidence was obtained by Razavilar et al. (2013);78.57%.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 entericai*s 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) (7.3%). But higher incidence 32%was reported by Egbebi (2016).In common carp (Cyprinus carpio) similar results was 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 (Table5). Nearly similar result was by Escarpulli et al. (2003) reporting incidences of A. salmoncidia and A. bestiarum 67.5% and 20.9%, respectively. Lower incidence was isolated 46.6%by Yagananth et al. (2009).*Aeromonas spp* isolated from 100% of the examined catfish samples (Table5). 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 carpsamples (Table5).Nearly similar result was found 99%by Kayıs 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(Table6). This result was nearly similar to Maimona et al. (2015), who observed 100%Pseudomonas spp. in all cultured samples. Lower incidences were 30.83% *Pseudomonas aeruginosa* and 20.3% *Pseudomonas fluorescense* by Eissa et al. (2010).

Pseudomonas spp. was isolated from 100% of the examined catfish samples (Table6). Similar result 100% was found by Maimona et al. (2015) in all cultured samples. Also, Kayıs 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 Egbebi (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 Kayıs 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

1. Abolagba, O.J. and Uwagbai, E.C. (2011): A Comparative Analysis of the Microbial Load of Smoke-Dried Fishes (Ethmalosa Fimbriation and Pseudotolithus Elongatus) Sold in Oba and Koko Markets in Edo and Delta States, Nigeria at Different Seasons. Australian Journal of Basic and Applied Sciences, 5(5): 544-550.
2. Adeshina, I.; Jenyo-Oni, A.; Ajani, E. K. and Adewale, Y. A. (2016a): Natural occurrence of Diplostomum spp. In farm-raised African catfish (Clariasgariepinus) from Oyo state, Nigeria. Intl. J. Vet. Sci. Med. 4: 41–45.
3. Akande, A. and Onyedibe, K. I. (2019): First report of enteropathogenic and enteroinvasive Escherichia coli with multiple antibiotic resistance indices from African catfish (Clariasglariepinus) in Nigeria. Afr. J. Clin. Exper. Microbiol. 20 (2): 95 – 103
4. Allen, M.J.; Edberg, S. C. Y and Reasoner, D.J. (2004): Heterotrophic plate count bacteria—what is their significance in drinking water. International Journal of Food Microbiology, 92: 265-274.
5. Alvarez, J.R.; Lamba, S. and Dyer, K.Y. (2006): An unusual case of urinary tract infection in a pregnant woman with Photobacterium damselae. Infectious Diseases in Obstetrics and Gynecology; 80682: 1-3.
6. Arni, M. M. (2012): The state of world fisheries and aquaculture Publishing Policy and Support Branch. Office of

knowledge exchange research and extension. FAO, Vialedelle Terme di Caracalla 00153 Rome, Italy.
7. Danba, E. P.; Bichi, A. H. and Ishaku, S.(2014): Occurrence of Pathogenic bacteria associated with Clariasgariepinus in selected fish farms of Kumbotso local Government area of Kano State, Nigeria. Bajopas. ; 7(2): 145-149.
8. Egbebi, A. O.; Muhammad, A. A.; Ugbodaga, M. and Oyama, M. O. (2016): Bacteriological analysis of catfish (Clariasgariepinus) in Owo area, Ondo state, Nigeria. J. Bio. Sci.; 2(10): 71-80.
9. Egbere, O. J.; Kadir, A.; Oyero, T.; Steve, K.; Odewumi, O. and Zakari, H. (2010): Bacteriological Quality of Catfish in Jos Metropolis, Nigeria. International Journal of Bioscience, 5 (2), 95-103.
10. Eissa, N.M.E.; Abou, E.E.N.; Shaheen, A.A. and Abbass, A. (2010): Characterization of Pseudomonas Species Isolated from Tilapia "Oreochromis niloticus" in Qaroun and Wadi-El-Rayan Lakes, Egypt. Global Veterinaria, 5, 116-121.
11. El-Hady, M.A. and Samy, A.A. (2011): Molecular typing of Pseudomonas species isolated from some cultured fishes in Egypt. Global Veterinaria, 7(6):576-580.
12. El-olemy, G.; Lobna, M.; Salem, G.; Nashwa, O.; Khalifa, S. and Mona, S. (2014): Detection of Some Bacterial Zoonosis in Market Fish in Qalyoubia Province and Their Control Benha Veterinary Medicine Journal 26:126- 136
13. Escarpulli, G.C.; Figueras, M.J.; Arreola, G.A.; Soler, L.; Rendon, E.F.; Aparicio, G.O., Guarra, j. and Chacon, M.R. (2003): Characterization of Aeromonas spp. isolated from frozen fish intended for human consumption in Mexico. International Journal of food Microbiology, 84:41-49.
