Use of a new multiplex PCR for the detection of *Mycobacterium tuberculosis* resistant to first- and second-line drugs in clinical strains and samples

B. Molina (1), A. Lacoma (1), C. Prat (1), E. Pimkina (2), J. Díaz (1), J. Maldonado (3), S. Samper (4), J. Ruiz Manzano (5), V. Ausina (1), J. Domínguez (1)

(1)Servei de Microbiologia, Hospital Universitari Germans Trias i Pujol, Badalona. Institut d'Investigació Germans Trias i Pujol. Universitat Autònoma de Barcelona. CIBERES, (2) National Tuberculosis and Infectious Diseases University Hospital, Vilnius, Lithuania, (3) Serveis Clínics, Barcelona, (4) Instituto Aragonés de Ciencias de la Salud, Zaragoza. Hospital Universitario Miguel Servet, Zaragoza. CIBERES. (5) Servei de Pneumologia, Hospital Universitari Germans Trias i Pujol, Badalona.

Introduction / 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: resistance to isoniazid (INH) and rifampicin (RIF).
- XDR TB: resistance to INH, RIF, fluoroquinolones (FLQ) and at least one of the second-line injectable drugs kanamycin, amikacin and capreomycin (KAN, AMK, CM).
- The objective of this study was to evaluate a multiplex PCR-based molecular method to detect MDR and XDR in clinical isolates and clinical samples.

Material and Methods

- Retrospective study
  - Sixty-one clinical isolates were tested for INH/RIF resistance and 60 isolates for FLQ/KAN/AMK/CM resistance. These strains were isolated in Hospital Germans Trias i Pujol and National Tuberculosis and Infectious Diseases University Hospital, Vilnius, Lituania.
  - Sixty respiratory samples were tested for INH/RIF resistance and 60 for FLQ/KAN/AMK/CM, corresponding to 34 patients diagnosed at Hospital Germans Trias i Pujol.
- Phenotypic drug susceptibility testing was performed by BACTEC system (Table 1).
- The multiplex PCR method (Anyplex II MTB/MDR/XDR [Seegene, Corea]) was performed to detect drug resistance in *M.tuberculosis.* This system consists on PCR amplification and detection of melting curves corresponding to *M.tuberculosis* and drug resistance.
  - MTB/MDR: resistance to INH and RIF → *katG* 315; *inhA* -15; *rpoB* 511, 513, del.513-516, 516, 522, 526, 531, 533.
  - MTB/XDR: resistance to FLQ, KAN, AMK, CM → *gyrA* 90, 91, 94; *rrs* 1401, 1402, 1484; *eis* -37, -14, -10.
- Genotypic results obtained by this method were compared to phenotypic results obtained by BACTEC.
- Discordant results between both methods were analyzed by alternative molecular methods (GenoType MTBDR*plus*, GenoType MTBDRs/ [Hain Lifescience, Germany] and/or pyrosequencing).

Table 1. Resistance pattern obtained by BACTEC for INH, RIF, FQ, KAN, AMK and CM for clinical strains and samples.

	Clinical strains						Clinical samples				
Drug	**INH**	**RIF**	**FQ**	**KAN**	**AMK***	**CM**	**INH**	**RIF**	**FQ**	**KAN**	**CM**
Resistant	51	36	27	27	12	7	45	44	10	24	24
Sensitive	10	25	33	33	25	53	15	16	50	36	36

* For 23 samples phenotypic DST for AMK was not performed.

Results

- Positive result for MTB
  - Clinical isolates: 100% (121/121)
  - Respiratory samples: 97.5% (117/120). 2 smear negative and one scanty smear samples.

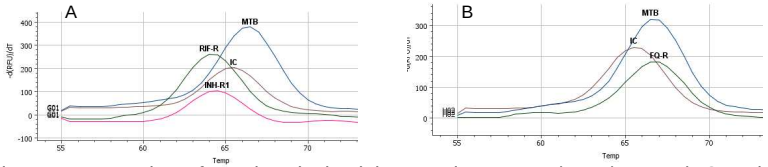

Figure 1. Examples of results obtained by Anyplex II MTB/MDR/XDR. A) Sample resistant to INH and RIF. B) Sample resistant to FQ and sensitive to KAN, AMK and CM.

Table 2. Sensitivity, specificity and agreement values between Anyplex II MTB/MDR/XDR and BACTEC for detecting drug resistance in clinical strains and samples[a].

	Clinical strains				Clinical samples			
Drug	**Anyplex II MTB/MDR/XDR**		**Agreement between Anyplex and BACTEC**		**Anyplex II MTB/MDR/XDR**		**Agreement between Anyplex and BACTEC**	
	Sensitivity (%)	**Specificity (%)**	**Agreement (%)**	**Kappa**	**Sensitivity (%)**	**Specificity (%)**	**Agreement (%)**	**Kappa**
INH	39/51 (76.5)	10/10 (100)	49/61 (80.3)	0.516	14/15 (93.3)	14/14 (100)	55/59 (93.2)	0.829
RIF	35/36 (97.2)	24/25 (96.0)	59/61 (96.7)	0.932	14/14 (100)	15/15 (100)	56/59 (94.9)	0.874
FQ	19/27 (70.4)	29/33 (87.9)	48/60 (80.0)	0.590	2/4 (50.0)	19/19 (100)	54/58 (93.1)	0.713
KAN	22/27 (81.5)	28/33 (84.8)	50/60 (83.3)	0.663	5/5 (100)	17/18 (94.4)	54/58 (93.1)	0.854
AMK	3/12 (25.0)	25/25 (100)	28/37 (75.7)	0.311	-	-	-	-
CM	6/7 (85.7)	51/53 (96.2)	57/60 (95.0)	0.772	5/5 (100)	17/18 (94.4)	54/58 (93.1)	0.854

[a] One sample per patient was considered for sensitivity and specificity calculations. AMK phenotypic DST for the clinical samples was not performed.

- Regarding the results that were discordant between BACTEC and Anyplex, and considering strains and samples together, alternative molecular methods confirmed genotypic results in 62.5% (10/16) of the cases for INH, 0% (0/5) for RIF, 100% (16/16) for FQ, 71.4% (10/14) for KAN, 100% (9/9) for AMK and 71.4% (5/7) for CM.

Conclusions

The multiplex PCR Anyplex II MTB/MDR/XDR may be a useful tool to detect drug resistance in clinical strains and samples in a timely manner. Nevertheless, for a correct managment of patients with tuberculosis, genotypic results must be confirmed by a phenotypic method.