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Brief Communication

RAPID DETECTION OF RIFAMPICIN RESISTANCE IN *M.TUBERCULOSIS* BY PHAGE ASSAY

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Abstract

Increase in multidrug-resistant *M.tuberculosis* (MDR-TB) has become a great cause of concern and rifampicin resistance is considered to be a good predictor of MDR-TB in many parts of the world. Its rapid detection will allow alteration in treatment regimens in time to reduce the spread of the disease. Detection of rifampicin resistance by phage assay is a useful tool as mycobacteriophages are specific for *M.tuberculosis complex* and detect viable cells only. In our study, we analyzed 85 samples for rifampicin resistance using a novel mycobacteriophage based test (Phage assay) and radiometric BACTEC 460 TB. Of the 85 samples, 70 (82.35%) were resistant and 12 (14.10%) were sensitive by both methods. Our study yielded a sensitivity and specificity of 100% and 80% respectively. A good correlation was observed with conventional LJ proportion method. We conclude that phage assay allows determination of rifampicin resistance within 48 hours from culture, reducing the time taken to define susceptibility results by BACTEC 460 TB and LJ proportion method (5-7 days and 6-8 weeks respectively).

Key words: *Rifampicin resistance, mycobacteriophages, M.tuberculosis*

Recent estimates of tuberculosis disease burden documented approximately 1.86 billion people infected with *M.tuberculosis* and 16.2 million cases of active disease.[1] Several publications have predicted approximately 10 million new tuberculosis cases annually by the year 2000 and 12 million by the year 2005.[2] The real threat to the success of national tuberculosis control programs globally, and the single most important risk factor for development of TB, is co-infection with human immunodeficiency virus (HIV). The problem is compounded by the rising incidence of drug resistance and particularly emergence of multidrug-resistant *M.tuberculosis* (MDR-TB).[3] MDR-TB is an emerging problem of great importance to public health. The fatality rates have increased to a greater extent as a result of MDR-TB than drug sensitive TB.[3]

Rifampicin and isoniazid remain the two most important drugs for treatment of tuberculosis and resistance to either drug represents a serious impediment to successful therapy.[4] Resistance to rifampicin exists at a rate of 1 in 10^8 bacilli,[5] the target being *rpo* gene, which encodes subunit of RNA polymerase. Rifampicin causes prokaryotic cell death by interacting with RNA polymerase and subsequently disrupting the process of transcription. Specific mutations in *rpo* gene diminishes the binding affinity of rifampicin for polymerase leading to drug resistance.[6]

Currently available methods for drug susceptibility testing include culture-based methods viz., LJ proportion method, absolute concentration method, radiometric BACTEC 460 TB, newer molecular methods like INNO-Lipa Rif-TB etc. The former two are slower and take months for results to arrive compared to radiometric BACTEC 460 TB.

Owing to the rampant spread of TB there is an urgent need of prompt and accurate diagnosis in addition to the requirement for rapid identification of drug resistant strains.[7] Phage assay, a novel mycobacteriophage based test allows determination of rifampicin resistance in strains of *M.tuberculosis* within 48 hours from culture, thus reducing the time to define susceptibility results from radiometric BACTEC 460 TB and conventional LJ proportion method which take 5-7 days and 6-8 weeks respectively. The assay is based on specific mycobacteriophages, which reflect presence of viable TB bacilli in drug containing sample compared with that in drug free control. On addition of these phages target bacteria are rapidly infected and on treatment with virucidal solution, all unadsorbed phages are killed. The only phages that remain are those, which are protected within viable TB bacilli. These phages will replicate and lyse the cells to release new progeny phages that are amplified by introduction of non-pathogenic fast growing *Msmegmatis*. Progeny phages undergo cycles of infection, replication and lysis, seen as clear areas (plaques) in a lawn of rapid growing cells.[8]

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In this study, we evaluated the use of phage assay for resistance to rifampicin drug and also compared it with radiometric BACTEC 460 TB and conventional LJ proportion method.

Materials and Methods

We analyzed 85 pre-grown clinical isolates of *M.tuberculosis* from BACTEC 460 TB which showed a growth index (GI) of about 500-800 for rapid detection of rifampicin resistance by the novel mycobacteriophage based test, phage assay.

Preparation of Controls

For positive control, serial dilutions upto 10^{-6} of rapidly growing *M.smegmatis* was prepared in phage medium. 1 mL of this final dilution was taken as positive control. Uninoculated phage medium (1 mL) was taken as a negative control.

Phage assay

The phage assay was performed using Fast Plaque TB-Rif [Biotec Laboratories Limited, UK] according to the manufacturer's instructions.

