INT J TUBERC LUNG DIS 19(12):1547–1552

http://dx.doi.org/10.5588/ijtld.15.0136

The thin-layer agar method for direct phenotypic detection of multi- and extensively drug-resistant tuberculosis

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SUMMARY

BACKGROUND: **Molecular techniques rapidly detect resistance to rifampicin (RMP) and isoniazid (INH), but do not eliminate the need for culture-based drug susceptibility testing (DST) against other drugs. The thin-layer agar (TLA) test, a non-commercial direct DST method, has demonstrated good performance for INH and RMP; however, evidence is still limited, and its applicability for DST of ofloxacin (OFX) and kanamycin (KM) is unknown.**

DESIGN: **We compared 279 TLA DST results with those of MGIT for INH and RMP, and 280 results for OFX and KM with those of the 7H11 agar proportion method, obtained from 320 smear-positive samples from 165 Georgian TB patients. Discrepancies were solved by comparison with a composite reference standard. The prevalence of multidrug-resistant tuberculosis (TB) was 30 of 164 patients (18.3%), 2 (6.7%) of whom had extensively drug-resistant TB.**

RESULTS: **TLA showed 94.7%, 98.2%, 100% and 78.9% sensitivity, respectively, for INH, RMP, OFX and KM, with 100% specificity. Average time to results was 7 days in TLA, 23 in MGIT and 49 for 7H11 agar.**

CONCLUSIONS: **In low-resource settings, TLA can be applied for the rapid detection of resistance to INH, RMP and fluoroquinolones. Further studies are necessary to improve sensitivity to KM and further assess its performance for OFX and other drugs and its applicability in field conditions.**

KEY WORDS: resistance detection; non-commercial method; tuberculosis; MDR-TB; XDR-TB

TO IMPROVE THE DETECTION of multidrug-resistant tuberculosis (MDR-TB), the World Health Organization (WHO) recommends the use of the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) and line-probe assays (LPA) such as GenoType® MTBDR*plus* (Hain LifeSciences, Nehren, Germany).[1,2] Whereas Xpert detects rifampicin (RMP) resistance directly from specimens, MTBDR*plus* detects resistance to both isoniazid (INH) and RMP; however, it can be applied directly only to decontaminated smear-positive samples. Nucleic acid amplification tests (NAAT) are highly sensitive, but do not eliminate the need for culture and drug susceptibility testing (DST) required to test other drugs and to monitor patients during treatment.[3] MTBDR*sl* (Hain LifeSciences) is the only commercial assay available for the rapid detection of second-line drug resistance. However, as its use in optimising individualised patient regimens has not been endorsed yet,[4] DST for these drugs still relies on phenotypic testing.[2]

In 2009, the WHO assessed the performance of non-commercial methods such as the colorimetric redox indicator (CRI) method, microscopic observation drug susceptibility (MODS) assay, nitrate reductase assay (NRA) and thin-layer agar (TLA). CRI, MODS and NRA have been recognised as valid methods, but were recommended only as interim solutions for reference laboratories.[5] The results for TLA were considered insufficient to draw conclusions about its performance and feasibility.[5]

The TLA method uses 7H11 agar plates directly inoculated with decontaminated smear-positive specimens. Reading by conventional light microscopy permits rapid detection of growth without opening the plates, thereby reducing the biohazard risk. Two studies report high performance of TLA in the isolation of *Mycobacterium tuberculosis* complex (MTC) and detection of direct resistance; however, either the sample size was limited,[6] or the plates were read by the naked eye, increasing time to detection.[7] Another study has used TLA DST as an indirect test for RMP, ofloxacin (OFX) and kanamycin (KM).[8] However, the performance of TLA in direct DST against OFX and KM remains unknown.

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Article submitted 10 February 2015. Final version accepted 3 July 2015.

In the present study, we assessed the performance of direct TLA DST in detecting INH and RMP resistance compared to indirect MGIT™ (Mycobacteria Growth Indicator Tube; BD, Sparks, MD, USA), and in detecting OFX and KM resistance compared to indirect 7H11 agar. Discrepant results were resolved using a composite reference standard (CRS) consisting of the determination of minimal inhibitory concentration (MIC) and target gene sequencing. In addition, we compared the processing time and the cost of TLA to gold standard methods.

MATERIALS AND METHODS

Study design

All patients aged ⩾15 years with at least one smear-positive sputum sample on Ziehl-Neelsen (ZN) staining in the National Reference Laboratory (NRL) of Tbilisi, Georgia, who had not received anti-tuberculosis treatment in the previous month, were eligible for the study. After providing informed consent, patients submitted two additional sputum samples which, if smear-positive, were refrigerated until shipment at ambient temperature to the Mycobacteriology Laboratory of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium. Only samples confirmed as smear-positive by the ITM were included in the study.

The study protocol was approved by the Institutional Review Board of the ITM, Antwerp, the Ethics Committee of the University Hospital of Antwerp, Belgium, and the Ethics Committee of the Georgian National Centre for Tuberculosis and Lung Diseases, Tbilisi, Georgia.

