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Combination of the MODS Assay with the Sensititre™ MYCOTB Plate for Rapid Detection of MDR- and XDR-TB

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Abstract

We combined the new Sensititre™ MYCOTB test with the MODS assay for detection of MDR- and XDR-TB. Categorical agreement of the MODS assay with the critical concentrations at 3 days of incubation was highest for INH (91.4%) and RIF (100%) and at 5 days 86.7% and 94.6% for the fluoroquinolones and aminoglycosides, respectively. By combining these two methods, it is possible to identify MDR-TB in as little as 3 days and XDR- or pre-XDR-TB within 5 days.

Keywords

Tuberculosis, Susceptibility Testing, MODS, MYCOTB, MDR-TB, XDR-TB

1. Introduction

Multidrug resistant tuberculosis (MDR-TB), extensively drug resistant (XDR-TB), and more recently, pre-XDR-TB threaten public health worldwide [1] [2]. MDR-TB is defined as TB resistant to isoniazid (INH) and rifampin (RIF) whereas XDR-TB is defined as TB resistant to INH and RIF plusany fluoroquinolone and at least one of three injectable second-line drugs (amikacin, kanamycin, or capreomycin). Pre-XDR TB is a relatively new category defined as TB resistant to INH and RIF and either a fluoroquinolone or a second-line injectable agent but not both. These threats are the largest in developing countries with limited resources. Unfortunately, drug susceptibility testing (DST) for detection of XDR-TB and pre-XDR-TB are not routinely performed in many settings in which TB is endemic.

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A relatively new susceptibility assay, the Sensititre™ MYCOTB plate (TREK Diagnostics), has been developed which provides for susceptibility testing of *Mycobacterium tuberculosis* (MTB) against twelve first- and second-line drugs in a 96-well, microtiter broth format. Drugs are lyophilized in the plates in a range of concentrations and are stable for up to 2 years at ambient temperature [3] [4]. Assay endpoints provide an actual minimum inhibitory concentration (MIC) versus testing of one or two critical concentrations typically used for other broth-based MTB susceptibility testing platforms. Determination of susceptibility or resistance is achieved by comparing the MYCOTB plate MIC to established critical concentrations for each drug using the following criteria: resistance to a given agent is indicated if the MIC result is greater than the broth-based, critical concentration; susceptibility is indicated if the MIC is less than or equal to the critical concentration. Recent studies have confirmed the performance characteristics of the MYCOTB plate assay including categorical agreement predictive of susceptibility or resistance which ranged from 96% to 100% for three first-line drugs, and 94% to 100% for nine second-line drugs. In addition, the sensitivity, specificity and precision of the MYCOTB plate assay were comparable to other commercially available susceptibility testing platforms [3] [4]. Reconstitution of the MYCOTB plates is done using a suspension of MTB in broth media followed by incubation and manual examination of the wells for growth using a mirror box and ambient light [3] [4]. Reading of the plates typically requires an average of 14 days for development of adequate growth in the wells visible to the naked eye. Such visible growth is necessary for accurate interpretation of results. Earlier visualization of growth in these plates using a more sensitive method than the naked eye, such as inverted light microscopy, would permit more rapid detection of MDR-, XDR- and pre-XDR-TB. Such a method has been in use for many years in resource-limited regions of the globe with a high burden of TB and is commonly known as the Microscopic Observation Drug-Susceptibility (MODS) assay [5] [6]. This assay involves cultivation of MTB in broth media either from growth in culture (indirect MODS) or from patient specimens (direct MODS) plus and minus various antibiotics such as INH and RIF with subsequent visualization of growth using an inverted light microscope [5] [6]. Use of an inverted light microscope permits visualization of MTB significantly earlier than can be done with the naked eye [5] [6]. Even though the MODS assay has proven to be an effective susceptibility test method, there are limitations to its use. Preparation of plates is labor intensive and requires quality control of the antibiotics being used [5] [6]. In addition, many laboratories do not have access to all first-and second-line drugs, thus only INH and RIF are typically tested [5] [6]. As a result, MDR-TB can be detected, whereas, XDR-TB or pre-XDR-TB may be missed.

In this study, we combined the MODS assay with the MYCOTB plate to assess the feasibility of using the two methods together. We postulated that application of the MODS assay, *i.e*. use of an inverted light microscope to examine the drug-containing and control wells in the MYCOTB plate, would permit more rapid determination of an MIC in ≤5 days versus the standard MYCOTB plate protocol which requires an average of 14 days for visualization of growth with the naked eye. Early determination of an MIC could then be compared to established critical concentrations for each drug to predict susceptibility or resistance to a given agent.

