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MICROBIOLOGICAL CHARACTERISTICS OF THE CONTAMINATION FISH OF TROPICAL SEAS

Abdelsalam Adoum Doutoum[1,4]*, Abdelsalam Tidjani[2], Hamadou Abba[3], Couba Faye[4], Mg. Seidy[4] & Bhen Sikina Toguebaye[5]

[1]Institut National des Sciences et Techniques d'Abéché (INSTA), Tchad
[2]Laboratoire de Recherches en Sciences des Aliments et Nutrition (LARSAN), Faculté des Sciences de la Santé Humaine (FSSH), Université de N'Djamena-Tchad
[3]Université Adam Barka d'Abéché
[4]Ecole Inter Etat des Sciences et Médicine Vétérinaires de Dakar, Laboratoire HIDAOA
[5]Université Cheikh Anta Diop du Sénégal
*Corresponding Author

ABSTRACT

The study of initial contamination of tropical sea fish has included 100 samples of red mullet (*Pseudupeneus prayensis*). The samples were taken from the flesh and the gills. The initial contamination bacteria are constituted by: Mesophile Flora Total Aerobic at 30 ° C (FMAT) and Aerobic Flora Psychrotrophe at 5 ° C (FMP), FMAT predominates in flesh and gills with an average of 2.6 x 10^2 CFU/g of meat versus 0.6 x 10^2-CFU/g of meat for FAP. At the gill level, an average of 1.2 x 10^4 CFU/g was obtained for the FMAT against 0.3x 10^4 CFU/g for the FAP.

Enterobacteriaceae absent in the flesh contaminate the gills with an average of 2.7.x 10^3 CFU/g. *E. coli* was isolated at a rate of 8% by the Mackenzie test.
Pseudomonas are almost absent in the flesh (2.4 CFU/g) and are more important in the gills (10^2 CFU/g).

Vibrios are present in the flesh and gills respectively in 51% and 76% of the samples. 59 samples carry *V. alginolyticus* in flesh and gills. 6 samples carry *V. parahaemolyticus* in the gills only.

In view of these results, the storage under ice of the fish must be done at an early stage, immediately after catching in order to avoid the proliferation of the flora.

The early delimbing of the fish makes it possible to avoid the spread of the germs towards the flesh.

Key words: *Initial contamination - Tropical seas - Mullet - Flesh - Gills – Germs.*

1. INTRODUCTION

The fishery is of significant economic importance to coastal African countries. Senegal is located in an area ranked among the most fishy in the world whose plankton is permanently renewed for marine currents. Fishing has been growing for a number of years and is thus helping to raise the trade balance.

Much of the catch is exported in the form of elaborate products to the European Union, Japan and Canada. Products for export must satisfy a number of microbiological criteria, based on data inherent in the types of bacteria encountered in temperate seas fish.

However, it remains to be seen whether these criteria are applicable to our products, especially since many studies claim that there is a difference in microbial composition between temperate (psychrotrophic) and tropical (predominantly mesophilic).

According to Ababouch (1995) and (Abdelrahim et al., 2012), fish is a source of animal protein of high nutritional value. Despite its nutritional importance, without hygiene precautions, these foods can cause food poisoning. The risk is increased with fish that is a very perishable commodity and a good medium for the development of pathogenic microorganisms or microorganisms indicative of non-compliance (Sylla and Seydi, 2003).

It is to contribute to a better knowledge of the flores hosted by our fishes, that we chose to deal with the following subject: Study of the initial contamination of tropical fishes.

2. MATERIAL AND METHODS

2.1. MATERIAL

The equipment used consists of biological or animal material and laboratory equipment.

2.1.1 Animal equipment

The animal material used for this work consists of whole red mullet (*Pseudupeneus prayensis*) captured that same day by canoes arriving at Hann beach. These fish are commonly called by the fishmongers of the "pieces of the day".

Justification for the choice of Hann beach

The Hann beach is located at Km 5, Centennial Boulevard of the Dakar Commune.

The choice of this beach as sampling site is justified by:

- Its proximity to the laboratory of analyzes (HIDAOA) of the EISMV;
- The advantage of finding fishmongers aware of the importance of ice storage of fish. Indeed, the latter provide the raw material to certain fishing companies of the beach;
- Ensure that the species used in the study can be found daily at the fishmonger. Fish status with purchase; Immediately after capture, red mullets are placed in PVC boxes containing ice of good bacteriological quality. Indeed, this one is produced by a factory which is in the bay of Hann.

2.1.2. Material and laboratory equipment

The work equipment is the one commonly used in all laboratories of food bacteriology:

- Sampling equipment: cooler, 3 dry ice generators, infrared thermometer;
- Material used for test samples: laminar flow hood, knives, scissors, pliers, electronic scales, sterile Stomacher sachets, crusher, bag holder;
- Analysis equipment: test tube, Petri dish, culture medium and reagents;
- Incubation equipment: ovens (at 30 ° C and 37 ° C) and refrigerators;
- Reading equipment: colony counter.

3. Methods

3.1. Sampling

Sampling is purchased on the day of the analysis. Their transport from the beach to the laboratory is done by taxi to minimize the time between catching fish and the start of the tests. Each sample consists of 5 randomly selected fish.

3.1.1. Material and laboratory equipment

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- Analysis equipment: test tube, Petri dish, culture medium and reagents;
- Incubation equipment: ovens (at 30°C and 37°C) and refrigerators. • Reading equipment: colony counter.

3.1.2. Transport of samples

Sampling is carried out without interruption of the cold chain. To do this, vials of dry ice and an isothermal enclosure (ice box) are used. This prevents pollution of germs.

3.1.3. Preparation of samples for analysis

- Temperature measurement

Each sample is placed in a stainless-steel tray, previously cleaned and disinfected. The temperature measurement is carried out using an infrared thermometer enabling the temperature to be obtained at the core. The core temperature of the fish, stored under ice, should be at most 7-8 ° C.

