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# Incidence of *Escherichia coli* and *Salmonella* Contamination in *Clarias lazera, Tetraodon fahaka* in Fish Marketing at Khartoum State

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**A B S T R A C T**

This study was carried out to assess the isolation and identification of contaminated bacteria *E. coli* and *salmonella* spp in fresh fish in two markets at Khartoum state. Fishes are a sensitive food which had a rapid rate of spoilage if they were not handled properly. The action of spoilage begins immediately after rigor mortis. When fish is caught, the subsequent steps of handling play great role on keeping quality and safety of fish. Two hundred of swab samples were collected, 100 sample from Central market and 100samples from Al-Mawrda market, each 100 samples were divided into, 50 samples type 1 (*Clarias Lazera*), 50 samples type 2 (*Tetradon Fahaka*), which were taken from five critical control points, fish skin, fish flesh, hands of labors, knives and tables of cutting. Then preserved in freezer at-18C° for (0, 14, 28 days). The isolates were two types of gram negative bacteria, *E. coli* and *salmonella spp.* Bacteriological analyses were performed using accredited methods, which showed highest contamination with *E. coli* recorded in central market 48 (80. 0) % in hands of labors, for type (1) *clarias lazera* 39 (65. 0) % in (fish skin and hands of labors) and for type (2) (*Tetradon fahaka*) 39 (65. 0) % in (fish flesh and knives). However, the highest contamination with *Salmonella* reported in Al-Mawrda market, in fish skin 40 (66. 7) %. The storage period was not affected significantly on samples contamination with either *E. coli* or *Salmonella.*

## Introduction

Fish which is the water bodies covers about two third of the earth surface (FAO, 1995). Estimates indicate that about 96% of fish capture in Sudan, comes from fresh water fisheries, of which 55% is from the white Nile, Blue Nile, Atbara River and luck Nubia (Carleton and Pena, 1982) Fish and fishery products are highly nutritious, in addition to the high percentages of animal protein, they provide several other nutrients such as vitamins A and B especially in the liver, and E and K vitamins, and they are good sources of some minerals

like calcium, phosphorus and iron (Lunven, 1982). Sandon (1953) reported that, in Sudan *Clarias* could survive for a relatively long period buried in the mud. The Family *Tetraodonitdaeowe*s its name to the world "*Tetraodon"*, which in Greek means "four teeth ". Fishes family possess characteristic jaws where in the teeth are fused into a beak –like dental plate with a median suture on each jaw (Talwar and J hingran, 1991)

The HACCP system was introduced in 1971 during the National food protection conference. Sea food processors must keep in mind that HACCP does not replace GMPs or guard against fraudulent practices (Russell *et al.,* 2004). Microorganisms can be used as an indicator of environmental pollution because they affect and influence the surrounding environment **(**Hans *et al.,* 2003 and Ali *et al.,* 2015).

To develop a better understanding of the microbiological problems associated with seafood processing and packaging, it became extremely necessary to apply the hazard analysis critical control point (HACCP) strategy. HACCP strategy identifies hazards associated with different stages of processing, packaging and handling, assesses the relative risk and identifies points where to control measures would be effective (Bryan, 1988; Ehiri *et al.,* 2001; Oranusi *et al.,* 2003).

Hazard Analysis and Critical Control Points (HACCP) is a systematic preventive approach to food safety and pharmaceutical safety that addresses physical, chemical, and biological hazards as a means of prevention rather than finished product inspection. HACCP is used in the food industry to identify potential food safety hazards, so that key actions, known as Critical Control Points (CCP's) can be taken to reduce or eliminate the risk of the hazards being realized. The system is used at all stages of food production and preparation processes including packaging, distribution, etc. The Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) use mandatory juice, seafood, meat and poultry HACCP programs as an effective approach to food safety and protecting public health. Meat and poultry HACCP systems are regulated by the USDA, while seafood and juice are regulated by the FDA. The use of HACCP is currently voluntary in other food industries (FSIS, 2007; USFDA, 2007; FSRIO, 2008).

