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Enumeration of *E. coli,* Coliform and Aerobic Bacteria in Marine Fish in Port Sudan, Red Sea State, Sudan

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Authors' contributions

This work was carried out in collaboration among all authors. Author IHAAA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AMAS and EAAA managed the analyses of the study. Author EMH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to detect the total bacterial count, coliform count and *E. coli* count through the production chain of marine fish (fish market, scaling, restaurant before cooking, restaurant after cooking and cooking and cooling for 24 h) in Port Sudan, Red Sea State, during the period from September to December 2018. A total of 50 fish samples were randomly selected from marine fish chain. Samples were subjected for bacteriological examination. The study showed that the mean count of *E. coli* in the five stages of the fish chain was $2.57\times10^{2}\pm0.6$ cfu, the mean coliform count was $2.01\times10^{3}\pm 0.7$ cfu/ g and the mean Aerobic Plate Count (APC) was found to be $3.6\times10^{6}\pm 0.09$ cfu/ g. *E. coli* showed higher mean count ($7.58\times10^{2)}$ cfu/ g in the scaling stage. The

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highest aerobic plate count (117×10^6) cfu/ g was detected also in the scaling stage, this may be due to the high number of workers involved in this process without using proper hygienic measurements. The highest coliform count 53.9×10^2 cfu/ g was detected in the stage of precooking, during this period fish were put on the surface of tables without being cooled. Since high coliform contamination takes place after evisceration, the count was in its highest limit after evisceration in the restaurants. By using ANOVA with confidence interval 95% there was a significant difference between the mean of coliform, APC and *E. coli* count among the five stages in fish production chain. In conclusion the results showed that the marine fish in Port Sudan city were grossly contaminated by *E. coli* and other coliform, but adequate cooking can kill all the *E. coli* and coliform.

Keywords: E. coli; fish; coliform; bacterial count.

1. INTRODUCTION

Marine fish has become an increasingly important source of protein, and other elements necessary for the maintenance of healthy body, and constitute an important food component for a large section of the world population [1]. The fisheries sector provides both food and employment for millions of people as well as fish for consumers who have a right to eat food which has been caught, handled and treated in a good way [2]. Fish contributes about60% of the world supply of protein, and 60% of the developing world derives more than 30% of their animal protein from fish [1]. The provision of safe wholesome and acceptable fish and fish products is essential from food safety point of view [2]. 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 [3]. Consumption of fish and shellfish may cause diseases due to infection or intoxication; some of these diseases have been specifically associated with pathogens which are resistant to antibiotics [4]. Microbial contamination on environmental surfaces may be transferred to the food products directly through surface contact or by vectors such as personnel, pests, air movements or cleaning system. Bacteria may also infect the fish from outside during careless handling of landed fish, its stowing and cutting. Among major external sources of bacterial contamination are ice and salt, crushed ice is known to carry heavy bacterial loads [2]. Many pathogenic and spoilage bacteria are able to attach on food contact surfaces, and remain viable even after cleaning and disinfection [2]. Control of microorganisms exerted through high level of hygiene and efficient cleaning, and disinfection practices during the processing and preservation procedures are essential to meet this goal to provide safe, wholesome, and acceptable food to the consumer [2].

This study was aiming at detecting *E. coli,* coliform and total bacterial load in marine fish in Port Sudan, Red Sea State, Sudan.

2. MATERIALS AND METHODS

2.1 Area of Study

This study was conducted in Port Sudan city, Red Sea State (Sudan), during the year 2018.

2.1.1 Source of samples

In this study which lasted for 3 months in the year 2018, a total of 50 fish samples were randomly selected from marine fish chain, in Port Sudan, Red Sea State (Sudan).

2.2 Sampling Procedure

Fish samples were put in ice box containing ice and transported to the laboratory of the Sudanese Standardization and Metrology Organization (SSMO) of the Red Sea State. In the laboratory samples were kept in a deep-freezer at -20°C. On the next day samples were removed from the deep freezer and left on the bench to thaw. Samples were then subjected to bacteriological analysis.