- Fish scaling

Chipping of the fish is done with a suitable knife.

Rinsing and draining follow the chipping process.

The aim of these operations is to reduce the microbial flora in the skin, in order to avoid cross contamination with the flesh during the test samples.

3.1.4. The desired microorganisms

For the study of the initial contamination of fish, we looked for four groups of bacteria, most often adapted to the living conditions of the aquatic environment. It's about:

- Vibrios;
- Enterobacteriaceae;
- of the Global Flora consisting of the Mesophile Total Aerobic Flora (FMAT) at 30°C and the FAP at 5°C
- of Pseudomonas.

Preparation of the test samples to the mother suspension

The test sample is the quality of flesh or gill taken for microbiological analysis.
Two types of sampling were carried out: in the flesh and in the gills.
These samples are taken starting with the least contaminated (flesh) cite to the most contaminated site (the gills).

- Flesh removal

Flesh removal is carried out under the laminar flow hood as follows:
Using a previously sterilized scalpel, the area to be removed is removed by incision of the skin. The flesh is then removed, at a depth near the central ridge, using a knife and sterile scissors.
The aim of these operations is to reduce the microbial flora in the skin, in order to avoid cross contamination with the flesh during the test samples.

- Gill sampling :

Gill sampling was performed using two methods:

- the first method consisted of swabbing. In this case, it is the mucus covering the gills which is recovered in swabs containing 5 ml of Bouillon Cervelle (BCC);

The whole is homogenized by mechanical agitation by vortex.
This branchial swab is used for the research of *Vibrionacae*.

- the second method consists in cutting the gills at the level of their insertion. The gills thus collected will be used to research the following types of flora: Enterobacteria, Global Flora and Pseudomonas.

• The withdrawal of the parent suspension

1 to g of flesh or gills are introduced into a sterile sachet stomacher. 90ml of Buffered Pitted Water (EPT) or BCC is added.
A 1 / 10th mother suspension is thus obtained. The contents of the sachet are homogenized by grinding for 3 minutes at the stomacher.
The suspension thus obtained is left to stand for 3 min, to ensure the revivification of the bacteria, stressed by the impact exerted during grinding.
The dilution of this parent suspension is obtained by establishing the ratio:

$$\frac{\text{Weight of food}}{\text{Total Volume (Thinner + Food)}}$$

Indeed, for liquid foods, it is considered that their density is close to 1, and consequently 1 gram is equivalent to a volume of 1 milliliter.

Search for Vibrios

Vibrios are typical germs that can be isolated from freshly caught fish and have not been manipulated. Their research is, therefore, of paramount importance in the study of the initial contamination of fish.

- Steps in Vibrios research

The search for bacteria of the genus Vibrio is done in 4 steps. At each of these stages corresponds a well-defined culture medium – enrichment.
The BCC is used as an enrichment medium at a rate of 90 ml per 10 g of flesh in the stomacher sachet.
The BCC is also placed in the 5ml swabs for Vibrios research at the gill level.
After homogenization; Swabs and sachets containing BCC are incubated at 37 ° C for 24 hours.

- Isolation

Using a pasteur pipette or a bone previously soaked in the enrichment solution, the insulation is produced by streaking, on the surface, on the TCBS medium – reading.
After incubation, two types of colony can be obtained on TCBS:

- Round colonies yellowish, positive sucrose: *V. alginolyticus; V.cholerae*;
- Round green colonies, negative sucrose: *V.parahaemolyticus.*

On some boxes one can have a coexistence of the 2types of colonies– purification.

The identification of Vibrios is based on the use of the morphological and biochemical characteristics of the family. Several techniques have, therefore, been used: - Gram staining

It was made using Gram kit comprising 4 elements:

- Phenolic gentian violet;
- iodo-iodide solution (lugo);
- acetone alcohol solution;
- diluted Ziehl Fuschin solution;

Immersion under the objective microscope at objective 100 X is colored bacteria in pink: Gram-negative;

- freshness.

The fresh state allows a direct observation of the Vibrions which, on the microscopic fields disappear and reappear.

These rapid movements attest to the mobility of the vibrios, thanks to the presence of a polar flagellum (mono-cheating bacterium). - the search for oxidase OX.

The search for oxidase makes it possible to differentiate within the negative Grams, the vibrios (OX +) and the Enterobacteria (OX-).

Disks impregnated with dimethyl-para-phenylenediamine oxalate have been used for the detection of oxidase. Procedure:

Colonies isolated on GNS will be used to carry out this test.

The disc is deposited with a forceps on a colony of the GNS agar. The presence of the oxidase is manifested by a violet coloration.

Thus, positive OX bacteria, including Vibrios, are present.

The absence of a violet coloration or late appearance in the case of false positives indicates that the bacteria are negative oxidases: Enterobacteriaceae.

In the case of a positive OX bacterium, identification is continued.

The vibriostatic compound 0/129

It makes it possible to make the differential diagnosis at the level of the Vibronacea family between the genus Aeromonas and Vibrio. The sensitivity or resistance to the vibriostatic action of 2,4-diamino-6,7-diisopropylpteridine, also called "vibriostatic compound 0/129", is sought in this test using impregnated discs.

Procedure

A Muller-Hington (MH) or GNS agar is inoculated by flooding. After drying after 15 minutes in an oven at 37.degree. C., a disk is placed, pressing lightly with a forceps, to ensure adhesion with the agar.

After incubation, the absence of a zone of inhibition around the disk makes it possible to conclude resistance to the vibriostatic compound: Aeromonas.

