Microbiological assessment of three types of fresh fish (*Tilapia niloticus*, *Labeo niloticus* and *Hydrocynus spp.*) sold in Ed Dueim, Sudan.

Arafat Mohammed Goja

Department of Food Science & Technology
Faculty of Agriculture & Natural Resources, University of Bakht Alruda, Ed Dueim,Sudan.
E-mail: arafatmohammed9@yahoo.com

Abstract: The aim of this study was to assess the microbial quality of fish and hygienic status of fisher market in Ed Dueim city, White Nile state, Sudan. Three fishes were selected, commonly named Polti (*Tilapia niloticus*), Debs (*Labeo niloticus*) and Kass (*Hydrocynus spp*.). Viable bacterial count was determined by Pour plate method, while coliform and fecal coliform were determined according to the Most Probable Number (MPN) techniques. Total Viable counts of bacteria and Staphylococci in fishes Skin were ranged from 2.8 x 10^3 to 9.8 x 10^4cfu/g and 0.0 to 7.2 x 10^2 cfu/g, respectively. The total count of Yeast and Moulds ranged from 0.0 to 5.3 x 10^2cfu/g while coliform and fecal coliforms were ranged from 15 to 120MPN/100g and 3 to 95MPN/100g, respectively. However, in fish's intestine the viable bacteria, Staphylococci and (Yeats & Moulds) counts were ranged from 1.5 x 10^3 to 8.4 x 10^4cfu/g, 0.0 to 8.0 x 10^2cfu/g and 0.0cfu/g to 3.7 x 10^3cfu/g, respectively. Total coliform and fecal coliform were ranged from 20 to 150MPN/100g and 0.0 to 75MPN/100g, respectively. The results revealed 8 genera of bacteria: *Enterobacteriaceae, Bacillus, Pseudomonas, Staphylococcus, Micrococcus, Streptococcus, Acinetobacter* and *Moraxella* in Skin's samples. The same genera were obtained from intestine samples. Among these isolates, *Enterobacteriaceae* [13(22.0%), 13(21.0%)] was the most prevalence isolated from skin and intestine, respectively. However, the least dominant isolate was *Acinetobacter* 2(3.4%) in skin and *Moraxella* 4(6.4%) in the intestine. The results also showed the detection of *Samlonella* and *Shigella* indifferent rate in some fish samples (skin and intestine). According to the finding from this study, the fishes examined were potentially contaminated with the pathogenic microorganisms. Therefore, fishes should be appropriate handling, cleaned, washed and cooked before consumption.

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1. Introduction

Fish and fishery products are highly perishable food, and its quick perishability has been the main hurdle in its preservation (Okoro et al., 2010; Dewi et al., 2011; Musa et al., 2010). Fish meats are very important sources of proteins, minerals, vitamin ….etc. However, fish meat spoil more quickly than other muscle foods, particularly when poor handled and such spoilage is primarily bacterial in nature; about 30% of landed fish are lost through microbial activity alone (Ghaly et al., 2010). Contamination of fish with microorganism reflected environment pollution (Adeyemo, 2003). So, the microbial flora associated with fish is a reflection of their aqueous environment. If the fish habitats are contaminated by pathogenic bacteria, the consumption of these fish may risk to the human health. Many studies of bacterial flora in the skin and intestine of fish have been conducted (e.g. Al-Harbi and Uddin, 2004; 2005; Okoro et al., 2010; Adebayo-Tayo et al., 2012a;b.; Yagoub, 2009; Das Trakroo and Agarwal, 2011). These studies have demonstrated variation in the microbial flora in fish species collected from different location in different countries. Bacteria such as *Pseudomonas fluorescens, Aeromonas hydrophila, Edwardsiella tarda, Vibrio spp.* and *Myxobacteria* are ubiquitous in the aquatic environment (Gilmour et al., 1976; Allen et al., 1983). However, pathogenic bacteria (eg. *Escherichia coli salmonella, Shigella*, etc.) were introduced to water bodies through human or animal faeces contaminant. In Sudan, recently some research has been carried out on the microbiological quality of fish and fish products (Ahmed and El Hag, 2011; El Hag et al., 2012). Moreover, *Enterobacteriaceae* and *Pseudomonas spp.* of raw fish in Khartoum market were investigated (Yagoub, 2009). These studies showed a wide range of microbial contamination of fish and fish products. Nowadays, in Sudan, the consumption of fish is relatively increased, especially in coastal cities, due to the rising of red meat prices as the main source of animal protein. Thus, increases the business activities in fish markets as well as the

consumption of fish. Ed Dueim town is one of the famous market's cities of fish and fish products in Sudan, located on the west of the White Nile River (200km South of Khartoum City). The fish is marketing early morning on the shore. Where are the anglers are landing? Due to lack of information on microbial quality, the consumers assess the quality of fish according to their visual observation based upon his experiences. Thus, the study was conducted to examine the hygienic status of the most abundant fresh fish sold in Ed Dueim market.

