

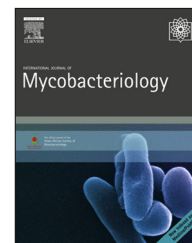


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## Proteome-scale MDR-TB-antibody responses for identification of putative biomarkers for the diagnosis of drug-resistant *Mycobacterium tuberculosis*

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### ABSTRACT

**Objective:** Multidrug-resistant tuberculosis (MDR-TB) is caused by *Mycobacterium tuberculosis* strains that do not respond to isoniazid and rifampicin, the two most effective first-line anti-TB drugs. Here, we designed and produced antibodies based on biomarkers that exist only in MDR-TB.

**Methods:** Bacilli were cultured for 4 weeks at 37 °C, and protein extraction was performed by sequential extraction. Bacterial cells were sonicated, centrifuged at 5000 rpm for 45 min, and the supernatant was collected and subjected to multiple rounds of treatment to prior to protein isolation. Protein concentration was determined using the Bradford method, and extracted proteins (50 µg) from each strain (drug-sensitive- and MDR-TB isolates) were visualized on polyacrylamide gels (5–15%) with Coomassie Brilliant Blue R-250 staining. Three extracts were mixed and dialyzed against 0.1 M ammonium bicarbonate (pH 8.0), followed by mass spectrometry. Specific polyclonal antibodies against purified MDR-TB proteins were purified by affinity chromatography and prepared in rabbits using three booster injections. The ELISA test was performed for evaluation the antibody production. The antibody was treated with normal oral flora to remove any non specificity and cross reactivity. Analyses of different protein patterns (drug-sensitive- and MDR-TB) were performed by western blot.

**Results:** Our revealed that the MDR-TB strains contained specific antigens, and that the protein profiles of drug-sensitive TB strains differed from those of the MDR-TB isolates. Five bands from the MDR-TB fractions were detected as diagnostic antigens and were not observed in drug-sensitive-TB fractions. Western blot results showed that the MDR-TB antigenic fractions showed immunogenic bands at 50.0 kDa and 70.0 kDa, with the five antigenic MDR-TB-specific bands were identified as Rv3248c, Rv0350, Rv0440, Rv0475, and Rv3588c.

**Conclusion:** Western blot data revealed dynamic properties of antibody responses that led to actionable findings for further research. Moreover, specific anti-mycobacterial antibodies, such as MDR-TB antibodies, can be essential tools in the identification of species-restricted antigens, such as drug-resistant TB antigens. The MDR-TB antibodies described here might promote identification of mycobacterial antigens during the course of infection, which could

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be helpful for the development of newer TB-vaccine candidates or therapeutic agents for improved TB treatment or diagnosis.

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### **Conflicts of interest**

The authors have no conflicts of interest to declare.