The appearance of an inhibition zone around the disc with a diameter greater than or equal to 15 mm shows the sensitivity to compound 0/129: Vibrio

- the halophilic test

It allows the identification of halophilic vibrios (such as *V. parahaemolyticus* and *V. alginolyticus*) with respect to non-halophilicity. The medium used is E.P.I medium (Indole-free Peptone water) at different concentrations of NaCl /

Procedure :

In each tube, 5 ml of PPE are introduced at a given concentration of NaCl.

At the same time, a suspension of vibrios is carried out as follows:

A colony taken from the GNS agar is placed in a tube containing 5 ml of sterile physiological water. After homogenization, a drop of this suspension serves to inoculate each PPE tube.

The reading is done after 24h of incubation.

Table I: interpretation of the halophile test

0% NaCl	3% NaCl	7% NaCl	10% NaCl	Matching Vibrion
-	+	+	-	*V. parahaemolyticus*
-	+	+	+	*V.alginolyticus*
+	-	-	-	*V.cholorae*

+ = growth ; - = Without growth

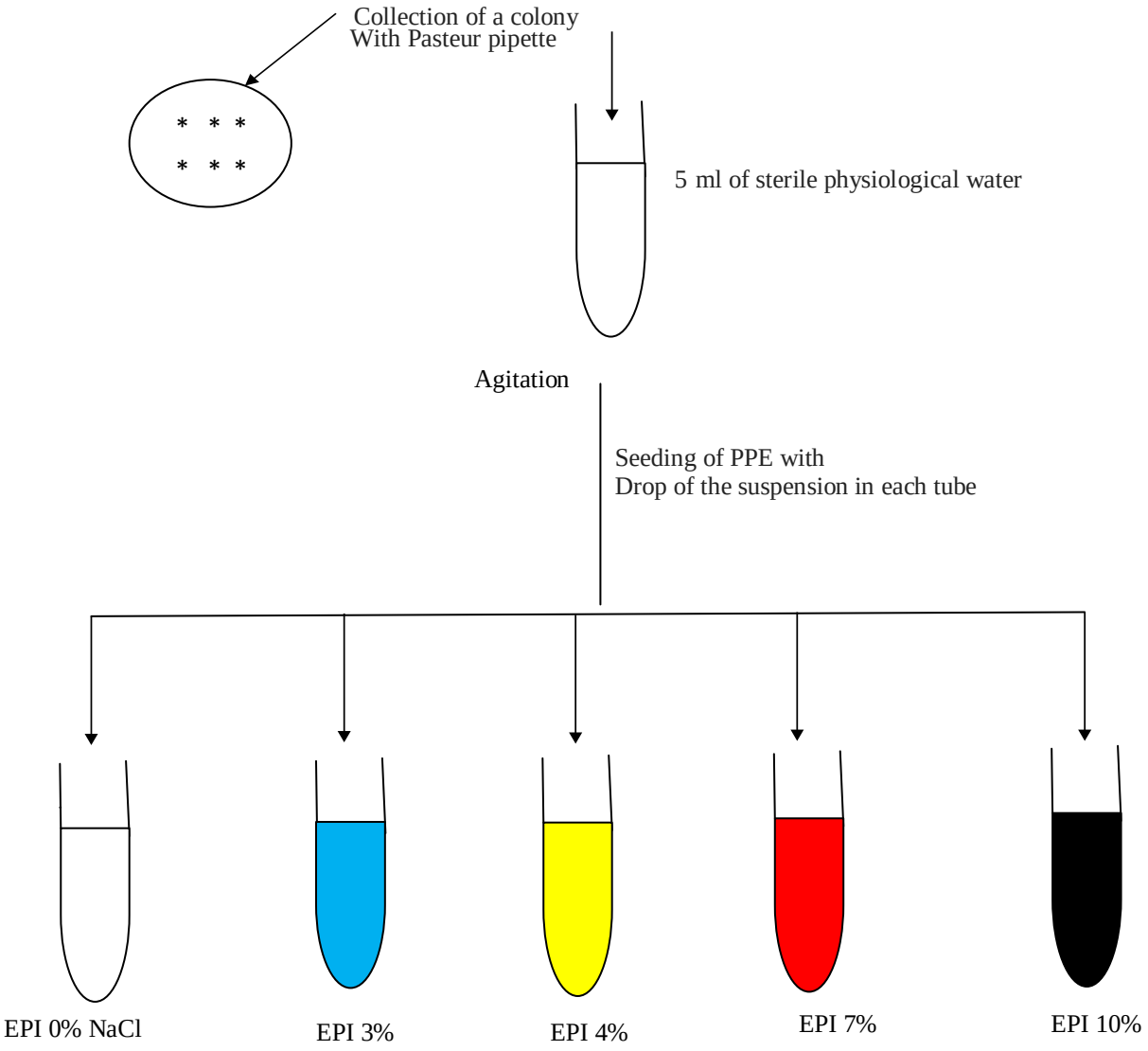

Figure 1: Halophilic Test Procedure

The chromagar agar

The colonies of vibrios, purified on GNS, are transplanted onto this agar at the surface. After 24 hours of incubation,

- or purple colonies: *V. parahaemolyticus*;
- or whitish colonies: *V.alginolyticus*.

Enumeration of marine flora

Two types of floras were counted, depending on the incubation temperature: FMAT at 30 ° C. and FAP at 5 ° C. Culture medium used:

The Agar Plate Count Agar (PCA) allowed the enumeration of two floras. Because of its low selectivity, it is used in a double layer, to avoid invading the surface of the box by contaminating germ like Proteus. Procedure

Dilutions 10^{-1} and 10^{-2} are carried out for the flesh while gills, which have a higher level of contamination, dilutions 10^{-2} and 10^{-3} are used.

A 1 ml of each dilution tube is removed aseptically and placed in the Petri dishes. 15 ml of PCA are then poured into each dish. The homogenization is done by circular movements to mix the inoculum with the PCA medium.

