J. Health Biol Sci. 2016; 4(3):193-197 doi:10.12662/2317-3076jhbs.v4i3.767.p193-197.2016

ORIGINAL ARTICLE

Characterization of the microbiota of the skin and oral cavity of *Oreochromis niloticus*

Caracterização da microbiota da pele e cavidade oral de *Oreochromis niloticus*

Edmar Maciel Lima Júnior[1], Tereza de Jesus Pinheiro Gomes Bandeira[2], Marcelo José Borges de Miranda[3] , Guilherme Emilio Ferreira[4], Ezequiel Aguiar Parente[4], Nelson Sarto Piccolo[5], Manoel Odorico de Moraes Filho[6]

1. Discente do Programa de Pós-graduação em Farmacologia da Universidade Federal do Ceará (UFC), Fortaleza, Ceará, Brasil. 2. Docente do Curso de Medicina do Centro Universitário Christus (UNICHRISTUS), Fortaleza, Ceará, Brasil. 3. Coordenador do Hospital São Marcos, Recife, Pernambuco, Brasil. 4. Discente do Curso de Medicina do Centro Universitário Christus (UNICHRISTUS), Fortaleza, Ceará, Brasil. 5. Chefe do Serviço de Cirurgia Plástica de Goiânia, Goiás, Brasil. 6. Docente do Programa de Pós-graduação em Farmacologia da Universidade Federal do Ceará (UFC), Fortaleza, Ceará, Brasil.

Abstract

Introduction: Fish are usually exposed to higher microbial loads than land or air animals. The microbiota of fish mostly consists of *Pseudomonas* spp., *Aeromonas* spp., Shewanella *putrefasciens*, *Acinetobacter* spp. and *Moraxella* spp. **Objective:** to analyze the oral cavity, and skin tissue microbiota on the Nile tilapia (*Oreochromis niloticus*), a fish species raised commercially in Brazil. **Methoda**: Samples were collected from the oral cavity and skin of 20 Nile tilapia specimens (*Oreochromis niloticus*), each weighing approximately 1,000 grams. The samples were cultures for quantitative analysis on sheep blood agar (SBA) and chromID™ CPS® agar (CPS). **Results**: Eleven different bacterial species were identified on CPS and SBA plates. Gram-negative species were the most prevalent, while gram-positive *Globicatella* spp, *Streptococcus* spp and *Enterococcus faecalis* were also found. *Pseudomonas aeruginosa* species were isolated from all samples. Gram-positive *Enterococcus faecalis* was found in 70 and 60% of the skin and oral samples, respectively. **Conclusion**: For all samples studied, the microbial load was less than 100,000 colony-forming units - CFU/g of tissue. This value is a cutoff standardized for the American Society of Microbiology to differentiate the causal agent from the colonizers. In light of this result and considering the absence of infectious signs in the fish samples, we conclude that the CFU values found in this study reflect a normal, non-infectious colonization/microbiota.

Keyworda: Microbiota. Nile tilapia. *Oreochromis niloticus*. Fish Farming in Brazil.

Reaumo

Introdução: Os peixes são normalmente expostos a cargas microbianas mais elevadas do que os animais em terra ou ar. O perfil da microbiota em peixes compreende principalmente *Pseudomonas* spp., *Aeromonas* spp., Shewanella *putrefasciens*, *Acinetobacter* spp., e *Moraxella* spp. **Objetivo:** analisar a microbiota da cavidade oral e do tecido da pele no Tilápia do Nilo (*Oreochromis niloticus*), comercialmente criado no Brasil. Métodos: Vinte espécimes de Tilápia do Nilo (*Oreochromis niloticus*), cada uma pesando cerca de 1.000 gramas, foram submetidas a coleta de amostras da cavidade oral e da pele. Estas amostras foram cultivadas quantitativamente em ágar sangue de carneiro (SBA) e chromID® CPS® agar (CPS). Resultados: Foram identificadas 11 diferentes espécies de bactérias em placas CPS e SBA. Os resultados mostram que bacilos gram-negativos são os mais prevalentes. Cocos gram-positivos como *Globicatella* spp, *Streptococcus* spp e *Enterococcus faecalis* também foram encontrados. Espécies de *Pseudomonas aeruginosa* foram isoladas a partir de todas as amostras. *Enterococcus faecalis* foi encontrado em 70 e 60% das amostras de pele e por via oral, respectivamente. Conclusão: Os resultados deste estudo mostram, para todas as amostras estudadas, uma carga de CFU de menos de 100.000 unidades formadoras de colonias - UFC / g de tecido. Este valor é um cutoff padronizado pela Sociedade Americana de Microbiologia, a fim de diferenciar o agente causal dos colonizadores. Diante destes resultados e considerando a ausência de sinais infecciosos nas amostras de peixes, conclui-se que os valores CFU's encontrados neste estudo consistem em colonização/microbiota.

