

**Identification of a *Rothia mucilaginosa* Strain in a Clinical Specimen Based on
PCR-Sequencing with *Mycobacterium hsp65* Primers**

ABSTRACT

Introduction: Tuberculosis (TB) is one of the top 10 causes of death worldwide. Xpert *Mycobacterium tuberculosis* (MTB)/ Rifampicin (RIF) has been recommended by World Health Organisation (WHO) to diagnose TB, while hsp65-Polymerase Chain Reaction (PCR) has been used for mycobacteria identification. Aim: To report a false positive result (*Rothia mucilaginosa*) using hsp65-PCR for detection of mycobacteria in a clinical specimen. Materials and Methods: A sputum sample from a 58-year-old, male, suspected-TB patient was studied by Xpert MTB/RIF and hsp65-PCR. The amplified hsp65-PCR product was sequenced and analysed using bioinformatics for bacterial identification and primer specificity. Results: Xpert MTB/RIF showed a negative result, while hsp65-PCR was positive, suggested the presence of Pulmonary NonTuberculous Mycobacterial (PNTM) Infection. The analysis of the sequencing result of the amplified hsp65-PCR fragment showed 98% similarity to *Rothia mucilaginosa*, a member of the normal flora of the human oropharynx and upper respiratory tract, which may cause pneumonia. Further analysis showed that the mycobacteria hsp65 primers used have high percentage of similarity with *Rothia mucilaginosa* gene sequence, resulting in non-specific detection. Conclusion: This study showed the possibility of false positive results in clinical specimens using PCR-hsp65 primers considered specific for mycobacteria, therefore, this test should be used in clinical samples with caution and it is suggested the need of its further re-optimisation.