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Sensititre MycoTB Plate Compared to Bactec MGIT 960 for First- and Second-Line Antituberculosis Drug Susceptibility Testing in Tanzania: a Call To Operationalize MICs

Scott K. Heysell,[a] Suporn Pholwat,[a] Stellah G. Mpagama,[b] Saumu J. Pazia,[c] Happy Kumburu,[c] Norah Ndusilo,[c] Jean Gratz,[a] Eric R. Houpt,[a] Gibson S. Kibiki[c]

Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, Virginia, USA[a]; Kibong'oto Infectious Diseases Hospital, Sanya Juu, Tanzania[b]; Kilimanjaro Clinical Research Institute, Moshi, Tanzania[c]

MIC testing for *Mycobacterium tuberculosis* is now commercially available. Drug susceptibility testing by the MycoTB MIC plate has not been directly compared to that by the Bactec MGIT 960. We describe a case of extensively drug-resistant tuberculosis (XDR-TB) in Tanzania where initial MIC testing may have prevented acquired resistance. From testing on archived isolates, the accuracy with the MycoTB plate was >90% for important first- and second-line drugs compared to that with the MGIT 960, and clinically useful quantitative interpretation was also provided.

Among other factors, the total numbers of drugs and drug class to which a *Mycobacterium tuberculosis* isolate is resistant predict the treatment outcomes (1, 2). Despite the advantage of rapid susceptibility testing results, the molecular mechanisms of drug resistance remain incomplete for many drugs used in the treatment of multidrug-resistant tuberculosis (MDR-TB) (3). Phenotypic methods rely on mycobacterial cultures, but the testing is ultimately qualitative, reporting a threshold of growth in the presence of a single "critical" drug concentration. In contrast to the quantitative results used in the testing of other rapidly growing pathogens, the critical concentrations for *M. tuberculosis* are based in part on the epidemiological breakpoints, which for some drugs are very near the MIC (4). The World Health Organization (WHO) endorsed phenotypic methods for drug susceptibility testing (DST), including the agar proportion method (APM) on solid medium and an automated system in liquid medium, the Bactec MGIT 960 (BD, Franklin Lakes, NJ, USA).

To overcome the prior limitations with MIC testing, a commercially available microtiter plate of lyophilized antituberculosis drugs (Sensititre MycoTB; Trek Diagnostics, Cleveland, OH, USA) was introduced (5). Prior comparisons of the MycoTB plate with the APM on Middlebrook agar found excellent agreement for most drugs (>94%) (5–7). To our knowledge, the MycoTB plate has not been compared to the Bactec MGIT 960.

The Kilimanjaro Clinical Research Institute in Moshi, Tanzania, supports a biosafety level 3 facility for *M. tuberculosis* research and receives study specimens from the national MDR-TB hospital, the Kibong'oto Infectious Diseases Hospital (KIDH). We hence compared DST results from the MycoTB plate and the Bactec MGIT 960 using archived specimens and now describe an illustrative clinical case from the KIDH.

Isolates were originally obtained from patients with known or suspected MDR-TB who had provided written informed consent for a protocol approved by the institutional review boards of the Tanzania National Institute for Medical Research and the University of Virginia. The *M. tuberculosis* complex was identified by Gen-Probe (San Diego, CA). Molecular testing results for *rpoB* or *inhA* and *katG* mutations by the Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) and the GenoType MTBDRplus (Hain Lifescience, Nehren, Germany), respectively, were recorded if the tests were performed for clinical purposes. In previous studies, the MIC plates were tested in duplicate with excellent agreement and rare discrepancies within one dilution (7). Thus, single MIC plates were inoculated as previously detailed from growth of *M. tuberculosis* on Lowenstein-Jensen agar (5) and read manually by the use of an inverted mirror at 10 and 21 days by two independent technicians. As reported previously, DST by the Bactec MGIT 960 was performed for all drugs, except amikacin and cycloserine (8, 9), on the MycoTB plate. The MGIT 960 was the only phenotypic DST comparator within the research lab, and second-line DST was available for only a subset of isolates. The sensitivity, specificity, and accuracy for the MycoTB plate were calculated using the MIC breakpoints reported for both the APM and the MGIT 960 when applicable (see Table 2). Tests with discordant results were not repeated.