Palavras-chave: Microbiota. Tilápia do Nilo. *Oreochromis niloticus*. Cultivo de peixe no Brasil.

INTRODUCTION

O Fish farming originated 2,500 years ago in China and it was introduced in Brazil in the 1930s. The country has several favorable conditions for large-scale fish production, such as large territorial area, tropical climate, and extensive river basins[1,2].

Fish farming in Brazil has been increasing in recent years. In 2010, the country was responsible for 82.3% of farmed fish production in South America. Commercial farming of Nile tilapia (*Oreochromis niloticus*) increased 105% between 2003 and 2009, and it is currently considered the species with the largest commercial production in Brazil[3].

According to previously published data the microbiota in fish farming is mainly composed of gram-negative bacteria[4,5,6]. They also evidence that microbiota diversity in different Nile tilapia farming systems, there have been found species from the following bacterial families: Enterobacteriaceae (45 samples), Micrococcaceae (87), Pseudomonadaceae (65) and *Vibrio*naceae (115)[7,8].

The significant increase in activities related to the cultivation of fish observed in Brazil, especially those hosts with potential for cultivation and marketing, has considerably enhanced the relevance of the studies carried out in order to

Correaponding Author: Tereza de Jesus Pinheiro Gomes Bandeira. Rua João Adolfo Gurgel, 133, Cocó. CEP: 60.190-060. Fortaleza,CE-Brazil. Email: bandtereza@gmail.com

Conflict of interest: The authors declare no conflict of interest.
Received: 2016 Jun 21; Revised: 2016 Jul 27, 2016 Ago 30; Accepted: 2016 Set 1

determine contamination of these animals with pathogenic microorganisms[9]. Thus, in intensive fish production systems, health is one of the most important aspects for the commercial breeding of any kind[1].

The success of fish farming enterprises depends on many factors, and one of the most important concerns is the health condition of the animals being cultivated[4].

In addition, the skin of Nile tilapia (*Oreochromis niloticus*) is being studied by researchers of our working group with the aim of using them as an occlusive biological graft in the treatment of burn rats (*Rattus norvegicus*) Wistar, with the intention of use in patients with large areas of burns (unpublished data).

Thus, monitoring fish health is essential to ensure the production of healthy animals and prevent loss in activity[10,11]. Consequently, the objective of this study was to analyze the oral cavity, skin and subcutaneous tissue microbiota of Nile tilapia (*Oreochromis niloticus*) commercially raised in net pens in Castanhão Reservoir, located in Jaguaribe, Ceara state, Brazil.

MATERIAL AND METHODS

This study was conducted by the Research Unit for Drug Development of Federal University of Ceará, at the Clinical Pathology Department of LabPasteur. The study was approved by the Ethics Committee on Animal Studies of Federal University of Ceará, under number 48/2016.

Twenty 10-month old Nile tilapia (*Oreochromis niloticus*) weighing approximately 1,000 grams were obtained from net pens kept in Castanhão Reservoir. These fish were raised at a density of 23 fish/2 m^3.

These specimens were retrieved from net pens and immediately placed individually in sterile plastic bags, which were sealed and immersed in ice at 0 °C. Upon arrival at the lab, they were placed on stainless steel tables with sinks, which had been previously disinfected with 70% ethanol.