Pathogenic bacteria associated with fishery product can be categorized into three main groups: *(i)* the indigenous bacteria that belong to the natural micro-flora of fish (*e. g., Clostridium botulinum, pathogenic Vibrio spp., Aeromonas hydrophila*); *(ii)* the enteric bacteria that are present due to fecal contamination (*e. g., Salmonella spp., Shigella spp.,* pathogenic *Escherichia coli*, *Staphylococcus aureus*); and *(iii)* the bacteria which contaminate during processing, storage or cooking (*e. g., Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Clostridium perfringens, Salmonella spp.)*. (Lyhs, 2009)

*Escherichia coli* is the most widely used bacteria as an indicator of sanitation because of these bacteria are commensal bacteria in the digestive and are pathogens. Based on identification process to several fishery products refers to (Indonesian National Standard number 01-2332. 1. (Jakarta: National Standard Institute).

*Salmonella* belongs to the *Enterobacteriaceae* family, characterized as a non sporulate gram-negative bacillus. It is routinely classified by serotype, based on the expression of three types of antigens: (O) somatic, (H) flagellar (vi) capsular, according to the Kauffmann-White scheme (Brasil, 2011). Salmonella infections in humans include mainly typhoid fever and these infections are commonly called as enteric fever which continues to be one of the most serious public health problems worldwide. The presence of higher level of *Salmonella* in fish causes several symptoms in human body like diarrhea, nausea, vomiting and abdominal pain Wyatt *et al.,* 1979, Suliman (2002) noted that when the marketing areas are near to the production area, high quality fish are offered for sale.

Freezing kill, some but not all of the microorganism present, and growth will take place after thawing if time permits. Fish carry floras storage and may be grow and product toxin when temperatures increase. With the advent of mechanical refrigeration, sharp freezing was employed and fish were (glazed). Ice with added salt was employed in older days. Quick freezing is applied to wrapped fillets or streaks, although whole smaller fish may be frozen (Fraizer, 1987). The freezing process partially or completely hinders disruptive microbiological or enzymatic reactions, nevertheless, it cannot repair damage already caused. The fish should be frozen after handling for a better quality. The great advantage of the freezing process is the ability to achieve stability without damaging the initial quality. The spoilage in fish can be reduced by maintain low temperature and good handling during unloading fish.

Therefore, this research was carried out to detect the isolation of *Salmonella spp* and *Ecoli* from two fish markets at Khartoum State in Sudan, and the effect of freezer storage on *Salmonella spp*. and *E. coli* contamination.

## Materials and Methods

Isolation and identification of *Salmonella* and *E. coli* were done by the conventional method that includes screening and biochemical tests.

## Sampling

A total of 200 swab samples were taken randomly from Al-Mawrda and central fish market for *Tetradon fahaka* and *Clarias lazera*

The collected swab samples were cultured. All plates were incubated at 37oc for 24 hours. The media used were Dexoycholate Citrate Agar for Salmonella spp. colonies, MaConkey Agar: for Escherichia coli Colonies.

## Statistical analysis

The data was reported as means of triplicate s± Standard Deviation (SD). For statistical analysis, the differences between the fish markets fish species, risk factor data were assessed using the SPSS 11. 5 at a degree of significance $P < 0.05$. The difference between the storage durations has been determined by analysis of variance (ANOVA).

## Results and Discussion

Fish is considered one of the most perishable foods, mainly due to the action of the bacteriological activity that occurs on the surface of the newly caught fish. In fish technology, the microbiological control is an essential for controlling the quality of raw fresh and other ingredients used in order to produce the final fishery products has being safe for the consumers and having good quality Gerasimov and Antonova, (1979) and ACPSFPI (2001). Microbial growth due to improper handling, long gap between harvesting and processing and poor storage conditions (Gram and Huss, 2000).

