Bacteriophage-based assays for the rapid detection of rifampicin resistance in Mycobacterium tuberculosis: a meta-analysis

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CRD summary
This review considered the accuracy of bacteriophage-based assays for the rapid detection of rifampicin-resistant tuberculosis. The authors concluded that the assay has high accuracy when used in culture isolates, but that there is a paucity of data on its accuracy when applied directly to sputum samples. This was a well-conducted review and these conclusions are likely to be reliable.

Authors' objectives
To evaluate the accuracy of bacteriophage-based assays for the rapid detection of rifampicin(RIF)-resistant tuberculosis (TB) in culture isolates and clinical specimens.

Searching
MEDLINE (1985 to 2004), EMBASE (1988 to 2004), BIOSIS Previews (1993 to 2004), the Science Citation Index (1985 to 2004), the Cochrane Library (2004), Web of Science (1985 to 2004) and Google Scholar (December 2005) were searched; the search terms were reported. Experts in the field and the manufacturers of the test kit were contacted for additional ongoing and unpublished studies. No language restrictions were applied.

Study selection
Study designs of evaluations included in the review
Diagnostic case-control or cross-sectional studies were eligible for inclusion.

Specific interventions included in the review
Studies evaluating the accuracy of the phage-based assays (either phage amplification or luciferase reporter phages) for detecting RIF-resistance in Mycobacterium tuberculosis in culture isolates or clinical specimens were eligible for inclusion. Both commercial tests (FASTPlaque-TB-RIF and FASTPlaque-TB-Response for phage-based assays) and in-house assays (D-29 phage-based) were used in the included studies. The included studies used different definitions of drug resistance.

Reference standard test against which the new test was compared
Studies that assessed the accuracy of the assay against a reference standard of any of the following drug susceptibility tests were eligible for inclusion: absolute concentration method, proportion method, resistance ratio method, radiometric BACTEC 460 method. Most of the included studies used BACTEC 460 or proportion methods as the reference standard test.

Participants included in the review
No inclusion criteria for the study participants were set.

Outcomes assessed in the review
Studies that assessed sensitivity and specificity were eligible for inclusion. Agreement between the results of the assay and the reference standard and the percentage of indeterminate results or contaminated samples were also reported.

How were decisions on the relevance of primary studies made?
Two reviewers independently screened all retrieved citations, with any disagreements resolved by consensus. The full texts of all relevant articles were examined.

Assessment of study quality
The methodological quality of the included studies was assessed using the following criteria: consecutive or random sampling; blind and independent comparison with the reference standard; partial or complete verification of test results using the reference standard. The quality assessment formed part of the data extraction process described below.

**Data extraction**

One reviewer extracted data from all of the included studies using a piloted data extraction form. A subset of one quarter of the included studies were independently data extracted by a second reviewer, and any disagreements were resolved by consensus. For each study, the sensitivity and specificity were calculated, along with 95% confidence intervals (CIs), from 2x2 contingency tables. Simple proportion agreement and kappa estimates were also calculated with 95% CIs. The reviewers used unresolved data where possible. Authors of the primary studies were contacted for additional information, when necessary.

**Methods of synthesis**

**How were the studies combined?**

Sensitivity and specificity data from individual studies were combined using summary receiver-operating characteristic (SROC) curves, fitted using the regression method of Moses et al., along with estimates of the Q* index and the area under the curve (AUC) and their standard errors (SEs).

**How were differences between studies investigated?**

Differences in the results between studies were illustrated using forest plots of sensitivity and specificity values with 95% CIs. The authors stated that they explored the effect on study results of variability in cut-points. Heterogeneity was explored using analyses stratified by assay method (phage amplification or luciferase reporter phage), source of assay (commercial or in-house) and source of sample (culture isolates or clinical samples). These subgroups were defined a priori.

**Results of the review**

Twenty-one studies with a total of 1,778 samples were included in the review. There were 14 studies of amplification-based assays and 7 studies of luciferase reporter phage assays.