2.3 Bacteriological Culture Media

Three bacteriological culture media were used. All culture media were obtained from Oxoid and Hi Media and prepared according to manufacturers' instructions. The used culture media were Plate Count Agar, for the Aerobic Plate Count T.B.X. (Tryptone bile x

XGlucuronide) for *E. coli* and Violet Red Bile Agar for coliform enumeration.

2.4 Enumeration of Total Aerobic Bacteria (APC)

APC was conducted according to the International Organization for Standardization [5]. From each fish sample 25 g of muscle were aseptically removed from the fish, minced and homogenized in 225 ml of buffer peptone water for 1 minute using stomacher blender (model 3500 THERMO, serial No. 40225, England). Tenfold dilution was then made by adding 1ml from the dilution to 9 ml of Peptone water. One ml of the homogenate was inoculated into tow Petri-dishes marked 1/10 to $1/10^5$. 45°C hot plate count agar was poured on the plates stirred and left to cool. Agar plates were incubated at 30°C for 48 hours and the number of Colony Forming Units (C.F.U) was calculated.

2.4.1 Counting of colonies

After incubation, colonies between 15 and 300 per plate were counted using the colony counter.

2.5 *E. coli* Isolation and Enumeration

Isolation and identification of *E. coli* was done according to the International Organization for Standardization [5]. Twenty five grams of fish muscle was homogenized with 225 ml buffer peptone water using a sterile pipette, transferred to a sterile Petri dish. One ml of the test sample initial dilution (1-10) inoculated in two plates per dilution; this procedure was repeated with the further decimal dilutions, and then poured into each Petri dish approximately 15 ml of the TBX medium, previously cooled at 44°C to 47°C in the water bath. Carefully the inoculum was mixed with the medium and allows the mixture to solidify, with the petri dishes standing on a cool horizontal surface. Inverted the inoculated dishes so that the bottom is uppermost and placed them in an incubator set at 44°C for 24 h. After the specified period of incubation the typical colonies were counted, Glucuronidase-positive *E. coli* gave rise to distinct blue- green *E. coli* colonies.

2.6 Coliform Enumeration

According to [5], 25 g of fish muscle were homogenized with 225 ml buffer peptone water using sterile pipette. One ml of the test sample from initial suspension (10^{-1} to 10^{-5} dilutions), each dilution was transferred to two Petri dishes, by mean of another sterile Pipette 1 Ml of each decimal dilutions were poured in about 15 ml of the Violet Red Bile Agar (VRB) at 44°C to 47°C into each petri dish, allowed to solidify then the plates were incubated at 37°C for 24h$\pm$2. Pink to red colored colonies were typical coliform colonies.

2.6.1 Data analysis

Data were analyzed using statistical package of social Sciences (SPSS). ANOVA analysis was conducted to compare the mean of coliform, APC and *E. coli* among fish chain and to assess if there were significant differences ($P<0.05$). Descriptive statistic was used to assess the frequencies and distribution of risk factors among Fish chain.

3. RESULTS

The mean aerobic plate count in the fish chain was found to be $3.6\times10^6\pm$ 0.09 cfu/ g. The minimum was $2.0.\times10^2$ cfu/ g and the maximum APC count was 29×10^6cfu. The mean coliform count in the fish chain was found to be 2.01 $\times10^3\pm$ 0.7 cfu/ g. The minimum count was (00) and the maximum coliform count was 28×10^3cfu. The mean *E. coli* count in fish chain was found to be $2.57\times10^2\pm$0.6 cfu. The minimum count was (00) and the maximum *E. coli* count was 20.1×10^2cfu (Table 1).

