Title: Standardization of primer extension reaction associated with mass spectrometry for rapid assessment of resistance to rifampicin in *Mycobacterium tuberculosis* isolates.

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Mass spectrometry has revolutionized many areas of knowledge including diagnostic medicine, as it is able to analyze organic compounds in blood, urine and other biological fluids, making it possible to diagnose metabolic diseases (diabetes, liver diseases, and so forth). The importance of this technique in the rapid detection of antibiotic resistance such as rifampicin in *Mycobacterium tuberculosis* isolates is also important, since the tests based on phenotypic analyses (proportional method and BACTEC MGIT 960) do not allow rapid profile resistance analysis of the isolates. Resistance to rifampicin occurs due to mutations in the *rpoB* gene that encodes the β subunit of RNA polymerase which is the target enzyme of the antibiotic. Molecular studies have demonstrated that approximately 96% of the mutations associated with this resistance are in an 81 bp region of the *rpoB* gene, with 68% frequency in codon 531. In this context, we standardize a primer extension reaction using a sensitive strain *M. tuberculosis* H37Rv (TCG at codon 531 of the *rpoB* gene) and a clinical isolate resistant to rifampicin (TTG at codon 531). Several parameters such as composition of buffer, primer concentrations, reaction volumes and annealing temperatures of the primer extension reaction were evaluated. Our results showed that the addition of ammonium sulfate to the reaction as well as a high concentration of the primer allows the amplification of the expected 22 bp fragments. These products of the primer extension reactions were analyzed by MALDI-TOF mass spectrometry and distinct spectra were obtained in the sensitive sample (6680 Da) and resistant isolate (6694 Da), making possible the differentiation between the phenotypes. In conclusion, these results reinforce that the method is promising in the identification of the antibiotic resistance in *M. tuberculosis*.

Key Words: *Mycobacterium tuberculosis,* resistance, rifampicin, mass spectrometry
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