BACTEC 460 TB

Rifampicin susceptibility was performed using radioactive BACTEC 460 TB when GI reached 500 - 800, the final concentration of the drug being 2 g/mL as previously described by Siddiqi.[9]

LJ proportion Method

Susceptibility testing to rifampicin was also performed by LJ proportion method (simplified variant) as previously described by Canetti *et al*,[10] the final concentration of the drug being 40g/mL.

Interpretation of Results

If the strain was resistant to rifampicin, its viability remained unaffected and it supported phage replication and resulted in plaque formation. On the other hand, if it was sensitive, its viability was affected and it did not support phage replication. Phage assay results were interpreted by number of plaques on RIF + plates as resistant (50 plaques), susceptible (10 plaques), or intermediate (11 - 49 plaques).

Results

After exposure of over 24 - 48 hours, plaque counts were obtained in phage assay. The results were compared with BACTEC 460 TB and LJ proportion method. The results are summarized in tables 1 and 2.

Table 1: Rifampicin susceptibility by Phage assay and BACTEC 460 TB

		Bactec 460 TB (n = 85)	
		Resistant	*Sensitive*
Phage assay (n=85)	*Resistant*	*70*	*0*
	Sensitive	*3*	*12*

Sensitivity = 100%, Specificity = 80%, Accuracy = 96.47%

Table 2: Rifampicin susceptibility by Phage assay and LJ proportion method

		LJ Proportion method (n=85)	
		Resistant	*Sensitive*
Phage assay (n=85)	*Resistant*	*70*	*0*
	Sensitive	*3*	*12*

Sensitivity = 100%, Specificity = 80%, Accuracy = 96.47%

The comparison of phage assay and BACTEC 460 TB is shown in table 1. Concordance was observed in 82 strains of 85 tested. In table 2, phage assay results were compared with LJ proportion method. Phage assay identified the strains that were resistant and sensitive correctly in all 82 cases. Three isolates out of 85 (3.53%) were sensitive by phage assay but were resistant by BACTEC 460 TB. Drug susceptibility testing of these three strains was performed using LJ proportion method by which they were confirmed as resistant.

Discussion

Resistance to rifampicin is often associated with resistance to other anti-tuberculosis drugs, and strains resistant to atleast both rifampicin and isoniazid are classified as MDR.[11] For this reason, rifampicin resistance is identified as a good predictor of MDR-TB in many parts of the world.[12] Due to growing demand for rapid diagnostic and susceptibility of tuberculosis we analyzed rifampicin resistance by phage assay and compared the results with radiometric BACTEC 460 TB and LJ proportion method.

Our results show that overall sensitivity, specificity and accuracy of phage assay compares well with BACTEC as well as LJ method. In the three cases where phage assay differed, radiometric BACTEC 460 TB and LJ proportion methods were in concordance. The discrepancies may be due to improperly homogenised suspension or low inoculum. The contents of the pre-grown culture vial (from BACTEC vial) have to be properly homogenised with a hypodermic syringe and a needle. Homogenisation enables breaking the clumps

of *M.tuberculosis* growth thus facilitating proper dispersion of organisms in the medium. If not properly homogenised, there are fair chances of less inoculum or larger clumps being pipetted out for the assay, both of which will lead to misinterpretation of the assay results. Use of low inoculum will mean that less number of viable organisms will be infected and less number of plaques will be visible for enumeration. Our results are in accordance with other studies.[12,13] Phage assay, because of its high sensitivity and specificity coupled with its cost effectiveness, could be used as a useful diagnostic tool. This assay was designed by Heidi *et al*[12] for developing countries due to its low cost and simplicity of use, which are not shared by newer techniques like BACTEC.

The results of phage assay were achieved more rapidly than the other methods (48 hours compared to 5-7 days for BACTEC 460 TB and 4-6 weeks for LJ method). Conventional culture based methods take several days for the results from receipt of the initial specimen which results in significant delay in receiving appropriate treatment for patients with MDR-TB. Due to rapid availability of the results, phage assay can be used as a diagnostic setting. On the other hand, phage assay utilizes basic microbiological equipment easily available in the laboratory, and does not require use of needles and syringes, thus reducing risk to laboratory personnel. Additional advantage of phage assay relates to safety i.e., during the course of the assay, a large number of percentage of *M.tuberculosis* bacilli are rendered non-infective through lysis by mycobacteriophages. This is in contrast to other rapid culture techniques in which the number of infective particles substantially increases.

This study shows a good correlation between phage assay and gold standard method for susceptibility testing. Phage assay has a potential to be used as a global assay for drug susceptibility testing in developed and developing countries.[13] Thus, phage assay allows the clinician to initiate timely and appropriate treatment enabling a positive impact on individual patient outcome and on spread of MDR-TB.

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