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Microbiological diversity aspects and hygienic-sanitary conditions of mussels sold in the Municipal market in the municipality of Belém do Pará

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***Abstract*—** *Foodborne Diseases are caused by the ingestion of food that is unsafe for consumption, occurring at any stage in the production chain or even in the preparation phase of the final product at the consumer's home. . A mussel marketing product in the State of Pará has an implementation and functioning to obtain this quality and insurance for public health. In order to identify the presence of Escherichia coli and coliforms for mussel mussels in natura in the Ver-o-Peso Belém do Pará market, microbiological examinations of the mexiethylene Kit Aquatest coli, and eosin blue culture medium were performed. The results show 100% of the samples confirms the presence of total coliforms and 90% of sample presence of E.coli, then the counts obtained results greater than > 100 CFU/ml. It is stated tat least 90% are outsideof the standards established by current legislation. The microbiological food standards of RDC 331 are indicated,In stages, microbiological food standards are indicated that are taken into account within the current legislation with a purpose of handling.*

I. INTRODUCTION

Mussel fishing is one of the most productive activities, being developed in various regions of the world, including Brazil and the State of Pará for production and consumption in different regions (Duarte et al., Rosa, 011). According to FAO (Food and Agriculture Organization of the United Nations), in 2009, the consumption and cultivation of bivalves had a relevant growth in the world.

For bacteria, the Brazilian legislation, through the Resolution of the Collegiate Directorate RDC 12/2001, of the Sanitary Surveillance Agency (BRASIL, 2001), demands sanitary microbiological standards for food, where coliforms must be tolerated at 5 x 10 /g in temperature of 45 ºC; Coagulase positive staphylococci/g: in quantity of 10^3; Absence of Salmonella sp. in 25g:, determining that Escherichia coli and/or total coliforms is an indicator of microbial contamination of fecal origin.

Fecal coliforms in water are highly correlated with fecal contamination by animals and humans, making them indicators of contamination, since mussels are filter feeders, concentrating microorganisms and are represented in particular by E. coli, whose presence corresponds to 90% of the checks (Lima, 2012).

According to the National Program for the Hygienic and Sanitary Control of Bivalve Mollusks (PNCMB) in conjunction with the Ministry of Fisheries and Aquaculture (MPA), it establishes analysis procedures in the collection of bivalve mollusk samples so that the food is

free of pathogenic microorganisms, toxins, or metabolites in quantities that cause damage to the consumer's health.

The genus Mytella belongs to the family Mytilidae and is native to Central and South America. They are filter-feeding organisms and, due to their physiological characteristics, are susceptible to the accumulation of various microorganisms and biotoxins (Martins, 2020).

Based on the above, the present work aims to evaluate the hygienic-sanitary conditions through microbiological analysis of fresh mussels sold at the Ver-o-Peso market in Belém, Pará.

II. MATERIAL AND METHODS

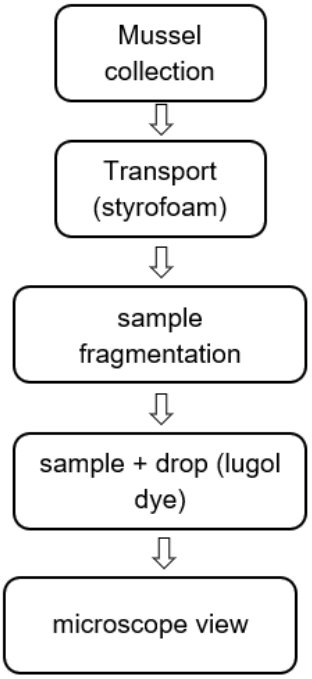
2.1 Location and sampling

The present study was carried out at Ver-o-Peso Market, located in the city of Belém-Pará. 900 g of raw mussel material (*Mytella charruana*) were collected, packed in an isothermal box at 4ºC and sent to the Bromatology and Clinical Analysis Laboratories of the University of Amazon - UNAMA (Belém-PA) and in the Rural Federal University of the Amazon for microbiological analysis.

2.2 Microbiological and parasitological analyzes

The presence and absence of total coliforms and E. coli in the water from the mussel raw material content (conservation ice + cell content), were evaluated with the Aquatest coli Kit, obtaining 25g of mussels in each sample and homogenized with 100 ml of saline water collected in sterile flasks added with 0.1ml of EDTA and incubated at 37ºC in the oven chamber for 24 hours in the Bromatology Laboratory of UNAMA. Samples positive for Total Coliforms are visually detected by viewing the yellow coloration and the presence of E. coli is detected by the blue-green fluorescent coloration when the sample is exposed to UV light from the (Vilber Lourmat Transilluminator) and also for confirmation, the colonies were counted on eosin blue methylene agar - EMB (Laborclin) culture medium, Gram staining was performed later with viewing under a microscope.

Approximately 10 mL of the first dilution of distilled water was used for each sample for parasitological analysis. Initially, direct examination was performed. For this, a drop of the samples from the bottom - the Falcon tube - was transferred to a glass slide, adding a drop of Lugol's dye, followed by observation under an optical microscope. Subsequently, The membranes were then washed by vigorous shaking with Tween 80 detergent and left to stand for three hours. The water resulting from washing the membranes was subjected to the centrifugation-flotation method in zinc sulfate (Faust's technique adapted for the investigation of parasites in water), and the floating material was stained with Lugol's stain and examined under a common optical microscope at 100- and 400-fold. The samples were taken in triplicate for reading by microscopy (FREITAS et al., 2015).



