

**TITLE:** IDENTIFICATION OF NONTUBERCULOUS MYCOBACTERIA IN COALHO CHEESE FROM THE STATE OF PARAIBA, BRAZIL

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**ABSTRACT:**

Unpasteurised milk and its byproducts have been associated with the transmission of infectious diseases and can carry pathogenic mycobacteria. Nontuberculous mycobacteria (NTM) are emerging pathogens which cause opportunistic infections in humans and animals. This study describes the identification of NTM in coalho artisanal cheese. Twenty - seven samples of cheese were collected at street markets in the State of Paraíba (municipalities of Campina Grande, João Pessoa, Juazeirinho, Conde and Soledade). Five grams of these samples were macerated with 5mL of physiological solution in Magna Lyser apparatus (Roche Life Science), and then decontaminated by Petroff's method, incubated at 37 ° C in Stonebrink medium and evaluated weekly for 90 days. The resulting colonies were inactivated at 87°C for 60 min and centrifuged at 14,000xg por 5 min. The supernatant was used for PCR, targeting *hsp65*. The amplicons were purified using the enzymes shrimp alkaline phosphatase and exonuclease I. The DNA sequencing reaction was performed with the Big Dye Terminator cycle sequencing kit (version 3.1, Applied Biosystems, Foster city, CA, USA). The purified PCR products were sequenced and the base calling of the DNA sequences was performed with the Sequence Scanner program (Applied Biosystems). The resulting sequences were submitted to Blastn identity search (NCBI). One sample (3.7%) presented NTM-compatible colonies. Three colonies from this sample were amplified by PCR. The resulting consensus DNA sequence presented as best hit the homologous fragment of *hsp65* in the isolate HNTM1 of *Mycobacterium novocastrense* (Sequence ID: HM807282.1), with an identity of 99% and an e-value 0.0. The identification of NMT in artisanal coalho cheese represents a risk to consumers and reinforces the need to intensify sanitary inspection of these products.

**Keywords:** Nontuberculous mycobacteria; PCR; molecular diagnosis; cheese.

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