Rapid And Accurate Detection Of Specific Volatile Organic Compounds From Clinical Isolates Of Pulmonary Mycobacterium Tuberculosis And Non-Tuberculosis Mycobacterium

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Introduction

A critical constraint in controlling active tuberculosis (TB) is the inadequacy of available diagnostic tools. In disease endemic areas, 90% of patients with suspected TB receive smear microscopy for diagnosis, which is not sensitive. Only 10% of patients are evaluated with the time consuming sputum culture, the current gold standard for confirming diagnosis. Polymerase chain reaction (PCR)-based assays that detect Mycobacterium tuberculosis (MTB) DNA have been developed for faster diagnosis of active TB. However, these tests are either too complex or still too expensive for developing countries. Diagnostic tools with better sensitivity than smear microscopy, faster speed than sputum culture, and lower cost and simplicity than PCR are needed.

Non-invasive detection of volatile organic compounds (VOCs) from exhaled breath of TB patients has shown potential promise. This study reports the detection of VOCs specific to MTB using advanced two-dimensional gas chromatography and mass spectrometry (2D-GC/MS).

Method

- 10 clinical isolates of MTB and 2 standard laboratory MTB strains from American Type Culture Collection (ATCC) were cultured in duplicate on Lowenstein-Jensen (LJ) media using Entech 60ml tubes.
- 7 strains of non-tuberculous mycobacteria (NTM) were also cultured on LJ media: M. avium, M. chelonae, M. gordonae, M. kansasi, M. smegmati, M. terrae, and M. xenopi.
- 5 blank samples of LJ media were assayed as negative control.
- Headspace of all tubes were sampled using Entech 7405 auto-sampler / 7150 pre-concentrator and tested by ZOEX 2D-GC/MS.
- Around 400 VOC peaks (above 10\(^{-5}\) signal threshold) were identified by MS spectrum using National Institute of Standards and Technology (NIST) library.

Analysis and Results

- Nonparametric Wilcoxon-rank test was utilized to identify differential expression VOCs.
- Two comparisons: MTBs vs. control and NTMs vs control, were performed independently using p-value cut-off of 0.01.
- 16 and 9 VOCs were identified as MTB- and NTM-specific markers. 3 VOCs were found in both MTBs and NTMs.
- Principal component analysis (PCA) was performed based on the identified specific VOCs, demonstrating clear separation between MTB, NTM, and control groups.

Prediction Power

- The blank tubes do not have TB- or NTM-specific VOCs.
- 85% (17 out of 20) of TB-tubes present ≥3 TB-specific VOCs and the remaining 15% (3 out of 20) have 2 TB VOCs, whereas all NTM-tubes present ≥2 TB-specific VOCs (Fig. 6a).
- 75% of TB-tubes presents no NTM-specific VOCs, whereas all NTM-tubes have ≥1 NTM-specific VOC (Fig. 6b).

Conclusion

- For the first time, specific VOCs of clinical MTB and NTM isolates are distinctly defined using advanced 2DGC/MS.
- The findings provide a strong scientific foundation towards detection of active TB infection by rapid exhaled breath sampling in patients.