# Broad-range PCR coupled with mass-spectrometry for the detection of *Mycobacterium tuberculosis* drug resistance

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## Abstract

**Background The need to limit the spread of drug-resistant *Mycobacterium tuberculosis* requires rapid detection of resistant strains. The present study aimed to evaluate a commercial assay using broad-range PCR coupled with electrospray ionization mass spectrometry (PCR/ESI-MS) for the rapid detection of isoniazid (INH) and rifampin (RIF) resistance in *M. tuberculosis* strains isolated from Romanian patients with pulmonary tuberculosis.**

**Methods PCR/ESI-MS was used to detect genotypic resistance to RIF and INH in a panel of 63 *M. tuberculosis* isolates phenotypically characterized using the absolute concentration method on Löwenstein-Jensen medium.**

**Results Thirty-eight (60%) strains were susceptible to both drugs, 22 (35%) were RIF and INH resistant, one was INH mono-resistant and two were RIF mono-resistant.**

**The sensitivity for INH and RIF resistance mutations detection were 100% and 92% respectively, with a specificity of more than 95% for each drug.**

**Conclusion PCR/ESI-MS is a good method for the detection of RIF and INH resistance and might represent an alternative to other rapid diagnostic tests for the detection of genetic markers of resistance in *M. tuberculosis* isolates.**


**Keywords *Mycobacterium tuberculosis*, broad-range PCR, electrospray ionization mass-spectrometry, MDR-TB**

## Background

Multidrug-resistant tuberculosis (MDR-TB) is an important public health concern worldwide. According to the World Health Organization, an estimated 480,000 new cases of MDR-TB occurred in 2013 resulting in 210,000 deaths.[1] The need to limit the spread of drug-resistant strains requires rapid detection of resistance; therefore several assays that identify *Mycobacterium tuberculosis* and drug resistance have been developed in the last years. Most of them are molecular assays, able to provide rapid detection of resistant *M. tuberculosis* strains.

A number of molecular tools can also classify *M. tuberculosis* isolates into phylogenetic groups, like principal genetic group (PGG) or lineage, based on single nucleotide polymorphisms (SNP) detected in specific loci.[2-4] These epidemiological data might be useful for TB control strategies.

Broad-range PCR coupled with electrospray ionization mass-spectrometry (PCR/ESI-MS) is a high-throughput method designed for the simultaneous identification of *Mycobacterium* species and the detection of resistance to isoniazid (INH), rifampin (RIF), ethambutol (EMB) and fluoroquinolones (FQ).[5]

Received: 24 August 2015; revised 03 October 2015; accepted: 07 October 2015

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Published March 2016

ISSN 2248 – 2997
ISSN – L = 2248 – 2997

In this study, we aimed to evaluate a commercial assay (MTB-DR, Abbott Molecular, Des Plaines, IL, USA) using PCR/ESI-MS for the rapid detection of INH and RIF resistance in *M. tuberculosis* strains isolated from Romanian patients with pulmonary TB.

## Methods

The study evaluated a panel of 63 *M. tuberculosis* isolates, previously characterized in the reference laboratory of 'Marius Nasta' National Institute of Pneumology (Bucharest, Romania) by phenotypic drug susceptibility test (DST) using the absolute concentration testing on Löwenstein-Jensen medium, (INH 0.2 µg/mL, RIF 40 µg/mL).[6]

Specific genomic regions associated with drug resistance were amplified using multilocus PCR and the amplicons were analyzed by ESI-MS, as previously described.[5] Briefly, the test detects a number of mutations in genes associated with resistance to INH (*katG*, *inhA* and its promoter, *ahpC* promoter), RIF (*rpoB*), EMB (*embB*) or FQ (*gyrA*). Detection of polymorphisms at *katG* codon 463 and *gyrA* codon 95 was used for identification of the PGG.

Statistical analysis was performed using the MedCalc software, version 14.8.1, (MedCalc Software, Ostend, Belgium).

The study protocol was approved by the ethics committees of the National Institute of Pneumology and the National Institute for Infectious Diseases.

## Results

According to the phenotypic DST results 38 strains of the panel (60%) were susceptible to both INH and RIF, 22 (35%) strains were RIF and INH resistant (MDR), one was INH mono-resistant and two were RIF mono-resistant. In all 23 INH resistant strains the PCR-ESI/MS assay detected a single mutation, located in *katG* gene (19 cases), *inhA* promoter (3 cases) or *ahpC* promoter (1 case). The mutation in *katG* was S315T, detected in 19 resistant isolates but also in 2 strains shown to be INH susceptible on phenotypic DST.