*E. coli* is an indicator of fecal contamination in small numbers and in large numbers it is an indicator of mishandling (Eze *et al.,* 2011). It is the only species in the coliform group that is found in the human intestinal tract and in the other warm blooded animals as a commensal and is subsequently excreted in large quantities in feces (Geldreich, 1983), Lobna and El-Newishy (2010). These contamination of fresh water fish with *E. coli* is indicator of fecal pollution of fresh water from which fish were harvested and or from subsequent un sanitary handling during catching, distribution and marketing practices of fish.

In this study as shown in Table 1, the highest contamination with *E. coli* recorded in hands of labors 48 (80. 0) %, the lowest in fish flesh at Central market 27 (45. 0) %. On the other hand highest contamination with *E. coli* recorded at Almawrda in skin fish36 (60. 0) %, the lowest in hands of labors18 (30. 0) %, which indicated poor hygiene in the central market compared with the Almawrda market.

Concerning types of fish, the highest numbers of *E. coli* for type (1) reported in skin of fish and hands of labors, the lowest in fish flesh, while for type (2), the highest contamination reported in fish flesh and knives and the lowest in hands of labors.

Imam *et al.,* (2022) estimated the count of *E. coli* through the fish production chain in port Sudan and found the highest count of *E. coli* in the second stage (fish scaling) (7. 58 x $10^2$ CFU)). Tilahun and Engidawork (2020) reported the isolation of *E. coli* from 11 of 131 (8. 4%) swab samples from the muscles of different species of fresh fish samples in Hawassa. The incidence of *Escherichia* spp. isolated from catfish fillets were 25%, 15%, 5% and 5% for African catfish fillet samples, bayad fillet samples, white basa fillet samples and red basa fillet samples, Noha *et al.,* (2023)

Claucas *et al.,* (1996) suggested that when present in food, pathogens such as *S. aureus, Salmonella, Shigella* and *Pseudomonas* are most likely to cause food-borne diseases, Stringent regulations and monitoring activities coupled with food safety training of suppliers (fishermen and traders) and ultimately the consumers on various aspects of Good Hygiene Practice (GHP), Good Manufacturing Practice (GMP) and HACCP is strongly recommended. Also found About 56. 3% of the mat samples collected and 38. 2% of the suspected isolates have been tested *Salmonella* positive. On the other hand, 75% of the collected samples and 36. 4% suspected isolates respectively were *E. coli* positive. Overall results revealed that there was no control to maintain bacteriological quality of fish markets.

The highest numbers of *Salmonella ssp* in this study, recorded in the central market in fish skin 34 (57. 7) %, while the lowest found in knives 10 (16. 7) %. Al-Mawrda market recorded the highest number of *Salmonella* in knives35 (58. 3) % and the lowest in skin fish and tables 24 (40. 0) %.

As for the two types of fish, highest numbers of *Salmonella spp.* for type (1) reported in fish skin 40 (66. 7) %while the lowest in hands of labors 21 (35. 0) %, however, type (2) recorded the highest number in hands of labors33 (55. 0) % and the lowest one in fish flesh and knives24 (40. 0) %..

The rate of spoilage occurs varies with species of fish, handling method, hygienic condition and chilling storage method. (Nanto *et al.,* 1993). *Salmonella* was examined qualitatively, 16% of yellowfin tuna, 19% of sailfish, 8% of sardine and 8% of shrimp showed the positive results, but none of the squid samples show the positive results. Aquatic environments are the major reservoirs of *Salmonella* and fish and shellfish appears to be passive carriers of Salmonella spp. Therefore, fishery products have been recognized as a major carrier of foodborne pathogens (Upadhyay *et al.,* 2010, Novotny *et al.,* 2004). Tesfaye, s. *et al.,* (2018) reported the finding of salmonellae in 5. 17% of the skin, gills, and intestine of fish samples from Lake Haike in northern parts of Ethiopia.