2. Materials and Methods

2.1. Collection of samples

The fishes sample Polti (*Tilapia niloticus*), Debs (*Labeo niloticus*) and Kass (*Hydrocynus spp.*) were aseptically collected from the Ed Dueim shore market on the White Nile River, Sudan. Then the samples immediately transferred to the microbiological lab for analysis.

2.2 Microbiological analysis

The bacterial counts on the skin and intestines of fish samples were determined as follows:

2.2.1. Skin

A sterile cotton swab was robbed all over the skin of the fish. The swab was immediately placed in a sterile bottle containing 100ml of 0.10% (w/v) peptone water. Then the bottle was mixed by hand shaking for 5minutes. The serial dilutions (10 fold) were made up to 10^5.

2.2.2. Intestine

From each fish samples 5g of the intestine were taken and homogenized in a mortar. The homogenate sample was transferred to the sterile bottle containing 95ml of 0.10% (w/v) peptone water. Then the bottle was mixed by hand shaking for 5minutes. The serial dilutions (10 fold) were made up to 10^5.

2.2.3. Total viable count (TVC)

Aseptically the appropriate serial dilutions of both parts of fishes (Skin and Intestine) were spread on poured plates of Plate count agar, Baird-Parker agar and Potato Dextrose Agar for counting of total viable bacteria count, staphylococci spp. count and for yeasts and moulds, respectively. All inoculated plates were incubated at 37 ^{o}C for 24-48 hours except Potato dextrose agar palates, which were incubated for 72 hours at 25 ^{o}C as described by (Harrigan, 1998).

2.2.4. Coliform and faecal coliform

This was done by Most Probable Number (MPN) technique as described in (APHA, 1995).

2.2.5 Detection of *salmonella* and *Shigella*:

10ml of homogenate solution (Skin and Intestine) were aseptically transferred to 90 ml of sterile nutrient broth bottle and incubated at 37^{o}C for 24 hours. 10 ml was taken aseptically and added to 100 ml selenite broth and incubated at 37 ^{o}C for 24 hours. Then with a loopful streaking was done on dried bismuth sulphite agar plates. The plates were incubated at 37 ^{o}C for 72 hours. Black metallic sheen separated colonies indicated the presence of salmonella. Then the conformation did by using a discrete black sheen colony and sub culturing it in a Triple sugar iron agar tubes. Production of a black colour at the bottom of the tube confirms the presence of Salmonella. For *Shigella*, a loopful selenite broth was streaking onto *Salamonella* and *Shigella* Agar (SSA) and incubated at 37 ^{o}C for 18-24 hours.

2.2.6. Isolation and identification of microorganism

Discrete colonies were picked from plate count agar and purified by streaking twice on nutrient agar, after purification, bacterial grouping according to morphological characteristics and then gram stain was carried out. All the purified isolates were examined for cell shape, motility and spores forming. The isolates were then subjected to biochemical tests as described in (Barrow and Gelthan, 1993).

3. Results

3.1. Bacterial load

Table 1 shows the total viable bacterial count (TVC), Staphylococci (STC) and Yeast & Moulds counts (Yst&Mds) in skins of three fish's types in Ed Dueim shore market, Sudan. They were ranged from 2.8 x 10^3 to 9.8 x 10^4cfu/g for TVC, 0.0 to 7.2 x 10^2 cfu/g for STC while the Yeast & Moulds counts ranged from 0.0 to 5.3 x 10^2cfu/g. For coliform and faecal coliform were ranged from 20 to 150MPN/100g and 0.0 to 75MPN/100g, respectively. Table 2 shows the total viable count (TVC), Staphylococci (STC) and Yeast & Moulds counts (Yst&Mds) in fish intestines. The viable bacterial count ranged from 1.5 x 10^3 to 8.4 x 10^4cfu/g, for STC 0.0 to 8.0 x 10^2cfu/g while the Yeast & Moulds counts ranged from 0.0 to 3.7 x 10^3cfu/g. For coliform and faecal coliform were ranged from 20 to 150MPN/100g and 0.0 to 75MPN/100g, respectively.

