



## **Microbial Assessment of Fresh and Frozen (For Four Days) Marine Najil Fish (*Plectropomus pessuliferus*)**

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### **Authors' contributions**

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

### **Article Information**

DOI: 10.9734/ajriz/2020/v3i3330095

Editor(s):

(1) Dr. Oluyomi A. Sowemimo, Obafemi Awolowo University, Nigeria.

Reviewers:

(1) Srishti Gupta, Vellore Institute of Technology, India.

(2) Muna Sameer Al-rawi, University of Baghdad, Iraq.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/61124>

**Original Research Article**

**Received 12 July 2020**  
**Accepted 18 September 2020**  
**Published 30 September 2020**

### **ABSTRACT**

The present study was conducted at Directorate General of Preventing Medicine and PHC, Epidemiology Dept, Ports and Quarantine Health Unit, Port Sudan, endeavors for Microbial evaluation (bacteriological load) of marine Najil fish (*Plectropomus pessuliferus*). And to determine the occurrence of some contaminated bacteria. 20 samples of Najil fish (*P. Pessuliferus*) were collected from Port Sudan fish market (in sterile plastic bags) and pre chilled with ice in thermostatic container. All the samples were tested immediately, and instantly froze for four days. Then the studied samples were tested microbiologically and results showed:

For fresh fish total bacterial count (TBC) was  $(5.68 \times 10^5)$  CFU/gm, and for frozen samples was  $(4.38 \times 10^5)$  CFU/gm, respectively.

The study concluded that, Najil fish (*P. Pessuliferus*) showed the highest level of contamination between fresh fish. While in frozen fish freeze seem have the higher effect (less number). And the results showed highly significant differences between fresh and frozen fish.

The samples were also tested for contaminant bacteria and the result indicated the presence of *Staphylococcus* and *E. coli*, while the *salmonella* and *vibrio* were absent at each of it.

**Keywords:** Microbial evaluation; frozen fishes; Najil (*Plectropomus pessuliferus*) fish.

## 1. INTRODUCTION

Fish accounts for approximately 17% of the global animal protein intake. Globally, the production of fish compete the growth of world population [1].

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, Also fish and Seafood supply a number of essential vitamins and minerals, including: vitamins A, B3, B6, and B12, and D, and the minerals calcium, iron, selenium, and zinc. Seafood consumption is linked with improvements in health conditions including: cardiovascular disease (stroke), Arthritis, Cognitive function, Hearing loss, Depression/mood, Osteoporosis, and Cancer. However, consumption of fish and shell fish may also cause diseases due to infection or intoxication, some of these diseases have been specifically associated with pathogens which are resistant to antibiotics [2].

Fisheries sector plays an important role in food security, poverty alleviation and economic development of Sudan. Inland and marine fisheries are the major fisheries sectors of Sudan. These sectors support not only the domestic needs but also contribute to world export markets [3].

In Sudan because of lacking facilities, bad handling and processing most of the fish landing sites where subjected to very poor conditions. Thus the competent authority that is supposed to intervene to improve this quality unfortunately has very limited role and effort as a result of lacking in technical and financial capabilities.

Some seafood commodities are more risky than others owing to many factors, including the nature of the environment from which they come, their mode of feeding, the season during which they are harvested, and how they are prepared and served. Fish are conditioned by their environment if the growing and harvesting environment of fish is polluted chemically or microbiologically, the fish will be also polluted [4].

When frozen seafood products are consumed raw, there is the likelihood of endangering the health of the consumer especially when the micro-organism present includes pathogenic ones [2]. However, as demands for and production of seafood increased, it becomes important to be more attention to seafood safety and seafood related diseases.

## 2. MATERIALS AND METHODS

### 2.1 Laboratory of Investigation

This study was carried out at Directorate general of preventing Medicine and PHC, Epidemiology Dept, Ports and Quarantine health unit, Port Sudan Laboratory, is an accredited laboratory which was accredited by [5]. The laboratory follows ISO 17025 standards.

### 2.2 Collections of Samples

A total of 20 samples of Najil (*Plectropomus pesulliferus*) fish were collected randomly from different sellers at Port Sudan fish market.