The isolation of *Salmonella, Shigella, and E. coli* indicate fecal and environmental pollution Yagoub (2004). Incidences of *Salmonella* species and *E. coli* in the fresh, raw fillet samples were 30% and 35%, respectively found by (Fasika, A. *et al.,* 2023).

As for fish markets, fish samples collected from different fish markets of Dinajpur district were contaminated with *Salmonella* and *E. coli* except one markets of Kaharol Upazilla. About 48. 9% of the samples collected and 28. 6 % of all the suspected isolates were tested *Salmonella* positive. On the other hand 75. 6% of all samples collected and 35. 8% of all the suspected isolates were tested *E. coli* positive Faridullah, *et al.,* (2019).

David *et al.,* (2009) collected ten fish from open-air markets and found *E. coli* (50%), *S. typhimurium* (20%), *S. paratyphi* (10%) and *S. typhi* (20%). Mhango *et al.,* (2010) Hossain and Barman, (2016) found, high microbial contamination of the fishes in retail markets that agree with the results in this research and it may be due to no or delay in icing, rough handling, compactness, lack of sanitation, contaminated water, contaminated ice, soiled surfaces and boxes.

El-olemy (2014) revealed that, the higher number of bacterial isolates were recovered from skin surface while muscles showed lower number of isolates at percentages of 3. 8% and 2. 3% for *O. niloticus* and 6. 8% and 3% for *C. gariepinus* respectively.

Shabarinath *et al.,* (2007) found *Salmonella* in 18. 9% of naturally contaminated shrimp samples and 21. 4% of crab, clam, mussel and oyster samples. 100 fish and shellfish samples obtained from the market and fish-landing center in India, where *Salmonella* was detected in 70% of fish, 59% of shrimp and 30% of oyster samples. *Escherichia coli* was detected in 1. 4, 1. 5, and 5. 9% of trout, salmon, and tilapia, respectively.

El-olemy (2014) found C. gariepinus samples had a significantly higher bacterial isolates (9. 8%) than O. niloticus samples (6%). Among the isolated bacteria *Streptococci* was detectable at higher percentage (13. 5%) followed by *Salmonella spp* (11. 5%), then Staph. aureus (4. 5%) and the lowest isolates were *E. coli* (2%). Two types of pathogenic bacteria Salmonella spp. and *Escherichia coli* were investigated and were more present on frozen tilapia from North region in Malawi (Bwanamudogo, 2022)

Freezing is a common practice in the meat, fish and other animal protein based industry, because it preserved the quality for an extended time and offers several advantages such as insignificant alterations in the product dimensions, and minimum deterioration in products color, flavor and texture (Obuz and Dikeman, 2003). The first and simplest method to both preserve and process fish is to keep it cool. Cool fish keeps longer than uncooled fish, although both will spoil in a matter of hours (Tawari and Abowei, 2011).

In this study storage period was not affected significantly on contamination with each *Salmonella* and *E. coli* at different risk factors for the two types *Clarias lazera and Tetradon fahaka* of fish at the two markets Centeral and Al-Mawrda, that differed from Can (2011) who established initial contamination of carp fillets with 3. 88 log cfu/g and increase in microbial counts during storage. Mahmoudzadeh, *et al.,* (2010) reported that, burgers from *P. elevatus* and *S. undosquamis* had low number of microorganisms at the end of storage period (-18 ºC).

