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Evaluation of the microscopic observation drug susceptibility assay for the rapid detection of MDR-TB and XDR-TB in China: a prospective multicentre study

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Objectives: To perform a multicentre study evaluating the performance of the microscopic observation drug susceptibility (MODS) assay for the detection of MDR-TB and XDR-TB in high-burden resource-limited settings.

Methods: We performed a prospective diagnostic accuracy study of drug-resistant TB suspects from outpatient and inpatient settings in five laboratories in China. Sputum was tested by smear microscopy, liquid [mycobacterial growth indicator tube (MGIT)] culture and the MODS assay at each site. Drug susceptibility testing (DST) was by MODS and an indirect 1% proportion method. The reference standard for *Mycobacterium tuberculosis* detection was growth on MGIT culture; the 1% proportion method was the reference standard for rifampicin, isoniazid, ofloxacin, kanamycin and capreomycin DST.

Results: *M. tuberculosis* was identified by reference standard culture among 213/532 (40.0%) drug-resistant TB suspects. Overall MODS sensitivity for *M. tuberculosis* detection was 87.8%–94.3% and specificity was 96.8%–100%. For drug-resistant TB diagnosis, excellent agreement was obtained for all drugs tested at the majority of sites. The accuracy was 87.1%–96.7% for rifampicin, 87.1%–93.3% for isoniazid, 92.7%–100% for ofloxacin, 90.9%–100% for kanamycin and 90.2%–100% for capreomycin. The median time to culture positivity was significantly shorter for MODS than for the MGIT liquid culture (8 days versus 11 days, $P<0.001$). The contamination rate ranged between 2.1% and 5.3%.

Conclusions: In the study settings, MODS provided high sensitivity and specificity for rapid diagnosis of TB and drug-resistant TB. We consider it to have a strong potential for timely detection of MDR-TB and XDR-TB in high-burden resource-limited settings.

Keywords: tuberculosis, diagnosis, drug resistance, second-line drugs

Introduction

MDR-TB and XDR-TB are major threats to TB control and represent a serious public health problem.[1] According to the WHO, 84 countries had reported at least one case of XDR-TB by 2012.[2] In China, which has the highest burden of MDR-TB in the world, ~8% of MDR cases are XDR-TB, most of which result from primary transmission.[3,4] Timely detection of these cases is crucial for patient management and control of further MDR and XDR transmission.

The WHO developed guidelines for drug susceptibility testing (DST) for first- and second-line drugs on Löwenstein–Jensen (LJ) medium or Middlebrook agar using the proportion method.[5] Unfortunately, conventional phenotypic DST methods based on LJ medium or Middlebrook agar are time-consuming, taking weeks to yield reliable results. In order to reduce this turnaround time, commercial broth-based systems and molecular tests have been developed.[6,7] However, these methods are beyond the reach of laboratories in most developing countries including China, due

to high cost and the need for complex infrastructure facilities. These settings still use conventional culture methods on agar or LJ medium that take months to obtain results. Therefore, the need for a rapid, affordable, accurate and easy-to-use test for MDR-TB and XDR-TB in resource-limited settings remains a priority.

The microscopic observation drug susceptibility (MODS) assay is a relatively low-cost and simple liquid culture method that relies on microscopic detection of the cording growth characteristic of *Mycobacterium tuberculosis*;[8–12] it has been endorsed by the WHO for the rapid detection of MDR-TB.[13] There are currently only a few studies evaluating the use of the MODS assay for the detection of resistance to second-line drugs. The objective of this study was to perform a multicentre evaluation of the performance of the MODS assay for the simultaneous detection of MDR-TB and XDR-TB through the detection of resistance to rifampicin, isoniazid, ofloxacin, kanamycin and capreomycin directly from sputum samples.

