Risk Associate with *E. coli* in Marine Fish in Port Sudan, Red Sea State, Sudan

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**ABSTRACT**

This study was carried to assess the risk associate with *E. coli* in marine fish in Port Sudan, Red Sea State, Sudan, through the production chain covering five stages. The stages were fish market, fish scaling, restaurant before cooking, and restaurant after cooking, and cooking and cooling for 24 h. A total of 50 fish samples were randomly selected from marine fish chain, in Port Sudan, Red Sea State, during the period from September to December 2018 to estimate the *E. coli* count using the TBX medium. In this study, the mean count of *E. coli* in the five stages of the fish chain was 257 ±0.6 cfu. In fish market the mean was 247 ±0.6 cfu in fish scaling stage, 283±0.6 cfu in restaurants before cooking, and 0.0 cfu after cooking and cooling for 24 hours after cooking. The chi-square test showed significant association between the load of *E. coli* among the fish chain and the most important potential risk factors (p-value ≤ 0.05), such as boxes cleaning (p-value= 0.000), hygienic practices (p-value= 0.002), waste disposal (p-value= 0.000), personal hygiene (p-value= 0.001), storage (p-value= 0.000) and general cleaning (p-value 0.002). The results of the frequencies of risk factors and their distribution in fish chain showed that 38% do not clean the boxes, 64% do not practice proper hygiene measures, 72% perform improper waste disposal, 72% had poor personal hygiene, 42% do not apply proper storage system, and 46% perform improper cleaning. All that will lead to medium overall risk estimation. Qualitative risk assessment for the *E. coli* in fish chain pathway was found to be high in the fish market and the exposure assessment in restaurant was found to be very low, so that the overall risk estimation for contamination with *E. coli* among the fish chain in Port Sudan was found to be Medium (The risky event is likely to occur more than once in the next three years).

**Keywords:** *E. coli*, marine fish, Port Sudan, risk analysis.

I. 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 provision of safe wholesome and acceptable fish and fish products is essential from food safety point of view [2]. 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 bac

Pathogenic *E. coli* strains can be isolated in massive numbers from the intestines of warm-blooded animals. However, many strains of *E. coli* are pathogenic to humans to varying degrees and these strains can be divided into six groups [5], Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterohemorrhagic *E. coli* (EHEC) or Shiga toxin-producing *E. coli* (STEC) [6], Enterog- gregarive E. coli (EAEC) or (EAggEc), and diffusely adherent *E. coli* (DAEC) [7]. *E. coli* is responsible for food poisoning, colitis, and Hemorrhagic Uremic Syndrome (HUS) by producing heat-stable endotoxins (STa) [8]. Spoilage is defined as a change in fish or fish products that renders it less acceptable, unacceptable or unsafe for human consumption [9]. Fish spoilage is a complex process, in which physical, chemical and microbiological mechanisms are implicated [10]. *E. coli* is one of the most important spoilage producing bacteria and can be isolated from fresh and spoiled fish and other sea foods [9]. Qualitative risk assessments are commonly used for screening risks to determine whether they merit further investigation, and can be useful in the preliminary risk management activities [11]. To ensure a logical chain of events in space and time a risk pathway is depicted graphically to provide a framework for the development of a mathematical model [12]. The main objectives of value chain analysis are to identify the main people, groups and
II. MATERIALS AND METHODS

A. Samples’ Collection and Processing

This study was conducted in Port Sudan, in the Red Sea State. Samples were collected during the period from September to December 2019; from ALSigala area which includes the fish market and some fish Restaurants. A total of 50 fish samples were randomly selected from the five stages of the fish chain (fish market, fish scaling, restaurant before cooking, restaurant after cooking and after cooking and cooling at 4°C for 24 hours). Ten (10) samples were collected from each stage. The samples were taken directly into a labeled sterile plastic container, kept in ice box and transported immediately for microbiological analysis at Sudanese Standard and Metrology organization-Red sea branch (SSMO) laboratory. Isolation and identification of *E. coli* was done according to the International Organization for Standardization [17]. Twenty five grams of fish muscle were homogenized with 225 ml buffer peptone water using a sterile pipette, transferred to a sterile container. One ml of the test sample initial dilution (1/10) was prepared and hence serial dilutions to $10^5$ were inoculated in two plates containing TBX media, 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. After incubation at 44°C for twenty four hours, the typical colonies were counted, positive *E. coli* gave rise to distinct blue-green colonies.