The fish samples were identified, measured and processed following the methods used by the association (personal communication). Then transported to the laboratory by collecting in sterilized plastic bag put into ice box (thermostatic containers) and directly analyzed as fresh, froze (put into freezer for 4days) and then analyzed.

### 2.3 Equipments and Materials

The following items were used for bacteriological count and these include: fish sample, plastic container, freezer to freeze fish sample, sensitive balance, sterile bottles, flasks, beakers, measuring cylinders, flame for sterilizing, sterile tubes (10 ml), sterile pipettes, normal saline, Petri dishes, glass (20×100 mm), Test-tube racks to hold tubes in incubator and during storage, Wire loops for inoculating media, Container for used pipettes, plate count agar, stomacher, autoclave, water bath, plate count agar, nutrient agar, incubator  $37^{\circ}\pm 1^{\circ}$ . All the used glass wares such as conical flasks, beakers, measuring

cylinders, test tubes were washed, dried and sterilized in autoclave at a temp of 121c for 15 min at 15 lb/inch pressures [6].

## 2.4 Processing of Fish Samples

Aseptic measures and conditions were undertaken during the sampling procedure to prevent contamination of the samples; 25g of each sample was weighted and homogenized by a stomacher blender for 2min at 160 revolution/min with 225ml. of peptone water (ph 7.0).

Each of three tubes was filled with nine milliliters of buffer saline solution (BS) of first tube to prepare  $10^{-1}$  dilutions. The 1ml was taken from the first tube and mixed to the second test tube to prepare  $10^{-2}$  dilutions. The  $10^{-3}$  dilution was prepared by these subsequent dilution techniques. Then sealed in polyethylene bags to store in frozen condition at  $-20^{\circ}$  c for further analysis, After 4 days fish samples were taken out of the refrigerator and thawed at room temperature. Then 20 g of each sample was blended with 180 ml of sterile bacteriological peptone water in a stomacher blender. Then 1 ml of this  $10^{-1}$  dilution was transferred to a screw cap vial containing 10 ml of sterile dilute of bacteriological peptone to make a dilution of  $10^{-2}$ . Then the vial was shaken gently. This process was repeated progressively to prepare of  $10^{-3}$ .

## 2.5 Bacteriological Examination: Any Culture Medium Must Contains

- A source of energy. -Sources of carbon, nitrogen, sulfur, phosphorus.
- Minerals, e.g.,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^{+}$ .
- Vitamins and growth factors.
- Water.

### 2.5.1 Culture media used for bacteriological evaluation

BPW (Bacteriological peptone water): Used for serial dilution of fish samples.

PCA (Plate Count Agar): Used for total bacterial count.

APW (Alkaline Peptone Water): Used for 1<sup>st</sup> enrichment of *Vibrio* spp.

TCBS (Thiosulphate Citrate Bile Salt Sucrose agar): Used for 2<sup>nd</sup> enrichment of *Vibrio* spp.

VRB (Violet Red Bile agar): Used for enrichment of *E.coli*.

LB (Lactose Broth): Used as pre-enrichment broth during testing of *Salmonella* spp.

BPW (Buffer Peptone Water): Used for 1<sup>st</sup> enrichment of *Salmonella* spp.

SCB (Selenite Systine Broth): Used for enrich the cultivation of while inhibiting other organisms.

XLDA (Xylose Lysine Deoxycholate): Used for 3<sup>rd</sup> enrichment of *Salmonella* spp.

### 2.5.2 Occurrence of total bacterial count

25grams of fish muscle was blended with 0.1% of 225ml peptone water in a sterile blender jar for 5 minutes. To make decimal dilution, 1ml mixture was transferred with the help of sterilized pipette to a test tube containing 9 ml of sterilized peptone water. In this way decimal dilutions of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  were prepared. One ml of sample from  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  dilution were pipetted into previously prepared agar plates. After mixing and solidification the media, the Petri-dishes were incubated at inverted position for 72 hours at  $30C^{\circ}$ . After 72 hour of incubation, forming colonies were counted which had a range between 30 and 300. As there is sufficient evidence that  $10^{-1}$  and  $10^{-2}$  dilution contain more than 300 colonies, these two dilutions were ignored for determining total bacterial load.