Comparative DST was performed for isoniazid (INH), rifampin, ethambutol, and streptomycin for 95 isolates, while 46 isolates were tested for rifabutin, ofloxacin, moxifloxacin, kanamycin, ethionamide, and *p*-aminosalicylic acid (PAS). For all drugs for which the critical concentrations differed between the APM breakpoint and the MGIT 960 breakpoint, the accuracy was superior when the APM breakpoint was used except for isoniazid (near equivalence) and for PAS (the MGIT 960 breakpoint was superior) (Table 1).

Of the eight isolates that were resistant to isoniazid by the

Received 11 May 2015 **Returned for modification** 27 June 2015
Accepted 20 August 2015

Accepted manuscript posted online 24 August 2015

Citation Heysell SK, Pholwat S, Mpagama SG, Pazia SJ, Kumburu H, Ndusilo N, Gratz J, Houpt ER, Kibiki GS. 2015. Sensititre MycoTB plate compared to Bactec MGIT 960 for first- and second-line antituberculosis drug susceptibility testing in Tanzania: a call to operationalize MICs. Antimicrob Agents Chemother 59:7104–7108. doi:10.1128/AAC.01117-15.

Address correspondence to Scott K. Heysell, skh8r@virginia.edu.

E.R.H. and G.S.K. contributed equally to this work.

TABLE 1 Comparative susceptibility testing by the MycoTB MIC plate and the MGIT 960 method

MycoTB plate MIC for medication (breakpoint)[a]	No. MGIT 960 resistant	No. MGIT 960 susceptible	Sensitivity (%)	Specificity (%)	Accuracy (%)
First-line drugs ($n = 95$)					
Isoniazid (0.12 μg/ml)			93.8	73.3	90.5
Resistant	75	4			
Discordant (μg/ml)[b]		0.13 (0.25, 4.0)			
Susceptible	5	11			
Discordant (μg/ml)[b]	0.06 (≤0.03, 0.12)				
Isoniazid (0.25 μg/ml)			90.0	86.7	89.5
Resistant	72	2			
Discordant (μg/ml)[c]		2.0, 4.0			
Susceptible	8	13			
Discordant (μg/ml)[b]	0.09 (≤0.03, 0.25)				
Rifampin (1.0 μg/ml)			95.7	92.0	94.7
Resistant	67	2			
Discordant (μg/ml)[c]		>16.0, >16.0			
Susceptible	3	23			
Discordant (μg/ml)[b]	1.0 (1.0, <0.12)				
Streptomycin (1.0 μg/ml)			90.5	64.1	75.7
Resistant	38	19			
Discordant (μg/ml)[b]		4.0 (2.0, 32.0)			
Susceptible	4	34			
Discordant (μg/ml)[b]	1.0 (0.5, 1.0)				
Streptomycin (2.0 μg/ml)			88.1	77.3	82.1
Resistant	37	12			
Discordant (μg/ml)[b]		20.0 (4.0, 32.0)			
Susceptible	5	41			
Discordant (μg/ml)[b]	1.0 (0.5, 4.0)				
Ethambutol (4.0 μg/ml)			72.0	68.1	69.1
Resistant	18	22			
Discordant (μg/ml)[b]		8.0 (8.0, 32.0)			
Susceptible	7	47			
Discordant (μg/ml)[b]	2.0 (1.0, 4.0)				
Ethambutol (8.0 μg/ml)			20.0	97.1	76.6
Resistant	5	2			
Discordant (μg/ml)[c]		16.0, 32.0			
Susceptible	20	67			
Discordant (μg/ml)[b]	8.0 (1.0, 8.0)				
Second-line drugs ($n = 46$)					
Rifabutin (0.5 μg/ml)			96.4	38.9	73.9
Resistant	27	11			
Discordant (μg/ml)[b]		2.0 (1.0, 16.0)			
Susceptible	1	7			
Discordant (μg/ml)	0.25				
Ofloxacin (2.0 μg/ml)			NA[d]	95.7	95.7
Resistant	0	2			
Discordant (μg/ml)[c]		4.0, 4.0			
Susceptible	0	44			
Moxifloxacin (0.25 μg/ml)			0	86.7	84.8
Resistant	0	6			
Discordant (μg/ml)		1.0 (0.5, 2.0)			
Susceptible	1	39			
Discordant (μg/ml)	0.12				
Moxifloxacin (0.5 μg/ml)			0	93.3	91.3
Resistant	0	3			
Discordant (μg/ml)[b]		2.0 (2.0, 2.0)			
Susceptible	1	42			
Discordant (μg/ml)	0.12				
Kanamycin (2.5 μg/ml)			NA	95.7	95.7
Resistant	0	2			
Discordant (μg/ml)[c]		5.0, 5.0			
Susceptible	0	44			