3.2. The number and percent of bacteria isolated

Table 3 and 4 showed the numbers and percentage of bacterial genera isolated from skin and

intestine. Fifty-nine isolates were recovered from fish skins, which were identified as 13(22.0%) *Enterobacteriaceae*, 10(16.9%) *Micrococcus*, 9(15.3%) *Pseudomonas*, 9(15.3%) *Staphylococcus,* 7(11.9%) *Streptococcus,* 6(10.1%) *Bacillus,* 3(5.1%) *Moraxella* and 2(3.4%) *Acinetobacter.*

Table 3. Sixty-two isolates were obtained from fish intestines, which were identified as 13(21.0%) Enterobacteriaceae, 8(12.9%) *Micrococcus,* 7(11.3%) *Pseudomonas,* 8(12.9%) *Staphylococcus*, 7(11.3%) *Streptococcus*, 9(14.5%) Bacillus, 4(6.4%) *Moraxella* and 6(9.7%) *Acinetobacter* Table 4.

Table 1. The microbiological examination of fish's Skin

Fish species	TVC cfu/g	STC cfu/g	Yst & Mds cfu/g	CC MPN/100g	FC MPN/100g
Tilapia niloticus	6.0×10^2 - 5.4×10^4	0.0 - 6.5×10^2	0.0 - 5.0×10^2	15 - 120	3 – 95
Labeo niloticus	3.6×10^3 - 9.8×10^4	0.0 - 5.8×10^2	0.0 - 5.2×10^2	26 - 120	11 – 93
Hydrocynus spp.	2.8 x 103 - 7.3×10^4	0.0 - 7.2×10^2	0.0 - 5.3×10^2	20 – 44	0 – 72

TVC ≡ Total viable count; STC ≡ Staphylococci count; Yst & Mds ≡ Yeast & Moulds count
CC ≡ Coliform count; FC ≡ fecal coliform count

Table 2. The microbiological examination of fish's Intestine

Fish species	TVC cfu/g	STC cfu/g	Yst & Mds cfu/g	CC MPN/100g	FC MPN/100g
Tilapia niloticus	4.2×10^3 - 8.4×10^4	0.0 - 8.0×10^2	0.0 - 5.6×10^2	24 – 150	11 – 75
Labeo niloticus	1.5 X x 10^3 - 3.6×10^4	0.0 - 7.0×10^2	NG	20 – 35	3 – 72
Hydrocynus spp.	7.6×10^3 - 6.7×10^4	0.0 - 6.0×10^2	0.0 - 3.7×10^3	19 – 75	0 – 14

TVC ≡ Total viable count; STC ≡ Staphylococci count; Yst & Mds ≡ Yeast & Moulds count
CC ≡ Coliform count; FC ≡ fecal coliform count, NG≡ No Growth

Table 3. Numbers and percentage of bacterial isolates from fish's skin surfaces.

Bacteria isolated	No. (%)	Fishes Species / No. (%)		
		Tilapia niloticus	*Labeo niloticus*	*Hydrocynus spp.*
Enterobacteriaceae	13(22.0)	4(30.8)	5(38.4)	4(30.8)
Staphylococcus	9(15.3)	3(33.3)	1(11.1)	5(55.6)
Bacillus	6(10.1)	2(33.3)	2(33.3)	2(33.3)
Pseudomonas	9(15.3)	3(33.3)	3(33.3)	3(33.3)
Micrococcus	10(16.9)	2(20.0)	4(40.0)	4(40.0)
Streptococcus	7(11.9)	2(28.6)	2(28.6)	3(42.8)
Acinetobacter	2(3.4)	1(50.0)	1(50.0)	0(0.0)
Moraxella	3(5.1)	0(0.0)	2(66.7)	1(33.3)
Total	59(100.0)	17(28.8)	20(33.8)	22(37.2)

Table 4. Numbers and percentage of bacterial isolates from fish's intestine.