From each tilapia, two 1.5 x 1.5 cm skin fragments were obtained, which weighed approximately 0.19 g. These fragments were seeded by imprinting both sides on sheep blood agar (SBA) and CPS agar (bioMérieux). Another skin fragment was transferred to an empty sterile Petri dish, to which 1 mL of sterile saline solution was added. Using a scalpel, this fragment was cut into several pieces and mixed with the saline solution until obtaining a dense suspension. An aliquot of 100 µL of this suspension was spread on SBA and CPS agar with microbiological loops, for further counting of colony-forming units (CFU). The remaining material was then transferred to a test tube with 3 mL of brain-heart-infusion (BHI) medium for further analysis. These plates and tubes were then incubated at 35 ºC (+/- 1 °C) for 24 hours.

In order to calculate the total number of organisms, we used the colony count times the dilution factor (homogenate dilution plus plates' subsequent dilution) divided by the weight of tissue. The CFU counts were analyzed according to Isemberg and Garcia (2010) in relation to the reference values of quantitative cultures of wound tissues. This parameter determines that for significant clinical isolates (pathogenic organisms), the CFU values should be more than 105 CFU/g of tissue. Hence, CFU counts below 100,000 CFU/g of tissue were considered suggestive of bacterial colonization[12,13].

Oral mucus was sampled from 20 fish randomly chosen from the tank population. Mucus was collected from the surface of the oral cavity region using sterile swabs, transferred to a tube containing saline and immediately spread plated. The swab was firmly rolled over the agar plate surface and was carefully streaked in the plate center and spread on the four quadrants using a platinum loop. When the swab samples were collected, the result was semi-quantitative, i.e., CFU/swab.

Colonies with morphologically distinct characteristics on SBA and CPS agar were selected and re-isolated. Pure colonies were then obtained from selective culture media and then submitted to identification through Gram staining and the automated VITEK® 2 (bioMérieux) method[14].

Statistical Analysis: The results obtained from the study were subjected to the Anova statistical analysis of variance (Table 1).

Table 1. Microorganism isolation proportion in 20 oral and 20 skin samples from Nile tilapia (Oreochromisniloticus), collected from fish arm net pens in Castanhão Reservoir, Fortaleza, Ceará, Brazil, in November 2015.

Microorganisms	Skin Samples(20) n (%)	Oral Cavity Samples (20) n (%)
Pseudomonas aeruginosa	20 (100)	20 (100)
Aeromonas sóbria	17 (85)	16 (80)
Klebsiella pneumonie	14 (85)	10 (50)
Enterococcus faecalis	14 (70)	12 (60)
Aeromonas hydrophila	8 (40)	9 (45)
Proteus mirabilis	6 (30)	10 (50)
Globicatella sanguinis	6 (30)	4 (20)
Aeromonas veronii	1 (5)	1 (5)
Streptococcus uberis	1 (5)	2 (10)
Candida parapsilosis	1 (5)	1 (5)
Streptococcus suis	1 (5)	1 (5)

n – number of positive samples

p-value < 0,001 - Nested Anova among species of both samples

RESULTS

The isolates obtained from the SBA and CPS plates are listed in Tables 1, 2 and 3. All 20 samples yielded bacterial growth after 48 hours of incubation. Eleven different bacterial species were identified on CPS and SBA plates. Gram-negative species were the most prevalent. *Pseudomonas aeruginosa* was isolated from all samples studied, i.e., prevalence of 100%, followed by *Aeromonas sobria* and *Klebsiella pneumoniae* with 17% and

14% respectively. The positive cocci isolates were represented, in descending order of prevalence, by *Enterococcus faecalis* (70%), *Globicatela sanguinis* (30%), *Streptococcus uberis* (5%) and *Streptococcus suis* (5%).

With respect to gram-positive bacteria, *Enterococcus faecalis* occurred in 70 and 60% of skin and oral cavity samples, respectively, and was in third position of prevalence in decreasing order.