(Continued on following page)

TABLE 1 (Continued)

MycoTB plate MIC for medication (breakpoint)[a]	No. MGIT 960 resistant	No. MGIT 960 susceptible	Sensitivity (%)	Specificity (%)	Accuracy (%)
Kanamycin (5.0 μg/ml)					
Resistant	0	0	NA	100	100
Susceptible	0	46			
Ethionamide (5.0 μg/ml)			46.2	84.8	73.9
Resistant	6	5			
Discordant (μg/ml)[b]		20.0 (10.0, 40.0)			
Susceptible	7	28			
Discordant (μg/ml)[b]	1.2 (0.6, 5.0)				
PAS (2.0 μg/ml)			0	95.6	93.5
Resistant	0	2			
Discordant (μg/ml)[c]		4.0, 4.0			
Susceptible	1	43			
Discordant (μg/ml)	0.5				
PAS (4.0 μg/ml)			0	100	97.8
Resistant	0	0			
Susceptible	1	45			
Discordant (μg/ml)	0.5				

[a] "Breakpoint" is the breakpoint for susceptibility (the MIC on the MycoTB plate). Where the critical concentrations differed between the MGIT 960 and the agar proportion method (APM), both are reported (the lesser concentration is for the MGIT 960, except for *p*-aminosalicylic acid [PAS]). Cycloserine and amikacin are reported for the MycoTB plate, but tests were not performed by the MGIT 960.
[b] For discordant results of four or more isolates, the MIC data are reported as median (minimum, maximum) values.
[c] When three or fewer isolates were discordant, all MIC values are displayed and separated by commas.
[d] NA, not available.

MGIT 960 but susceptible by the MycoTB plate using the INH breakpoint of 0.25 μg/ml (MICs of ≤0.03, 0.06, 0.06, 0.06, 0.12, 0.25, 0.25, and 0.25 μg/ml), only two were tested by molecular DST and both were *katG* mutated (MICs of ≤0.03 and 0.25 μg/ml). Two isolates were susceptible to isoniazid by the MGIT 960 but resistant by the MycoTB plate (MICs of 2.0 and 4.0 μg/ml), and the one isolate tested was found to have a *inhA* mutation only (MIC of 2.0 μg/ml).