14. FAO (2019): The state of world fisheries and aquaculture. FAO Fisheries department, fisheries information, data and statistics unit, Rome.
15. Food and Drug Administration "FDA" (2012): https://www.centerwatch.com/drug-information/ FDA-approved-drugs/year/2012.
16. General Authority for Fish Resources Development "GAFRD"(2013): fisheries statistics year book 2012. Cairo, Egypt.
17. Goja, A.M.; Ahmed, T.A.A.; Saeed, S.A.M. and Dirar, H.A. (2013): Isolation and identification of Staphylococcus spp. in fresh beef. Pakistan J. of Nutrition, 12(2): 114-120.
18. Guerra, I. M. F.; Fadanelli, R.; Figueiro, M.; Schreiner, F.; Delamare, A.P. L.; Wollheim, C.; Costa, S. P. O. and Echeverrigaray, S.(2007):Aeromonas associated diarrhoeal disease in South Brazil: prevalence, virulence factors and antimicrobial resistance. Brazilian J. Microbiology, 38: 638–643.
19. Hanson, B.M.; Dressler, A.E.; Harper, A.L.; Scheibel, R.P.; Wardyn, S.E.;Roberts, L.K.; Kroeger, J.S. and Smith, T.C. (2011): Prevalence of *Staph. aureus* and *MRSA* on retail meat in Iowa. J. Infect. Public Health, 4:169-174.
20. Hardi, H. E.; Rudy, A. N.; Gina, S.; RIA, S.; Maulina, A. and Mira, M. (2018): Identification of potentially pathogenic bacteria from tilapia (Oreochromis niloticus) and channel catfish (Clariasbatrachus) culture in Samarinda, East Kalimantan, Indonesia. BIODIVERSITAS 19 (2): 480-488.
21. Hassan, A.H.M.; Noor Eldeen, A.E.; Galal, H.M.; SohadDorgham, M.; Bakry, M.A. and Hakim, A.S. (2012):Further characterization of Enterobacteriaceae isolated from cultured fresh water fish in Kafr El sheikh governorate, Clinical, Biochemical and Histopathological study with emphasis on treatment trials. Global Veterinaria, 9(5):617-629.
22. International Organization for Standardization"ISO" 7251: 2005(E): Microbiology of food and animal feeding stuffs – Detection and enumeration of presumptive *Escherichia coli* – Most probable number technique.
23. International Organization for Standardization"ISO" 6579: (2002) (E): 4th ed. Microbiology - General guidance on methods for the detection of Salmonella, International Organization for Standardization, Geneve, Switzerland.
24. International Organization for Standardization"ISO" 6888: (2003): Microbiology - General guidance on methods for the detection of Coagulase-positive staphylococci, International Organization for Standardization, Geneve, Switzerland.
25. International Organization of Standardization "ISO"7218: (2007): Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations.
26. Jiang, H.; Tang, D.; Liu, Y.; Zhang, X.; Zeng, Z.; Xu, L. and Hawkey, P. (2012): Prevalence and characteristics of B-lactamase and plasmid-mediated quinolone resistance genes in Escherichia coli isolated from farmed fish in China. J. Antimicrob. Chemotherapy, 67(10): 2350-2353.
27. Kayıs, S.; Duzgun, A. and Er, A. (2018): Bacterial and Parasitic Pathogens Isolated from Some Wild Cyprinid Fishes, El-Cezerî Journal of Science and Engineering, 5(3); 763-772.
28. Kok, T.; Worswich, D. and Gowans, E. (1996): Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill Livingstone, UK.
29. Hanna, M.I.; El-Hady, M.A.; Ahmed, H.A.; Elmeadawy, S.A. and Kenwy, A.M.(2014): A contribution on Pseudomonas aeruginosa infection in African Catfish (Clariasgariepinus). RJPBCS 5(5) Page No. 575. ISSN: 0975-8585.
30. Maimona, Samia and Sabiel, Y. A (2015): Detection of the causative agents of bacterial fish septicemia of tilapia and clarais in Khartoum state. International Journal of Recent Scientific Research Vol. 6, Issue, 6, 4374-4377.
31. Maysoon, S. A. (2014): Isolation of bacteria from fish. International Journal of Advanced Research, 2, Issue 3: 274-279. ISSN 2320-5407.
32. McFadden, J.F. (2000): Biochemical testes for identification of medical bacteria. Waverly press Inc., Baltimore, med. 21202, USA.