Table 2 reports the CFU/gram values of tissue samples and Table 3 shows the semi-quantitative results of oral cavity samples reported as CFU/swab. *Pseudomonas aeruginosa* species showed the highest numbers of CFU/gram of tissue (31.85) and grew in 100% of the skin samples, followed by *Aeromonas sobria* and *Klebsiella pneumoniae* in decreasing order in both groups of samples.

Quantitatively, as shown in Tables 2 and 3, *P. aeruginosa*, besides being isolated in all samples, also yielded the highest number of CFU/gram of tissue, with an average of 31.85 CFU/gram of tissue in skin samples.

Comparing the skin samples with the oral cavity samples, we did not observe statistically significant differences, since $p < 0.05$ for each isolate.

Yeasts of the *Candida parapsilosis* species complex were the only species of fungi isolated in both groups of samples, with on 5% prevalence (Table 1).

Table 2. Colony-forming unit counts obtained from skin samples of 20 Nile tilapia (*Oreochromis niloticus*) collected from net pens in Castanhão Reservoir, Fortaleza, Ceara, Brazil.

Isolates	CFU/sample																				CFU/g tissue[2]
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	AVG[1]	
P. aeruginosa	50	58	30	18	15	21	45	22	36	28	45	31	35	37	27	48	45	12	15	19	31.85	16.76
A. sóbria	0	0	15	15	12	10	6	5	16	23	8	0	12	0	5	20	8	18	14	0	9.35	4.92
K. pneumoniae	10	0	0	0	0	12	19	12	14	11	13	0	12	13	15	13	0	11	12	0	8.35	4.39
P. mirabilis	4	6	4	4	1	0	11	0	8	5	0	0	10	4	11	9	8	0	6	0	4.55	2.39
A. hydrophila	10	12	0	0	0	0	0	20	6	0	0	6	0	19	0	0	0	0	8	8	4.45	2.34
E. faecalis	0	0	0	0	0	0	3	6	4	5	6	5	4	3	2	2	6	6	6	4	3.1	1.63
G. sanguinis	2	2	3	3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.65	0.34
A. veronii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0.4	0.21
S. uberis	0	0	0	0	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0.25	0.13
C. parapsilosis	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.1	0.05
S. suis	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0.05	0.03

CFU: Colony Forming Unit.
[1]Average.
[2]Results derived from the average of CFU/sample.

Table 3. Colony-forming unit counts obtained from oral swabs of 20 Nile tilapia (*Oreochromis niloticus*) collected from net pens in Castanhão Reservoir, Fortaleza, Ceara, Brazil.

Isolates	CFUs /swab																				AVG[1] of CFU/swab
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15	A16	A17	A18	A19	A20	
P. aeruginosa	80	68	61	74	36	36	27	36	36	25	27	22	35	27	29	32	18	34	27	29	37.95
A. sóbria	0	0	8	8	17	11	8	5	11	10	8	0	14	21	8	18	8	9	12	0	8.80
K. penumoniae	3	1	5	5	4	6	10	0	8	6	17	2	15	15	2	9	2	16	15	9	7.50
P. mirailis	0	0	0	0	0	0	15	0	9	4	6	0	0	0	11	12	6	10	0	0	3.65
E. faecalis	0	0	0	0	0	0	0	5	2	7	6	2	8	2	6	4	6	6	6	0	3.00
A. hydrophila	2	16	0	0	0	0	0	11	5	0	0	8	0	0	0	0	0	0	0	12	2.70
G. sanguinis	2	3	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.40
A. veronii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0.40
S. uberis	0	0	0	0	0	0	3	0	0	0	0	0	3	0	0	0	0	0	0	0	0.30
C. parapsilosis	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.10
S. suis	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0.10

CFU: Colony Forming Unit.
[1]Average

DISCUSSION

According to Tiamiyu et al. (2015), the density of bacteria in fish bodies may also be due to contamination during handling and storage. The predominant bacteria isolated from fish skin and stomach samples in their study were strains of *Staphylococcus aureus*, *Escherichia coli*, *Proteus* species, *Bacillus* species, *Klebsiella* species, *Micrococcus* species, *Serratia* species, *Pseudomonas* species, *Salmonella* species, and *Streptococcus* spp[15].