Table 1. Mean of bacterial total plate, coliform and *E. coli* count of fish in Port Sudan city

Descriptive statistics	N	Minimum	Maximum	Mean	Std. error of mean
E .coli	50	0.00 cfu/ g	20.1 ×102 cfu/ g	2.57X102 cfu/ g	0.6
Coliform	50	0.00 cfu/ g	28×103 cfu/ g	2.01 X103 cfu/ g	0.7
APC	50	2.0 ×102 cfu/ g	29 ×106 cfu/ g	3.69 ×106 cfu/ g	0.09
Valid N (list wise) 50	50				

The mean aerobic bacteria, coliform and *E. coli* count in the fish market were 2.47×10^2, 7.1×10^2 and 5.7×10^6 cfu/ g respectively. The mean aerobic bacteria, coliform and *E. coli* count in the fish scaling were 7.58×10^2 cfu/ g , 39.5×10^2 cfu/ g and 117×10^6 cfu/ g respectively. The mean aerobic bacteria, coliform and *E. coli* count in the restaurant before cooking were 2.83×10^2 cfu/ g ,53.9×10^2 cfu/ g and 9.3×10^5 respectively. The mean aerobic bacteria, coliform and *E. coli* count in the restaurant after cooking were 0.0 cfu/ g, 0.0 cfu/ g and 2.87×10^2 cfu/ g respectively. The mean aerobic bacteria, coliform and *E. coli* count in cooled fish after 24 hours from cooking were 0.0 cfu/ g, 0.0 cfu/ g and $2.\times10^2$ cfu/ g respectively (Table 2).

By using ANOVA analysis with confidence interval 95%, there was a significant difference between the mean of APC count in fish market (5.7×10^6 cfu/ g), fish scaling (117×10^6 cfu/ g), restaurant before cooking (9.3×10^5 cfu/ g), restaurant After cooking (2.87×10^2 cfu/ g) and at 24 hours cooling after cooking ($2.\times10^2$). Also there was a significant difference between the mean of coliform count in fish market (7.1×10^2), fish scaling (39.5×10^2 cfu/ g), restaurant before cooking (53.9×10^2 cfu/ g), restaurant after cooking (00 cfu/ g), and after 24 hours cooling of cooked fish (00 cfu/ g). Also there was a significant difference between the mean of *E. coli* count in Fish market (2.47×10^2 cfu/ g), fish scaling (7.58×10^2 cfu/ g), restaurant before cooking (2.83×10^2 cfu/ g), restaurant After cooking (00 cfu/ g) and after 24 hours cooling of cooked fish (00 cfu/ g) (Table 3).

4. DISCUSSION

This study was conducted to detect the total bacterial limits, coliform count and estimate the count of *E. coli* through the fish production chain in Port Sudan city. It was shown that samples of fish in fish chain used in this study were grossly contaminated by pathogenic organisms. The difference in the mean bacteria counts of samples obtained from the five stages may be due to the hygienic measures practiced in each step. The highest count of *E. coli* 7.58×10^2 cfu was detected in second stage (fish scaling). Also the highest APC mean 117×10^6 cfu/ g was detected in this stage, while the highest coliform count 7.58×10^2 cfu/ g was detected in restaurant before cooking, this may be due to the improper hygienic practices during scaling, and hence high coliform contamination takes place after evisceration, the count was in its highest limit after evisceration in the restaurants. The mean *E. coli* count in fish chain in the five stages was found to be 2.57×102 + .6cfu/ g. [1] stated that the most dominant isolates from gills, skin, muscles and intestine of randomly collected fishes were *E. coli*, *Citrobacter sp*., *Enterobacter sp.* and *Klebsiella sp*. These microorganisms

Table 2. Mean counts of APC, coliform, and *E. coli* of fish production chain

Stage	*E. coli*	Coliform	APC
1. Fish market	2.47×102 cfu/ g	7.1×102 cfu/ g	5.7×106 cfu/ g
2. Fish Scaling	7.58×102 cfu/ g	39.5×102 cfu/ g	117×106 cfu/ g
3. Restaurant before cooking	2.83×10^2 cfu/ g	53.9×10^2 cfu/ g	9.3×10^5 cfu/ g
4. Restaurant After cooking	0.0 cfu/ g	0.0 cfu/ g	2.87×102 cfu/ g
5. After 24hcooling post cooking	0.0 cfu/ g	0.0 cfu/ g	2×102 cfu/ g

Table 3. Comparison between APC, coliform and *E. coli* count in different stages of fish chain