2. Methods

A total of 75 MTB strains were used for this study including clinically derived susceptible (n = 36), mono-resistant (n = 15), MDR-(n = 9), pre-XDR-(n = 3), and XDR-TB (n = 1). Eleven strains were resistant to 2 or more drugs, but by definition were neither MDR- nor XDR-TB. H37Rv (American Type Culture Collection, 27294) was used as the susceptible control isolate. Drug susceptibility testing (DST) was performed according to the manufacturer's instructions for use of the MYCOTB plates (Thermo Fisher Scientific, TREK Diagnostic Systems, Oakwood Village, Ohio) [7]. Susceptibility testing of all strains used in this study was initially done using the standard MYCOTB assay (S-MYCOTB), previously validated in our laboratory using agar proportion as the reference method for all first- and second-line TB drugs. Drugs and concentrations contained in the MYCOTB plates are as follows: ofloxacin (OFL, 0.25 µg/ml to 32 µg/ml), moxifloxacin (MOX, 0.06 µg/ml to 8 µg/ml), RIF(0.12 µg/ml to 16 µg/ml), amikacin (AMI, 0.12 µg/ml to 16 µg/ml), streptomycin (STR, 0.25 µg/ml to 32 µg/ml), rifabutin (RBT, 0.12 µg/ml to 16 µg/ml), *p*-aminosalicyclic acid (PAS, 0.5 µg/ml to 64 µg/ml), ethionamide (ETH, 0.3 µg/ml to 40 µg/ml) cycloserine (CYC, 2 µg/ml to 256 µg/ml), INH (0.03 µg/ml to 4 µg/ml), kanamycin (KAN, 0.6 µg/ml to 40 µg/ml), and ethambutol (EMB, 0.5 µg/ml to 32 µg/ml). Susceptibility profiles for all MDR-, XDR-, and pre-XDR-TB strains tested are presented in **Table 1**. All strains were maintained on Lowenstein-Jensen agar slants (Becton Dickinson, Sparks, Maryland) in an atmosphere of 5% CO_2 at 37˚C. For the S-MYCOTB assay, all plates were incubated at 37˚C and examined for growth (turbidity in

Table 1. Susceptibility profiles for MDR/XDR-TB/pre-XDR-TB strains used in this study.

MTB Strain	Drug Susceptibility Profile										
	INH	RIF	EMB	STR	AMI	KAN	OFL	MOX	ETH	PAS	RBT
37, 58	R	R	R	R					R		R
48	R	R		R					R		
52	R	R									R
53	R	R		R	R	R					R
67	R	R	R	R	R	R	R		R		R
68, 71	R	R	R	R							R
70, 74	R	R	R								R
72	R	R	R	R	R	R					R
73	R	R		R							R
75	R	R		R	R	R			R		R

the wells) using a mirror box per the manufacturer's instructions on days 10, 14 and 21 [7]. MICs for the S-MYCOTB assay were recorded when control wells contained sufficient growth as to be easily visible with the mirror box (typically on day 14) and were defined as the lowest concentration of each drug which completely inhibited growth [5]-[7]. For the combined MODS-MYCOTB assay (M-MYCOTB), plates were read with an inverted light microscope (Olympus CK2) using the 20X objective on days 1 through 10 to determine the earliest time-point each drug could be read which correlated with results obtained using the S-MYCOTB assay. Growth in the positive control wells on each day was compared to growth in the drug-containing wells and the M-MYCOTBMIC was defined as the well with the lowest concentration of drug exhibiting nearly complete inhibition (>80%) versus the untreated controls. All MODS-based MICs were recorded and compared to those obtained with the S-MYCOTB plate protocol for days 10, 14, or 21. Susceptibility or resistance for each strain-drug combination was determined by comparing individual MICs from both the S-MYCOTB and M-MYCOTB methods with critical concentrations for each drug established with commercial shorter incubation liquid media systems such as the Mycobacterial Growth Indicator Tube (MGIT 960, Becton Dickinson, Sparks, Maryland) [8]. Strains were considered resistant if the MIC result was greater than the broth-based, critical concentration; susceptible if the MIC was less than or equal to the critical concentration. For the M-MYCOTB assay, this process was repeated and the optimal time-point determined for each individual drug and first- and second-line drugs considered together. All assays were performed in duplicate and all plates for both the S-MYCOTB and M-MYCOTB methods were blinded and examined by two independent technologists.