Methods

Study design and settings

This prospective study was carried out in five laboratories situated in China: Province Tuberculosis Reference Laboratory, Jiangxi Chest Hospital, Nanchang, Jiangxi; Department of Clinical Laboratory, First Teaching Hospital of Tianjin University of TCM, Tianjin; Clinical Microbiology Laboratory, Shanghai First People's Hospital, Shanghai; Clinical Microbiology Laboratory, University of Hong Kong-Shenzhen Hospital, Shenzhen, Guangdong; and Department of Clinical Laboratory, Third Affiliated Hospital of Kunming Medical University, Kunming, Yunnan. Study inclusion criteria were age ≥12 years, cough for ≥3 weeks, informed consent and categorization by the treating clinician into one of the following categories: (i) suspected treatment failure, (ii) suspected relapse, (iii) treatment default or (iv) contact with an individual with known or suspected drug-resistant TB.[13] All subjects submitted a single 'spot' sputum sample. Sputum samples were collected prospectively at the five sites from January 2012 to September 2013. Each sample was divided into two aliquots: the first aliquot underwent sputum acid-fast bacilli (AFB) smear examination, decontamination, mycobacterial growth indicator tube (MGIT) culture and DST; and the second aliquot underwent MODS testing. All participating laboratories had experience with the MODS assay or were previously trained.[14,15] With the exception of centrifugation, all test procedures, including sample processing and inoculation, were handled in a class II biosafety cabinet. Before centrifugation, all samples were sealed in centrifuge tubes with a screw cap.

Informed consent was obtained from all subjects. This study was approved by Institutional Review Boards at the Jiangxi Chest Hospital, First Teaching Hospital of Tianjin University of TCM, Shanghai First People's Hospital, the University of Hong Kong-Shenzhen Hospital and the Third Affiliated Hospital of Kunming Medical University.

Specimen processing and inoculum preparation

Sputum specimens were digested and decontaminated using the *N*-acetyl-L-cysteine-sodium hydroxide method; the final sodium hydroxide concentration was 2%.[16] Sediments were resuspended in a final volume of 3 mL and used immediately for inoculation of culture media.

MGIT culture

Culture was performed according to the manufacturer's instructions for the MGIT 960 automated system (Becton Dickinson).[17] The inoculation volume was 0.5 mL per tube. MGIT cultures that were contaminated prior to 42 days were redecontaminated and recultured. All positive cultures by MGIT were identified as *M. tuberculosis* complex by niacin and nitrate reductase tests.

MODS culture

The MODS assay was performed in accordance with published standard operating procedures, with minor modifications by adding a sterility control with only MODS liquid medium in each culture plate.[18] MODS medium was prepared using Middlebrook 7H9 broth base (Becton Dickinson), 0.31% glycerol, 10% oleic albumin–dextrose–catalase (Becton Dickinson) and PANTA (Becton Dickinson). Cultures were prepared in 24-well tissue culture plates. For each processed sample, two drug-free wells (control wells), one rifampicin-containing well, one isoniazid-containing well, one ofloxacin-containing well, one kanamycin-containing well and one capreomycin-containing well were set up. Nine-hundred microlitres of this sample/broth mixture was aliquotted into each of seven wells in a 24-well microtitre plate. Next, 100 μL of distilled water was added into the control wells. Finally, 100 μL of drug was added to each drug-containing well. The drug concentrations in each well were 1 mg/L rifampicin, 0.4 mg/L isoniazid, 2 mg/L ofloxacin, 5 mg/L kanamycin and 2.5 mg/L capreomycin.[19,20] A sterility control with only MODS liquid medium and a susceptible control well with MODS liquid medium plus bacteria (H37Rv) were included. Two or three drug-resistant *M. tuberculosis* clinical isolates with definite drug resistance characteristics, depending on the resistant model of strains used at different sites, were included as drug-resistant control strains in each study site. These drug-resistant control strains were cultured in a separate culture plate in each run of the MODS test to confirm the effectiveness of the drugs. Plates were sealed with tape and ziplock bags and incubated at 37°C. Mycobacterial growth was observed daily with an inverted light microscope at ×40 magnification from the 3rd to the 15th day of incubation. After 15 days of incubation, observation was limited to twice a week. Positive MODS cultures were defined by the presence of the characteristic cord formation at the time of detection of growth. Any isolate with growth in both the control and drug-containing wells was recorded as resistant. If growth was observed in control wells but not in the drug-containing wells, a susceptible result was recorded for the relevant drug.