Eight of the 21 included studies used random or consecutive sampling methods. Twelve studies reported blinded interpretation of either the phage-based assay or the reference standard. None of the studies had the potential for verification bias. The included studies reported the proportion of indeterminate results or contaminated samples: this ranged from zero to 17%.

**Accuracy of phage amplification assays (14 studies).**

Most studies used culture isolates; 2 studies directly applied FASTPlaque-TB assays to sputum specimens. For the commercial kits (8 studies), the sensitivity ranged from 81 to 100% and the specificity from 73% to 100%; the AUC, derived from the summary ROC curve, was 0.99 (SE=0.0105) and the Q* index was 0.95 (SE=0.0232). Of the 2 studies that assayed sputum samples directly, one reported sensitivity and specificity values of 100% and 99%, respectively, while the other reported a sensitivity of 86% and a specificity of 73%. For the in-house assays (6 studies), the sensitivity ranged from 97 to 100% and the specificity from 84 to 100%; the AUC, derived from the summary ROC curve, was 0.99 (SE=0.0016) and the Q* index was 0.98 (SE=0.0056).

**Accuracy of luciferase reporter phage assays (7 studies).**

All of these studies were of in-house assays applied to culture isolates. With the exception of one study, which had a sensitivity of 92%, all of the studies had sensitivity estimates of 100%. The specificity estimates ranged from 89 to 100%. The AUC, derived from the summary ROC curve, was 0.98 (SE=0.0042) and the Q* index was 0.95 (SE=0.0085).

**RIF resistance as a marker for multi-drug resistant TB (7 studies).**
Seven studies determined the number of RIF-resistant samples that were also isoniazid-resistant (the criteria for multi-drug resistant TB). On average, 96% of RIF-resistant samples were also isoniazid-resistant.

Authors’ conclusions
Phage-based assays appear to have relatively high sensitivity and specificity when applied to culture isolates; however, specificity estimates are relatively lower and more variable, with the resultant potential for over-diagnosis in low prevalence settings. There was a lack of evidence on the accuracy of phage-based assays applied directly to sputum specimens. Several issues need to be addressed before these assays can be applied in routine clinical practice.

CRD commentary
This review article had a clearly stated aim and relevant inclusion criteria. Extensive literature searches were reported, no language restrictions were applied, and attempts were made to minimise publication bias. Measures were taken to avoid the introduction of reviewer error or bias in the review process (study selection, validity assessment and data extraction). The methodological quality of the included studies was assessed using criteria relevant to diagnostic accuracy studies. The methods used to summarise the data were appropriate and clearly described. Data were appropriately stratified for analysis, although no formal test of heterogeneity was described. This was a well-conducted review, and the authors’ conclusions follow from the data presented and are likely to be robust.

Implications of the review for practice and research
Practice: The authors stated that phage-based assays may have a potential role in detecting RIF-resistance when applied to culture isolates. However, they stated that several issues need to be addressed before these assays can be applied in routine clinical practice: unexplained low estimates of sensitivity and specificity in some studies; the potential for contamination and indeterminate results; variability in the cut-points used to determine test validity; and drug resistance.

Research: The authors stated that additional studies are needed to establish the accuracy of phage-based assays when used directly on sputum specimens (an application that has the potential to produce significantly reduced diagnostic turnaround times). Future studies on phage-based assays should: employ consecutive or random sampling methods; apply blinded interpretation of both phage assay and reference standard results; include an adequate number of patients with confirmed RIF-resistance; report the cut-points used to determine test validity and drug resistance, as well as the drug concentrations used; report the proportion of indeterminate test results and the effect of their exclusion on accuracy measures; and provide data on the prevalence of non-tuberculosis mycobacteria and the effect of their isolation on phage assay specificity. Studies are also needed to determine the clinical usefulness of rapid diagnosis of RIF-resistant TB using a phage-based assay in terms of its effect on clinical outcomes and transmission rates. Finally, studies are needed to assess the cost-effectiveness of phage-based assays in comparison with conventional drug susceptibility testing.

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