33. Mitov, I.; Tanya, S. and Boyka, M. (2010): Prevalence of virulence genes among Bulgarian nosocomial and cystic fibrosis isolates of Pseudomonas aeruginosa. Brazilian Journal of Microbiology 41: 588-595.
34. Ahmed, O.M.. (2019): Bacteria Associated with Fresh-water Aquaculture Tilapia Fish (Oreochromis niloticus) in Suez, Egypt. Food Sci Nutr Res; 2(1): 1-7.
35. Onyango, M.D.; Wandili, S.; Kakai, R. and Waindi, E.N. (2009): Isolation of Salmonella and Shigella from fish harvested from the Winam Gulf of Lake Victoria, Kenya. J. Infect. Dev. Ctries., 3(2): 99-104.
36. Parker, J.L. and Shaw, J.G. (2011): Aeromonas spp. Clinical microbiology and disease. J. Infect. 62:109–118.
37. Quinn, P.J.: Carter, M.E.: Markey, B.K.: Donnelly, W.J.C. and Leonard, F.C. (2002): Veterinary Microbiology and Microbial diseases. MPG Book 1st ed. Bodmin, Cornwall, UK.
38. RahayuKusdarwati; KismiyatiSudarno; Hendi Kurniawan and YudhaTeguhPrayogi (2017): Isolation and Identification of Aeromonas hydrophila and Saprolegnia sp. on Catfish (Clariasgariepinus) in Floating cages in Bozem Moro Krembangan Surabaya. IOP Conf. Ser. Earth Environ. Sci. 55(1): 012038.
39. Razavilar, V.; Khani, M. R. and Motallebi, A. A. (2013): Bacteriological study of cultured silver carp. Iranian Journal of Fisheries Sciences, 12(3): 689-701.
40. Salem, M.A.A. (2015): Studies on Some Predominant Bacterial Diseases in Some Fresh Water Fishes in Dakahlian Farms. M. V. Sc. Thesis, Faculty of Veterinary Medicine, Mansoura University, Egypt.
41. Silva, N.; Junqueira, V.A.; Silveira, N.A.; Taniwaki, M.H.; Santos, R.S. and Gomes, R.R. and Okazaki M.M. (2007): Manual of methods for microbiological analysis of food. 3th ed. São Paulo: Varela.

42. Sivakami, R.; Premkishore, G. and Chandran, M.R. (2008): Occurrence and distribution of potentially pathogenic Enterobacteriaceae in carps and pond water in Tamil, Nadu, India. Aquaculture Research, 27, 375– 378.
43. Tayo, A.B.C.; Odunn, I.N.J.P.N. and Okonko, I.O. (2012): Microbiological and Physiochemical level of fresh catfish (Arius hendelotic) from different markets in AkwaIbm state, Nigeria, New York Science Journal., 5(4):38-45
44. Valenzuela-Armenta, J.A.; Sylvia, P.D.; Julio, A.C.; Magdalena, D.U.; María D.C.; Ignacio, O. and María, E.B. (2018): microbiological analysis of tilapia and water in aquaculture farms from Sinaloa. Microbiological analysis of tilapia Biotecnia 20(1): 20-26.
45. Van, T.H.; Nguyen, H.K.; Smooker, P.M. and Coloe, P.J.(2012):The antibiotic resistance characteristics of nontyphoidal Salmonella enterica isolated from food producing animals, retail meat and humans in South East Asia. Int J. Food Microbiol, 154: 98-106.
46. Wamala, K S. P.; Mugimba, K.; Mutoloki, S.; Evensen, Ø.; Mdegela, R.; Byarugaba, D. K. and Sørum H. (2018): Occurrence and antibiotic susceptibility of fish bacteria isolated from Oreochromis niloticus (Nile tilapia) and Clariasgariepinus (African catfish) in Uganda. Fisheries and Aquatic Sciences 21(6):1-10
47. Wedekind, C. M.; Gessner, F.; Vazquez, M. and Maerkiand, D. S. (2010): Elevated resource availability sufficient to turn opportunistic into virulent fish pathogens. Ecology, 91(5) 1251-1256.
48. Wong, S.; Street, D.; Delgado, S.I. and Klontz, K.C. (2000): Recalls of foods and cosmetics due to microbial contamination reported to the U.S. Food and Drug Administration. J. Food Prot., 63:1116-1113.
49. Yogananth, N.; Bhakyaraj, R.; Chanthuru, A.; Anbalagan, T. and Nila, M. (2009): Detection of virulence gene in Aeromonas hydrophilia isolated from fish samples using PCR technique. Global journal of Biotechnology and Biochemistry, 4(1):51-53.