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Poster Session III
Recent advances in molecular diagnosis of M. tuberculosis

USE OF A NEW MULTIPLEX PCR FOR THE DETECTION OF MYCOBACTERIUM TUBERCULOSIS RESISTANT TO FIRST- AND SECOND-LINE DRUGS IN CLINICAL STRAINS AND SAMPLES

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Objectives: Drug resistant tuberculosis (TB) is a global threat due to spreading of multidrug resistant (MDR) and extensively drug resistant (XDR) *Mycobacterium tuberculosis* strains. MDR TB strains are resistant to isoniazid (INH) and rifampicin (RIF), and XDR TB strains are additionally resistant to fluoroquinolones (FQ) and at least one of the second-line injectable drugs kanamycin, amikacin and capreomycin (KM/AM/CM). The objective of this study is to evaluate a multiplex PCR-based molecular method to detect MDR and XDR in both clinical isolates and clinical samples.

Methods: One hundred fourteen clinical strains, isolated from 114 patients, and 60 clinical specimens from 36 patients were retrospectively selected. The multiplex PCR method (Anyplex II MTB/MDR/XDR [Seegene, Corea]) performed detects the presence of *M.tuberculosis* complex and mutations associated to resistance to INH, RIF, FQ and KM/AM/CM. The genotypic results obtained by multiplex PCR were compared to those obtained by BACTEC 460TB or MGIT960. Discordant results obtained by both methods were analized by alternative molecular methods (sequencing, GenoType MTBDR*plus*, GenoType MTBDR*sl* [Hain Lifescience, Alemania] and/or pyrosequencing).

Results: The global concordance rates between results of both Anyplex II MTB/MDR/XDR and BACTEC 460TB/MGIT for detecting resistance to INH, RIF, FQ and KM/AM/CM in clinical isolates are 80.3% (49/61), 96.7% (59/61), 80.0% (48/60) and 85.0% (51/60), respectively. In the case of discordant results obtained by both methods, all the alternative molecular methods confirmed the genotypic result in 91.7% of the cases for INH resistance, in 83.3% of the cases for FQ resistance, and 77.8% for KM/AM/CM resistance. For the remaining discordant results for INH, RIF and FQ, the result obtained by Anyplex II was confirmed by one of the alternative molecular methods but not with the others. In the case of KM/AM/CM resistance, the two remaining discordant results corresponded to strains that harbored a mutation in *eis* promoter, a region not explored by the currently available molecular methods. The global concordance rates for detecting resistance to INH, RIF, FQ and KM/AM/CM in clinical samples are 93.2% (55/59), 94.9% (56/59), 93.1% (54/58) and 93.1% (54/58), respectively. In the case of discordant results obtained by both methods, alternative molecular methods confirmed the genotypic result in 50.0% of the cases for INH resistance, in all the cases for FQ resistance but in any case for both RIF and KM/AM/CM detection. One of the discordant results for KM/AM/CM resistance detection corresponded to a strain that harbored a mutation in *eis* promoter.

Conclusions: The multiplex PCR method Anyplex II MTB/MDR/XDR is useful for the rapid identification of drug resistance TB in both clinical isolates and clinical specimens, thus allowing a initial therapeutic approach. Nevertheless, for a correct management of drug resistant TB patients, the results should be confirmed by a phenotypic method.