

Molecular Identification of the species that cause leprosy

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KEYWORDS

Leprosy, Mycobacterium leprae, Mycobacterium lepromatosis, Hansen's disease

ABSTRACT

Leprosy or Hansen's disease is a chronic granulomatous disease that mainly affects the skin and peripheral nerves, caused by *Mycobacterium leprae*, from the point of view of the bacteriological index by means of bacilloscopies or histopathology, with Ziehl Neelsen or Kinyoun staining, it is classified in multibacillary patients, those patients whose bacilloscopy contains more than 2 bacilli in 100 fields observed under the microscope in 100X objective. (BB, BL and LL) or paucibacillary (PB) with less than 2 bacilli, (BB, BT and TT) (1-4).

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1. Background

In 2008, a new mycobacteria causing the disease, *M. lepromatosis*, was discovered in two patients with diffuse LL and Lucio phenomenon and was confirmed to be genetically distinct from *M. leprae* (3, 5).

Since then, molecular studies have been conducted in various parts of the world to determine the frequency with which *M. lepromatosis* causes the disease.

2. Results

Lanes from left to right: molecular weight marker (100bp), positive control: sample from armadillo infected by *Mycobacterium leprae*, sample from patient with clinical diagnosis of lepromatous leprosy amplicon size 440 bp compatible with *M. leprae* and negative control (Figure 1).

In the period from June 1, 2019 to June 1, 2020,

30 patients with multibacillary leprosy and positive bacilloscopy were included, where 28 (93.33%) achieved molecular identification. Of these, 4 (13.3%) were identified as *M. leprae* by specific primers RLEP 7 and 8 and by means of the specific primer LPM-244 to *M. lepromatosis* with a total of 17 (56.7%) (Chart 1).

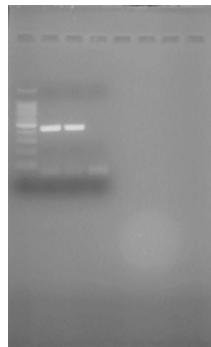


Figure 1. Polymerase Chain Reaction (PCR) amplification for *Mycobacterium leprae* identification.

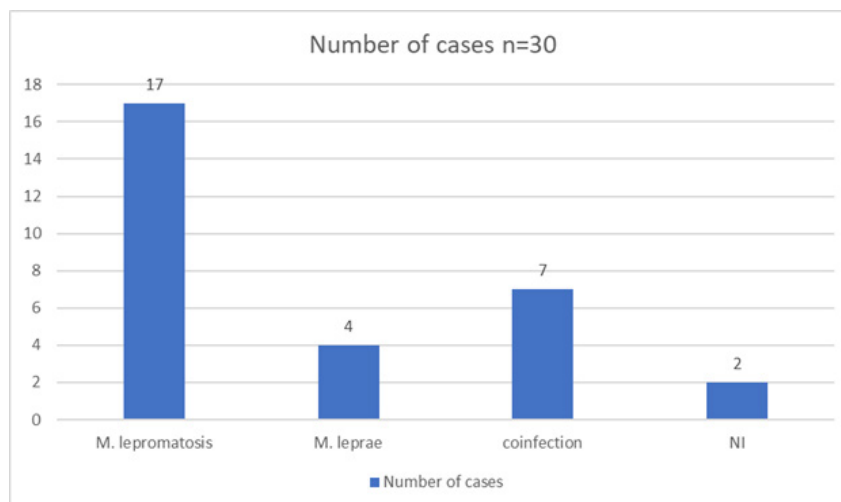


Chart 1. Molecular identification of *Mycobacterium leprae* and *Mycobacterium lepromatosis* among multibacillary leprosy patients ($n = 30$).

Of the 30 bacilloscopic samples taken from patients with a clinical and microbiological diagnosis of leprosy, by PCR analysis we detected a clear predominance of infections by *M. lepromatosis* with a total of 17 samples (56.7%), compared to those who presented infection by *M. leprae* where 4 were amplified (13.3%), which reinforces the hypothesis that it is the main causal agent of lepromatous leprosy in Mexico (Figures 2a and 2b).

In the present study, a total of thirty patients were recruited with a ratio of 2.33:1. Regarding the association of the species found and sex, a predominance

of *M. lepromatosis* was demonstrated in 13 (43.4%) of the 21 males included, followed by co-infection in 6 (20%), ($p < 0.0001$, Shapiro Wilks normality test and student t test for statistical significance, in addition, the squared χ^2 test was performed to determine if there was an association between the diagnosis of leprosy and sex, $p = 0.462$).

While of the 9 females, 4 (13.3%) were identified as *M. lepromatosis*, followed by 3 (10%) for *M. leprae*.

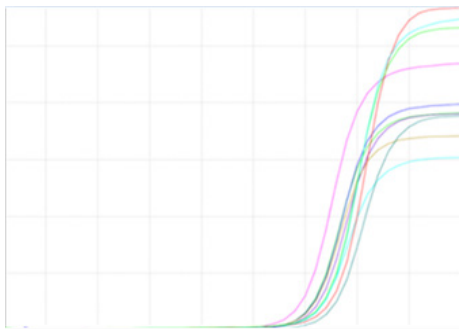


Figure 2a. Amplification curve.

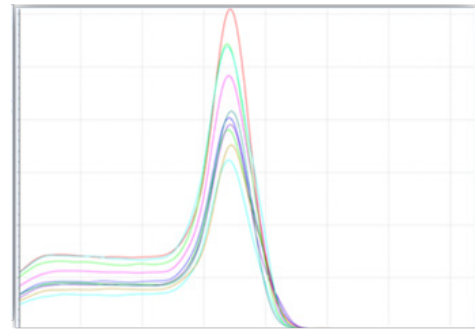


Figure 2b. Melting curve.

3. Conclusion

M. lepromatosis is the predominant causal agent of leprosy in patients from the Dermatological Institute of Jalisco recruited by CIINDE, in turn, this

species seems to be clinically associated with lepromatous leprosy, however, we did not find statistical significance in these findings ($p = 0.462$).

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