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Microbiological quality of trahira fish (*Hoplias malabaricus*) from Baixada Maranhense, municipality of São Bento, MA

*Qualidade microbiológica de peixe traíra (***Hoplias malabaricus***) proveniente da Baixada Maranhense, município de São Bento, MA*

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ABSTRACT: Fish are considered rich sources of nutrients. Health care throughout its production chain aims to ensure quality, minimizing the risks of transmission of foodborne diseases. In order to evaluate the microbiological quality of trahira fish (*H. malabaricus*), 40 samples were analysed for Most Probable Number (MPN) of coliforms at 45ºC, counts of aerobic mesophilic bacteria and *Staphylococcus* spp., identification of *E. coli, Salmonella* spp. and *Aeromonas* spp.. Analyses were conducted according to official methods, procedures, and recommendations. Microbiological results showed coliform values at 45 °C ranging from <3 to > 1.1 × 10^3 MPN/g, presence of *E. coli* in 14 (35%) samples, counts of mesophilic aerobic bacteria from 9 × 10^2 to 10^9 CFU/g and absence of coagulase positive *Staphylococcus aureus*. *Salmonella* spp. was detected in 2 (5%) samples, which is in disagreement with the standards required by the RDC Nº 12 of January, 2001 (ANVISA) regarding *Aeromonas* spp. In total, 36 (90%) samples were contaminated, 7 (19.4%) by *A. cavie* and 29 (80.6%) by *A. hidrophila*. The results of this research showed unsatisfactory hygienic and sanitary conditions of fish from the municipality of São Bento (MA), exposing consumers to the risk of foodborne diseases.

KEYWORDS: *Aeromonas* spp.; *E. coli*; *Salmonella* spp.; hygiene; sanity.

RESUMO: Os peixes são considerados fontes ricas de nutrientes. Cuidados sanitários durante toda a sua cadeia produtiva visam garantir a qualidade, minimizando os riscos de transmissão de doenças alimentares. Com objetivo de avaliar a qualidade microbiológica de peixes traíra (*H. malabaricus*), foram analisadas 40 amostras quanto à determinação do Número Mais Provável (NPM) de coliformes a 45 ºC, contagem de bactérias aeróbias mesófilas e *Staphylococcus* spp. e identificação de *E. coli*; *Salmonella* spp. e *Aeromonas* spp. As análises foram processadas conforme métodos, procedimentos e recomendações oficiais. Os resultados microbiológicos mostraram valores de coliformes a 45 ºC variando de < 3 a > 1,1 × 10^3 NMP/g, presença de *E. coli* em 14 (35%) amostras, contagens de bactérias aeróbias mesófilas de 9 × 10^2 a 10^9 UFC/g e ausência de *Staphylococcus aureus* coagulase positivo. Detectou-se *Salmonella* spp. em 2 (5%) amostras, portanto, em desacordo com os padrões exigidos pela RDC Nº 12 de janeiro de 2001 (ANVISA) e para *Aeromonas* spp. No total, 36 (90%) amostras estavam contaminadas, sendo 7 (19,4%) por *A. cavie* e 29 (80,6%) por *A. hidrophila.* Os resultados da pesquisa demonstram que os peixes provenientes do município de São Bento (MA) apresentaram condições higiênico-sanitárias insatisfatórias, expondo os consumidores a risco de doenças veiculadas por alimentos.

PALAVRAS-CHAVE: *Aeromonas* spp.; *E. coli*; *Salmonella* spp.; higiene; sanidade.

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INTRODUCTION

Among the native species from the wetlands of Baixada Maranhense, *Hoplias malabaricus,* Bloch, 1794, stands out; popularly known as "trahira", this is a neotropical fish belonging to the *Erythrinidae* family, which is world-wide distributed (FOWLER, 1950; NELSON, 1994). For human nutrition, fish is a source of proteins of high biological value; however, being a highly perishable food, it requires much care in handling all the way: from the capture process and storage on fishing boats to commercialization (FAO, 2012; LEITÃO et al., 1997).

Fish also play an important role in transmission of foodborne diseases. Therefore, for the control and prevention of these diseases, indicators of environmental and fecal contamination, through enumeration of coliforms and research of pathogens such as *Salmonella* spp. and *Staphylococcus aureus*, are of great importance for public health, and such investigation is essential to determine the quality of the product (LIMA; REIS, 2002).