Bacteria isolated	No. (%)	Fishes Species / No. (%)		
		Tilapia niloticus	*Labeo niloticus*	*Hydrocynus spp.*
Enterobacteriaceae	13(21.0)	5(27.8)	4(18.2)	4(18.2)
Staphylococcus	8(12.9)	2(11.1)	2(9.1)	4(18.2)
Bacillus	9(14.5)	2(11.1)	4(18.2)	3(13.6)
Pseudomonas	7(11.3)	2(11.1)	2(9.1)	3(13.6)
Micrococcus	8(12.9)	2(11.1)	3(13.6)	3(13.6)
Streptococcus	7(11.3)	3(16.6)	2(9.1)	2(9.1)
Acinetobacter	6(9.7)	1(5.6)	3(13.6)	2(9.1)
Moraxella	4(6.4)	1(5.6)	2(9.1)	1(4.5)
Total	62(100.0)	18(29.0)	22(35.5)	22(35.5)

From the tables, *Enterobacteriaceae* was the most frequency bacterial isolates [(22.0%), (21.0%)] in both skins and intestines, respectively. *Acinetobacter* was least dominant (3.4%) in skins While *Moraxella* (6.4%) in intestines

3.3. *Salmonella* and *Shigella* detection

Table 5 showed the detection percentage of *Salmonella* and *Shigella* in fish parts. *Salmonella* was recorded high and low percent (46.7%), (33.3%) in intestine and skin of *Tilapia niloticus* samples, respectively.

Table 5. Representative Percent of Salmonella and Shigella in the fish samples

Fish species	No.of Samples	*Salmonella* %		*Shigella* %	
		Skin	Intestine	Skin	Intestine
Tilapia niloticus	15	33.3	46.7	20.0	20.0
Labeo niloticus	15	40. 0	40.0	26.7	26.7
Hydrocynus spp.	11	45.5	36.4	27.3	36.4

However, *Shigella* was recorded higher percent (36.4%) in an intestine of *Hydrocynus spp.* samples while the lower percent (20.0%) was observed in both skin and intestine of *Tilapia niloticus* samples.

4. Discussion

This is the first study on the microbial quality of shore market fishes in Ed Dueim, Sudan. The results showed that the bacterial load varied in skin Table 1 and intestine Table 2. Bacterial counts of fish from skin parts were ranged from .8 x 10^3 to 9.8 x 10^4cfu/g, staphylococci count 0.0 to 7.2 x 10^2 cfu/g while the total Yeast & Moulds counts ranged from 0.0 to 5.3 x 10^2cfu/g. The total viable bacterial count of the samples from intestine parts ranged from1.5 x 10^3 to 8.4 x 10^4cfu/g, staphylococci count 0.0 to 8.0 x 10^2cfu/g while the Yeast & Moulds counts ranged from 0.0 to 3.7 x 10^3cfu/g. According to Surendran et al. (2006), the acceptable limit of bacterial load in fresh fish is 5 x10^5 /g at 37°C. The bacterial load in all samples comes within the acceptable range, which were ranged from 1.5 x 10^3 to 9.8 x 10^4. Similar results obtained by (Al Ghabshi, 2012 and Prakash et al. 2011). They found 1.54 x 10^4 cu/g in fresh fish 5.7 x 10^4cfu/g (as maximum) in dried seafood's in different seasons. Adams and Moses (2008) reported that the total bacterial load of the surface slime of fish can range from 10^2 to 10^7 cfu/cm2 and the gills and Intestines can range up to 10^3 and 10^7 cfu/g respectively. The finding also within the range of the maximum limit (5×10^5 cfu/g) as recommended by International Commission on the Microbiological Specification of Foods (ICMSF, 1982).