Twenty-two of the 24 RIF-resistant strains had at least one *rpoB* mutation: S531L was predominant (14 cases), but mutations in other codons (511, 513, 516, 526 or 533) were also detected. A discordant result was registered for 3 isolates (2 strains with a resistant phenotype but with no detected mutations and 1 susceptible strain with S531L mutation). The diagnostic accuracy of PCR/ESI-MS compared to phenotypic DST is shown in the Table.

**Table. PCR/ESI-MS performance in detecting RIF and INH resistance**

| | Isoniazid | Rifampin |
|---|---|---|
| Sensitivity % (95% CI) | 100 (85-100) | 92 (74-98) |
| Specificity % (95% CI) | 95 (83-99) | 97 (87-99) |

CI – confidence interval.

A phenotypic DST result for EMB or FQ was available for only 17 and 13 isolates, respectively. A nucleotide substitution in codon 306 of the *embB* gene was detected in 5 of the 11 EMB resistant strains and in none of the susceptible ones. Five isolates with MDR phenotypes had mutations in *katG*, *rpoB* but also in *gyrA*.

One strain (2%) belonged to the PGG 1, 33 (52%) to PGG2 and 28 (44%) to PGG 3; in one isolate the PGG could not be assessed.

## Discussion

PCR/ESI-MS is an automated method that can identify *M. tuberculosis* as well as non-tuberculous mycobacteria to the species level and detect drug resistance profiles of *M. tuberculosis* for 12 samples in less than 8 hours.[5] A large study performed on 1340 samples originating from different populations showed very good results for drug resistance detection with PCR/ESI-MS, with sensitivities of 96.4% for RIF and 89.2% for INH and specificities of 98.6% and 95.8% for RIF and INH, respectively.[5] In another study performed on 48 samples the sensitivity and specificity values were 100% and 93.8% for INH and 100% and 92.3% for RIF when PCR/ESI-MS was compared to phenotypic DST.[7] Similarly, in the present study PCR/ESI-MS had sensitivities in

detecting INH and RIF resistance of 100% and 92% respectively and specificities exceeding 95% to both RIF and INH. Several factors, like the sample size or the phenotypic method used as gold standard, might explain the differences between the results of these studies. Discordant results between phenotypic and genotypic results were described for several genotypic methods, including PCR-ESI/MS.[5,7] Several causes might explain these discordances, like the limited number of targeted genes and mutations, the detection of mutations associated with an elevated MIC but lower than the critical concentration, the challenges of phenotypic DST for some drugs, like ETB, and the incomplete understanding of the molecular mechanisms of drug resistance.

An important advantage of this method is the simultaneous detection of resistance to INH, RIF, EMB and FQ. In the present study five isolates with MDR phenotypes showed additional FQ resistance.

Interestingly, all but one isolates belonged to the Euro-American lineage (PGG 2 and 3); the only PGG1 strain was isolated from a non-Caucasian patient. This low proportion of PGG1 and consequently of Beijing type observed in the studied group is similar to that reported in Central and Western Europe[3] and in contrast with the higher prevalence rates from neighbouring Ukraine and North-Western Russia.[5] This observation needs to be confirmed by further studies, since the present study was not designed for prevalence estimation.

The present study is limited by its small sample size; however, we found that PCR/ESI-MS provides rapid and reliable results and might be a useful tool for the detection of drug-resistant TB. However the high costs of this technology may limit its use outside reference laboratories.

## Conclusion

PCR/ESI-MS seems to provide good detection of RIF and INH resistance when DST is used as gold standard; this method might represent an alternative to other rapid diagnostic tests for the detection of genetic markers of resistance in *M. tuberculosis* isolates.

**Note:** Partially communicated at the 52nd Interscience Conference of Antimicrobial Agents and Chemotherapy (ICAAC) San Francisco, USA, 9-12 Sep 2012

**Authors' contributions statement:** DF contributed to the idea, concept and design of the manuscript, the analysis and interpretation of the data, drafting and revising of the article and approved the final version of the draft for publication. DO contributed to the design of the manuscript, drafting and revising the article, and approved the final version of the draft for publication. IDO contributed to the design of the manuscript, the interpretation of data, drafting and revising the article, and approved the final version of the draft for publication. AH contributed to the idea, concept and design of the manuscript, the interpretation of data, drafting and revising the article, and approved the final version of the draft for publication. All authors read and approved the final version of the manuscript.

**Conflicts of interest:** All authors – none to declare.

**Acknowledgment:** DF was supported by the Sectoral Operational Program Human Resources Development financed from the European Social Fund and by the Romanian Government, CERO, under the contract no. POSDRU/159/1.5/S/135760.

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Please cite this article as:
Florea D, Oțelea D, Olaru ID, Hristea A. Broad-range PCR coupled with mass-spectrometry for the detection of *Mycobacterium tuberculosis* drug resistance. GERMS 2016;6(1):10-13. doi: 10.11599/germs.2016.1083