#### 2.5.2.1 Plate count agar media preparation

For isolation of TBC (Total Bacterial Count), Bacteriological peptone plate count agar (BPCA) media was used. To dilute the fish sample Bacteriological peptone water was prepared by mixing 1g of media with 1000 ml distilled water (according to the manufacturer's instructions). Then PCA was prepared by mixing 11.75 g of medium with 500 ml of distilled water and heated properly (according to the manufacturer's instructions). Both of the media were, then autoclaved at  $121^{\circ}C$  for 15 minutes.

#### 2.5.2.2 Test procedures

- I. Each of 1 ml of solution from  $10^{-3}$ ,  $10^{-4}$  and  $10^{-5}$  dilutions was plated by pipette into sterile plates.
- II. About 15 ml of sterile PCA was poured into the plates.
- III. After solidification of the media, the plates were inverted and incubated in incubator at  $30^{\circ}C$  for 72 hours.
- IV. Bacterial colonies were counted by using digital colony counter; and the total number of bacteria per gram of sample was obtained by multiplying the average number

of colonies on Petri dishes by the respective dilution factor.

### 2.5.3 Occurrence of *Vibrio* spp.

To know the presence or absence of *Vibrio* spp. in the sample, 25 grams of fish sample was homogenized with 225 ml. of Alkaline Peptone Water (APW) and kept for enrichment over night in an incubator at  $37^{\circ}\text{C} \pm 10^{\circ}\text{C}$ . Later a loop full of enriched sample was streaked on to the Thiosulphate Citrate Bile Salt Sucrose agar (TCBS) plates and incubated at  $37^{\circ}\text{C} \pm 10^{\circ}\text{C}$  for 24 hrs for the appearance of positive colonies.

#### 2.5.3.1 Media preparation

For *vibrio* spp. isolation, Alkaline Peptone Water (ABW) was prepared by mixing 20mg of media with 1000ml of distilled water. Then 225ml of the first enrichment was transferred to culture bottles and sterilized at  $121^{\circ}\text{C}$  for 15minutes.

#### 2.5.3.2 Test procedures

- I. For preparation of first enrichment, 25g of sample was taken and diluted with 225ml of sterile APW(Alkaline Peptone Water ).This suspension was incubated at  $37^{\circ}\text{C}$  for 6hours.
- II. For selective enrichment, TCBS (Thiosulphate Citrate Bile Salt Sucrose) agar was prepared and sterilized. Then the culture of the second enrichment was streaked by means of a loop on the surface of TCBS agar plates.
- III. After streaking, the plates were inverted and incubated in an incubator at  $37^{\circ}\text{C}$  for 24hours.
- IV. After incubation, the presence of typical colonies of *vibrio* spp. were examined and marked on the bottom of the dish (typical colonies of *vibrio cholera* growth on TCBS agar are smooth, yellow and 2mm to 3mm in diameter.

### 2.5.4 Occurrence of *E.coli*.

#### 2.5.4.1 Media preparation

For *E.coli* spp. isolation, VRB (Violet Red Bile) agar was prepared by mixing 41,53gms of media with 1000ml of DW (Distilled Water), and then 225ml of the first enrichment was transferred to culture bottle and sterilized at  $121^{\circ}\text{C}$  for 15minutes.

#### 2.5.4.2 Test procedures

- I. For preparation of initial suspensions, 25g of sample was taken and diluted with 225ml of sterile peptone water. Then it was incubated at  $37^{\circ}\text{C}$  for 24hours.
- II. 1ml of each three suspensions (containing sample) was transferred to duplicate Petri dishes, VRB broth was added to this Petri dishes.
- III. Then it was incubated at  $35^{\circ}\text{C}$  for 24hours.
- IV. After incubation *E. coli* colonies was grown under the media and can easily be isolated.