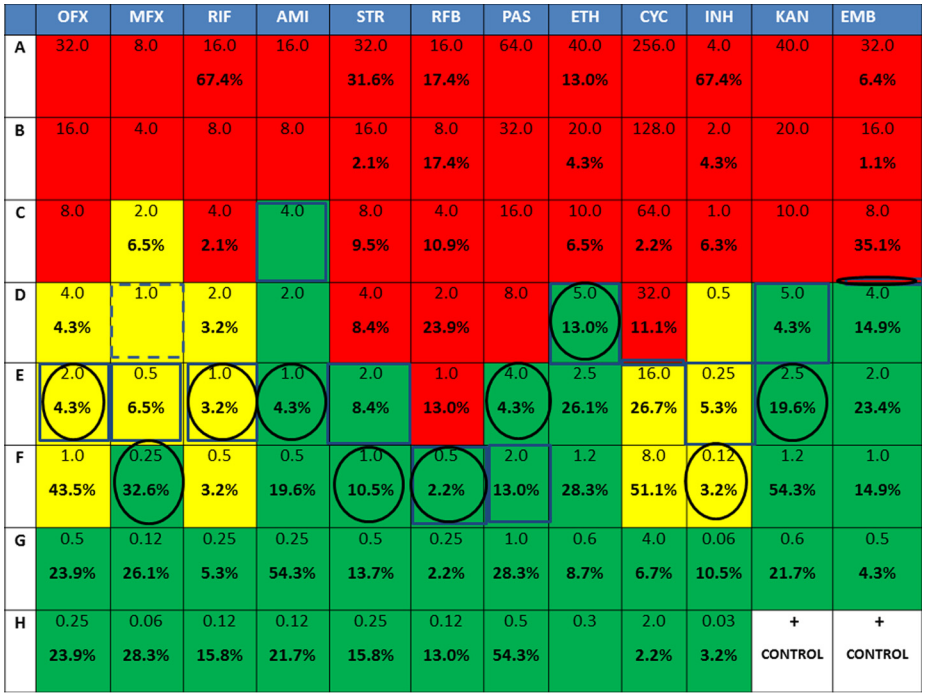

FIG 1 MycoTB plate with proportions of isolates (percentages in bold) distributed at each MIC. Colors indicate the following: green, susceptible; yellow, borderline; red, resistant. Borderline shading is plus or minus one dilution around the agar proportion breakpoint for medications where a dose increase could be tried or for cycloserine where the dose could be reduced when an isolate is highly susceptible. The squares with solid blue outlines are the susceptibility breakpoints by agar proportion, and a narrow rectangle is drawn when this breakpoint falls between the recorded MICs (for ethambutol and cycloserine). The square with a dashed blue outline represents the proposed breakpoint for moxifloxacin according to Lee et al. (7). The solid black circles cover breakpoints by the MGIT 960. OFX, ofloxacin; MFX, moxifloxacin; RIF, rifampin; AMI, amikacin; STR, streptomycin; RFB, rifabutin; PAS, *p*-aminosalicylic acid; ETH, ethionamide; CYC, cycloserine; INH, isoniazid; KAN, kanamycin; EMB, ethambutol.

Of the three isolates that were resistant to rifampin by the MGIT 960 but susceptible by the MycoTB plate, all were *rpoB* mutated but two had MICs at the breakpoint (MICs of <0.12, 1.0, and 1.0 μg/ml). The two isolates susceptible to rifampin by the MGIT 960 but resistant by the MycoTB plate both had MICs of >16.0 μg/ml, and one was *rpoB* mutated, while the other was the wild type.

Two isolates were resistant to ofloxacin by the MycoTB plate (both with MICs of 4.0 μg/ml), and three were resistant to moxifloxacin by the MycoTB plate (all with MICs of 2.0 μg/ml). Lee et al. had suggested the moxifloxacin breakpoint of 1.0 μg/ml by receiver operating characteristic (ROC) analysis (7). Employing this breakpoint for moxifloxacin, we found that all isolates resistant to the fluoroquinolones by the MycoTB plate but susceptible by MGIT 960 were only one dilution above the breakpoint. The MICs for other medications such as ethambutol were found to cluster around the APM breakpoint (plus or minus one dilution) (Fig. 1). As such, the figure was colored to reflect how a clinician may act upon a MIC result to treat a patient with MDR-TB within a resource-limited setting. "Borderline" results might trigger a dose increase (e.g., fluoroquinolones). For other medications, adherence to the susceptible and resistant categorization only is prudent, given the dose-related toxicities (e.g., aminoglycosides) or the existence of similarly potent alternatives (e.g., ethambutol).

In a clinical case, a 30-year-old man who was HIV negative and had had prior episodes of TB treatment was admitted to the KIDH after his isolate was found to be resistant to isoniazid, rifampin, and streptomycin but susceptible to ofloxacin and kanamycin by the APM at the national laboratory. He was started on levofloxacin, kanamycin, ethionamide, cycloserine, and pyrazinamide. The patient remained culture positive after 6 months. At this time, repeat DST was performed on a pretreatment isolate by the MGIT 960 and MycoTB plate, and it was found to be susceptible to ofloxacin and kanamycin by the MGIT 960 but with an ofloxacin MIC of 4.0 μg/ml (one dilution higher than the proposed breakpoint) and high-level resistance to ethionamide (Table 2). DST from the isolate obtained after 6 months of treatment revealed resistance to ofloxacin and kanamycin by the MGIT 960 with correspondent increases in MICs. The patient is currently culture negative while being treated with an extensively drug-resistant (XDR)-TB regimen informed by the MIC testing.