After solidification of the first layer, the second layer is poured, in the proportion of 5 ml of PCA. When incubated, the cans are turned over, lids towards the oven shelf, at 30 ° C for the FMAT for 48 to 72 hours and at 5 ° C for the refrigerator for the FAP for 5 to 10 days. At the end of this period the reading takes place.

Reading

The reading is done on 2 plates seeded with successive dilutions using a colony counter with a magnifying glass or with the naked eye.

Observed colonies are white-milky in color with a rice grain shape and grow deep.

The number of germs per gram of product is obtained according to the following formula:

$$N = \frac{\sum C}{V\,(n_1 + 0{,}1\,n_2) \times d}$$

N = number of germs per gram of product
ΣC = sum of the characteristic colonies on the two boxes retained
V = volume of incubation applied to each dish (in ml)
D = dilution rate corresponding to the first dilution retained
n_1 = number of boxes read at the first dilution
n_2 = number of boxes read at the 2nd dilution

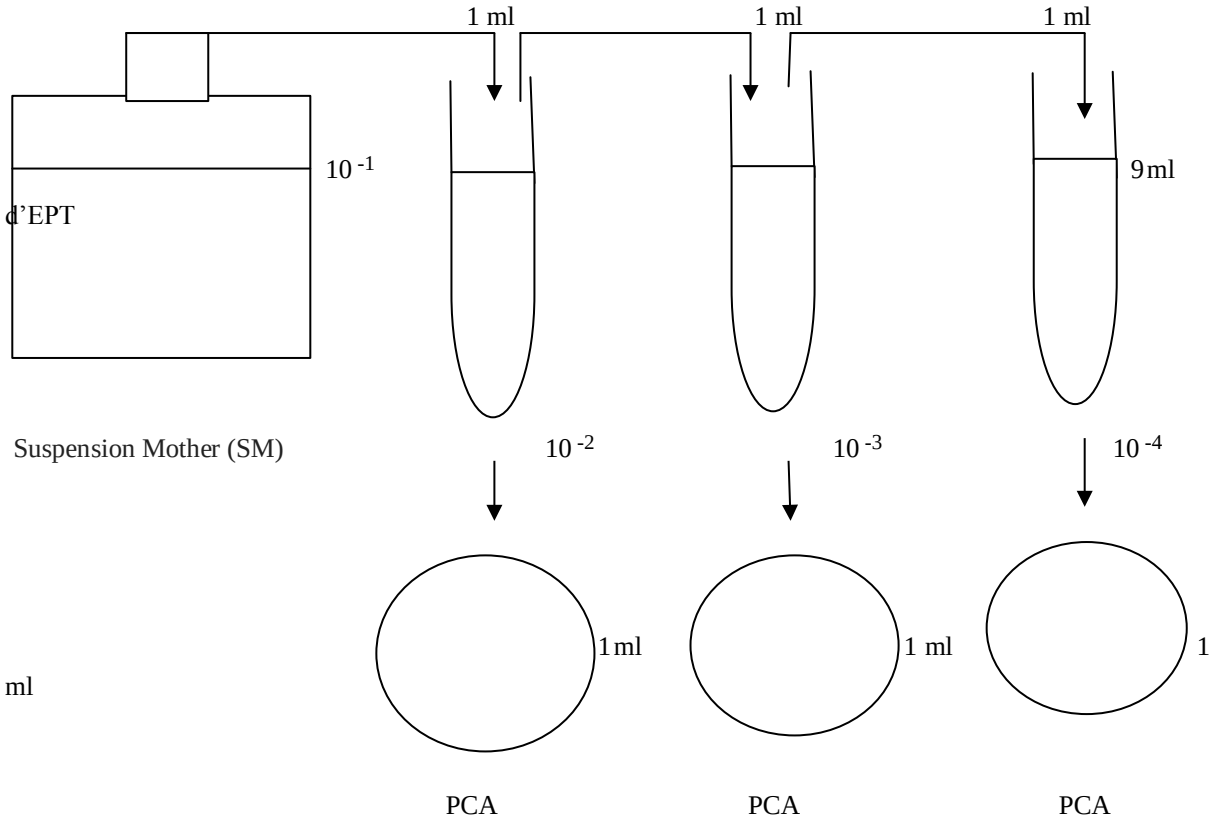

Figure 2: Enumeration of FMAT at 30 ° C and FAP at 5 ° C

Incubation is at 30 ° C for FMAT and 5 ° C for FAP

3.1.5.1.4. Count of Enterobacteriaceae

The bacteria of the family Enterobacteriaceae were searched both at the level of the flesh and at the level of the gills

Culture medium used

The bile glucose agar with the violet crystal and the neutral red used for enumerating Enterobacteriaceae (VRBG). It inhibits the growth of Gram-positive bacteria and practically that of other bacteria) Gram-negative.

Dilutions 10^{-1} and 10^{-2} were used for the search for Enterobacteriaceae in the flesh, while for their gill search the dilutions were pushed to 10^{-3}.

After 24 hr incubation, violet red round colonies with a diameter of at least 0.5 mm are counted on the VRBG agar.

The result is reported to the product unit (1g) according to the previous formula.