DST by the agar proportion method

All cultures positive by MGIT were subcultured on LJ slant. Bacterial colonies on LJ slant were then used to prepare bacterial suspension for indirect DST by using an agar-based proportion method. The agar proportion method was performed on 7H10 agar according to the standard procedure, with the recommended critical concentrations of 1 mg/L rifampicin, 0.2 mg/L isoniazid, 2 mg/L ofloxacin, 5 mg/L kanamycin and 10 mg/L capreomycin.[5]

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for all specimens to assess the performance of the MODS assay. For categorical variables, we compared proportions using χ^2 tests; for continuous variables, we compared medians using the Wilcoxon rank-sum test. Data analysis was performed using SPSS 17.0. All *P* values were two-sided with $\alpha = 0.05$ as the significance level.

Results

Patients and samples

A total of 540 sputum specimens were collected from 540 drug-resistant TB suspects. Figure 1 shows a flow diagram of

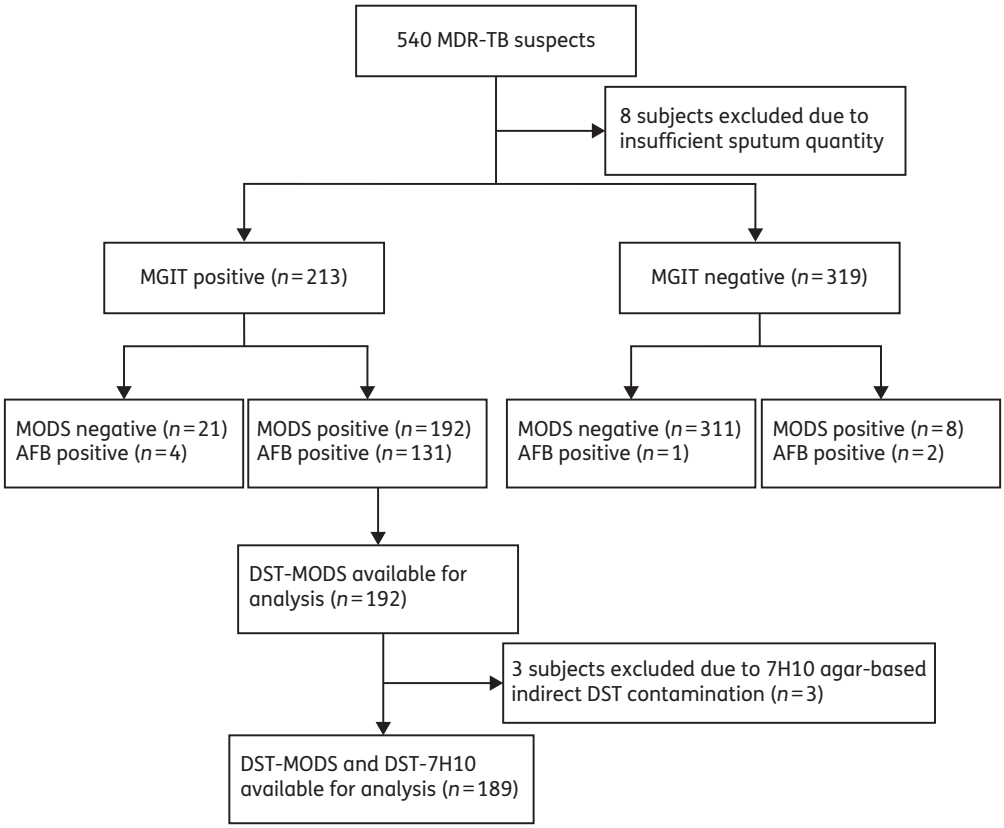

Figure 1. Study flow diagram.

study subjects at each site. Eight subjects were excluded from further analysis due to insufficient sputum quantity. The demographic and clinical characteristics of the 532 subjects are shown in Table 1.