III. RESULTS

3.1 Microbiology Analysis

The results obtained in the analysis with the Aquatest Coli kit, 100% of the samples visually presented yellow color confirming the presence coliforms and 90% of sample presented blue-green fluorescence collor detecting *E.coli*, only the mex 6 didn´t show this bacteria, as results in Figure I.

In the microbiological analysis by inoculation on EMB agar medium, all samples showed results greater than > 100 (CFU), as shown in Figure II.

Fig.I. Samples visualized through the Vilber Lourmat Transilluminator (UFRA)

Photo: The authors

Fig.II. Growth of sample colonies on EMB agar medium (Laboclin)

Photo: The authors

To confirm the colonies *of E. coli* bacteria, the colonies were viewed under a microscope, as the results showed that 100% had colonies of Gram negative bacteria, as shown in figure III.

Fig.III. Presence of Gram negative bacilli colonies visualized under the microscope

Photo: personal file

Table 2. Results of the 11 mussel samples for presence and absence of E.coli

ID Amostra	Presença de Coliformes totais	Presença de *Escherichia coli*	Contagem de colônias
MEX 1	YES	YES	>100
MEX 2	YES	YES	>100
MEX 3	YES	YES	>100
MEX 4	YES	YES	>100

MEX 5	YES	NO	>100
MEX 6	YES	NO	-
MEX 7	YES	YES	>100
MEX 8	YES	YES	>100
MEX 9	YES	YES	>100
MEX 10	YES	YES	>100
MEX 11	YES	**YES**	>100

Source: personal file

3.2 Parasitological Analysis

Presence of Nematopsis spp. belonging to the phylum Apicomplexa were observed in parasitophorous vacuoles of phagocytic cells (phagocytes), found mainly in the mantle of *Mytella charruana.(figure IV)*

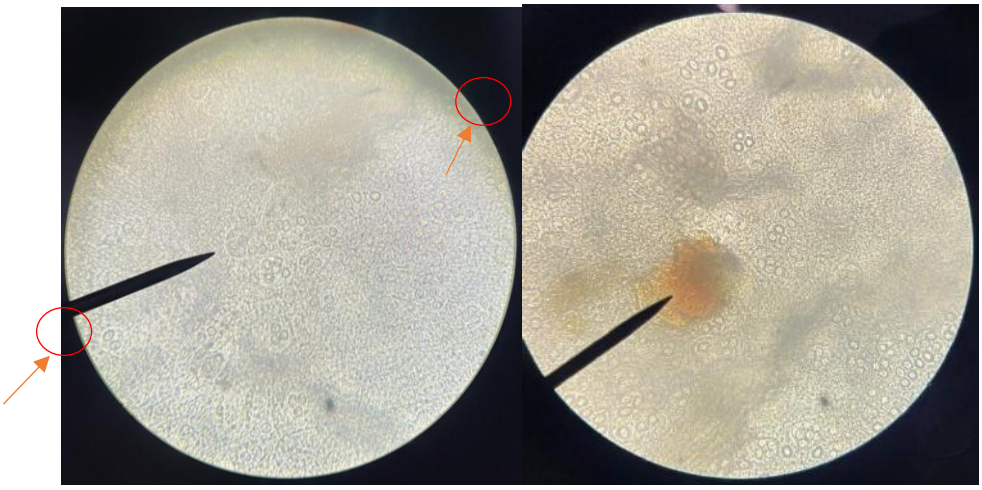


Fig.IV: Nematopsis is a gregarine genus Apicomplexan in the family Porosporidae

Source: personal file

IV. DISCUSSION

The samples analyzed are above the standards set by current legislation, which allows up to 7 CFU/g in one sample. According to the most current RDC 331 of 2019, it is mandatory to record the presence of E. coli, through an analytical report.

According to the PNCMB(National Program. Hygienic-Sanitary Control of Bivalve Molluscs), the Interministerial Normative Instruction No. 7, determines that thermotolerant coliforms are indicators of food contamination at room temperature, in addition to indicating in the temperature control during transport and storage processes (Oliveira, 2020).

Microbiological species included parasites and bacteria: Nematopsia and *E.coli*, respectively. As reported by Boehs 2012, parasites of the genus Apicomplexan and bacterial contaminants in culture water are commonly found in mussels. The impacts of the presence of these contaminants can be significant to the sustainability of aquaculture production (SHINN, et al., 2015).

The presence of total and thermotolerant coliforms in the samples may be related to several factors, such as hygienic conditions, sanitary quality of the water from which the shellfish are taken, handling and conservation of the product (Nascimento, 2011).

Regarding microbiological diversity, many aquatic animals, when presenting parasitic infections, present bacterial infections. Bacteria in the liver have a symbiotic relationship with external and internal parasites, among the functions; nutritional role, immunomodulatory property and contribution to pathogenesis (Rausch et al., 2013)

V. CONCLUSION

In view of the results found, it is suggested visibility in the Brazilian legislation on the criteria for evaluation of the quality of bivalves according to the PNCMB allied to ANVISA, regulatory bodies of sanitary production and evaluation at public health level, of animal products. It is recommended that this evaluation be based on analyses of the bacteriological contamination of these bivalves, both for exploitation and marketing and for consumption.

Since animals filtering biological particles from the water are possible, with bacteria and contaminants of superior quality to a microbiological quality.

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