

**TITLE:** THE BIOPROSPECTION OF GRAM-NEGATIVE BACTERIA RESISTANT TO B-LACTAM ANTIBIOTIC IN LAKE BOLONHA, PARÁ – BRAZIL

**AUTHORS:** ALVES, J.T.C.<sup>1</sup>; ARAÚJO, C.L.A.<sup>1</sup>; DIAS, L.M.<sup>1</sup>; COSTA, W.L.O.<sup>1</sup>; BARRETO, D. F.<sup>1</sup>; CASTRO, I. R. R.<sup>2</sup>; BARAUNA, R..A.<sup>1</sup>; SILVA, A.<sup>1</sup>; FOLADOR, A.R.C<sup>1</sup>.

**INSTITUTION:** <sup>1</sup> LABORATÓRIO DE GENÔMICA E BIOINFORMÁTICA, CENTRO DE GENÔMICA E BIOLOGIA DE SISTEMAS, UNIVERSIDADE FEDERAL DO PARÁ – UFPA (RUA AUGUSTO CORRÊA, 1 – CEP 66075-110, BELÉM – PA, BRAZIL); <sup>2</sup> UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA – UFRA (AV. PERIMETRAL, 2501 – CEP 66077-830, BELÉM - PA, BRAZIL).

**ABSTRACT:**

Antibiotic resistance has been observed in several aquatic environments including rivers, domestic and hospital sewage, sediments, surface waters, lakes, oceans and drinking water, as well as in soils. The antibiotics released to the environment through wastewater effluents can influence the local bacterial community by horizontal gene transfer from pathogenic and environmental bacteria causing dissemination of resistance genes. Thus, the present study aims to bioprospection of Gram-negative bacteria resistant to the antibiotics cefotaxime and imipenem present in Lake Bolonha (Belém, Pará), which is one of the main water sources for public supply in the metropolitan region of Belém. Three distinct points were selected in Lake Bolonha, where for each point two liters of water were collected, intended for physical-chemical parameters analysis, and then study of bacterial communities by cultivation-dependent method (bacterial strains isolation). For the isolation, two culture dependent methods were used. In the seeding technique, 100 µl of the water sample was serially diluted in volumes 10<sup>0</sup>, 10<sup>1</sup>, 10<sup>2</sup>. Later, they were plated with MacConkey Agar culture medium supplemented with antibiotic cefotaxime and imipenem. Afterwards, 1:5, 1:10, 1:50 water samples were filtered in 0.22 µm membranes, which were plated with MacConkey Agar culture medium supplemented with antibiotic cefotaxime and imipenem. A total of 74 isolates resistant to cefotaxime and 61 isolates resistant to imipenem were obtained. The isolates were identified by sequencing the 16S rRNA gene, in which the samples were classified as belonging to the Enterobacteriaceae family. The results observed through the physical-chemical parameters analysis showed that the dissolved oxygen, electrical conductivity, nitrogen and total phosphorus were higher than the reference values defined by the Conama Resolution 357/2005. Indicating pollution by industrial and domestic effluents, and eutrophication of the lake. In addition, the presence of bacteria resistant to the antibiotics of last resort revealed that the Lake Bolonha can be considered a reservoir and a place of transmission of resistant bacteria.

**Keywords:** antibiotic resistance, imipenem, cefotaxime, lake Bolonha.

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