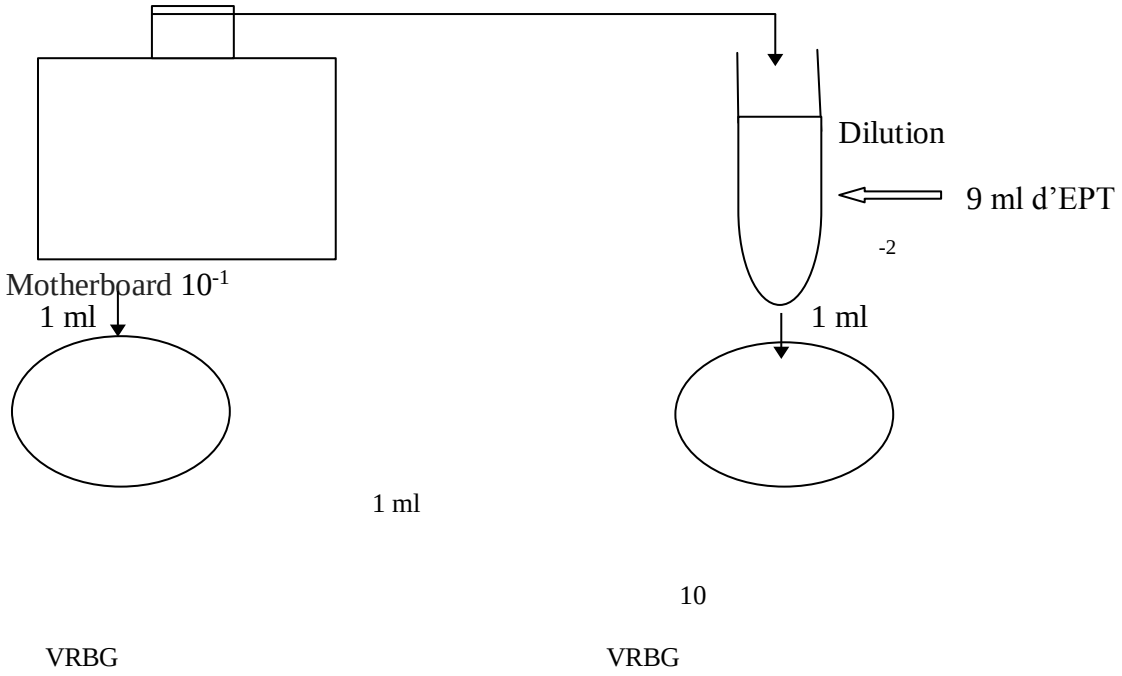

Figure 3: Counting of Enterobacteriaceae

The Mackenzie Test :

E. coli can be characterized by the Mackenzie test (fermentation of lactose and release of gas) Production of indole: it is demonstrated from a bacterial suspension. One drop of this suspension is introduced into a BLBVB tube containing 1 Durham bell and into a sterile PPE tube.

After incubation of BLBVB for 24 h at 44 ° C and 4 EPI for 24 h at 37 ° C, the following procedure is performed:

- the BLBVB tube is positive (presence of E. coli) when there is bacterial growth at the bottom of the tube, medium turbidity and gassing in the Durham bell.

The search for indole production is carried out by introducing a few drops of KOVACS reagent into the PPE tube. A positive result is manifested by the appearance of a red halo.

3.4 The search for Pseudomonas

Pseudomonas are the main germs of alteration of fish stored under ice. For the isolation of *Pseudomonas aeruginosa* or *Pyocyanic bacillus*, Pyocyanosel agar was used.

The previously cast boxes are inoculated at the surface using dilutions 10^{-1} and 10^{-2} .They are then incubated at 37 ° C. for 24 hours.

Reading :

The colonies obtained are green; this coloring is due to the production of a green pigment, pyoverdin

4. Results and Discussion

4.1 Results

Table II: contamination of red mullet by FMAT

Values in number of germs per gram	Flesh	Gills
Minimum value	$0.1x10^2$	$1.1x10^3$
Average	$2.6x10^2$	$1.2x10^4$
Maximum value	$1.2x10^3$	$3.7x10^4$

Table III: contamination of red mullet by FAP

Values in number of germs per gram	Flesh	Gills
Minimum value	0	$0.2x10^2$
Average	$0.6x10^2$	$0.3x10^4$
Maximum value	$0.6x10^3$	$1.1x10^4$

Table IV: Contamination of red mullets by bacteria of the genus *Pseudomonas*

Values in number of germs per gram	Flesh	Gills
Minimum value	0	0
Average	$0.2x10^1$	10^2
Maximum value	$3.0x10^1$	$0.7x10^4$

Table V: Red mullet contamination by Enterobacteriaceae

Values in number of germs per gram	Flesh	Gills
Minimum value	-	$0.3x10^2$
Average	-	$2.7x10^3$
Maximum value	-	$1.4x10^4$

Table VI: Contamination of red mullet by bacteria of the genus Vibrio

Corresponding Percentage	Flesh	Gills
Presence	51%	76%
Absence	49%	24%

Table VII: Global contamination of red mullets by bacteria of the genus Vibrio

100 samples of mullet			
Positives			Negatives
		76 samples	24 samples
V.alginolyticus	*V. parahaemolyticus*	NI	Total absence
59 samples	6 samples	11 samples	24 samples

NI = unidentified (the colonies obtained on TCBS are characteristic, are also sensitive to compound 0/129, are Gram negative, grow on saline EPI, but could not be identified by the other tests (API gallery 20e).

4.2. Discussion

4.2.1. Methodology

The study of the initial contamination of fish in the tropics has never been considered before, hence the difficulties in choosing a methodology.

The choice of methodology first focused on the types of sampling to be carried out on the fish, then on the types of bacteria to be investigated and on the incubation temperature.

However, previous studies carried out in temperate zones have guided us in the choice of the methodology adopted.

Thus, the types of samples selected concerned the flesh, the consumer product and the gills.

Gill sampling is justified by the fact that, as numerous authors have asserted (Guiraud, 1980, Huss, 1988 and Ndiaye, 1998), the bacteria found in the flesh originate from the initial sites of contamination (digestive tract and gills).

