Researchers develop new technology to easily detect active TB

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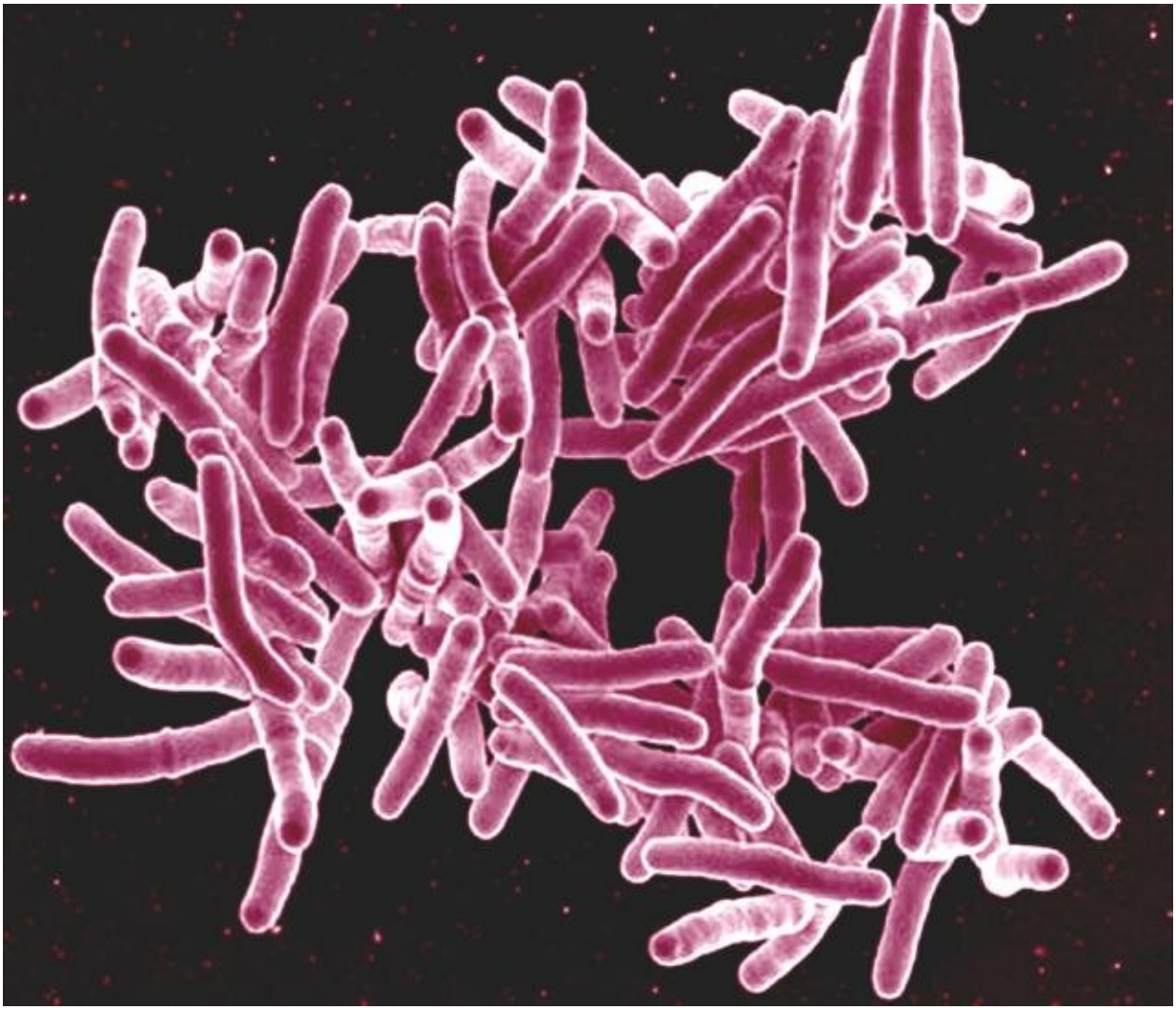

Scanning electron micrograph of *Mycobacterium tuberculosis* bacteria, which cause TB. Credit: NIAID

A team of faculty from Wayne State University has discovered new technology that will quickly and easily detect active Mycobacterium tuberculosis (TB) infection antibodies. Their work, "Discovery of Novel Transketolase Epitopes and the Development of IgG-Based Tuberculosis Serodiagnostics," was published in a recent edition of *Microbiology Spectrum*.