M. tuberculosis detection

Among 532 specimens, 138 (25.9%) had smear results positive for AFB, 200 (37.6%) were positive for *M. tuberculosis* by the MODS assay and 213 (40.0%) were positive for *M. tuberculosis* by culture on MGIT. The sensitivity and specificity of the MODS assay for *M. tuberculosis* detection at each study site are shown in Table 2. The accuracy of MODS for *M. tuberculosis* detection ranged between 93.0% and 96.7%.

DST

Of 192 specimens positive by both MODS and reference standard MGIT culture, three were agar proportion method indeterminate due to contamination of subculture. Therefore, 189 directly inoculated patient specimens had concurrent MODS isoniazid, rifampicin, ofloxacin, kanamycin and capreomycin wells for comparison with the agar proportion method. Table 3 shows for each site the number of resistant and susceptible samples detected by the MODS assay compared with results obtained by the reference method used at the site.

Table 1. Characteristics of study subjects

Characteristic	Total, $n=532$
Study site	
Site 1	156
Site 2	98
Site 3	114
Site 4	72
Site 5	92
Age (years), median (IQR)	39 (26–47)
Sex	
male	318
female	214
HIV infection status	
positive	8
negative	495
unknown	29
Reason for referral	
default	46
relapse	169
treatment failure	254
contact with known/suspected MDR case	63

Table 2. Sensitivity and specificity of MODS in detection of *M. tuberculosis*

	Culture on MGIT 960 automated system											
	total		Site 1		Site 2		Site 3		Site 4		Site 5	
Culture on MODS	positive	negative	positive	negative	positive	negative	positive	negative	positive	negative	positive	negative
Positive	192	8	55	3	31	2	43	2	33	1	30	0
Negative	21	311	7	91	3	62	6	63	2	36	3	59
Sensitivity (%)	90.1		88.7		91.2		87.8		94.3		90.9	
Specificity (%)	97.5		96.8		96.9		96.9		97.3		100	
PPV (%)	96.0		94.8		93.9		95.6		97.1		100	
NPV (%)	93.7		92.9		95.4		91.3		94.7		95.2	
Accuracy (%)	94.5		93.6		94.9		93.0		95.8		96.7	

Table 3. Susceptibility results for the MODS assay compared with the agar proportion method for each site

		Agar proportion method									
		rifampicin		isoniazid		ofloxacin		kanamycin		capreomycin	
Site	MODS	R	S	R	S	R	S	R	S	R	S
1	R	29	0	31	1	17	1	11	2	8	3
	S	2	23	4	18	2	34	1	40	1	42
2	R	17	1	19	0	10	0	4	2	3	0
	S	3	10	4	8	0	21	0	25	1	27
3	R	24	1	20	1	14	2	5	1	4	4
	S	1	15	2	18	1	24	1	34	0	33
4	R	21	1	23	1	13	0	3	2	3	1
	S	2	9	2	7	0	20	1	27	0	29
5	R	17	1	19	1	12	1	5	0	2	0
	S	0	12	1	9	1	16	0	25	0	28

R, resistant; S, susceptible.

Table 4. Specificity and sensitivity of the MODS assay for rifampicin and isoniazid obtained at each site

	Rifampicin (%)					Isoniazid (%)				
Site	sensitivity	specificity	PPV	NPV	accuracy	sensitivity	specificity	PPV	NPV	accuracy
1	93.5	100	100	92.0	96.3	88.6	94.7	96.9	81.8	90.7
2	85.0	90.9	94.4	76.9	87.1	82.6	100	100	66.7	87.1
3	96.0	93.8	96.0	93.8	95.1	90.9	94.7	95.2	90.0	92.7
4	91.3	90.0	95.5	81.8	90.9	92.0	87.5	95.8	77.8	90.9
5	100	92.3	94.4	100	96.7	95.0	90.0	95.0	90.0	93.3
Overall	93.1	94.5	96.4	89.6	93.7	89.6	93.8	96.6	82.2	91.0

Sensitivity, specificity, PPV and NPV are shown in Table 4 for rifampicin and isoniazid and in Table 5 for ofloxacin, kanamycin and capreomycin. For rifampicin, the accuracy ranged between 87.1% and 96.7%; the overall accuracy was 93.7%. Four sites (Sites 2–5) had one false resistant result each, while Sites 1 and 4 had two false susceptible results each, Site 2 had three false susceptible results and Site 3 had one false susceptible result. For isoniazid, the accuracy ranged between 87.1% and 93.3%; the overall accuracy was 91.0%. All sites had false susceptible results and four sites (Sites 1 and 3–5) had one false resistant result each.

