BACTERIOPHAGE-BASED TESTS FOR TUBERCULOSIS

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Despite the enormous global burden of tuberculosis (TB), and the overall low rates of case detection, conventional TB diagnostics center on sputum microscopy and culture, tests that have been used for more than a 100 years. Although microscopy is simple, specific, and rapid, its low sensitivity is a major cause for concern, particularly in populations with high prevalence of HIV infection.1 Mycobacterial cultures are more sensitive and allow for the determination of drug susceptibility, but they are time consuming and not easily accessible.1 Conventional drug susceptibility tests (DST) include the absolute concentration, proportion, and resistance ratio methods. These tests are typically performed on cultures isolated from clinical specimens. Newer liquid media-based techniques (e.g. BACTEC® and MGIT® systems) are more rapid than agar-based methods.1 However, they are expensive, and require sophisticated laboratory infrastructure.

Substantial effort has been made to develop alternative tools for TB diagnosis and DST.1 Unfortunately, serological tests and nucleic acid amplification (NAA) assays have not lived up to their initial promise.1,2 Although molecular (genotypic) tests for DST (e.g. line probe assays, molecular beacons, DNA sequencing, and NAA tests) appear to be highly accurate,1 they are expensive and poorly suited for high burden settings. Recently, interferon-g assays have emerged as new immunodiagnostic tools.1 But these assays detect latent rather than active TB. Among the other alternatives, tests based on bacteriophages have shown some promise.4,5

Phage-based assays have been evaluated for diagnosis of TB, as well as DST.4,5 They use mycobacteriophages to infect live Mycobacterium tuberculosis and detect the bacilli using one of two methods. In the first method, the key principle is amplification of phages after their infection of M. tuberculosis, followed by detection of progeny phages as plaques on a lawn of M. smegmatis. In the second method, the principle is detection of light (using luminometry or photographic films) produced by luciferase reporter phages (LRP), phages with fire-fly luciferase genes inserted within their genome, after their infection of live M. tuberculosis.4,5 Amplification based assays are commercially available, whereas LRP tests are still under development. In general, phage assays have a turn around time of 48 hours, and require a laboratory infrastructure similar to that required for performing mycobacterial cultures.4

Phage-based assays are now available as commercial kits (e.g. FASTPlaque-TB® [Biotec Laboratories Ltd, Ipswich, UK], and PhageTek MB®, a name variant of FASTPlaque-TB) and as in-house (“home-brew” or laboratory-developed) assays.4,5 For diagnosis, phage assays are directly used on sputum specimens. Certain phage assays (e.g. FASTPlaque-TB-MDR®, previously named FASTPlaque-TB-RIF®) are designed to detect rifampicin resistance in culture isolates.4,5 Newer versions of this kit are being developed for the detection of drug resistance directly from sputum specimens (FASTPlaque-TB-Response®). Drug resistance is diagnosed when M. tuberculosis is detected in samples that contain the drug (e.g. rifampicin). When phage-based assays do not detect M. tuberculosis in drug containing samples, the strains are classified as drug-sensitive.4,5

What is the accuracy of phage-based assays and what role can they play in TB diagnosis and DST? In two previous meta-analyses, we summarized the accuracy of phage-based assays for the detection of M. tuberculosis in clinical specimens (i.e. for diagnosis), and accuracy of phage-based assays for detection of rifampicin resistance.7 Together, these meta-analyses synthesized data from 33 publications (with 34 studies, of which 4 studies were from India) on phage assays and offer the current best evidence on this topic. In this editorial, we summarize the key findings of these meta-analyses and their implications.

Data from the first meta-analysis on diagnosis6 (13 studies) suggest that phage-based assays have high specificity (range 83% to 100%), but modest and variable sensitivity (range 21% to 88%). Analyses of studies that directly compared phage tests with microscopy against culture as a common reference standard suggested that overall accuracy of phage-based assays is slightly higher than smear microscopy in head-to-head comparisons.6 Overall, it appears that phage-based assays have high specificity but lower and variable sensitivity.6 Their diagnostic performance characteristics, therefore, are fairly similar to microscopy.5 In contrast to microscopy, phage-based assays are more complex and resource-intensive and thus cannot be performed in field and primary-care settings. Another issue of concern is the potential for contamination and/or indeterminate results in the
phage assays. In all, phage assays, as of now, cannot replace conventional diagnostic tests such as microscopy and culture. Research is required to identify strategies to enhance the sensitivity of phage assays without compromising the high specificity.

Data from the meta-analysis on rifampin resistance showed that when performed on culture isolates (19 studies), phage assays appear to have relatively high sensitivity and specificity. Eleven of 19 (58%) studies reported sensitivity and specificity estimates in ≥95%. Specificity estimates were slightly lower and more variable than sensitivity; 5 of 19 (26%) studies reported specificity <90%. Only two studies performed phage assays directly on sputum specimens; although one study from South Africa reported sensitivity and specificity of 100% and 99%, respectively, another study from Pakistan reported sensitivity of 86% and specificity of 73%. Current evidence, therefore, is mostly restricted to the use of phage assays for the detection of rifampicin resistance in culture isolates. When applied to isolates, these assays have relatively high sensitivity and specificity. This is expected because of the large numbers of bacilli in the isolates. However, the need for primary isolation reduces the applicability of this assay. Further, specificity estimates appear to be more variable than sensitivity, and this can potentially result in over-diagnosis of drug resistant TB in low prevalence settings. In contrast to the evidence on phage assays when applied to culture isolates, data are lacking on the accuracy of these assays when they are directly applied to sputum specimens. As seen in the meta-analysis on diagnosis, when applied directly to clinical specimens, phage assays seem to have lower sensitivity, presumably because of the lower bacillary concentration. Phage-based assays can be directly used on clinical specimens and if they are shown to have high sensitivity and specificity, they have the potential to improve the diagnosis and management of drug resistant TB.

In conclusion, phage-based assays show some promise, particularly for the detection of rifampicin resistance, but currently not accurate enough to replace conventional TB diagnostics. Efforts are ongoing to improve their performance characteristics, especially for the rapid direct detection of rifampicin resistance in clinical specimens. These initiatives will help to better define the accuracy and clinical applicability of phage-based assays.

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References