The team is led by Lobelia Samavati, M.D., professor in the Center for Molecular Medicine and Genetics in the School of Medicine. Samavati was joined by Jaya Talreja, Ph.D, and Changya Peng, research scientists in Wayne State's Department of Internal Medicine.

TB remains a global health threat, with 10 million new cases and 1.7 million deaths annually. According to the latest World Health Organization report, TB is the 13th leading cause of death and the second leading infectious killer after COVID-19. Latent tuberculous infection (LTBI) is considered a reservoir for TB bacteria and subjects can progress to active TB. One-third of the world's population is infected with TB, and on average, 5 to 10% of those infected with LTBI will develop active TB disease over the course of their lives, usually within the first five years after initial infection.

The gold standard tests to determine whether an infection is active TB are the sputum smear and culture tests. However, these methods require collecting sputum, which is time-consuming, expensive, requires trained personnel, and lacks sensitivity. The current conventional tests differentiating LTBI from uninfected controls—such as tuberculin skin tests (TST) and/or interferongamma release assay (IGRA)—do not differentiate between active TB infection and latent TB. Despite advances in rapid molecular techniques for TB diagnostics, there is an unmet need for a simple inexpensive point-of-care (POC), rapid non-sputum-based test.

Samavati's research group has worked for more than 15 years to develop technology for detection of antibodies in various respiratory diseases. Her lab has developed a novel non-sputum based technology and has discovered several novel immune-epitopes that differentially bind to specific immunoglobulin (IgG) in TB-infected subjects. The levels of epitope-specific IgG in serum can differentiate active TB from LTBI subjects, healthy controls and other respiratory diseases. This technology can be used as a simple serum assay non-sputum based serological POC-TB test, which is highly specific and sensitive, to differentiate active TB from LTBI.

"Previously, we developed a T7 phage antigen display platform and after immunoscreening of large sets of serum samples, identified 10 clones that differentially bind to serum antibody (IgG) of active TB patients differentiating TB from other respiratory diseases," said Samavati.

"One of these high-performance clones had homology to the Transketolase (TKT) enzyme of TB bacteria that is an essential enzyme required for the intracellular growth of the bacteria in a host. We hypothesized that abundance of IgG in sera against the identified novel neoantigen that we named as TKTμ may differentiate between active TB, LTBI and other non-TB granulomatous lung diseases such as sarcoidosis. We developed a novel direct Peptide ELISA test to quantify the levels of IgG in serum samples against TKTμ. We designed two additional overlapping M.tb TKT-peptide homologs with potential antigenicity corresponding to M.tb-specific transketolase (M.tb-TKT1 and M.tb-TKT3) and hence standardized three Peptide ELISA (TKTμ, M.tb TKT1 and M.tb TKT3) for the TB serodiagnosis."

After development and standardization of a direct peptide ELISA for three peptides, the research team tested 292 subjects, and their TKT-peptide ELISA results show that TB patients have significantly higher levels of TKT-specific antibodies compared to patients who were

healthy controls and with LTBI. The increased levels of TKT-specific antibodies is presumably associated with growing M.tb bacteria in active TB patients. TKT plays a key role in the switch from the dormancy to proliferative phase and TKT specific IgG may uncover the differences between active TB and LTBI. Thus, IgG-based serodiagnosis of TB with TKT-peptide ELISA is promising.

Currently, commercially available serological TB tests show poor sensitivity and specificity. The ELISA results obtained with the Wayne State team's discovered TKT peptides yielded high specificity and sensitivity. Their results show that IgG antibodies against transketolase can discriminate active tuberculosis.

"Our TKT peptide ELISA test requires chemically synthesized TKT peptides to coat the wells in the ELISA plate, less than 100µl blood serum sample from patient, detection reagents and an ELISA plate reader," said Samavati. "We are extremely enthusiastic about our technology and the fact that with a simple test we can differentiate active TB from LTBI and other respiratory diseases. We believe that our method and TKT peptide ELISA can fit the requirements of the World Health Organization and the Centers for Disease Control and Prevention as a POC screening method."

More information: Jaya Talreja et al, Discovery of Novel Transketolase Epitopes and the Development of IgG-Based Tuberculosis Serodiagnostics, *Microbiology Spectrum* (2023). DOI: 10.1128/spectrum.03377-22

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