### 2.5.5 Occurrence of *staphylococcus* spp.

#### 2.5.5.1 Media preparation

For *staphylococcus* spp. isolation, BBAB (Baired Barker Agar Base) was prepared by mixing 36gms of media with 950ml DW (Distilled Water).heat to boiling, sterilized in autoclave at 151lbs,pressure ( $121^{\circ}\text{C}$ ) for 15minutes,cool to  $55^{\circ}\text{C}$  then supplement called egg telluride emulsion yolk (50ml) was added.

#### 2.5.5.2 Test procedures

- I. For preparation of initial suspensions, 25g of sample was taken and diluted with 225ml of sterile peptone water (first dilution),10ml of first dilution was transferred to bottle containing 90ml peptone water (second dilution), finally 2ml of second dilution was transferred to bottle containing 18ml peptone water (third dilution).
- II. 0.1ml of this three diluents was transferred to ready duplicate dishes contain Baired parker agar media and incubated at  $35^{\circ}\text{C}$  for 24hours.
- III. After incubation, *staphylococcus* spp. (*staphylococcus aureus*) colonies were growth and can easily be isolated.

### 2.5.6 Occurrence of *salmonella* spp.

To know the presence or absence of *Salmonella* in the sample, primary enrichment was done in lactose broth (LB). Secondary enrichment was done using Fluid Selenite Cystine broth (SCB). Later a loop full of enriched sample was streaked on Brilliant Green Agar (BGA) plates and incubated at  $37^{\circ}\text{C} \pm 10^{\circ}\text{C}$  for 24 hrs for the appearance of positive colonies.

### 2.5.6.1 Media preparation

For *salmonella* spp. isolation, pre-enrichment media BPW (Buffered Peptone Water) was prepared by mixing 20g of media with 1000ml of DW (Distilled Water). Then 225ml of the pre-enrichment broth was transferred to culture bottle and sterilized at 121<sup>0</sup>c for 15minutes. For LB (Lactose Broth) preparation, 13gm of media mixed with 1000ml DW (Distilled Water), heat, sterilized by autoclave at 121<sup>0</sup>c for 15 minutes.

### 2.5.6.2 Test procedures

- I. For preparation of initial suspension, 25g of sample was taken and diluted with 225ml of sterile LB (Lactose Broth) and incubated at 37c0 for 24hours.
- II. 1ml this suspension (containing sample) was transferred to tube contain 90ml of SCB (Selenite Cystine Broth) and also incubated for another 24hours.
- III. By loop, sample from this tube was taken and cultured in Petri dish with XLD (Xylose Lysine Deoxycholate), then incubated for 24hours at 37<sup>0</sup>c.
- IV. For identification of salmonella spp. solid media (XLD) was prepared, and then the culture obtained after incubation of SCB was streaked by means of a loop, on the surface of XLD plate.
- V. After streaking, the plates were inverted and incubated at 37c0 for 24hours.
- VI. After incubation, the presence of typical colonies of *salmonella* spp. Was examined on the bottom of the dish (typical colonies of *salmonella* spp. growth on XLD agar plate have a black centre and a lightly transparent zone of reddish colour.

## 2.6 Bacterial Identification

### 2.6.1 Culture method

#### 2.6.1.1 First isolation

Fish was isolated in nutrient agar. Supplemented plates were incubated at 37<sup>0</sup>c for 24 hours depending on the appearance of bacterial growth on the surface of the media.

#### 2.6.1.2. Preparation of nutrient agar

32g of nutrient agar dissolved in 400ml distill water then, it was sterilized by autoclaving at 15 pound per square inch pressure (121<sup>0</sup>c) for 15 minutes.

## 2.7 Biochemical Test

To identify bacteria, we must rely heavily on biochemical testing. The types of biochemical reactions each organism undergoes act as a "thumbprint" for its identification.

### 2.7.1 Catalase test

Enzymes that decompose hydrogen peroxide into water and oxygen. Hydrogen peroxide forms as one of the oxidative end products of aerobic carbohydrate metabolism. If this is allowed to accumulate in the bacterial cells it becomes lethal to the bacteria.