In terms of the types of bacteria we were looking for, we chose to study FG which, along with Vibrionaceae, is the dominant flora of the primary contamination of fish 95% (Azibe, 1991). In addition, the studies carried out in Senegal by Seydi et al. (Small, 1987), Japan and the USA (ICM, 1980) have led us to seek and give particular importance to the Vibrionacae family. The search for Enterobacteriaceae is justified by the good survival of salmonellae and coliforms in the marine environment. However, this research would be more interesting in the study of secondary contamination of handled foods such as nets.

Four other studies have examined fishes in the seas, but have always listed Vibrionaceace (either Vibrio cholerae or Vibrio parahaemolyticus): Tangkranakul, 2000; Vuddhakal, 2008 and Suthienkal, 2000.

However, Elyounoussi et al. In 2015 have access to their research on Enterobacteria. Thus, they found that the bacterial strains belonging to Enterobacteriaceae could contaminate fish were isolated and identified: sardine (*Sardina pilchardus*), Sole (*Solea vulgaris*), mackerel (*Scomber scombrus*), mackerel (*Trachurus trachurus*) Sale of Casablanca. Microbiological analyzes revealed that 50% of the fish are contaminated, in which case the description of 23 bacterial strains belonging to the family of Enterobacteriaceae "7 *Escherichia Coli*, *Citrobacter Braakii*, 2 *Cloaceae Enterobacter*, *Serratia fonticola*, 4 *Raoutella terrigena*" (*Sardina pilchardus*), Sole (*Solea vulgaris*), mackerel (*Scomber scombrus*), mackerel (*Trachurus trachurus*), Elyounoussi et al. (2015) have continued their studies on fish markets in Casablanca), Resulting in a contamination rate of 50%. Enterobacteria (*Escherichia coli*, *Citrobacter Braakii*, *Cloaceae Enterobacter*, *Serratia fonticola* and *Raoutella terrigena*). These results are much higher than ours and this could be explained by the diversity of the fishes analyzed.

Their results are different from our ones with very high coliform rates. On the other hand, they did not report *Vibrionaceae*.

4.2.2. Bacterial contamination of red mullet

The discussion of the results of the bacteriological analyzes will consist, on the one hand, in assessing the level of contamination of the fish for each research germ group, compared to the sampling sites (flesh and gills), and on the other hand to compare the results obtained At the level of the two types of psychotropic and mesophilic bacterial flora.

It should be pointed out that there are no microbiological criteria for the flesh of whole fish, so in some cases we will use microbiological criteria for chilled or fresh fillets for our comparisons.

4.2.2. 1. Assessment of the level of contamination of mullet by Enterobacteria, and bacteria of the genus Pseudomonas

4.2.2.2. Contamination of flesh by Enterobacteriaceae

The flesh of all the samples analyzed does not contain Enterobacteriaceae.

This confirms the work of many authors (Huss, 1988; and Seydi, 1985) who assert that the flesh of the living fish, or just caught, is quite sterile.

Other works carried out by TAYLOR quoted by PETIT (Ndiaye, 1997) have shown that Enterobacteria are not found in the flesh or in the intestines of fish.

These are bacteria of fecal origin, which is found in the mucus of the skin and the gills.

According to the same author, fish and net in particular undergo numerous manipulations, after capture and before consumption; This causes its contamination by microorganisms, mainly of fecal origin.

Indeed, all the work, which focused on the search for Enterobacteriaceae and coliforms in particular, in the fish fillets, allowed to detect contamination rates sometimes exceeding the fixed standards (10 germs / gram for the refrigerated fillets), (Azibe, 1991) obtained, for 66.9% of the samples analyzed, an average of $6.25x10^2$ germs per gram of nets. This average is higher than that found by Ndiaye (Lesne, 1998) for chilled fillets with an average of $2.92x10^2$ fecal coliforms / gram of product.

All these results make it possible to confirm that the fish chair does not contain originally Enterobacteria. The presence of bacteria in the nets is due to the intervention of humans in general and the workers of the fish processing companies in particular.

4.2.2. 3. Contamination of mullet with bacteria of the genus Pseudomonas

Pseudomonas are the main germs of alteration of fish. Their presence in large numbers on a considerable economic importance on the market value of the product.

Pseudomonas are with bacteria such as Aeromonas, Moraxella and Alcaligenes represent 95% of the flora of the aquatic environment (Lesne, 1998).

Of the 100 samples of chair analyzed, we noted a rarity, or even a near absence of Pseudomonas. Only 4 samples allowed to isolate Pseudomonas with a negligible number of colonies, on average 2.4 CFU/g of flesh, against an average of 10^2 CFU/g gills.

Indeed, this confirms the work of numerous authors (Guiraud, 1980; Huss, 1988 and Ndiaye, 1997) which show the initial localization of the alteration flora of the fish in particular of the Pseudomonas, is mainly branchial or intestinal. It is during alteration that bacteria invade the chair through the collagen fibers.

The isolated bacteria belongs to the fluorescent group, with *Pseudomonas aeriginosa*, Putaci and fluorescens.

4.2.2.4. Gill contamination by Enterobacteriaceae

Bacteriological analysis of 100 gill samples yielded an average of 2.7 x 10^2 CFU/g. The results obtained for initial gill contamination are included in the range given by some authors such as Seydi, 1985: 10^3 to 10^6 CFU/g of gills.

The gills, as well as the skin, are the sites that reflect the most, the degree of pollution of the marine environment. Indeed, they function as true filters retaining the bacteria present in the sea water (Doutoum et al., 2003). The mucus produced at this level favors this localization.

4.2.2. 5. Assessment of the level of contamination of the flesh by the FMAT

An average of 2.6x10^2 CFU/g of meat was obtained for the FMAT. Compared to the results on refrigerated fillets on the one hand, and frozen on the other hand, this average is far lower.