The presence of these microorganisms in the raw material may be related to their environment of development, contamination during landing, processing or storage, which contributes to a poor-quality product (DIAS et al., 2010). Contamination may also occur in improper handling and processing, or by the use of contaminated equipment and utensils, also important factors related to presence of bacteria in fish. The most common types are *Aeromonas, Salmonella* spp., *Escherichia coli, S. aureus, Bacillus cereus, Clostridium perfringens* and *Clostridium botulinum* (MURATORI, 2000; VIEIRA, 2004).

Other pathogenic microorganisms found in fish are bacteria of the genus *Aeromonas,* which can be found in soil, fresh and salt water, chlorinated water, and animal feces (SOUZA, 2003). These bacteria carry several virulence factors, which justify their status of threat as a human pathogen, since they are responsible for diarrhea, extra-intestinal infections, and, in immunocompromised patients, septicemia, meningitis, and death (YAMADA et al., 1997). Gastroenteritis is the most common form of human infection caused by *Aeromonas*. In fish, the disease can show a variety of clinical signs, such as exophthalmos, fin erosion and hemorrhagic septicemia, leading to death. Aeromonas are associated with opportunistic infections in humans, as well as in homeothermic animals and in fish, and may be resistant to multiple drugs (JANDA; ABBOTT, 1998; PALU et al., 2006).

The region of Baixada Maranhense is an complex ecosystem in which human beings play an important role for management, use, and conservation of several of its components. As a consequence of abundant regional water resources, fishing is possibly the most important socioeconomic activity (ARAÚJO; PINHEIRO, 2008). Considering the economic importance of the artisanal fishing activity in the region, the great availability of fish resulting from floodwaters in the region and the availability of the product for sale in local markets, this research aimed to evaluate the hygienic sanitary conditions and microbiological aspects of trahira fish (*H.malabaricus*) found in the municipality of São Bento (MA).

MATERIAL AND METHODS

Fish were slaughtered and not eviscerated, acquired in fairs and markets of the municipality of São Bento (MA), between July and December, 2011. Four samples of trahira (*H. malabaricus*) were collected, each sample consisting of three units (average weight of 250 g per piece) from the same supplier. The samples were separated into sterile polyethylene bags, identified, and packaged in isothermal boxes, then transported to the Laboratory of Microbiology of Food and Water of the Veterinary Medicine Course at Universidade Estadual do Maranhão (UEMA), where they were analyzed according to the American Public Health Association (APHA, 2001), (VANDERZANT; SPLITTSTOESSER, 1992; SILVA et al., 2001).

Dilution of samples

For the dilution of samples, 25g of product were aseptically weighed and then added to bottles containing 225 mL of 0.1% peptone water, constituting the dilution 10^{-1}. Then, 1mL of the first dilution was pipetted into tubes containing 9mL of the same diluent, obtaining successive dilutions up to 10^{-6}.

Determination of Most Probable Number of Coliforms at 45 ºC per gram (MPN/g)

Initially, 1mL of the previously prepared dilutions was withdrawn and transferred to three tubes containing 10 mL of Tryptose Lauryl Sulfate Broth (TLS), which were incubated at 35 ºC/48 h. Aliquots were withdrawn from the gas-formed tubes, which were then inoculated into tubes containing Brilliant Green-bile 2% broth (BG). BG-positive cultures were transferred to tubes with *Escherichia coli* Broth (EC) and incubated at 44,5–45 ºC/24 h for detection of coliforms at 45 ºC. The results were interpreted using the table of Most Probable Number (MPN). Positive cultures in EC medium were seeded in Eosin-Methylene Blue Agar (EMB) at 35–37 ºC/24 h. Colonies with blackened centers and metallic green sheen were submitted to biochemical tests (Indol production, voges-proskaer test, red methyl test and citrate test) for identification of *E. coli*, abiding by the American Public Health Association (APHA) standards, as described by SILVA et al. (2001).

Standard Count on Mesophilic Aerobic Bacteria Plate

Aliquots of 1 mL of each dilution were pipetted into sterile Petri plates containing Standard Agar for Counting (SAC) incubated at 35–37 ºC/48 h. For counting, plates of the same dilution presenting 30 to 300 colonies were considered; the result was expressed in colony-forming units (CFU)/mL (SILVA et al., 2001).