Corroborating the findings of Tiamiyu et al. (2015), our results show that gram-negative bacteria were more prevalent than gram-positive ones, and the most frequent gram-negative species was *P. aeruginosa*. Species of the genus *Aeromonas* were the second most frequent, with *Aeromonas sobria*, *Aeromonas hydrophila* and *Aeromonas veronii* being isolated, representing 17, 8 and 1% of skin samples, and 16, 9 and 1% of oral samples, respectively[15].

This is also similar to what Rodrigues (2007) reported, using different selective culture media for Nile tilapia samples of freshwater fish microbiota in specimens collected in a global study. The microbiota contained a higher frequency of gram-negative species, and was in most cases composed of four genera. In 420 plates, she observed the following species: *Aeromonas* spp., *Acinetobacter* spp., *Micrococcus* spp. (gram-positive), *Pseudomonas* spp. and some coliform species. The author, using different selective culture media for Nile tilapia samples, also identified four different bacterial genera. In 420 plates, she observed these species as follows: *Aeromonas* spp. 286 (68.1%), *Vibrio* spp. 69 (16.4%), Enterobacteriaceae 51 (12.2%) and *Pseudomonas* spp. 14 (3.3%). When compared to this study, the results are similar except for the *Vibrio* spp[16].

Silva et al. (2015) isolated and identified the main bacterial groups present in farmed Nile tilapia. Among the isolates, the most frequent genera were *Pseudomonas* spp., *Aeromonas* spp., *Staphylococcus* spp., *Bacillus* spp., *Mycobacterium* spp., *Micrococcus* spp., and *Corybacterium* spp[17].

In Brazil, Molinar et al. (2003) measured total bacterial numbers in the gastrointestinal tract of semi-intensively cultured tilapia. Gram-negative bacilli were the most isolated group of bacteria and *Aeromonas hydrophila*, *Aeromonas* veronii, *Burkholderia cepacia, Chromobacterium violaceum, Citrobacter freundii, Escherichia coli*, *Flavimonas oryzihabitans* and *Plesiomonas shigelloides* were the most frequently isolated among than[7].

Regarding to gram-positive cocci, this study demonstrated the isolation of *Enterococcus faecalis* and *Streptococcus* species according to results found in previous publications[18].

Our result provides that the *Globicatella* genus was present in 6 (30%) skin samples and in 4 (20%) oral cavity samples. Surprisingly, in a PubMed survey we only found 13 articles mentioning *Globicatella* sanguinis, and of these, 10 were associated with human diseases and only 3 in animals (2 in sheep and 1 in horses)[19,20,21].

Although some authors have reported significant isolation of Candida species collected from aquatic animals, our study showed only two species of the *Candida parapsilosis* species complex, one recovered from skin samples and the other from the oral cavity swab[22,23,24].

CONCLUSION

Although some scientific publications have shown that the role of many of these fish-associated bacteria is unclear and the relationship bacteria/fish uncertain, we performed in our work a quantitative culture that shows in all samples studied, a CFU load less than 100,000 CFU/g of tissue. This value is a cutoff standardized by the American Society of Microbiology to differentiate the causal agent from colonizer isolates in human wounds. In light of these results and considering the absence of infectious signs in the fish samples, we conclude that the CFU values found in this study indicate the presence of normal, non-infectious microbiota.

REFERENCES

1. Zago A.C. Análise parasitological e microbiológica de tilápias do Nilo (*Oreochromis niloticus*), criadas em tanques-rede no reservatório de Água Vermelha- SP e suas inter-relações com as variáveis imunológicas e fase de criação [dissertation]. Botucatu (SP): UNESP; 2012.

2. Chechim, F.E. Características morfológicas do epitélio intestinal e desempenho de tilápia do Nilo, *Oreochromis niloticus*, suplementada com mananoligossacarídeo (MOS) [dissertation]. Dois Vizinhos [PR]: UTFPR; 2013.