- in the case of chilled fillets, Ndiaye, (1997) obtained an average of 0.41x10^6 of net. This average is slightly higher than that found on the same product by Bernadac et al. Cited by Ouattara (1986) (9.3 x 10^6 CFU/g of nets)
- for frozen fillets, higher contamination levels were obtained by Azibe (1991) with an average of 1.8 x 10^6 CFU/g of fillets. Ouattara (1986) found, itself, a lower average, of the order of 1.73 x 10^5 CFU/g of frozen fillets. These last two results corroborate with those obtained on the same type of product by NDIAYE (3.17 x 10^5 germination CFU/g of nets).

The low contamination of the flesh of fresh fish compared to chilled fillets on the one hand and frozen on the other hand is not surprising, especially since, as many authors have previously pointed out, flesh of the fish is very poorly contaminated at the outset. To this internal flora will be added an external flora, resulting from the different technological manipulations that the flesh undergoes during its transformation into nets.

Compared to current standards (10^5 CFU/g of chilled fillets). The results obtained at the level of the flesh are in conformity whereas those obtained at the level of the fillets are more often acceptable or non-compliant.

4.2.2.6. Assessment of the level of contamination of flesh by both types of floras (FMAT and FAP)

The mean FMAT obtained at the level of the flesh is 2.6x10^2 CFU/g, whereas for the FAP, it is 0.6x10^2 CFU/g of flesh therefore by far lower.

The mesophilic flora is about 4X higher than the psychotropic flora.

These results confirm the work of many authors (Ouattara, 1998; Small, 1987, Seydi, 1985) according to which the fish flora of the temperate seas is dominant psychotropic whereas in the tropical seas the microflora of the fish is rather mesophilic.

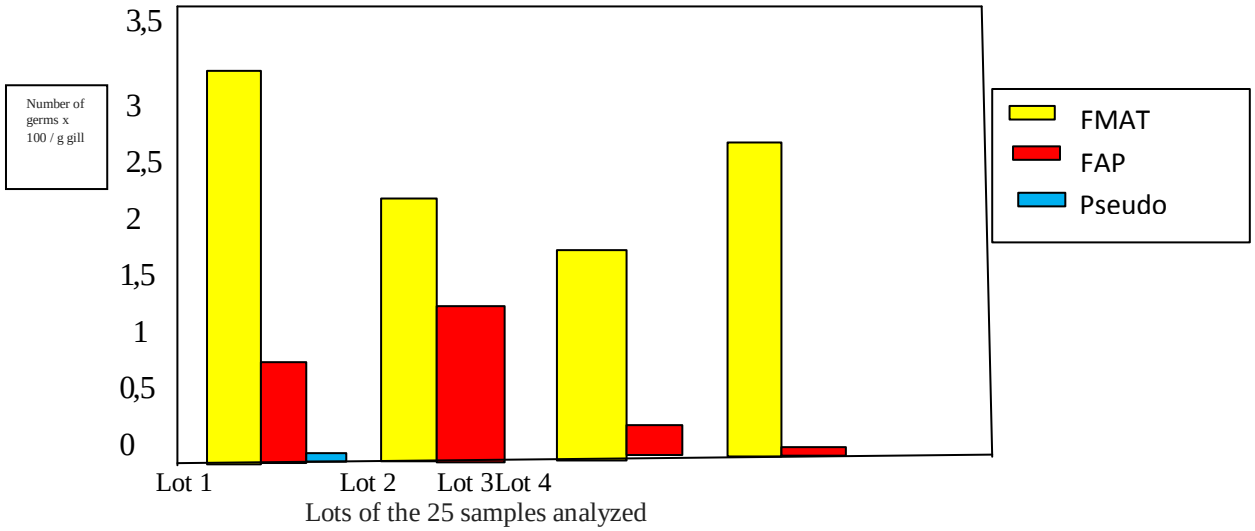

Figure 4: Contamination of red mullet flesh by global flora and *Pseudomonas sp*

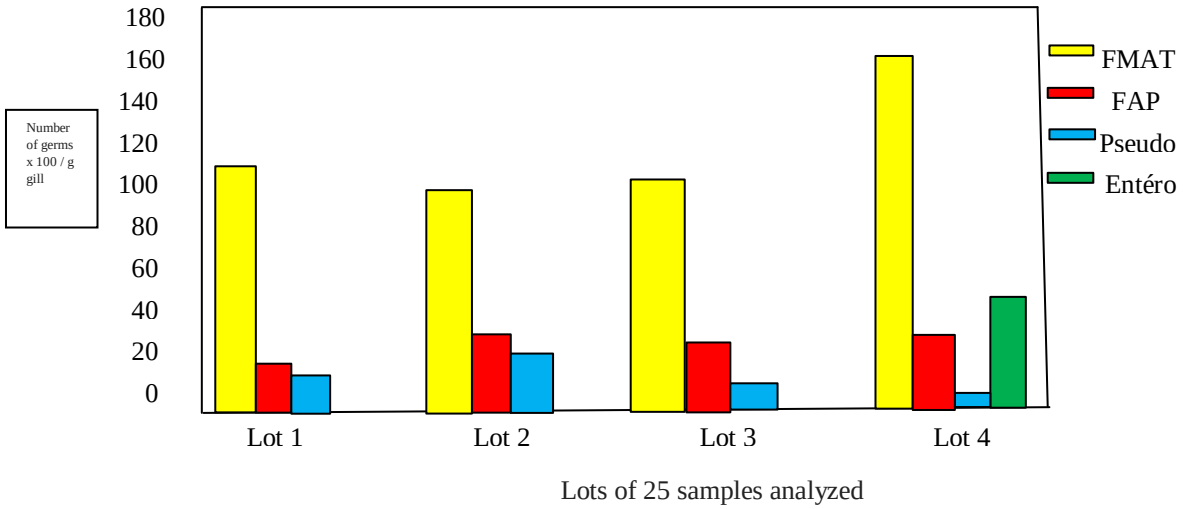

Figure 5: Contamination of red mullets with global flora, *Pseudomonas sp* and Enterobacteria

According to the same author, only 5% of the flora of fish in the temperate countries would be mesophilic, whereas for those caught off the Mauritanian coast, mesophiles and psychrotrophes represent respectively 55% and 45%.

