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## Assessment of bacteriological contamination of fish in some fish farms at Khartoum State

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### المستخلص:

أجريت هذه الدراسة المقطعية في الفترة من أبريل إلى يوليو 2014 في ولاية الخرطوم ، السودان ، للتحقيق في البكتيريا الهوائية لجلد الأسماك في 3 مناطق مختلفة في الخرطوم. تم جمع 150 عينة من الجلد والخياشيم. واستخدمت وسائل الاستزراع السائلة وشبه الصلبة والصلبة مثل مرق المغذيات وأجار الدم وأجار ماکونكي وسيط سترات سيمون لعزل وتحديد البكتيريا والفطريات. الى جانب ذلك ، تم إجراء تلويح غرام وتوصيف كيميائي حيوي. كشفت زراعة العينات التي تم جمعها عن نمو البكتيريا من جميع العينات التي تم جمعها وأظهرت الخياشيم أعلى نسبة تلوث في السالمونيلا. البكتيريا معزولة ، أساسا أنواع السالمونيلا ، و E.coli. كما تم استخدام استبيان. كان لدى غالبية المجيبين مستوى جيد من المعرفة والموقف الإيجابي بشأن النظافة الغذائية ، ولم يكن سوى قاصر من المجيبين على مستوى جيد من الممارسة الصحية. وقد تبين أن 64.7 ٪ و 46 ٪ من المستطلعين تلقوا تدريباً على صحة الأغذية والتفتيش عامل الصحة البيئية على التوالي. كما تم الكشف عن وجود علاقات بين المعرفة والموقف والتدريب الرسمي على نظافة الأغذية ومستوى ممارسات صحة الغذاء. تم العثور على جلد السمك والخياشيم لإيواء العديد من البكتيريا المسببة للأمراض وقد يكون مصدرًا للعدوى ويمكن أن ينقل العديد من مسببات الأمراض للعاملين في مزارع الأسماك والمستهلكين. لذلك ، يوصى بالتفتيش الروتيني الشامل والدقيق للأسماك لضمان السلامة وعدم وجود مخاطر جسيمة للمستهلكين. للسيطرة على التلوث يمكن استخدام تدابير رقابة صارمة مثل نظام تحليل المخاطر ونقطة مراقبة سلامة الأغذية الأخرى.

الكلمات المفتاحية: البكتريولوجية ، التلوث ، الأسماك.

### ABSTRACT:

This cross-sectional study was conducted from April to July 2014 in Khartoum state, the Sudan, to investigate aerobic bacteria of skin of fish in 3 different areas in Khartoum. A total of 150 samples were collected from the skin and gills. Liquid, semi-solid, and solid culture media like nutrient broth, blood agar, MacConkey agar and Simmon's citrate medium were used for the isolation and identification of bacteria and fungi. Besides, Gram staining and biochemical characterization were also conducted. Culturing of the collected samples revealed growth of bacteria from all samples collected and gills showed the *Salmonella* highest contamination. Bacteria isolated, mainly *Salmonella* species, and *E.coli*. Also a questionnaire was used. A majority of the respondents had a good level of knowledge and positive attitude about food hygiene, only minor of the respondents had a good level of hygienic practice. It was revealed that 64.7% and 46% of the respondents received training on food hygiene and environmental health worker inspection respectively. It was also revealed that, there were relationships between

knowledge, attitude, formal training on food hygiene and the level of food hygienic practices. Fishes skin and gill were found to harbor many pathogenic bacteria and may be a source of infections and can transmit several pathogens to workers in fish farms and consumers. Therefore, thorough and strict routine inspection of fish is recommended to ensure safety and that there are no serious risks to consumers. For controlling the contamination strict control measure can be used such as HACCP and other food safety management system.