3. Sousa A.D.L. Mananoligossacarídeo e B-glucano na suplementação dietária para juvenis de tilápia do Nilo mantidos em tanque-rede [thesis]. Jaboticabal [SP]: UNESP; 2010.

4. Pavanelli, G. C.; Eiras, J. C.; Takemoto, R. M. (2008). Doenças de peixes: profilaxia, diagnóstico e tratamento. 3rd. Maringá: Eduem; 2008.

5. Lanzarin M., Almeida Filho E.S., Ritter D.O., Mello C.A., Corrêa C.S.S., Ignácio C.M.S. Ocorrência de *Aeromonas* sp. e microrganismos psicrotróficos e estimativa do prazo de validade comercial de filé de pintado (Pseudoplatystoma coruscans) mantidos sob refrigeração. Arq. Bras. Med. Vet. Zootec. 2011 Dec; 63(6):1541-1546. doi: http://dx.doi.org/10.1590/S0102-09352011000600035.

6. Giatsis C., Sipkema D., Smidt H., Heilig H., Benvenuti G., Verreth J., Verdegem M. Impact of rearing environment on the development of gut microbiota in tilapia larvae. Scientifc Repports. 2015 Dec 11 [access 2016 Apr 20]; 5(18206): 1-15. Available from: http://www.nature.com/articles/srep18206. DOI: 10.1038/srep18206.

7. Molinari L.M., Scoaris D.O., Pedroso R.B., Bittencourt N.L.R., Nakamura C.V., Uedanakamura T., et al. Bacterial microbiota in the gastrointestinal tract of Nile tilapia, *Oreochromis niloticus* cultured in a semi-intensive system. Acta Scientiarum. Biological Sciences. 2003; 25(2):267-271.

8. Parkingking R., Palma P., Usero R. Quantitative and qualitative analyses of the bacterial microbiota of tilapia (*Oreochromis niloticus*) cultured in earthen ponds in the Philippines. World Microbiol Biotechnol. 2015 Fev; 31(2):265-275. doi:

10.1007/s11274-014-1758-1. PubMed PMID: 25555375.

9. Barony G.M., Tavares G.C., Assis G.B.N., Luz R.K., Figueiredo H.C., Leal C.A. New hosts and genetic diversity of Flavobaterium columnare isolated from Brazilian Native species and Nile tilapia. Dis Aquat Organ. 2015 Nov 17; 117(1):1-11. doi: 10.3354/dao02931.

10. Austin, B. The bacterial microflora of fish, revised. ScientificWorldJournal. 2006 Aug 11. 6: 931–945.

11. Huicab-Pech Z.G., Landeros-Sánchez C., Castañeda-Chávez M.R., Lango-Reynoso F., López-Collado C.J., Platas Rosado D.E. Current state of bacteria pathogenicity and their relatioship with host and environment in tilapia *Oreochromis niloticus*. Aquac. 2016; 7(5):1-10. doi: 10.4172/2155-9546.1000428.

12. Agência Nacional de Vigilância Sanitária. Microbiologia clínica para o controle de infecção relacionada à assistência à saúde. Módulo 4: procedimentos laboratoriais: da requisição do exame à análise microbiológica e laudo final. Brasília: ANVISA; 2013. 95 p.

13. Garcia, L. S.; Isenberg H. D. Clinical Microbiology Procedures Handbook. 3rd. ed. Washington: American Society for Microbiology; 2010.

14. Pincus, D.H. Microbial identification using the bioMerieux VITEK 2 System. In: Miller, M. Encyclopedia of Rapid Microbiological Methods. Bethesda: PDA/DHI; 2006. p 1-32.

15. Tiamiyu, A.M., Soladoye, M.O., Adegboyega, T.T. and Adetona, M.O. Occurrence and Antibiotic Sensitivity of Bacterial Strains Isolated from Nile Tilapia, *Oreochromis niloticus* Obtained in Ibadan, Southwest Nigeria. Journal of Biosciences and Medicines. 2015; 3: 19-26. doi: http://dx.doi.org/10.4236/jbm.2015.35003.