ANOVA	Sum of squares	df	Mean square	F	Sig.
Between Groups	249397803.280	4	62349450.820	2.592	.049
Coliform within Groups	1082519236.900	45	24055983.042		
Total	1331917040.180	49			
Between Groups	3838732.000	4	959683.000	6.033	.001
E. coli within Groups	7157730.000	45	159060.667		
Total	10996462.000	49			
Between Groups	1039745602458782.100	4	259936400614695.530	9.303	.000
APC within Groups	1257399131558652.000	45	27942202923525.600		
Total	2297144734017434.000	49			

occurring on fish products as a result of contamination from the animal/ human reservoir. This contamination has normally been associated with faecal contamination or pollution of natural waters or water environments, where these organisms may survive for a long time (months) or through direct contamination of products during processing [6]. Nearly similar results were obtained by [7,8] who reported that fish samples were contaminated with *E. coli*. The presence of such microorganisms in fish which are not the part of macrobiotic of these aquatic organisms indicates the occurrence of food contamination due to poor hygiene in handling and lack of preservation techniques [7]. The mean coliform count was $2.01 \times 10^3 \pm 0.7$ cfu/ g and the mean of the Aerobic Plate Count was found to be $3.6 \times 10^6 \pm 0.09$ cfu/ g. The highest coliform count 53.9×10^2 cfu/ g was detected in restaurants before cooking followed by the count in fish scaling 39.5×10^2. coliform count in fish market was 7.1×10^2 cfu/ g while the count is zero after cooking and after twenty four hours post cooking this difference was found to be statistically significant using ANOVA with p=0.05. [9] reported that the MPN value of the seafood samples varied with different seasons. He found total coliform and during summer varied from 3 to 65/ 100 g to 45 to 115 / 100 g of the fish meat in post-monsoon and between 65 to 150 /100 g in monsoon, respectively. Our results were not in agreement with [10] findings which stated 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 acceptable because they are not normal flora of fish [11]. The APC was highest 117×10^6 cfu/ g in the fish scaling stage, followed by the stage of fish market 5.7×10^6 then restaurant before cooking stage 9.3×10^5 cfu/ g, then after cooking 2.87×10^2 cfu/ g and the least count was detected in cooked fish 24 hours post cooking. Statistically there was significant differences (p=0.05) in the mean count of the five stages. [12] examined 3 species of marine fish for total viable count and prevalence of Salmonella, *E. coli*, Staphylococcus sp. and Vibrio sp. She detected a count of 5×10^5 in fish markets which is lower than the findings of this study. [13] reported that the total bacterial load of the surface slime of fish can range from 10^2 to 10^7cfu. This finding was in line with the present study. A higher range of bacterial count was obtained by [13] who reported a count range of 5.5×10^6 cfu/ g to 9.8×10^9cfu/ g in Nigerian marine fish. [14] reported higher counts from different type of fishes ranging between 8.7×10^5- 2.2×10^9 cfu/ g in freshwater fishes. [6] in raw fish sold in Khartoum State reported that the total bacterial count in skin and intestine ranged from 3×10^7 cfu/ g to 4×10^9 cfu/ g and 1.5×10^5 cfu/ g to 1.6×10^8cfu/ g, which was higher than this study. This could be due to the differences in market's situation and locations. Results was obtained by [3] detected bacterial load between 8×10^3 cfu/ g to 9.8×10^4cfu/ g in Ed Dueim fish market. Similar results were obtained by [15] and [9] who reported APC between 1.54×10^4cfu/ g and 5.7×10^4cfu/ g (as maximum) in fresh water fish, which is lower than what was observed in this study.

5. CONCLUSION AND RECOMMENDATIONS

The results showed that the marine fish in Port Sudan city were grossly contaminated by *E. coli* and other coliform, but adequate cooking can kill all the *E. coli* and coliform.

From this study we recommend training and extension for fisher men and trader who work in market of fish about possessing and handing of fish, All containers and boxes used have to be made of an easy to clean materials, The ice used should be crushed and come from an ice processing plant and transported in clean containers, using only new ice and not the dirty one remaining from the precedent fishing trips and The refrigeration facilities have to be adequate to ensure proper product temperature. Also hygienic measures have to be followed at different parts of fish production chain and HACCP should be implicated. Adequate cooking is the ideal way for reducing cases of fish poisoning cases.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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