TITLE: EVALUATION OF *MYCOBACTERIUM ABSCESSUS* ISOLATES WITH INDUCED RESISTANCE MECHANISM IN THE STATE OF SÃO PAULO, BRAZIL.

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ABSTRACT:

The *Mycobacterium abscessus* group (MAG) is the major cause of lung and extrapulmonary infections among the rapid growing mycobacteria. To determine phenotypic resistance to clarithromycin (CLA), the basis of combined therapy, minimum inhibitory concentration (MIC) method can be performed, but readings are taken after three and fourteen days of incubation to detect induced resistance (IR). The IR can also be differentiate by the identification of a 274pb deletion in the *erm*(41) gene. The present study aims to evaluate the molecular basis of CLA resistance in MAG isolates. A total of 227 MAG isolates, which met the bacteriological criteria according to the American Thoracic Society, were received at the Adolfo Lutz Institute, São Paulo, Brazil, between 2010 and 2012. Isolates were identified by the PRA-h*sp*65 method as *M. abscessus* type 1-Mab1 (n=148, 65.2%) or *M. abscessus* type 2-Mab2 (n=79, 34.8%). Amplification of *erm*(41) indicated that 38 isolates (16.7%) showed the 274bp deletion in this gene. By MIC method, eight isolates (3.5%) presented resistance (R) on the 3rd-day reading, while 162 isolates (71.4%) presented growth only on the 14th-day reading, classified as IR and 57 (25.1%) were susceptible (S) after the 14 days incubation. The concordance between the MIC and PCR detection of CLA was 89.2% for Mab1 and 92.4% for Mab2 isolates; overall concordance was 90.3%. For the following analysis, the gold standard was *erm*(41) gene sequencing; the sensitivity and specificity of the PCR and MIC resulted in 67% and 99% for PCR and 96% and 80% for MIC, respectively, and PCR had 93% and 94% of Positive and Negative Predictive Values. Among the 16 discordant Mab1, all isolates were S by MIC; however only four presented susceptible genotype in the *erm*(41) gene sequencing, likely indicating the presence of another (but unidentified) mechanism of reversion to susceptibility. Among the six discordant Mab2, four isolates were S by MIC and presented the intact *erm*(41) gene with IR genotype detected; one isolate was R and without deletion, also likely associated with another resistance mechanism; and one isolate presented IR with deletion. In conclusion, the tests when associated were able to detect the IR to CLA, and PCR can be used as a fast and reliable screening to predict MIC results. Deletion detection can predict a reliable CLA susceptibility; on the other hand, an intact *erm*(41) gene demands a MIC result to conduct the more appropriate treatment.

Keywords: nontuberculous mycobacteria, drug resistance, minimal inhibitory concentration, polymerase chain reaction.