**Keywords:** bacteriological ,contamination, fish

### **Introduction:**

Seafood and fish constitute an important food component for a large section of world population (Wafaa *et al.*, 2011). They come after meat and poultry as staple animal protein foods where fish forms a cheap source of protein (Wafaa *et al.*, 2011). Sea foods have traditionally being a popular part of the diet in many parts of the world and in some countries constituted the main supply of animal protein. Today, even more people are turning to fish as a healthy alternative to real meat (Adebayo-Tayo *et al.*, 2012). Sudan is one of the largest countries in Africa with an area of 1861500 km. The contribution of fisheries to the gross domestic product (GDP) is currently marginal, however Sudan is endowed with water resources and lands that can support vigorous capture fisheries and aquaculture (FAO,2014). Tropical fish can be either salt water as marine or fresh water as Nile , species are available in huge variety of colour and size (Russell *et al* 2014) . On a global scale, fish and fish products are the most important source of protein and it is estimated that more than 30% of fish for human consumption comes from aquaculture (Hastein *et al* 2006) . In Africa, fish supplies 17% of protein and it is one of the cheapest source of protein (Calculus and Ward 1996) . The advantage of fish as food is as a result of its easy digestibility and high nutritional value. Fish should be viewed not only as food, but also as a really source of income in the small holder farming sector (Smith and Yoshida , 2000) . Fish is easy to digest and taste delicious can be prepared easily in many food industries , fish oil also important and necessary to children (Kamel *et al.*, 2013) ,also fish is good food for the brain as well as contain protein and a wide variety of vitamins and minerals including vitamin A and D , phosphorus , magnesium , selenium and iodine (FAO ,2004). The vast majority of outbreaks of food-related illness are due to pathogenic microorganisms, rather than to chemical or physical contaminants. As they are generally undetectable by the unaided human senses (i.e.they do not usually cause colour changes or produce off-flavours or taints in the food) and they are capable of rapid growth under favourable storage conditions (Lelieveld *et al.*, 2003). *E.coli* is the most common contaminant and is often encounter in high number that reported by Thampuran *et al.*, (2005). Morales *et al.*,(2004) found that *Salmonella* spp is most in the external surface of Tilapia. HACCP is management system in which food safety is addressed through analysis and control of biological, chemical and physical hazards from raw material, production, procurement and handling to manufacturing, distribution and consumption of the finished product (NACMCF, 1992). The need for HACCP, to successfully implement HACCP in food supply system, authorities responsible for food safety should be aware to system such as HACCP. In a survey conducted to find out whether HACCP was more effective strategy than their current or other methods industry groups had used to secure food hygiene 41% strongly agreed 50% agreed , while only 9% did not think that the strategy was more effective than their current provision (Ehiri *et al.*, 1997) . This study conducted to isolate the gram-negative bacteria and to discuss the hazards from environmental factor that effect fish farm in Khartoum state .

**Materials and Methods:****Study area:**

A cross-sectional study was carried out from April to July 2014 in Khartoum state. Aquiculture farms in three localities were investigated, namely; Khartoum, Khartoum North (Bahry) and Omdurman.

**Sample size:**

A total of 150 samples were selected randomly from 3 fish farms. Selection of the farms was based on the willingness of the owners to participate in the study. That means not all the farms has the same chance for being selected and this was called Non-probability sampling methods as described by Thrusfield (2007).

**Samples collection:**

A total number of 150 samples were collected from the skin and gill of fish. Samples were collected as described by Buller (2004). For investigation of bacteria, the samples were taken with a cotton swab from 10 cm<sup>2</sup> - depending on size- fish skin area near to the lateral line of the body. For gill samples, the operculum was opened firstly and then the gills were swabbed with cotton swab. All samples were placed in an ice container and transported to the laboratory of the Department of Microbiology, College of Veterinary Medicine, Sudan University of Science and Technology, and were cultured within 2 hours of collection.

**Culture media and reagents:**

Different types of culture media including liquid, semi-solid, and solid media, and chemicals, and reagents were used for isolation and identification of bacteria all were either bought from companies ready-to-use or prepared according to Barrow and Feltham, 2003; Ochei and Kolhatkar 2000). The culture media were peptone water, nutrient broth, glucose phosphate peptone broth, nutrient agar, blood agar, MacConkey agar, starch agar, nutrient gelatin media, urea agar, Simmon's citrate medium, Hugh and Leifson's (O-F) medium, motility medium, and Arginine media. Chemicals and reagents included Gram solution, crystal violet, Lugol's iodine, decolorizing reagent, counter stain, Tetra methyl-p-phenylenediamine dihydrochloride, and Alpha-naphthol solution, as well as Voges-Proskauer (VP) test, Methyl red, Kovac's reagent and Nessler's, Andrade's indicator, Neutral red, Phenol red, Bromothymol blue, and Lead acetate paper.

**Culturing:**

Collected samples were first inoculated into liquid media and incubated at 37°C for 24 h, then they were streaked onto blood and MacConkey agars and incubated aerobically at 37°C for 24 h. Also further incubation was continued for another 24 h and if no growth was evident, then the plates were discarded and the sample was considered negative. All cultures were examined by naked eye for growth, colony morphology, and any changes in the medium. Purification of isolates was done by sub-culturing of a single well separated colony onto blood agar or nutrient agar and finally pure cultures were stored at 4°C.