Staphylococcus spp. count

From the initial up to 10^{-3} dilutions, 0,1 mL were inoculated onto Baird-Parker Agar plates, incubated at 35–37 ºC for 45–48 h. Soon after, the typical colonies were counted and confirmed by the coagulase test. The results were expressed in CFU/g (SILVA et al., 2001).

Research of *Salmonella* spp.

25 g of the sample unit were weighed, homogenized in 225 mL of peptone water and incubated at 37 ± 1ºC / 18 ± 2 h. Subsequently, 0.1 mL of the sample was transferred to 10 mL of Rappaport-Vassilidis Soy Broth (RVS) and 1mL to 10 mL of Cystine Selenite Broth. Incubation occurred at 37 ºC/24 h. From each culture, Xylose Lysine Deoxycholate Agar (XLD), Hectoen Enteric Agar (HE) and Brilliant Green-bile (BG) broths were prepared. The XLD, HE and BG plates were incubated upside down at 37 ± 1ºC/24 ± 3 h. After incubation, colonies typical of *Salmonella* spp. were seen, which were later inoculated in Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA) for biochemical proof. Confirmation was made by serological tests, using polyvalent anti-*Salmonella* sp. antigen, according to ICMSF (1988).

Identification of *Aeromonas* spp.

For pre-enrichment, Tripticase Soy Broth (TSB) and ampicillin (30 mg/L) were used. Aliquots of the cultures grown in TSB were stretched onto plates containing Phenol Red Agar amide-ampicillin (PALUMBO et al. 1985; MAJEED et al. 1990) and Dextrin-ampicillin Agar, as suggested by HAVELAAR; VONK (1988), added of ampicillin (10 mg/L) and incubated at 28 ºC for 24 h. Then, colonies were seeded in Trypticase Soy Agar (TSA) and incubated at 28 ºC for 24 h. Gram staining and replication were performed on Triple Sugar Iron Agar (TSI) (SAAD et al., 1995). Positive cultures were submitted to oxidase tests, catalase test and resistance to O/129 vibriostatic agent (2,4-diamino-6,7-diisopropylpteridine phosphate). Strains characterized as belonging to the genus *Aeromonas* were seeded in TSA so the colonies could be kept viable for subsequent analysis by biochemical methods, according to Aerokey II classification key (CARNAHAN et al., 1991).

RESULTS

All 40 samples of trahira analyzed had coliform values at 45 ºC, ranging from < 3 to > 1.1 × 10^3 MPN/g. Regarding *E. coli* identification, 14 (36.9%) samples were contaminated by this pathogen, which is concerning from the sanitary point of view. In 2 (5%) samples *Salmonella* spp. was detected, which is in disagreement with standards established by RDC Nº 12, January 200, Agência Nacional de Vigilância Sanitária (ANVISA). Aerobic mesophilic microorganisms counting ranged from 9 × 10^2 to 10^9 CFU/g (Table 1).

Staphylococcus spp. was present in 18 (45%) samples, with population ranging from 10^2 to 1.8 × 10^8 CFU/g, being all negative upon coagulase test (Table 2).

Research of *Aeromonas* spp showed 36 (90%) samples of trahiras contaminated: 7 (19.4%) by *Aeromonas cavie* and 29 (80.6%) by *Aeromonas hydrophilic* (Table 3).

Table 2. Number and percentage of trahira samples (*H. malabaricus*) contaminated by *Staphylococcus* spp. and coagulase test, São Bento, MA.

(CFU/g)	*Staphylococcus* spp.		
	n	%	Coagulase Test
Absence	22	55.0	-
10^2–10^3	4	10.0	Negative
10^4–10^5	8	20.0	Negative
10^6–10^7	4	10.0	Negative
1.8 × 10^8	2	5.0	Negative
Total	40	100	-

CFU/g: colony-forming units per gram.

Table 3. Number and percentage of trahira samples (*H. malabaricus*) contaminated by *A. cavie* and *A. hidrofila*, São Bento, MA.

Species	*Aeromonas* spp.	
	n	%
cavie	7	19.4
hidrofila	29	80.6
Total	36	100

Table 1. Number and percentage of trahira (*H. malabaricus*) samples contaminated by coliforms at 45ºC, *E. coli*, *Salmonella* spp. and aerobic mesophiles. São Bento, MA.