16. Rodrigues, E. Pesquisa de *Aeromonas* spp. em tilápia (*Oreochromis niloticus*), cultivada no estado do Rio de Janeiro-Brasil: isolamento, identificação de espécies e avaliação da sensibilidade antimicrobiana [tese]. Niterói (RJ): Universidade Federal Fluminense; 2007.

17. Silva, J.L.S.; Cavalcante, D.H.; Carvalho, F.C.T.; Vieira, R.H.S.F.; Sá M.V.C. and Sousa, O.V. Aquatic microbiota diversity in the culture of Nile tilapia (*Oreochromis niloticus*) using bioflocs or periphyton: virulence factors and biofilm formation. Acta Scientiarum. Animal Sciences. 2016 Jul-Sep; 38(3): 233-241. doi: http://dx.doi.org/10.4025/actascianimsci.v38i3.31910.

18. Anshary H., Kurniawan R.A., Sriwulan S., Ramli R., Baxa D.V. Isolation and molecular identification of the etiological agents of *Streptococcus* in Nile tilapia (*Oreochromis niloticus*) cultured in net cages in Lake Sentani, Papua, Indonesia. Springerplus. 2014 Oct 24; 3(627):1-11. doi: 10.1186/2193-1801-3-627. PubMed PMID: 25392797.

19. Elsinghorst T.A. First cases of animal diseases published since 2000.Vet Q. 2003;25(4):165-169. doi: 10.1080/01652176.2003.9695160. PubMed PMID: 14714740.

20. Vela A.I., Fernández E., Las Heras A., Lawson P.A., Domínguez L., Collins M.D., Fernandez-Garayzabal J.F. Meningoencephalitis associated with *Globicatella* sanguinis infection in lambs. J Clin Microbiol. 2000 Nov; 38(11):4254-4255. PubMed Central PMCID: PMC87575.

21. Collins M.D., Rodriguez J.M., Lawson P.A., Falsen E., Foster G. Characterization of a novel gram-positive, catalase-negative coccus from horses: description of Eremococcus coleocola gen. nov., sp. nov. Int J Syst Bacteriol. 1999 Oct; 49(Pt 4):1381-1385. doi: 10.1099/00207713-49-4-1381. PubMed PMID: 10555316.

22. Brilhante R.S., Castelo-Branco D.S., Soares, G.D., Astete-Medrano D.J., Monteiro A.J., Cordeiro R.A., et al. Characterization of the gastrointestinal yeast microbiota of cockatiels (Nymphicus hollandicus): a potential hazard to human health. J Med Microbiol. 2010 Jun; 59(Pt 6): 718–723. doi: 10.1099/jmm.0.017426-0. PubMed PMID: 20150318.

23. Brilhante R.S., Jesus S.R.T., Souza C.M.C.B., Teixeira C.E., Brito M.R., Bandeira S.P., et al. *Candida parapsilosis* complex from animals and its antifungal susceptibility and virulence attributes. Med Microbiol. 2014 Nov; 63(Pt 11): 1568-1572. doi: 10.1099/jmm.0.076216-0. PubMed PMID: 25190736.

24. Sidrim J.J., Carvalho V.L., Castelo-Branco D.S., Brilhante R.S., Bandeira T.J., Cordeiro R.A., et al. Yeast microbiota of natural cavities of manatees (Trichechus inunguis and Trichechus manatus) in Brazil and its relevance for animal health and management in captivity. Can J Microbiol. 2015 Oct. 61(10): 763-769.

Como citar este artigo/How to cite this article:
Lima EM Júnior, Bandeira TJPG, Miranda MJB, Moraes MO, Ferreira GE, Parente EA, Picollo NS. Characterization of the microbiota of the skin and oral cavity of *Oreochromis niloticus.* J Health Biol Sci. 2016 Abr-Jun; 4(3):193-197.