**Identification of isolates:**

Gram staining was carried out according to Barrow and Feltham (2003). The prepared slides were examined microscopically under oil emersion objective lens. Biochemical tests including catalase test, oxidase test, motility test, and oxidation fermentation (O/F) test were conducted. Other biochemical tests were sugar fermentation test, gelatin hydrolysis, arginine hydrolysis, starch hydrolysis, slide and tube coagulase test, indole production test, H<sub>2</sub>S production test, urease test, citrate utilization test, Voges-Proskaur (VP) test, and methyl red test.

**Questionnaire:**

A structured questionnaire was adopted from previous published research articles in order to meet the objective of this study (Adesokan and Raji, 2014; Soares *et al.*, 2012). The language of the questionnaire was translated to the local language (Arabic) in which all the participants can communicate. After pre-testing the questionnaire with 20 of fish farm workers the last version was prepared.

**Data analysis:**

The statistical analyses of the data were performed by using SPSS (Statistical Package for the Social Sciences) software version 20. Descriptive statistics such as frequency (%) for categorical and mean and standard deviation (SD) for numerical data were used to sum up the data. ANOVA test was also used to find the relationship between the variables. P-value less than 0.05 were considered statistically significant.

**Results:**

As shown in table (1) out of 150 collected samples 50 (33.3%) showed positive isolation for *E.coli*, whereas 60(40%) showed *Salmonella* spp. from the total, and mixed isolation (*E.coli* and *Salmonella* spp.) were recorded about 40(26.7%) of the positive samples.

Table (1) the number and percentage of bacteria isolated from fish in Khartoum state

Locality	<i>Salmonella</i>	<i>E.coli</i>	Mixed
	No (%)	No (%)	No.
Khartoum	15 (30)	17 (34)	18 (36)
Omdruman	18 (36)	19 (38)	13 (26)
Khartoum North	27 (54)	14(28)	14 (28)

No=number, %=percent

The mean of the total plate count (TPC) for all the fish (skin and gills) samples ranged between 6.89 -7.27 bacteria as shown in table (2) out of the 150 fish samples analysis for TPC the gills had the highest number of bacteria.

Table (2) the mean  $\pm$  standard deviation of bacteria isolated from gills and skins in some farms in Khartoum state

Locality	Number of bacteria	Percent %	Mean $\pm$ Sd	Significant
<b>Khartoum</b>				
Gill	25	16.7 %	6.89 $\pm$ 0.20	NS
Skin	25	16.7%	6.92 $\pm$ 0.23	NS
<b>Omdurman</b>				
Gill	25	16.7%	7.27 $\pm$ 0.24	NS
Skin	25	16.7%	7.19 $\pm$ 0.09	NS

**Khartoum****North**

Gill	25	16.7%	7.24 ± 0.18	NS
Skin	25	16.7%	7.27 ± 0.17	NS

On the other hand, the critical points that associated with environment in fish farms were obtained from respondents, as shown in table (3)

Table (3) Personnel data, food safety knowledge, practice and attitude among some fish workers in fish farm investigated in Khartoum state

Factor	Frequency	Percent %
1-Sex male	33	97.1
female	1	2.9
2-Age 20- 30	7	20.6
31-40	10	29.4
> 40	17	50
3- Training simple	22	64.7
High	12	35.3
4- Education literate	23	67.6
illiterate	11	32.4
5- Fish size large	3	8.8
Medium	27	79.4
Small	4	11.
6- Fish pond one	6	17.6
2-5	15	44.2
>6	13	38.2
7- Production <5	16	47.1
6-9	5	14.7
>10	13	38.2
8-Uniform yes	19	55.9
no	15	44.1
9-Hand washing yes	2	5.9
No	2	5.9
Missing system	30	88.2
10-Untensil wash yes	12	35.3
No	5	14.7
Some times	17	50.0
11-Sellplace instate	29	85.3
Outstate	5	14.7
12-Fish cooling yes	16	47.1
no	18	52.9
13-Fishtransport after	12	35.3
Before clean	22	64.7
14-Roten fish unclean	34	100
15-Fish preserve chillin	8	23.5
Freezed	23	67.6
salted	3	8.8
16-Preferseason	17	50.0
Summer	11	32.4
Winter	6	17.6
Autoum		
17-Bath after yes	25	73.5
No	9	26.5
18-Diseased yes	6	17.6

No	28	82.4
19-Nail cutting yes	13	38.2
No	21	61.8

The results showed that the age of the farm-workers in these farms were over 40 and they had simple training percent (64.7%). Also was 79.4% from the total . The production in more farms about 5 ton in the year 47.1%. Fish ponds arranged between 2-5 in most farms .The worker whom had uniform for their work were 55.9 % , also they were doing hand washing is less (5.9% ). Some of the owners washed the utensils (50%). The distribution of fish in Khartoum state was most common (85.3%) , and the fish cooling was also recorded (table 3). Most of the respondents were transported fish without washing (64.7%) and preserved those frozen (67.6%). The majority of owners had a bath (73.5%) after working.