MPN/g	Coliforms at 45ºC		*E. coli*		*Salmonella* spp.		Aerobic mesophiles		
	n	%	n	%	n	%	CFU/g	n	%
< 3	5	12.5	-	-	-	-	9 ×10^2	1	2.5
3.6 to 9.3 × 10^1	15	37.5	5	12.5	-	-	10^3–10^4	7	17.5
1.5 to 4.6 × 10^2	10	25.0	3	7.5	-	-	10^5–10^6	17	42.5
10 × 10^2	-	-	-	-	-	-	10^7–10^8	10	25.0
> 1.1 × 10^3	10	25.0	6	15.0	2	5.0	10^9	5	12.5
Total	40	100	14	36.9	2	5.0	-	40	100

MPN/g: most probable number per gram; CFU/g: colony-forming units per gram.

DISCUSSION

Isolation of pathogens and/or indicator organisms contributes to evaluate the quality and safety of food, allowing sanitary control. The RD Nº 12 of January, 2001, ANVISA, determines absence of *Salmonella* spp. in 25 g and values of up to 10^2 MPN/g for coliforms at 45ºC as the standard for fish, the latter only for raw consume (BRASIL, 2001). The high density of coliforms at 45 ºC and the presence of *E. coli* in 14 trahira samples (35%) reflect poor hygiene conditions and displays the degree of contamination this food is exposed to. This contributes to food deterioration and represents a risk to consumer's health, since five *E. coli* groups can lead to gastroenteritis in humans (enterotoxigenic *E. coli*, enteropathogenic *E. coli*, enterohemorrhagic *E. coli*, enteroinvasive *E. coli*, and enteroaggregative *E. coli*) (VILA et al., 2009). The results obtained from these indicators can be attributed to fishing activities in polluted areas, exposure to unsanitary conditions such as sources of sewage, increasing the risk of contamination and interfering in the quality of products commercialized. ALVES et al. (2001) consider that the high density may indicate contamination, inefficient heat treatment or multiplication during storage.

Similar results were observed by LORENZON et al. (2010) in a research involving fish culture, in which coliforms were found at 45 ºC, ranging from < 3 to 5.1×10^3 MPN/g. An experiment carried out in Egypt, with investigation of pathogenic and potentially pathogenic organisms in tilapia units from the Nile detected *E. coli* in 7.9% of the samples (YOUSSEF et al., 1992). FERREIRA et al. (2014) found inferior results (14%) by researching *E. coli* in sawfish and considered ice as the source of cross-contamination.

Salmonella spp. was detected in 2 (5%) samples, characterizing the product as improper for consumption and in disagreement with the current legislation, which request absence of these microorganisms in 25 g. Contamination by *Salmonella* spp. in fishery products may result from the contamination of the environment where the animals were reared or the lack of hygienic habits in handling and processing them (SANATH KUMAR et al., 2003). Several authors consider the presence of *Salmonella* in the gastrointestinal tract of fish a concern, as they can contaminate meat during its handling. In Brazil, a study carried out in Botucatu, SP, analyzed intestinal contents of 77 freshwater fish from farmers and isolated different microorganisms from the group of enterobacteria, including *Salmonella thyphimurium* (2.28%), (LANGONI, 1999). According to MARTINS (2006), cooking eliminates the risk of this pathogen, but in some cases, it takes very few *Salmonella*-infected cells (from 1 to 10 cells for some serotypes) to cause symptoms in humans (LINDER et al., 2011).

For mesophilic aerobic bacteria counting, the legislation does not determine a standard, however, excessive count contributes to a higher deterioration of the product, besides paving the way to pathogenic bacteria. In this research, the high counts did not determine macroscopic changes of the fish, since most animals had been freshly caught and showed characteristics consistent with the state of freshness. As stated by FURLAN et al. (2007), deteriorating microorganisms are responsible for reduced shelf life of the fish because of their proteolytic, pectinolytic, lipolytic capacity. A way to minimize high counts is the rapid cooling of the fish, since the mesophilic microbiota multiplies rapidly at room temperature. AGNESE et al. (2001) Report that, values of mesophilic microorganisms higher than 10^6 CFU/g in fish are considered critical to the freshness level. However, LIRA et al. (2001) observed that some fish presenting more than 106 CFU/g had not alteration in its characteristics, whereas others with lower number were disqualified upon sensorial analysis, similar to what happened in the present study.