B. Data Collection

Check lists were used to collect data about some potential risk factors that might have an association with the *E. coli* contamination in the fish chain in Port Sudan- from market to direct consumer, determining the risks at different steps in the fish chain. The Check lists included general questions covering the practices performed at every stage of the five stages in the fish chain.

C. Data Collection

All data of the check list and the laboratory results were analyzed using statistical package of social Sciences (SPSS). Analysis by using chi-square test for the association between different risk factors, and ANOVA to study the differences between the different stages in the fish chain.

D. Risk Assessment for *E. coli* in the Fish Chain

Hazard identification: A hazard is something potentially harmful to animals, human, plant and environment [13]. In this study the hazard is the *E. coli*.

The Study risk question: What is the risk associated with *E. coli* contaminating the fish for fish chain consumers in Port Sudan?

Release assessment pathway: It describes the biological pathway for introducing hazard to the animals and estimates the probability of its occurring [18]. In this study the release assessment estimated the probability or likelihood of introducing *E. coli* contaminating the fish in fish chain.

Exposure assessment pathway: It describes the biological pathways necessary for exposure of animals to the hazard [18]. In this study the exposure assessment estimated the probability or likelihood of the exposure of fish to *E. coli*, as well as exposure of human population to the *E. coli* contaminated fish.

E. Qualitative estimation for the Probability (Likelihood)

It involves two steps: 1-Information (derived from collected data) was put together with the risk pathway in a tabular frame work in order to make a systematic process and evidence-based assessment and encourage transparency. 2- Logical conclusions were extracted by comparing the requirements for each step with the actual situation [18].

F. Tools used in Qualitative Approach for Estimation the Likelihood (Probability)

- Identification and characterization of risk factors within value chains.
- Scoring methods.
- Risk ranking based on scores.

The risk scoring of the Department of environment, food and rural affaires agency (Defra) in the United Kingdom was used in this study for estimating the likelihood as shown in Table I.

| Likelihood | Description |
|------------|-------------|
| VL: Very low | Rare (the risky event may occur in exceptional circumstances). |
| L: Low | Possible (the risky event may occur in the next three years). |
| M: Medium | Likely (the risky event is likely to occur more than once in the next three years). |
| H: High | Almost certain (the risky event is likely to occur this year or in frequent intervals). |

G. Using Tabular Framework for Risk Pathway, Value Chain Description and Risk Factors

The tabular framework explains the key information for describing the location of risk in the pathway and geographically referring to the value chain mapping. Also it contains detailed description for the main risk factors which
were organized using tabular framework. The risk factors represent all the factors which influence the steps of risk pathway, either increasing the risk or decreasing it, for scoring and estimating the risk [18].

**H. Overall Risk Estimation**

Containing the results from release, exposure and consequence assessments to produce overall measures of risks associated with hazard identified at the outset [18]. The overall level of risk is defined as the product of the likelihood of an unwanted outcome occurring and the impact resulting should it occur:

\[
\text{Risk} = \text{likelihood} \times \text{impact} \quad [13]
\]

A qualitative risk assessment scheme used by Defra in UK was used to estimate the overall risk Table II.

Fish market risk assessment pathway and exposure assessment were illustrated in Fig. 1 and 2.