**Table.1** Relationship between studied risk factors and contamination with E. coli for fish species in Central and AL-Mawrda fish Markets

| | Risk factor | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fish skin | | Fish flesh | | Knives | | Hands of labors | | Tables | |
| | No. of positive (%) | P. value | No. of positive (%) | P. Value | No. Of positive (%) | P. Value | No. of Positive (%) | P. Value | No. of positive (%) | P. Value |
| **Fish source (markets)** | | | | | | | | | | |
| **Central** | 33 (55. 0) | 0. 580 N. S | 27 (45. 0) | 0. 237 | 46 (76. 7) | 0. 000** | 48 (80. 0) | 0. 000** | 34 (56. 7) | 0. 853 N. S |
| **Al-Mawrda** | 36 (60. 0) | | 33 (55. 0) | | 26 (43. 3) | | 18 (30. 0) | | 35 (58. 3) | |
| **Fish species** | | | | | | | | | | |
| **Type1** | 39 (65. 5) | 0. 097 N. S | 21 (45. 0) | 0. 001** | 33. (55. 0) | 0. 264 N. S | 39 (65. 0) | 0. 028* | 36 (60. 0) | 0. 58O N. S |
| **Type2** | 30 (50. 0) | | 39. 65. 0) | | 39 (65. 0) | | 27 (45. 0) | | 33 (55. 0) | |

Type 1 = *Clarias Lazera* Type 2 = *Tetradon Fahaka*
**=Significant difference at P<0. 01 *= Significant difference at P<0. 05 N. S=No significant differences

**Table.2** Effect of freezer storage on risk factors contamination with *E. coli*

| | Risk factor | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fish skin | | Fish flesh | | Knives | | Hands of labors | | Tables | |
| | No. of positive (%) | P. Value | No. of positive (%) | P. Value | No. of positive (%) | P. Value | No. of positive (%) | P. Value | No. of positive (%) | P. Value |
| **Storage period (days)** | | | | | | | | | | |
| **0** | 23 (57. 0) | 1. 000 N. S | 20 (50. 0) | 1. 000 N. S | 25 (62. 5) | 0. 901 N. | 22 (55. 0) | 1. 00 N. S | 22 (55. 5) | 0. 903 |
| **14** | 23 (57. 0) | | 20 (50. 0) | | 23 (57. 5) | | 22 (55. 0) | | 23 (57. 0) | |
| **24** | 23 (57. 0) | | 20 (50. 0) | | 24 (60. 0) | | 22 (55. 0) | | 24 (60. 0) | |

N. S=No significant differences

**Table.3** Relationship between risk factors and contamination with *Salmonella* for fish species in Central and Al-Mawrda fish markets

| | Risk factor | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fish skin | | Fish flesh | | Knives | | Hand of labors | | Table | |
| | No. of positive (%) | P. Value | No. of positive (%) | P. Value | No of positive (%) | P. Value | No. of positive (%) | P. Value | No. of positive (%) | P. Value |
| **Fish source (markets)** | | | | | | | | | | |
| **Central** | 34 (57. 7) | 0. 200$^{NS}$ | 31 (46. 7) | 0. 201$^{NS}$ | 10 (16. 7) | 0. 000** | 12 (20. 0) | 0. 000** | 30 (50. 0) | 0. 271$^{NS}$ |
| **Al-Mawrda** | 24 (40. 0) | | 27 (45. 0) | | 35 (58. 3) | | 24 (70. 0) | | 24 (40. 0) | |
| **Fish species** | | | | | | | | | | |
| **Type1** | 40 (66. 7) | 0. 200 N. S | 24 (40. 0) | 0. 001* | 24 (40. 0) | 0. 572 N. S | 21 (35. 0) | 0. 028* | 27 (45. 0) | 1. 000 N. S |
| **Type2** | 31 (50. 0) | | 21 (35. 0) | | 21 (35. 0) | | 33 (55. 0) | | 27 (45. 0) | |

Type 1= *Clarias lazera.* Type 2 = *Tetradon fahaka*
**=significant different at p<0. 01 *= significant different at p<0. 05 N. S=No significant differences