For ofloxacin, the accuracy ranged between 92.7% and 100%; the overall accuracy was 95.8%. All results were concordant at

Table 5. Specificity and sensitivity of the MODS assay for ofloxacin, kanamycin and capreomycin obtained at each site

Site	Ofloxacin (%)					Kanamycin (%)					Capreomycin (%)				
	sensitivity	specificity	PPV	NPV	accuracy	sensitivity	specificity	PPV	NPV	accuracy	sensitivity	specificity	PPV	NPV	accuracy
1	89.5	97.1	94.4	94.4	94.4	91.7	95.2	84.6	97.6	94.4	88.9	93.3	72.7	97.7	92.6
2	100	100	100	100	100	100	92.6	66.7	100	93.5	75.0	100	100	96.4	96.8
3	93.3	92.3	87.5	96.0	92.7	83.3	97.1	83.3	97.1	95.1	100	89.2	50.0	100	90.2
4	100	100	100	100	100	75.0	93.1	60.0	96.4	90.9	100	96.7	75.0	100	97.0
5	92.3	94.1	92.3	94.1	93.3	100	100	100	100	100	100	100	100	100	100
Overall	94.3	96.6	94.3	96.6	95.8	90.3	95.6	80.0	98.1	94.7	90.9	95.2	71.4	98.8	94.7

Sites 2 and 4. For kanamycin, the accuracy ranged between 90.9% and 100%; the overall accuracy was 94.7%. Three sites (Sites 1, 2 and 4) had two false resistant results each and Site 3 had one false resistant result, while three sites (Sites 1, 3 and 4) had one false susceptible result each. For capreomycin, the accuracy ranged between 90.2% and 100%; the overall accuracy was 94.7%. Site 3 had four false resistant results, Site 1 had three false resistant results and Site 4 had one false resistant result, while two sites (Sites 1 and 2) had one false susceptible result each. Because of the very low number of strains resistant to capreomycin at Site 2, the sensitivity of MODS was lower for this drug.

Culture contamination

The proportion of contaminated cultures was similar for MODS culture [22 (4.1%) of 532 samples] and MGIT culture [33 (6.2%) of 532 samples; $P=0.13$]. The proportion of contaminated cultures on MODS was 8 (5.1%) of 156 cultures for Site 1, 3 (3.1%) of 98 for Site 2, 6 (5.3%) of 114 for Site 3, 3 (4.2%) of 72 for Site 4 and 2 (2.1%) of 94 for Site 5. During MODS testing, none of the negative control wells showed mycobacterial growth. The contamination rate was between 2.2% and 9.2% for the MGIT.

Time to positivity for detection of M. tuberculosis and drug resistance

Overall, the median time to culture positivity was significantly shorter for MODS than for MGIT culture [MODS 8 days (IQR 7–14 days) versus MGIT 11 days (IQR 7–16 days); $P<0.001$]. Median times for growth using the MODS assay were the same (8 days) for four study sites (Sites 1–4), while it was 7 days at Site 5. Median time to positivity for MODS XDR-TB diagnosis [8 days (IQR 7–14 days)] was significantly shorter than that for the agar proportion method, which was up to 70 days (IQR 52–75 days; $P<0.001$) when including the time for primary isolation in MGIT.

Discussion

This was the first known prospective multicentre study to assess the performance of the direct MODS assay for the detection of MDR-TB and XDR-TB in sputum samples. MODS detected *M. tuberculosis* and associated drug resistance with high sensitivity and shorter time to positivity compared with MGIT liquid culture and DST methods among drug-resistant TB suspects.

