Evaluation of the microscopic observation drug susceptibility assay for the detection of first- and second-line drug susceptibility for *Mycobacterium tuberculosis*

To the Editor:

The End Tuberculosis Strategy of the World Health Organization (WHO) calls for the early diagnosis of tuberculosis (TB) and use of universal drug susceptibility testing (DST) in order to start patients on the most effective treatment regimen as early as possible. DST using solid or liquid media and rapid molecular-based DST to detect drug-resistant TB are available and endorsed by WHO; however, in 2015, WHO reported that among new cases of bacteriologically confirmed TB, only 12% had undergone DST [1]. Most national tuberculosis programmes do not offer universal DST [2], and in 2015, 12 countries reported no capacity to perform phenotypic DST [1] while 28 countries had neither in-country capacity nor a linkage with a partner laboratory for second-line DST.

Molecular-based DST is both costly and technically demanding to administer, thus is not widely implemented in many low-income countries [2]. Noncommercial DST methods such as the microscopic observation drug susceptibility (MODS) assay have the potential to help close this gap in diagnostic capacity because they can be implemented in resource-poor settings at low cost and with little training [3, 4]. MODS has been endorsed by WHO as DST for isoniazid (INH) and rifampicin (RIF) [5], and there is enormous potential for MODS to be used as DST for second-line drugs [6]. The objective of this study was to evaluate the performance of MODS for the simultaneous detection of resistance to seven different anti-TB drugs and to evaluate cut-off points for capreomycin (CAP).

Specimens were collected as part of an observational cohort study (ClinicalTrials registration number NCT02170441) conducted by the Global Consortium for Drug-resistant TB Diagnostics (GCDD). Descriptions of the protocols have been published previously [7].

The Mycobacterium Growth Indicator Tube (MGIT) 960 DST was selected as the phenotypic reference standard, using the critical drug concentrations recommended by the WHO at the time of the study: INH 0.1, RIF 1.0, moxifloxacin (MOX) 0.25, ofloxacin (OFX) 2.0, amikacin (AMK) 1.0, kanamycin (KAN) 2.5 and CAP 2.5 µg·mL^{-1} [8, 9].

The MODS assay [6] was performed with drug concentrations of INH 0.4, RIF 1.0, MOX 0.5, OFX 1.0, AMK 2.0 and KAN 5.0 µg·mL^{-1} [6, 10], and multiple concentrations of CAP: 1.25, 2.5, 5.0 and 10.0 µg·mL^{-1}. The pyrosequencing platform [11, 12] scanned regions associated with mutations in the *rrs* (positions 1397–1406) and *eis* promoter regions (positions −5 to −47) to investigate resistance to injectable drugs (KAN, AMK and CAP).

The isolates used by TROLLIP *et al.* [6] were not the same samples collected in this prospective study; however, as the methods and staff employed were the same, it was deemed appropriate to pool those results with the current results.

Of 1128 study participants enrolled between April 2012 and June 2013, 213 (18.9%) were culture-negative, one (0.1%) was culture-contaminated and 914 (81.0%) were culture-positive (demographic data have been published previously [7]). In total, 826 (73%) samples were smear-positive while 302 (27%) were smear-negative.

Of the 1128 MODS tests, 731 (64.8%) were positive, 288 (25.5%) were negative, 55 (4.9%) were contaminated and 43 (3.8%) were indeterminate. 11 (1.1%) had some discordance between the two drug-free control wells. Overall sensitivity for MODS to detect *Mycobacterium tuberculosis* compared with MGIT was 84.9% and specificity was 98.8%. For smear-positive samples, overall sensitivity was 91.8% and specificity was 97.4%, while for smear-negative samples, sensitivity was 46.6% and specificity was 99.2%.

MODS and MGIT DST results were available for 729 samples. The overall sensitivity was high: INH 97.0%, RIF 99.6%, AMK 90.0%, KAN 61.9%, MOX 97.8% and OFX 98.2%. The overall specificity was excellent: INH 98.7%, RIF 97.8%, AMK 99.5%, KAN 99.8%, MOX 97.1% and OFX 98.0%. Of the 18 non-CAP site/drug combinations, only six had sensitivity lower than 95% (India-KAN; Moldova-AMK, KAN, MOX, OFX; South Africa-INH) and only four had specificity lower than 98% (India-INH, MOX, OFX; South Africa-RIF).

All MODS-discordant KAN results in Moldova (n=40) were false positives, of which 26 (65%) had mutations in the *eis* promoter region scanned. Of the six discordant results in India, two (33%) had had mutations in the *eis* promoter region. There were no discordant results for KAN in South Africa, and no South African samples had mutations in the *eis* promoter region.

TROLLIP *et al.* [6] reported provisional cut-off points for CAP, and our results showed that moving from a 2.5 µg·mL^{-1} to a 1.25 µg·mL^{-1} breakpoint yielded an increase of 6% in sensitivity but a concomitant decrease of 2.1% in specificity.