**Reagent:** 3% hydrogen peroxide stored in dark brown bottle under refrigeration, 18 to 24 hrs. culture of the organism to be tested. Example: Positive: *Staphylococcus aureus*

Drop of hydrogen peroxide was placed on microscopic slide. Using sterile glass. Rod small part of isolates colony was taken and emulsified in the hydrogen peroxide drop. The production gas bubbles were considered to be an immediate apposite a positive reaction. The test described by the [7].

### 2.7.2 Indol test

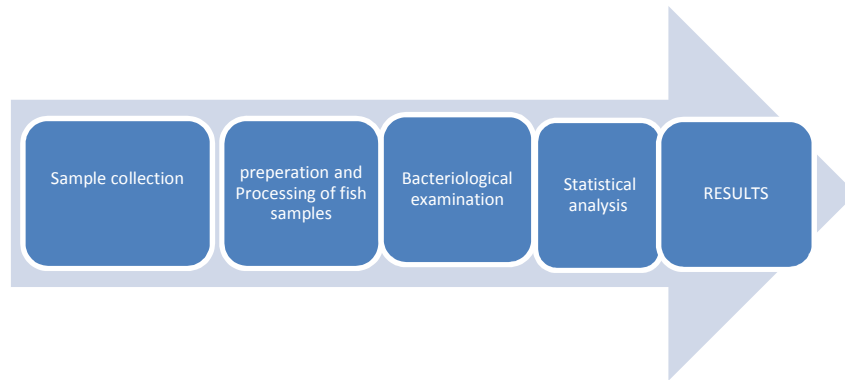
IMViC: it's a group of tests used mainly to identify Enterobacteriaceae members which include Indol test. Indole is a component of the amino acid tryptophan. Some bacteria have the ability to break down tryptophan for nutritional needs using the enzyme tryptophanase. When tryptophan is broken down, the presence of indole can be detected through the use of Kovacs' reagent. Kovac's reagent, which is yellow, reacts with indole and produces a red color on the surface of the test tube.

**Results:** Indole-Positive reaction: red color ex. *E. coli*. Using sterile tube isolated and incubated at 37c<sup>0</sup> and examined to addition covac's solution was occur red ring in the surface of tube.

The test was performed according to the method described by [7].

## 2.8 Statistical Analysis

The Obtained results were analyzed statistically using IBM6 SPSS statistic version (23).



**Fig. 1. Flowchart explaining the process of the evaluation and methods undertaken to perform the experiment**

### 3. RESULTS

The result obtained that the bacterial count for fresh Najil fish (*P. Pessuliferus*) was  $5.68 \pm 1.57 \times 10^5$ , and for frozen Najil fish (*P. Pessuliferus*) was  $4.38 \pm 1.96 \times 10^5$  cfu/g respectively. And indicates that *Staphylococcus aureus* and *E.coli* were isolated as contaminant bacteria; while *salmonella* and *vibrio* were not isolated from both fresh and frozen Najil (*P.*

*Pessuliferus*) fish. As the result shown in Tables 1 and 2.

Paired sample T- test was used to test the difference between the load of bacteria as fresh (before freezing) and after freeze fish samples. It's revealed that there was highly significant difference in total bacterial count ( $p < 0.05$ ) between fresh and frozen Najil (*P. Pessuliferus*) fish.

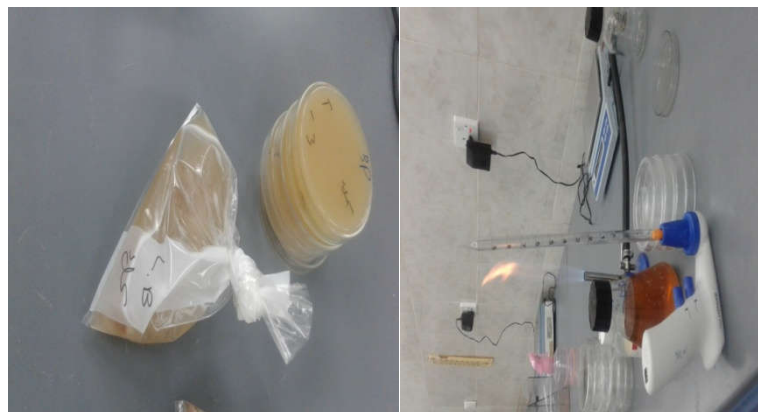
**Table 1. Microbial load for fresh and frozen Najil (*P. Pessuliferus*) fish**