**III. RESULTS**

**A. Mean Counts of \(E.\ coli\) of the Five Stages of the Fish Chain**

This study was carried to assess the risk implicated from \(E.\ coli\) in marine fish in Port Sudan, through the production chain. The mean \(E.\ coli\) count in fish chain in the five stages was found to be \((2.57 \times 10^2 + 0.6)\) cfu, the minimum count was \((0.0)\) and the maximum was \((20.1 \times 10^2)\). In fish market the mean was \(247 + 0.6\) cfu, in fish scaling stage was \(758 + 0.6\) cfu, in restaurants before cooking it was \(283 + 0.6\) cfu and the mean count was \(0.0\) in restaurants after cooking and in 24 hours post cooking (Table III).

**TABLE III: MEAN COUNTS OF \(E.\ coli\) OF THE FIVE STAGES OF THE FISH CHAIN**

| Stage                                | \(E.\ coli\) mean count | Significance |
|--------------------------------------|--------------------------|-------------|
| 1. Fish market                       | \(2.47 \times 10^2\)     | 0.032*      |
| 2. Fish Scaling                      | \(7.58 \times 10^2\)     | 0.011*      |
| 3. Restaurant before cooling         | \(2.83 \times 10^2\)     | 0.00**      |
| 4. Restaurant after cooking          | 0.0                      | 0.00**      |
| 5. After 24 hrs cooling after cooking| 0.0                      |             |

Statically there was significant differences between the mean of \(E.\ coli\) in Fish market \((2.47 \times 10^2)\), fish scaling \((7.58 \times 10^2)\), restaurant before cooking \((2.83 \times 10^2)\). Restaurant After cooking \((0.0)\) and after cooking and cooling for 24 hours \((0.0)\), with confidence interval 95% (Table III).

Forty percent of fish were kept in clean boxes in fish market and during transportation; proper waste disposal was practiced by 28% of the fish seller or the farmer of the fish market. In the restaurant 54% of the restaurants' workers process the fish in a clean environment and 28% of the workers follow the personal hygiene properly (Fig. 3). The level of the risk factor in the fish chain is regarded as low if the likelihood is less than 50%, medium between 50%-75% and the likelihood is regarded as high if it scores more than 75%. There is a significant association between the level of \(E.\ coli\) and the six risk factors evaluated with \(P\)-value \(\leq 0.05\) (Fig. 3).
B. *E. coli* Risk Scoring among Risk Pathway

In this study *E. coli* risk scoring was medium I the stage of fish market, high in fish scalding, medium in restaurant before cooking, very low in restaurant after cooking and after 24 hours cooling after cooking (Fig. 4).

C. Qualitative Risk Assessment

Risk of *E. coli* was assessed using the tabular frame for the Fish market pathway as shown in Fig. 5, and the Probability of releasing *E. coli* amid fish market, fish scaling, restaurant before and after cooking and after cooling the cooked fish for twenty four hours was found to be as follows:

Fish market pathway: Medium × High = High

Restaurant pathway: Medium × V. Low × V. Low = V. Low

Fig. 4. Risk pathway analysis. *E. coli* was qualified as follows:

- 0.0 cfu - less than 10 cfu = V. low risk
- 10 cfu – less than 100 cfu = Low risk
- 100 — less than 300 cfu = Medium risk
- 300 and more cfu = High risk

IV. DISCUSSION

This study was conducted to estimate the count of *E. coli* through the fish production chain and to assess the risk of *E. coli* in the fish chain in Port Sudan. The highest count of *E. coli* was detected in the second stage (fish scaling) (7.58×10^2 cfu) this may be due to the improper hygienic practices during scaling, and hence high contamination that takes place after evisceration, the count was in its highest limit after 24 hours cooling after cooking (Fig. 4).