Drug-resistant TB, especially MDR-TB and XDR-TB, is now a major threat to global TB control. China has been described as a global 'hot spot' for drug-resistant TB. The national TB control programmes in China are however unable to routinely screen or conduct surveillance for MDR-TB and XDR-TB due to lack of affordable rapid tests. Therefore, the need for a rapid, affordable, accurate and easy-to-use test for MDR-TB and XDR-TB in resource-limited settings remains a priority. Automated liquid culture such as MGIT is a recognized reference method (or gold standard) in both *M. tuberculosis* detection and DST, but it is too expensive for developing countries. In this study, MGIT culture was used as a reference method in *M. tuberculosis* detection. The 7H10 agar-based indirect agar proportion method, which is also a reference method recommended by WHO, was used as a reference standard in DST due to the affordability of the test.

In recent years, several rapid assays for drug-resistant TB diagnosis have been developed.[21–23] Among them, the MODS assay is a simple, rapid, low-cost method that holds great promise for resource-limited settings. A number of studies have reported the usefulness of the MODS assay for determining susceptibility or resistance to rifampicin and isoniazid, the two most important drugs for the treatment of TB, and have shown high sensitivity and specificity. The MODS assay also has the potential to be used for the detection of resistance to second-line drugs. In 2009, Devasia *et al*.[24] reported the first evaluation of the MODS assay for the detection of ofloxacin resistance and found complete concordance with the proportion method. More recently, Fitzwater *et al*.[19] and Trollip *et al*.[20] explored the candidate critical concentrations for second-line DST for *M. tuberculosis* using the MODS assay. They obtained accuracy that ranged between 59% and 100%. However, the correctness and suitability of these candidate critical concentrations should be tested in a range of epidemiological settings.

In the present study, the sensitivity and specificity for detecting resistance to rifampicin, isoniazid and second-line drugs were excellent. The sensitivity for detection of isoniazid and rifampicin resistance was similar to previously reported studies.[8,9,11,12] The contamination rate of MODS culture was relatively lower, although it was not significantly different, than MGIT culture, which is mainly due to the use of PANTA antibiotic supplement in MODS liquid medium. This is the first known multicentre study that confirms that direct MODS can also be used to screen XDR-TB. Moreover, a quick turnaround time with DST is important for ensuring the patient receives an appropriate treatment regimen. As expected, the MODS assay provided far more rapid results than the conventional DST method.

Compared with the other available non-commercial culture and DST methods for the rapid diagnosis of XDR-TB, such as the nitrate reductase assay (NRA) and colorimetric redox indicator (CRI) assay, MODS shows several advantages. First, MODS can simultaneously detect *M. tuberculosis* and *M. tuberculosis* drug resistance directly from sputum using liquid broth media. Although NRA can be performed on both culture isolates and smear-positive ($\geq$1+) sputum specimens, more evidence is required regarding the accuracy of NRA applied directly on specimens. Second, the MODS assay is performed directly on processed sputum in a sealed plastic bag that does not require further manipulation once the specimen has been inoculated; however, in the NRA and CRI assays, reagents such as freshly prepared Griess reagent or resazurin solution must be added to the culture for some time after incubation, increasing the risk that the mycobacteria will escape. Therefore, MODS is actually considerably safer than all indirect TB drug susceptibility methods and a properly maintained class II biosafety cabinet is more than adequate.[13,25] Finally, the MODS assay relies on microscopic detection of the cording growth that is characteristic of *M. tuberculosis*. The NRA and CRI assays do not reflect the mycobacterial growth in real time. Therefore, the direct MODS assay can be used as a rapid and low-cost screening method to detect *M. tuberculosis* and associated drug resistance.

In conclusion, MODS detected *M. tuberculosis* and *M. tuberculosis* drug resistance with high sensitivity and a more rapid time to positivity compared with standard culture and DST methods. This study promotes a wider use of the MODS assay as a new and rapid phenotypic diagnostic test for the detection of MDR-TB and XDR-TB, especially in developing countries where rapid and inexpensive methods are urgently needed.

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Transparency declarations

None to declare.

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