Analysis of receiver operating characteristic (ROC) curves (figure 1) showed agreement between this study and two previously published studies on breakpoints using MODS to test for *M. tuberculosis* resistance to capreomycin [6, 13]. TROLLIP *et al.* [6] reported CAP results for four concentrations (n=55, 29 of which were CAP-resistant) and FITZWATER *et al.* [13] reported CAP results for six concentrations (n=94, of which 51 were CAP-resistant).

ROC curve analysis supported the lowering of the critical concentration CAP for MODS to 1.25 µg·mL^{-1} (figure 1). We pooled our study results with those of TROLLIP *et al.* [6], which showed that if the breakpoint concentration for CAP is lowered to 1.25 µg·mL^{-1}, a combined sensitivity for CAP of 92.7% (95% CI 86–97%) and specificity of 96.1% (95% CI 94–97%) would be achieved.

This is the first multinational study to prospectively assess the clinical performance of the MODS assay for the detection of multi/extensively resistant TB (M/XDR-TB). The findings from our large study (n=1128) significantly bolster the current WHO policy guidance, which endorses MODS to screen patients suspected of having MDR-TB [5], and adds to the growing body of literature on the use of MODS to detect resistance to second-line anti-TB drugs.
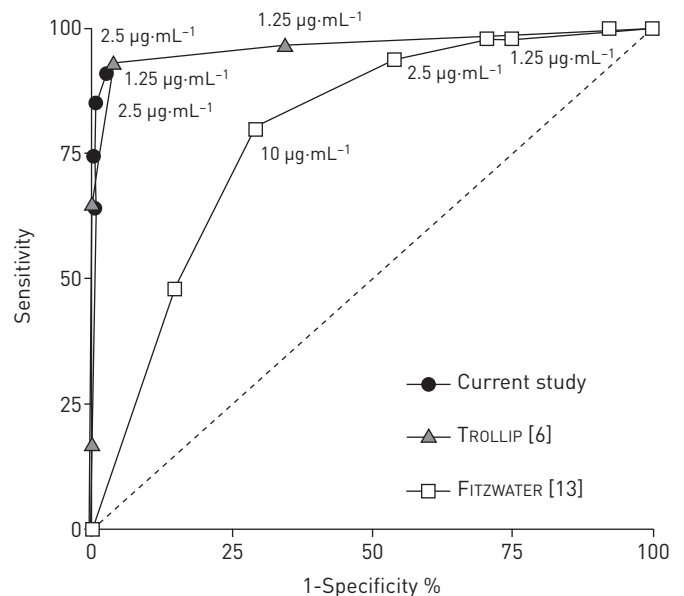

FIGURE 1 Receiver operating characteristic curves for capreomycin. In the current study and that of TROLLIP *et al.* [6], the reference drug susceptibility testing (DST) method was MGIT960 with a critical concentration of 2.5 µg·mL^{-1} (n=729 and n=55, respectively); in the study of FITZWATER *et al.* [13], the reference DST method was a proportional method with an unreported critical concentration (n=94).

The MODS assay demonstrated a clear dose–response relationship between smear grade/culture and production of a positive, negative or indeterminate result (not shown). The lower sensitivity of MODS could be due to our use of presumptive identification of *M. tuberculosis* with the MODS assay while employing a confirmatory identification of *M. tuberculosis* for our reference standard. It is likely that combining the MODS assay with a test to confirm *M. tuberculosis* (*e.g. para*-nitrobenzoic acid or MPT64) would decrease the discordance between MODS and MGIT culture.

In the present study, the 95% confidence intervals surrounding MODS sensitivity and specificity as a DST for INH, RIF, AMK, MOX and OFX [7] meet and/or exceed the WHO target product profiles for a tuberculosis diagnostic [14]. We found higher sensitivity and specificities than those in the only other prospective study of MODS (n=540 in one country) for INH, RIF, OFX and CAP, although our estimates were lower for KAN [15]. Our low sensitivity for AMK was driven by a low number of AMK-resistant samples (n=10). Clear performance increases were observed when the CAP breakpoint was lowered to 1.25 μg·mL^{-1}.

Investigation of genetic mutations known to confer resistance to KAN revealed that mutation status of samples can have a dramatic effect on accuracy, especially sensitivity, owing to the presence of mutations in *rrs* and *eis* promoter regions, which confer high-level (*rrs*) or low-level (*eis* promoter) resistance to KAN.

The MODS assay yielded comparable results to MGIT first- and second-line DST, and results are obtained much faster than MGIT (but not as fast as the MTBDR*sl* [7]), thus we believe that MODS can be deployed in resource-poor laboratory settings. Given its noncommercial nature, MODS is a low-cost alternative to other WHO-endorsed DST methods, and operational research would be helpful to determine the cost/benefits of implementing MODS in individual resource-poor laboratory settings.

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