3. Results

Overall agreement for determination of an MIC (±1 doubling dilution) using the S-MYCOTB protocol and the M-MYCOTB assay was 91.1%. However, this agreement did vary by time-point and drug. For instance, overall agreement on Days 3, 4, 5, 7, and 10 was 79.5%, 86.5%, 93.8%, 94.9%, and 99.7%, respectively. Overall agreement was highest for INH (94%), RIF (92.5%), RFB (93.5%), AMI (95.2%), STR (91.6%), and KAN (95.9%). For the FQ's, MOX and OFL, agreement was 84.3% and 86.2%, respectively. Similar agreement was observed for ETH (88.7%), EMB (88.7%), and CYC (85.0%). The lowest agreement was noted for PAS (73.9%).

Categorical agreement of the M-MYCOTB assay with the critical concentrations, indicative of susceptibility or resistance is varied by drug and time-point (**Table 2**). On Day 3 of incubation, agreement was highest for INH (91.4%) and RIF (100%). For the remaining drugs at the same time-point, including the fluoroquinolones (FQ), MOX and OFL, and the aminoglycosides (AG), AMI and KAN, agreement ranged from 52.3% to 93.1%, respectively. However, by Day 5, categorical agreement with the critical concentrations for the FQ improved to 86.7% and for the AG, 94.6%. Two drugs, PAS and ETH, were difficult to read at both time-points with concordance at 3 and 5 days of 40.8% and 78.7%, and 79.2% and 85.8%, respectively. By Day 7, categorical agreement had increased to ≥92.5% for all drugs tested (**Table 2**). Importantly, at the same time-points of 3, 4, 5 and 7 days, no growth could be seen in any of the wells including the untreated controls when visually examined using the mirrorbox. Thus visual interpretation of the plates for the S-MYCOTB assay was not possible at these early time-points and required an average of 14 days of incubation.

Table 2. Percent agreement for the M-MYCOTB assay versus critical concentrations predictive of susceptibility or resistance.

Drugs	Overall	Day 3	Day 4	Day5	Day 7	Day
INH	97.6%	91.4%	97.1%	99.4%	100%	100%
RIF	100%	100%	100%	100%	100%	100%
OFL	83.0%	57.5%	68.8%	87%	98.9%	100%
MXF	81.5%	52.3%	69.6%	86.3%	98.3%	98.9%
AMI	96.0%	93.1%	96.4%	96.4%	97.1%	97.1%
STR	87.7%	77.0%	83.3%	88.7%	93.7%	94.8%
KAN	90.7%	90.9%	91.7%	93%	93.1%	93.7%
EMB	92.9%	85.1%	92.0%	95.2%	96.6%	95.4%
RFB	95.7%	91.5%	94.2%	96.4%	97.7%	97.7%
ETH	87.9%	78.7%	89.1%	85.8%	92.5%	92.5%
PAS	75.8%	40.8%	63.8%	79.2%	94.8%	92.5%

4. Discussion

Early detection of MDR- and XDR-TB is essential for effective clinical management of active TB and initiation of appropriate treatment. Rapid, inexpensive diagnostic assays are thus necessary to provide for timely diagnosis and prevention of transmission. The MODS assay is simple to perform and only requires the use of an inverted light microscope to visualize growth days earlier than can be done with the naked eye. For this reason, the MODS assay is currently used in many TB endemic, resource-poor settings [3] [4]. Limitations include too few drugs in the plates, especially with regard to second-line agents, the labor required for preparation, and issues related to quality assurance. Until now, no commercial platform was available which provided the number of anti-TB drugs contained in the MYCOTB plates. Determination of an MIC with subsequent comparison to established critical concentrations for each drug with other broth-based methods provides for determination of categorical agreement for the combined (M-MYCOTB) assay. By combining these two methods, it is possible to identify MDR-TB in as little as 3 days and XDR- or pre-XDR-TB within 5 days. The faster turn-around-time and concomitant testing of 1st and 2nd-line drugs in a single platform provides for more rapid detection of MDR-, XDR-, and pre-XDR-TB, resulting in more targeted treatment regimens, decreased morbidity and mortality, and improved TB control.

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