4.2.2.7. Assessment of the level of contamination of red mullet by both types of The averages obtained for the FMAT are $2.6x10^2$ for the flesh and $1.2x10^4$ for the gills.

The gills are therefore more contaminated than the flesh.

The results obtained for the FAP are similar to the previous one with an average of $0.6x10^2$ for the flesh and $0.3x10^4$ for the gills.

4.2.2.8. Assessment of the overall level of contamination of samples by Vibrios.

The vibrios are present at a rate of 51% in the flesh while for the gills the contamination is more important with a carrying rate of 76%.

The identification tests carried out on all the samples showed that:

- 59 samples carry *V. alginolyticus* both in the flesh and on the gills;
- 6 samples carry V. parahaemolyticus on the gills only.

The vibrios are finally absent in 24 samples.

The rate of *V. parahaemoluticus* (6%) in red mullet is comparable to that found by SEYDI et al. (Ouattara, 1986, Small, 1987) which could isolate it, in different species, with a rate of 7.64% slightly higher than the rate obtained.

This, as the results obtained in Senegal by Cisse et al. (1987) and in Kenya by Binta and Nyaga quoted by Seydi (1985), *V. parahaemolyticus* isolated from fish are non-hemolytic apathogenic donors.

The strains encountered in food are no haemolytic, while those isolated from human diarrheal stools are still (Shewan, 1962 and Shewan, 1967).

V. alginolyticus was isolated from flesh as well as gills for 76% of the samples whereas isolation from the flesh was possible only for 51% of samples carrying *V. alginolyticus*.

This confirms the work of Seydi et al., In 170 samples analyzed, isolated *V.parahaemolyticus* in 5 cellar samples and 4 gill samples (Seydi, 1985)

The high frequency of vibrios in the red mullet is related to its biology? Indeed, the latter is a benthic limestone species, which lives in permanent contact with marine mud, true gites for marine bacteria including vibrios (Shewan, 1967). The biology of the species seems not to be the only factor determining the contamination of fish by marine vibrios. Indeed, work carried out in Senegal, on several different species of red mullet, made it possible to obtain contamination rates of 11.1% in Pomadasydae, 10.8% in Clupeideae and 7.4% in Carangideae (Seydi, 1985).

Environmental factors such as temperature and salinity, among others, are not to be neglected and can also be decisive in the contamination of fish by Vibrio.

Conclusion

The exploitation of fishery products is a significant source of foreign exchange for Senegal. It has an annual surplus of 24 billion CFA francs (63).

The microbiological quality of fishery products is the main limiting factor in the Senegalese and African fishing industry in general.

Indeed, these products come from a medium, whose balance is constantly disturbed, by the contributions of polluting elements residues of human and animal activity.

The initial contamination of the fish is that which occurs during its lifetime or just after its capture. Criteria for fishery products were derived from microflora data from temperate countries. However, according to many authors, the composition of the initial flora of fish from temperate seas is quite different from that of tropical seas.

The study of initial fish contamination at tropical seas in the HIDAOA laboratory and EISMV focused on the analysis of 100 samples of red mullet.

Of the samples, 200 samples were taken including 100 at the flesh level and 100 at the gill level. The search for the germs concerned the Global Flora which includes the FMAT and the FAP, the Enterobacteria, Pseudomonas and Vibrio.

From analyzes, it appears that the flesh of fresh fish contains a Global Flora, composed both of mesophiles and of psychrotrophs, with a predominance of mesophiles. The average level of contamination is $2.6x10^2$ germs per gram for the FMAT and $0.6x10^2$ for the FAP.

However, the location of this flora is rather branchial. At this level, very high rates of about $1.2x10^4$ for the FMAT and $0.3x10^4$ for the FAP were counted.

The results of the analyzes led to the conclusion that there was a complete absence of Enterobacteria in the flesh and a fairly high contamination, with an average of $2.7x10^3$ CFU/g in the gills. The Mackenzie test, carried out on 61 samples, made it possible to isolate E. coli in 8% of the cases. The research of Pseudomonas showed that these bacteria were initially localized in the gills and that they invaded the flesh later, causing its alteration. Quasi absent from the flesh, it is found right, 10^2 CFU/g on average in the gills.

As for the bacteria of the genus Vibrio, they were isolated from the flesh as well as from the gills with a predominance in the gills.

- 76 samples out of 100 have been used to isolate Vibrio in both flesh and gills. 67% of the 76 samples were contaminated in the flesh only.
- 24 samples are free from Vibrio
-

The identification of the 76 samples contaminated with Vibrio gave the following results:

- 8% of the samples carry *V. parahaemolyticus* at the gill level
- 78% are identified as *V. alginolyticus* in both flesh and gills with predominance in gills.
- 14% of the samples did not allow identification of the species of the genus vibrio.

In view of these results, we can say that the bacteria which predominate for the initial contamination of the fish are the vibrios and the mesophilic flora. Enterobacteriaceae and Pseudomonas occur mainly in the gills. The absence of Enterobacteria, on the level of the flesh, shows that the latter contaminate the pulpit secondary.

The use of appropriate harvesting methods combined with good handling on board fishing gear and the early and continuous use of the cold chain will result in products with reduced microbial load at the inlet of the fishing gear, factory.

However, the first use of microbial pauci is not sufficient to guarantee the bacteriological quality of the finished product; There are a number of factors, such as Good Manufacturing Practices (GMP), Good Hygienic Practice (GHP) and Good Laboratory Practice (GLP), which are the main pillars of a fishing industry More and more conquering.

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