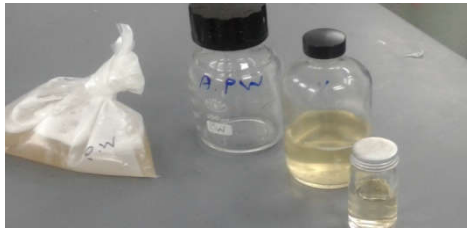
Items	Microbial load for fresh Mean±Std. Deviation	Microbial load for frozen Mean± Std. Deviation
N=20	5.68±1.57 cfu/g	4.38±1.69 cfu/g
Significant	highly significant	**

\*\*= highly Significant at ( $P < 0.05$ ).

**Table 2. Pathogens that have been isolated from Najil fish (*P. Pessuliferus*) samples**

Organism Treatment	Staphylococcus	salmonella	E.coli	vibrio
Fresh fish	+ ve	- ve	+ ve	- ve
Frozen fish	+ ve	- ve	+ ve	- ve







**Fig. 2. Sample preparations**

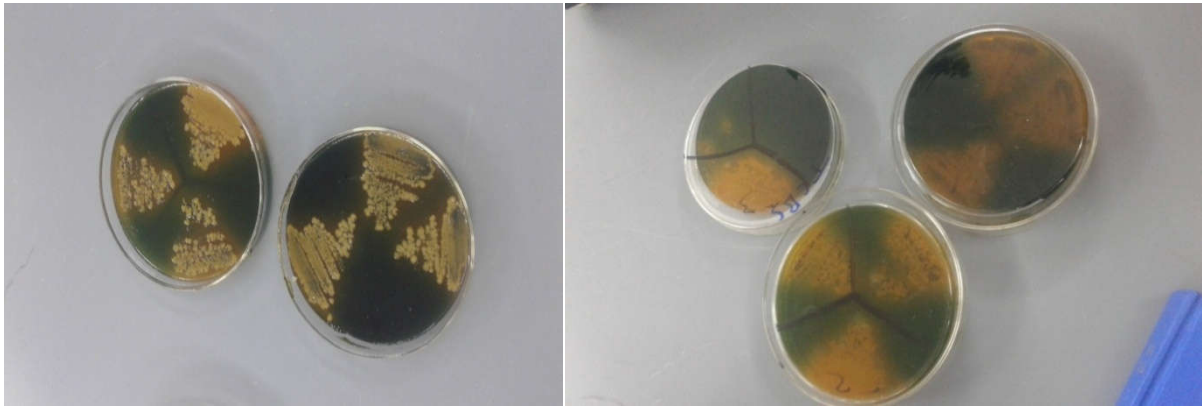


**Fig. 3. Staph colonies at Baird Parker (BP) agar plates for fresh Najil (*P. Pessuliferus*) fish**



**Fig. 4. *E.coli* colony at violet red bile (VRB) agar plates for fresh Najil (*P. Pessuliferus*) fish**

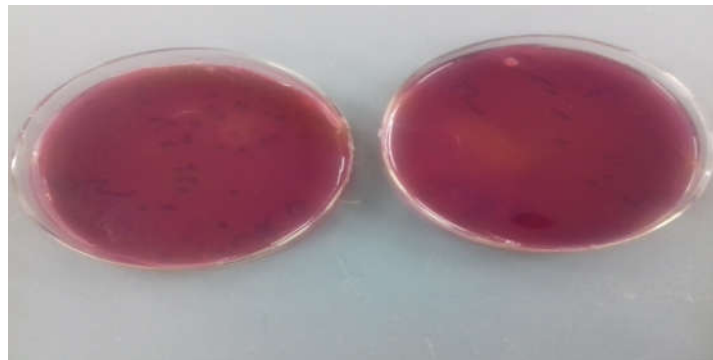




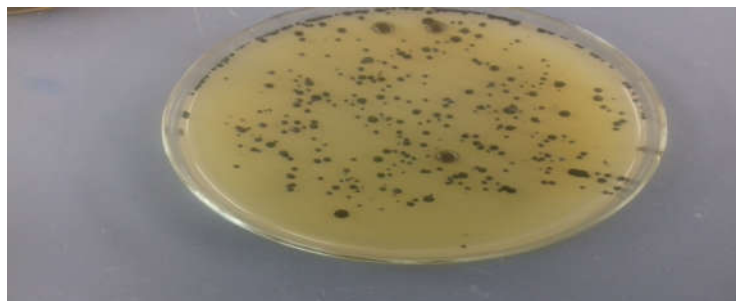
**Fig. 5. Colonies of *V. SPP.* on Thiosulphate Citrate Bile Salt Sucrose (TCBS) after Incubation for fresh Najil (*P. Pessuliferus*) fish**



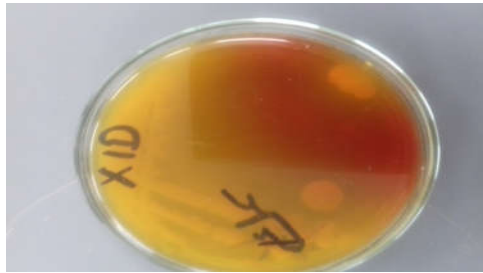
**Fig. 6. *Salmonella* colonies at Xylose Lysine Deoxycholate (XLD) agar plates for fresh Najil (*P. Pessuliferus*) fish**



**Fig. 7. *E.coli* colonies at Violet Red Bile (VRB) agar plates for frozen Najil (*P. Pessuliferus*) fish**



**Fig. 8. *Staph* colonies at Baird Parker (BP) agar plates for frozen Najil (*P. Pessuliferus*) fish**



**Fig. 9. *Salmonella* colonies at Xylose Lysine Deoxycholate (XLD) agar plates for frozen Najil (*P. Pessuliferus*) fish**

**Biochemical tests:**

Table 3. Revealed a biochemical test of the *Eshcerichia coli* and *Staphylococcus aureus* respectively

**Table 3. Biochemical test for fresh and frozen Najil (*p. pessuliferus*) fish**

Type of bacteria	Shape	Indole test	Catalase
<i>Staphylococcus aureus</i>	Sphere	- ve	+ ve
<i>E.coli</i>	Rod	+ ve	- ve