Given these findings, we call for rigorous study of the application of MIC testing to individualize MDR-TB regimens. While accuracy for the MycoTB plate for certain drugs was similar to that for conventional qualitative DST (5–7), in this case compared against the MGIT 960, our interpretation was limited by the presence of only a few isolates with fluoroquinolone resistance and none with aminoglycoside resistance. As such, we did not perform additional ROC analyses to corroborate breakpoints proposed by others (7). Additional limitations include the lack of simultaneous comparison to the APM and sequencing for drug resistance mutations.

Nevertheless, we view the discordance among the DST methods observed here as expected for routine practice (10) and instead welcome the reporting of MIC results within a clinically actionable borderline range (Fig. 1). In our prior studies of plasma drug activity among TB patients at the KIDH, we have demonstrated that low circulating drug concentrations are common and, relative to increased MICs, can lead to poor activity *in vitro* and a possible negative impact on the treatment outcome (11, 12).

TABLE 2 Drug susceptibility testing by different methodology in a case of acquired drug resistance while on a standardized MDR-TB regimen

Medication[a]	Pretreatment	During treatment
Ofloxacin		
APM	Susceptible	NA[b]
MGIT 960	Susceptible	Resistant
MIC (breakpoint, 2.0 μg/ml) (μg/ml)	4.0	8.0
Moxifloxacin		
APM	NA	NA
MGIT 960	Susceptible	Resistant
MIC (breakpoint, 1.0 μg/ml) (μg/ml)	2.0	4.0
Kanamycin		
APM	Susceptible	NA
MGIT 960	Susceptible	Resistant
MIC (breakpoint, 5.0 μg/ml) (μg/ml)	1.2	20.0
Amikacin		
APM	NA	NA
MGIT 960	NA	NA
MIC (breakpoint, 4.0 μg/ml) (μg/ml)	0.25	>16.0
Ethionamide		
APM	NA	NA
MGIT 960	Resistant	Resistant
MIC (breakpoint, 5.0 μg/ml) (μg/ml)	40.0	10.0
p-Aminosalicylic acid		
APM	NA	NA
MGIT 960	Susceptible	Susceptible
MIC (breakpoint, 2.0 μg/ml) (μg/ml)	≤0.5	2.0
Cycloserine		
APM	NA	NA
MGIT 960	NA	NA
MIC (breakpoint, 16.0 μg/ml) (μg/ml)	16.0	32.0
Pyrazinamide		
APM	NA	NA
MGIT 960	Resistant	Resistant
MIC	NA	NA

[a] APM, agar proportion method on solid agar at the national referral laboratory. The proposed breakpoints are for MIC testing on the MycoTB plate whereby visible growth at the concentration represents conventional resistance.
[b] NA, not available.

While adjunctive measures, such as plasma therapeutic drug monitoring, are not available for routine practice, in response to a borderline MIC, a clinician may cautiously increase the dose of a key medication, such as levofloxacin or moxifloxacin, or even consider dose reductions for drugs with exposure-related toxicity, such as cycloserine, in the presence of a very susceptible MIC (13).

An interventional approach as envisioned in the colors in Fig. 1 can be prospectively studied in many laboratories capable of TB culture (14) and represents a step toward individualized management of the disease (15). Additionally, other newer or third-line medications might be customized to be used for the MycoTB plate. To conclude, had MIC testing been performed on the initial isolate from the clinical case, an MDR-TB regimen might have included high-dose levofloxacin or moxifloxacin, amikacin (or capreomycin), PAS, and third-line agents such as clofazimine or

linezolid, given the borderline resistance to cycloserine and resistance to pyrazinamide. Such a regimen may have provided the best opportunity to avoid acquired XDR-TB.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health/Fogarty training grant D43 TW008270 (to E.R.H. and G.S.K.), National Institutes of Health grant R01AI093358 (to E.R.H.), and National Institutes of Health grant K23AI099019 (to S.K.H.).

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