Fig. 5. Risk pathway analysis.
The presence of such aquatic organisms indicates the occurrence of food contamination due to poor hygiene in handling and lack of preservation techniques [20]. In this study, six, potential risk factors were found to be associated with the count of E. coli among fish chain using Chi square, these factors included boxes cleaning (p-value= 0.000), hygienic practices (p-value= 0.002), waste disposal (p-value= 0.001), personal hygiene (p-value= 0.001), storage (p-value= 0.000) and cleaning of equipments (p-value= 0.002). The overall risk for contamination throughout the food chain by E. coli was found to be medium. This means the risky event is likely to occur more than once in the next three years, according to Defra risk scoring. To prevent the contamination with E. coli in fish chain proper processing, storage, and handling procedures of fish should be employed. Adequate treatment methods before consumption of fish should be applied in all steps of fish chain to minimize the risk. Contamination with E. coli in fish market stage in fish chain was found to be High which means that almost certain the risky event (E. coli count) is likely to occur this year or in frequent intervals. To assess the risk we need to assess the two events, the first one is to assess the risk of the fresh or chilled fish received from fishermen, which had E. coli count (247 cfu) this was assessed to be Medium (Fig. 4). The second one in the fish market where fish scaling process, handling and storing, usually take place was assessed to be high (Fig. 4). For the direct effect of the main risk factors, improper storage 58%, improper personal hygiene in 72%, improper waste disposal 72% in addition to count of E. coli was the highest in the scaling process. The Probability of selling fish that contaminated with E. coli was assessed to be high (Fig. 5), that means almost certain the risky event is likely to occur this year or in frequent intervals (Table I). The assessment of high risk fish market pathway indicates that poor hygienic measures and practices in scaling and handling process. Bacteria may also infect the fish from outside during careless handling of landed fish. It is stowing and cutting together with other external sources such as ice and salt. Crushed ice is known to carry heavy bacterial loads [2]. The exposure pathway of E. coli in restaurant in the fish chain was assessed by three events, the first event in this pathway is the fish handling before cooking which may lead to contamination with E. coli and affection by the most prominent risk factors with frequency of 50%-75%. The qualitative estimation of the count of E. coli was assessed to be medium so that the risk in this event was assessed to be Medium (Fig. 4). The second event in the same pathway is the probability that fish handling during and after cooking was contaminated with E. coli. E. coli count after cooking was estimated to be zero which indicated adequate cooking in this stage leading to destruction of E. coli, so that the risk was assessed to be V. Low (Fig. 4). The third event in the same pathway is the probability of E. coli cross contamination during handling and cold storing after 24 hours post cooking. This was assessed to be V. Low, (the risky event may occur in exceptional circumstances) (Fig. 4). [22] stated that adequate cooking kills most pathogens; however, unlike other foods, such as meat and poultry, that are usually fully cooked, seafood is often consumed raw or prepared in ways that do not kill all organisms. In the present study the overall risk estimation for contamination with E. coli among fish market, and restaurant (Alsigala) in Port Sudan was assessed to be Medium (the risky event is likely to occur more than once in the next three years (Table I). The results of the frequencies of risk factors and their distribution in fish chain showed that 38% did not apply boxes cleaning, 64% don’t apply proper hygiene practices, 72% don’t apply proper waste disposal, 72% had poor personal hygiene, 42% don’t apply proper storage and 46% don’t apply proper cleaning which all lead to Medium overall Risk Estimation. Moreover, risk management decisions should take into account the whole food chain from primary production to consumption and should be implemented in the context of appropriate food safety infrastructures, such as regulatory enforcement, food product tracing and traceability systems. In the fish processing chain, managing risks factors should be based on scientific knowledge of the microbiological hazards and the understanding of the primary production, processing and manufacturing technologies and handling during food preparation, storage and transport, retail and catering [23].

V. CONCLUSION

The results showed that the marine fish in Port Sudan were grossly contaminated by E. coli, but adequate cooking can kill all the E. coli. The overall estimated risk for E. coli in fish chain was found to be Medium. This means the risky event is likely to occur more than once in the next three years. The most effective risk management measures were continual chilling of raw fish or freezing and adequate cooking immediately before consumption. And that the effective way for reducing or eliminating risk of fresh fish borne disease.

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CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.
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