**4. DISCUSSION**

Registered bacterial species in fresh and processed fish samples warn high risk situation for human health due to ingest of fish, especially when it is consumed raw or with short baking time, and also because the presence of *enterobacteria* was predominant [8].

A t-test comparing the fresh and frozen conditions of Najil (*P. pessuliferus*) showed that there was a highly significant different. On the other hand, the total bacterial count of Najil (*P. pessuliferus*) fish stored under frozen condition for four days showed a significant decrease through the time of preservation. The bacterial load showed decreases from  $5.68 \times 10^5$  to  $4.38 \times 10^5$  cfu/g. This numbers was in the acceptable limit mentioned by SSMO (Sudanese Standards Metrology Organization, SDS357) which was  $5 \times 10^5 - 10^6$  for fish products.

The International Commission on Microbiological Specifications for Food (ICMSF) recommends that during fish storage at lower temperature, the total plate count should never exceed log mean 7 log CFU/g of wet weight [9]. According to the result, Najil fish did not loss its microbiological quality acceptance level (microbiological based shelf-life). The initial load ( $5.86 \times 10^5$  cfu/g) may have contributed to the accepted number (acceptable level of ICMSF) of total bacterial count in short storage time.

This result is coincide with finding of [10] who studied the microbial quality of frozen fishes: Shinna (*Auxisthazard*), Bonga (*Ethmalosafimbriata*) and Mackerel (*Scomber scombrus*) obtained from three different markets were carried out using standard methods revealed that, The total heterotrophic bacterial count ranged from  $3.0 \times 10^5$  to  $5.0 \times 10^5$  cfu/g,  $3.0 \times 10^5$  to  $4.8 \times 10^5$  cfu/g and  $3.0 \times 10^5$  to  $6.3 \times 10^5$  cfu/g for Shinna, Bonga and Mackerel respectively.