A greater range of bacterial count than this study have been obtained by other researchers (Okoro et al 2010; Chowdhury et al. 1989; Al-Harbia and Uddin, 2004; 2005.) from intestines in different fishes. They were found 8.7 x 10^5 Cfu/g in Mullet, Nigerian marine fish, 5.5 x 10^6 to 9.8 x 10^9 cfu /g in tilapia and 8.9+1.8 x 10^5 to 1.3+2.2 x 10^9, 6.8 x 10^6 to 7.5 x 10^7cfu/g in freshwater tilapia, respectively. A previous study carried out in Sudan by Yagoub (2009) in raw fish sold in Khartoum State. She found that the total bacterial count in skin and intestine were 3 x 10^7 to 4 x 10^9 and 1.5 x 10^5 to 1.6 x 10^8 cuf/ml, which greater than this study. This could be due to the differences in market's situation and locations. Regarding to the Tables 1 and 2, staphylococci count and Yeast & Moulds counts, were ranged from 0.0 to 8.0 x 10^2 cu/g and 0.0 to 3.7 x 10^3, respectively. This value is within the range of values of fresh fish meat as reported by Microbiological Criteria for Arabia and Egyptian Standard Food and by (Gillespie et al., 2000; Jackson et al., 2001 and Eleftheriadou et al., 2002). A study reported Yeast & Moulds and staphylococci counts as 1.0x10^1 to 6.6x10^1 and 2.1x10^1 to 2.2x10^2 cfu/g of fresh fish meat sample's storage at room temperature, respectively El-Shamery (2010) that is within the range of the present study. The coliform and faecal coliform bacteria in all samples ranged from 15 to 150MPN/100g and 0.0 to 95MPN/100g, respectively Tables 1 & 2. Prakash *et al.* (2011) reported that the MPN value of the seafood samples varied with different seasons. He founds total coliforms and faecal coliforms during summer varied from 3 to 65 and 10 to 30 / 100 g, 45 to 115 and 15 to 95 / 100 g in post-monsoon and between 65 to 150 and 25 to 95 / 100 g in monsoon, respectively. Our results were not in agreement with Hood *et al*. (1983) findings. He found that fecal coliform levels were above the recommended wholesale level suggested by the National Shellfish Sanitation Program (less than or equal to 230/100 g).

Generally, the presence of coliform and faecal coliform is not the normal flora of bacteria in fish (Mandal et al., 2009). This is reflecting the contamination of fish habitat with the human and animal faeces. Peoples in Ed Dueim city they use shore as open defecation, washing, bathing, swimming is daily activities along the shore of the White Nile River and dumping of house waste directly into the water.The present study revealed 8 genera of bacteria in both skin and intestine samples: *Enterobacteriaceae, Micrococcus, Pseudomonas, Staphylococcus, Streptococcus, Bacillus, Moraxella* and *Acinetobacter* in different rate as shown in Tables 3 and 4. In addition, *Salmonella* and *Shigella* were also detected Table 5. Bacterial genera detected in skins and intestines from fishes were most similar. *Enterobacteriaceae, Micrococcus, Staphylococcus* and *Pseudomonas* were the most common and dominant bacteria in skin as shown in Table 3. *Enterobacteriaceae, Bacillus, Micrococcus* and

Staphylococcus were dominated bacterial flora obtained from intestine Table 4.

Among these isolates *Enterobacteriaceae* represented most dominant isolates [(22.0%), (21.0%)] in both skin and intestine samples, respectively. Similar results obtained by Yagoub (2009) who was isolated *Enterobacteriaceae* in more than 50% of raw fish samples collected from Khartoum market. Some organisms of isolated (Tables 3, 4 and5) are of public health concern. Shigellosis and Salmonellosis are food borne diseases caused by *Shigella* and *Salmonella*, respectively. *Staphylococcus spp.* It is associated with food poisoning, produced toxin, which makes man sick, usually associated with the nausea, vomiting and diarrhea after eating the staphylococci infected food (O'connell, 2002). Adebayo-Tayo et al.(2012a) reported that Bacillus spp. is known to be human food poisoning causes a toxin-medicated disease rather than an infection.

5. Conclusion

Good fish quality should have a total count of bacteria less than 10 per gram and coliforms and faecal coliforms should not exceed 100/gm and 10/gm, respectively (FAO, 1979). Based on our findings it can be concluding that, although the bacterial load, coliform and faecal coliform counts were come within the limit standard. However, the detection of *Salmonella* and *Shigella* in fresh fish samples will cause health risks to the fish consumers. The presence of *Salmonella* and *Shigella* in these fishes indicates the contaminant environment habitats of fish and poor personal hygiene of sellers and fishermen. Thus, the following recommendations are made: fishes should be appropriate handling, cleaned, washed and cooked before consumption, fishermen should be educated on the adverse effect of lack of proper personnel, environmental hygiene and sanitation and the Public health authorities in Ed Dueim should inspect the market and fishes before sold to the consumers.

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Corresponding Author

Dr. Arafat Mohammed Goja
Department of Food Science and Technology
University of Bakht Alruda
White Nile State, Ed Dueim, Sudan
E-mail:arafatmohammed9@yahoo.com

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