Coagulase-positive *Staphylococci* were not detected in analyses, therefore, the samples met the standard required by the current legislation for these microorganisms. Nevertheless, coagulase-negative *Staphylococci* were seen in 18 (45%) samples, with counts ranging from 10^2 to 1.8×10^8 CFU/g (Table 2). High values of coagulase-negative *Staphylococci* can be attributed to inadequate manipulation by fishermen and sellers bearing the bacterium on their mucosae and skin surface. Emphasis is placed on the implementation of good practices, guiding the seller regarding personal hygiene, thus improving product quality.

SANTOS (2003) described the toxicity of 63.2% of *Staphylococcus epidermidis* strains and 84.6% of *Staphylococcus cohnii* strains isolated from food handlers involved in outbreaks of food poisoning. Current legislation does not establish a standard for coagulase negative *Staphylococcus*, although these bacteria produce enterotoxins responsible for food poisoning. LAMAITA (2003) emphasizes that legal standards for food specify the presence of coagulase positive species only and reinforces the need of revision in the existing legislation of food hygiene by official services, including standards for coagulase-negative *Staphylococci*.

Aeromonas was found in 36 (90%) samples, of which 7 (19.4%) were identified as *A. cavie* and 29 (80.6%) as *A. hydrophilic*. The genus *Aeromonas* is autochthonous of the aquatic environment and the mobile and mesophilic species are found worldwide in fresh and brackish waters, both typical of the region under study. Besides being an important deterioration factor in fish, contamination by such species poses risk to the consumers' health, since these are recognized as pathogenic to humans due to the production of cytotoxins, enterotoxins, and hemolysins, besides causing skin infections, mainly in immunocompromised patients. (GONZÁLEZ-SERRANO et al., 2002).

Among the species of *Aeromonas* spp., *A. caviae* and *A. veronii* biovar sobria are more commonly associated with infections in humans and are responsible for more than 85% of all clinical cases related to this genus (AL-BENWAN et

al., 2007; TSAI et al., 2009). In Brazil, there are reports of isolation of different species of *Aeromonas* spp. in clinical samples from cases of children diarrhea, hospital infection or gastroenteritis after fish, meat, and vegetables intake (MARQUES, 2011). *A. hydrophila* was reported as the agent causing diarrhea and pneumonia and further evolution to sepsis in a child exposed to contaminated water (RODRÍGUEZ et al., 2005). These data reinforce the need to implement new hygienic-sanitary measures for people directly dealing with food in order to reduce this problem.

SILVA (2010) evaluated the presence of *Aeromonas* in tambaqui (*Colossoma macropomum)*, tambacu (*C. macropomum* x *Piaractus mesopotamicus*), tilapia (*Oreochromis niloticus*) and curimatá (*Prochilodus lineatus*) units from fish farms in the Baixada Maranhense region, and found out that these properties were an important natural reservoir of *Aeromonas* at the time of investigation, also posing risk to animals raised in the surroundings. The isolates of *A. hydrophila* found in this research were obtained from fish without clinical signs, which suggests that these specimens were carriers of these bacteria, and that intaking contaminated products could lead to gastroenteritis.

The results of *A. hydrophila* and *A. cavie* research corroborate the findings by RALL et al. (1998), who found *Aeromonas* contamination in 48% of the samples of pintado (*Pseudoplatystoma* sp.) collected in supermarkets of the city of São Paulo, *A. cavie* being the most frequent (60%), followed by *A. hydrophila* (50%) and *A. sobria* (12%), emphasizing the relevance of these species in fish.

Fish units evaluated in this work were commercialized in fairs often under poor hygienic conditions, which represents a potential risk to consumers in case of contamination.

CONCLUSION

Trahiras from the municipality of São Bento (MA) have unsatisfactory hygienic-sanitary conditions and can carry *Salmonella* spp. and species of *Aeromonas* spp. for the consumers. A revision of RDC Nº 12, January 2001, ANVISA is necessary for the inclusion of parameters for coagulase-negative *Staphylococci*, since these may produce toxins that cause damage to human health.

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