#### Discussion:

One hundred and fifty samples were collected from gills and skins of fish and cultured. All samples showed bacterial growth and gave isolated . The bacteria pathogens that were isolated and identified include *E.coli* , *Salomonella*. The highest isolation of *E.coli* was in Omdurman due to environment habitats, feeding habits .The high incidence of *Salmonella* was recorded in Khartoum North might be due to precipitation ,environmental condition prevailing high degree of another cause to contamination. The isolation of these groups of organisms indicated fecal and environmental pollution and these supported the finding of Yagoub *et al* ( 2009) who isolated pathogenic and potential pathogenic organisms from tap water that originated from Nile River . This also confirms the finding of Koutsoumanis and Nychas (2000), Gonzalez-Podriguez *et al* (2001) and Herrera *et al* (2006) who isolated similar organisms from fish and fish products . *E .coli* was isolated from gills and skins of samples fish in the present investigation this is in agreement with previous study in Sudan by Hnadi (2008) who reported the presence of *E.coli* in gills and intestines. Also according to published microbiological guidelines by Gilbert *et al.*, (1996) the results suggest that microbiological quality of the fish examined is unacceptable levels. The total number of bacterial count for fresh fish was ranged between 7.00 and 7.18×10 for *Salmonella* and 7.08 to 7.16 ×10 for *E.coli* , this number was not accepted limit mentioned by SSMO ( Sudanese Standards and Metrology Organization ,SDs357) which was 5×10 to 5×10 cfu/g for fresh fish products This results in contrast with the finding of Kapute *et al.*, (2013) who found that the total bacterial count reach 2.1×10 . This means that *Salmonella* revealed the highest prevalence in these farms. In this study, the critical points depend on the environment habitats and the other point the feeding of fish .This shows the import of control the pathogenic bacteria which might cause serious infection leading to considerable economic losses in fishes when environmental condition altered in ponds and fish's resistance was reduced. This study showed that fish farm in Khartoum state could be a source of food borne bacterial pathogens. Improvement in handling and processing are needed to minimize the prevalence of the pathogenic bacteria .This need to build up the quality control and food safety measures system such HACCP and food safety management system. .

#### References:

- 1.Adebayo-Tayo B.C; Ody N.N; Anyamele L.M. (2012). Microbial quality of frozen fish sold in Uyo Metropolis. Nature and Science, 10(3): 71-77
- 2.Adesokan, H.K; Raji ,A.O.Q.(2014). Safe meat-handling knowledge, attitudes and practices of private and government meat processing
- 3.at retail in Cochin, India. J. Food Prot. 68(10): 2208-2211.