**Table.4** Effect of freezer storage on risk factors contamination with *Salmonella*

| Risk factor | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Fish skin | | Fish flesh | | | Knives | | Hand of labors | | Table | |
| | No. of positive (%) | P. Value | No. of positive (%) | P. Value | No. of positive (%) | P. Value | No. of positive (%) | P. Value | No. of positive (%) | P. Value |
| **Storage period** | | | | | | | | | | |
| **0** | 21 (52. 5) | 0. 584 N. S | 21 (52. 0) | 0. 967 N. S | 12 (30. 0) | 0. 474 N. S | 18 (45. 5) | 1. 000 N. S | 21 (525. 5) | 0. 493 N. S |
| **14** | 20 (50. 0) | | 17 (42. 5) | | 17 (42. 5) | | 18 (45. 5) | | 17 (42. 5) | |
| **24** | 20 (50. 0) | | 17 (42. 5) | | 16 (40. 0) | | 18 (45. 5) | | 16 (40. 0) | |

NS=No significant differences

Means with different superscript letters in the same column significantly differenet at p<0. 05.

(El-Sherif *et al.,* 2011) found that the total bacterial count (TBC) of raw Mullet fish during storage period at -18°C were 2. 01, 4. 32, 3. 05 and 2. 65 $log_{10}$ cfu /g sample after 0, 60, 120 and 180 days of storage, respectively, while TBC of raw Tilapia fish were 2. 35, 5. 75, 4. 15 and 2. 95 $log_{10}$ cfu /g sample after the same periods of frozen storage.

Microbial contamination causes fish deterioration and leads to the end of its shelf-life when reaches levels between $10^7$ and $10^9$ cfu/g-1 (Scheleguedaa *et al.,* 2016)

Thatcher and Clark (1973) reported that, the kind and number of microorganisms found on frozen fish is affected with the source of the fish, additional contamination introduced in the fishing boat, freezing temperature during storage, the severity of freezing process, contamination of handlers and market sellers, therefore, great attention to handle fish in more hygienic ways to keep up to the microbiological standard of the use of fishing equipment which often serves as a route of contamination of frozen fish and storage environment.

Arannilewa S. T, *et al.,* (2006) found total bacterial increase 3. 0 x $10^3$ -5. 6 x $10^4$ - 6. 5 x $10^4$ - 6. 7 x $10^4$ -4. 0x $10^6$ -7. 5 x $10^6$ in 0day to 60 days respectively the increasing total coliform counts of fish is best before frozen storage and that quality of frozen fish is better achieved in the first ten days of storage. Ohalete *et al.,* (2011) who isolated 20% of *Salmonella* spp. from frozen fish samples. Wendwesen Teka (2015) Finding 42. 5% of frozen raw Nile tilapia fillet samples carried *E. coli.*

So, we could clarify and recommend the following preventive and corrective hygienic measures, to be employed, to avoid enter pathogens contamination and ensure safety of freshwater fish to be fit for human consumption: Prevention of sewage drain into fresh water, to prohibit the main source of contamination.

- Application of obligatory hygienic training programs, personal hygiene and good hygienic practices, for fishermen and fish sellers - periodical hygienic inspection of fish markets to ensure perfect cleaning and removal of waste, continual upgrading of hygienic certificates for sellers - awareness of customers for hygienic practices in handling.

The study revealed that high microbial contamination with *E. coli* and *Salmonella* spp were frequently found in markets.

This phenomenon is caused by poor icing, rough handling, lack of sanitation, contaminated water and ice, surfaces and containers. Poor hygiene practices followed during marketing help to make fish highly hazardous food. Fish preservation and processing is a very important aspect for the fisheries

## Recommendations

-Fish should not be put on the ground during marketing, susceptible to air and dust, and use approved containers with covers to avoid contamination.

-Applying strict laws to organize and monitors fish handling activates in fish markets.

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## Author Contributions

D. H. Enas: Investigation, formal analysis, writing—original draft. M. M. Maha: Validation, methodology, writing—reviewing. M. A. Abdalla:—Formal analysis, writing—review and editing. A. S. Ali: Investigation, writing—reviewing. K. O. Abderahman: Resources, investigation writing—reviewing. Ramzy A. Yousif: Validation, formal analysis, writing—reviewing.

## Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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