This in differ from the finding of [11] who studied the prevalence of pathogenic microflora along the two major sea fish samples, Rupchanda (*Pampuschinensis*) and Surmai (*Scomberom orusguttatus*) and reported that the total bacterial count was  $2.5 \times 10^6$  cfu./g in fish blend samples. Also it is differed from the result of [12] who studied microbiological qualities of some frozen fishes available in some reputable supermarkets in Lagos State and reported that total bacterial count ranged between  $2.0 \times 10^3$  to  $7.4 \times 10^3$  cfu/g.

The result it is in partial agreement with result obtained by [13] who studied that the total bacterial count of marketed Mola (*Amblypharyngodon mola*) ranged from  $1.8 \pm 0.25 \times 10^4$  to  $6.5 \pm 0.75 \times 10^6$  cfu/g for fresh sample and  $5.5 \pm 0.55 \times 10^3$  to  $7.0 \pm 0.80 \times 10^5$  cfu/g for frozen .

According to [14] good quality fish should have count of total bacteria less than  $10^5$  per gram. This study within the acceptable limit recommended by food and agricultural organization. However, in this study the fish samples of Najil (*P. pessuliferus*) were contaminated with bacteria load, this might be due to the contamination of water that fish found or might be due the contamination during the time of handling as well as transporting of fishes in boats, preservation boxes materials or contaminated ice.

## 5. CONCLUSION

Food borne pathogens are a growing concern for human illness and death [15].

The total viable counts for fresh samples of Najil (*p. pessuliferus*) was ( $5.68 \times 10^5$ ) CFU/gm, and for frozen samples was ( $4.38 \times 10^5$ ) CFU/gm is strongly suggest the urgent need to improve the quality control and assurance systems. It has also shown that samples of fresh fish and frozen fishes used in this study were grossly contaminated by pathogenic organisms such as *Staphylococcus aureus*, *Escherichia coli*, which indicated post-harvest contamination probably due to mishandling, improper storage and using of contaminated containers during transportation, and thus, constitute potential public health hazard due to the unhygienic nature of fish handlers which predisposes frozen fishes to contamination by pathogenic microorganisms. This call for public health concerns and improvements in handling and processing are needed to minimize the prevalence of the pathogens.

Higher rate of *Staphylococcus aureus* and *Escherichia coli* was isolated from the fresh and frozen fish, which pose high risk of food borne illness, as this fish commonly eaten by high numbers of consumers as first class fish.

Also it was concluded from this study that fish markets harbored bacteria of zoonotic importance that may constitute potential hazards to fish handlers.

The results of this study also constitute an indicator of bacteriological contamination of one of important marine fishes. However, fishes should be properly cooked before consumption and good quality control measures should be adopted in culturing, processing, harvesting and consumption of sea foods.

Frozen condition storage could maintain the fish fillet qualities for longer time. In general, the results obtained showed that storing at frozen condition can significantly improve the shelf life of fish quality compared to fresh storage. Besides this, fish catching, handling and processing need to be evaluated and improved to assure quality and safety, and to minimize loss.

To limit the microbial loads of frozen fish and fish product, I suggest the provision of the adequate storage facilities i.e. refrigerator by retailer so as to avoid the multiplication of microbes under atmospheric temperature in the market.

Finally, messages warning consumers of the potential risks of infection associated with consumption of good and hygienic fish and sea food products should be considered as educational strategies. Also fish handler's education of the importance of personal hygiene should be implemented as control measures to prevent contamination during postharvest handling, processing and distribution of fish.

This finding may be considered as additional knowledge to enhance proper controlling of the storage life of fish, and fish products quality in Constantine. Safety of this kind of seafood can be guaranteed mainly by preventive measures and application of appropriate procedures of hygiene, because the surveillance of potential contaminant bacteria in harvested seafood is crucial for sustenance of public health.

## ACKNOWLEDGEMENT

Foremost, my heartfelt appreciation to my parents (bdraldein and nabih), my brother Mohammed and sisters Shimaa and Fatima, reverent teachers and staff of the Directorate general of preventing medicine and PHC, Epidemiology Dept for providing a good working environment .

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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