4. Barrow, G. and Feltham, R.K. (2003). Cowan and Steels, Manual for the identification of medical bacteria (3rd ed). Cambridge University Press, Cambridge, UK.
5. Buller, N.B. (2004). Bacterial diseases. A Textbook of bacteria from fish and other aquatic animals: a practical identification manual. 2nd Edn., Biddles Ltd, King's Lynn, the British Library, London, UK; pp 1-75.
6. characterization of typical and atypical *Escherichia coli* from fish sold
7. Clacus, I J, ward, A .R. (1996) .Post-harvest fishery development .Aguide to handling ,preservation , processing and quality . Charthan , Maritime , Kent M E 44T B , United Kingdom .
8. Ehiri et al: Ehrir , J . E : Morris , G , P , and Mc Ewen , J . (1997). A survey of HACCP Implementation In Glasgow , Is the information reaching the target , International Journal of Environmental Health Research, 7 : 71 -84 .
9. FAO , (2014). The State of World Fisheries and Aquaculture 2014 . Rome 223 .
10. FAO. (2004). www . fao . org /f1 / FCP /-F /CP-SDN .
11. Gilbert, R.J., de Louvois, J., Donovan, T. (1996). Microbiological guidelines for some ready to eat foods sampled at the point of sale. *PHLS Microbiol. Digest.*, 13: 41-43.
12. Gonzalez-Rodriguez, M.N., Sanz, J.J., Santos, J.A., Otero, A., Garcia-lopez, M.L. (2001). Bacteriological Quality of aquaculture freshwater fish portions in prepackaged trays stored at 3 degrees C. *J. Food Prot.* 64 (9): 1399 – 1404
13. Hastein T, Hjeltnes B ,lille haug A ,Utne Skare J Berntssen Mand Lunde bye K. (2006) . Food safety hazards that occur during the production stage , challenges for fish farming and the fishing industry Review Science . Technology , 25 : 607 -625 .
14. Herrera, F.C., Santos, J.A., Otero, A., Garcia-Lopez, M.L. (2006). Occurrence of Foodborne pathogenic bacteria in retail prepackage portions of marine fish in Spain. *J. Appl. Microbiol.* 100 (3): 527-36.
15. Hnadi ,G.B .B .(2008). Microbiological contaminations of some fresh water fish species (*Oreochromis niloticus* , *Clarias* spp and *Synodontis* spp ) Bsc (Hon) Department of Fisheries , University of Juba .
16. Kamel, E .A. (2001) .Characterization of *Tilapia* species (lecture) . A training program on Improving fish farm management . Organized by ICLARM . The World fish centre and the multi sector support program , MSSP ,of European community . Abassa , Egypt ,20 -24 February 2001 .
17. Kapute f ,likongwe j ,kang j , kiyukia, C. (2013). shelflife of whole fresh lake Malawi *Tilapia* (*Oreochromis niloticus* ) stored in ice ,African journal of food , Agriculture ,nutrition and development (ajfand) ,31 (1) =7156-7138
18. Koutsoumanis K., Nychas, G.J.(2000). Application of systemic experimental procedure to develop a microbial model for rapid fish shelf life predictions. *Int. J. Food Microbiol.* 60 (2-3): 171-184.
19. Lelieveld, H.L.M.; Mostert, M.A.; Holah, J. and White, B.(2003).Hygiene in Food Processing. Cambridge, Woodhead Publishing Limited.
20. Morales, G, Blanco, L, Arias, M and Chaves. C. (2004). Bacteriological evaluation of fresh *tilapia* coming from the Northern Region of Costa Rica. *Archive Latinoam Nutrition.* 54: 433 – 437.
21. NACMCF. (1992). National Advisory Committee on Microbiological Criteria for Foods ( NACMCF) (1992) : Hazard Analysis Critical Control Points . International Journal of food Microbiology 16 : 1 -23 .

22. Nunes IL.(2012). Knowledge, attitudes and practices in food safety and the presence of coagulase-positive staphylococci on hands of food handlers in the schools of Camacari, Brazil. *Food Control* ;27:206-13.
23. Ochei, J. and A. Kolhatkar. (2000).*Medical Laboratory Science. Theory and Practice*. Tata Mcgraw-Hill Publishing Company Limited: New Delhi. 2nd Edition, pp. 331-349.
24. Pearson ,A .M . and Dusion , T. R .(1995) . HACCP in meat , Poultry and fish processing . Black in Academic & Professional , Bishop- briggs , United Kingdom .
25. Russel,F.E,; Maretic, Z. (1986).Scombroid poisoning: mini-review with case histories. *Toxicon*, 24 pp. 967-973.
26. Smith ,G A , Yoshida .(2000) . Sustainable integrated Kysei nature farming E M technology and food security in Africa .
27. Soares LS, Almeida RCC, Cerqueira ES, Carvalho JS,
28. SSMO ,Sudanese Standards and Metrology Organization ,SDs357. For fresh or frozen fish and its products .Fishing products and seafoods *Trends food science Technology* , 81 : 258 - 265 .
29. Thampuran N; Surendraraj, A.; Surendran, P.K. (2005). Prevalence and characterization of typical and atypical *Escherichia coli* from fish sold at retail in India. *J.Food Prot.* 68(10):2208-11.
30. Thrusfield, M. (2007) *Veterinary Epidemiology*. 3rd ed., Ch. 13. Black Well Science Ltd., United Kingdom. p228-246.
31. Wafaa MK Bakr, Walaa A Hazzah, Amani F Abaza. 2011. Detection of *Salmonella* and *Vibrio* species in some seafood in Alexandria. *Journal of American Science*;7 (9):663-668.
32. Yagoub , S.O. (2009). Isolation of Enterobacteria and *Pseudomonas* . SPP from raw fish sold in fish market in Khartoum State . *J. Bacteriol . Res. .* 1 (7), 85 -88.