Laboratory procedures

In ITM, the samples were decontaminated with a final concentration of 1.5% *N*-acetyl-L-cysteine-sodium hydroxide. After centrifugation for 20 min at 3000 *g*, the sediment was re-suspended in 1 ml distilled water. One drop was used to prepare a ZN smear and 500 µl was inoculated in MGIT culture according to the manufacturer's protocol. The remaining suspension was diluted 1:5 in sterile distilled water, and 100 µl was inoculated into each of the six wells of a TLA plate (Corning Costar 3516; Sigma-Aldrich, St Louis, MI, USA), which consisted of one well for growth control (GC), one well for each drug and one well for *p*-nitrobenzoic acid (PNB) to differentiate between MTC and non-tuberculous mycobacteria (NTM). Plates, prepared at ITM, contained 7H11 agar and oleic albumin dextrose catalase (BD 211886), plus amphotericin, piperacillin and trimethoprim (all at 4 µg/ml concentration) to reduce contamination.[9] Drug-containing wells contained final concentrations of 0.2 µg/ml INH, 1 µg/ml RMP, 2.0 µg/ml OFX (Sigma-Aldrich) and 6.0 µg/ml KM (ICN Biomedicals Inc, Aurora, OH, USA). MTC growth in MGIT, confirmed using SD-Bioline TB Ag MPT64 (Standard Diagnostic, Gyeonggi-do, Republic of Korea), was inoculated for INH and RMP DST in MGIT, following the manufacturer's instructions, and in parallel on Löwenstein-Jensen (LJ) for strain storage and for OFX and KM DST by the 7H11 agar indirect proportion method, according to international standards. The drug concentrations for DST on 7H11 agar were the same as for TLA, with resistance defined at a cut-off of 2.0 µg/ml for OFX and 6.0 µg/mg for KM.

After inoculation, TLA plates were read on days 5, 7, 10, 13, 15, 20, 25, 30, 35, up to 40 days, adjusted to working days, using a conventional light microscope (objective 10×). Growth in the GC well paired with inhibition in the PNB well was considered positive for MTC.[9] DST was interpreted if at least 10 colonies were present in the GC well; otherwise it was considered invalid. Drug resistance was defined as any growth in the drug wells.[7] TLA plates were read blinded to the MGIT and DST 7H11 agar results.

Discordant results were compared to a CRS consisting of MTBDR*plus* for INH and RMP, MTBDR*sl* for OFX, target gene sequencing (*rpo*B, *kat*G, *inh*A, *gyr*AB and *rrs* 1400 region) and MIC, performed on LJ for INH and RMP, 7H11 agar for OFX and resazurin microtiter assay (REMA) for KM, with cut-offs at respectively 0.2 µg/ml, 40 µg/ml, 2 µg/ml and 2.5 µg/ml.[10] Finally, the CRS also included comparison of the results from the paired sample of the same patient, confirmed to have the same genotype by spoligotyping, to exclude laboratory errors. Test costs were calculated using the manufacturer's price list and the FIND (Foundation for Innovative New Diagnostics, Geneva, Switzerland) website. Time for each test performance was calculated based on the ITM laboratory technician's estimates for each step.

RESULTS

Between November 2010 and February 2012, 362 smear-positive samples were received at the ITM from 183 consecutive patients (the Figure). Of these, 42 (11.6%) were found to be smear-negative (S–) and excluded. The samples were decontaminated after a median of 12 days (interquartile range [IQR] 9–14) from collection. Of the 305 (95.3%) MTC-positive TLA plates, seven showed partial contamination, which did not prevent the interpretation of DST results. On MGIT, 306 (95.6%) cultures were positive, increasing to 313 (97.8%) after re-decontamination of seven partially contaminated MGIT cultures. Of 320 smear-positive samples, direct TLA yielded valid DST results in 89%, indirect MGIT in 97.5% and indirect 7H11 agar in 97.2% of the samples (Table 1).

In total, 279 DST results were available for

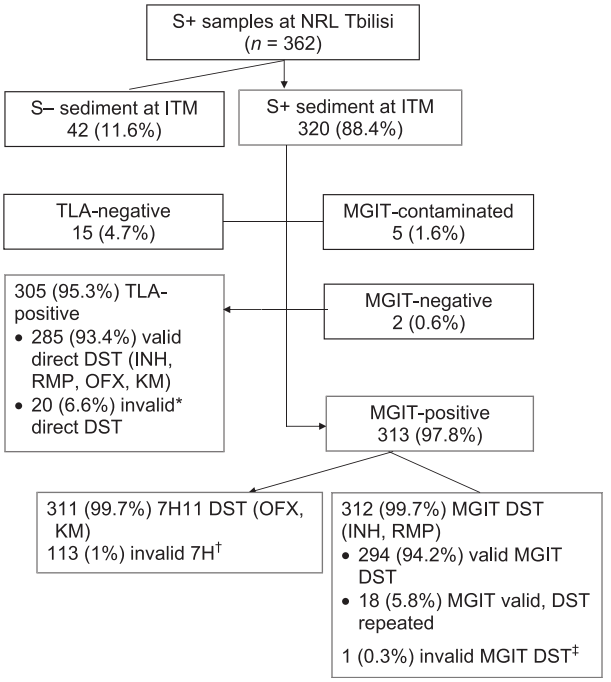

Figure TLA culture results. *GC <10 colonies. †No growth on GC. ‡Rapid growth on GC tube. NRL = National Reference Laboratory; S+ = smear-positive; ITM = Institute of Tropical Medicine; S– = smear-negative; TLA = thin-layer agar; MGIT™ = Mycobacteria Growth Indicator Tube; DST = drug susceptibility testing; INH = isoniazid; RMP = rifampicin; OFX = ofloxacin; KM = kanamycin; GC = growth control.

comparison for INH and RMP, with 35.1% resistance to either or both drugs, and 280 DST results for OFX and KM, with 8.2% resistant to either or both drugs. TLA sensitivity compared to indirect DST prior to resolution of discordances was respectively 92.3%, 96.4%, 83.3% and 57.9% for INH, RMP, OFX and KM, while specificity was 100% for all drugs, except OFX (99.6%) (Table 2). Discrepant results between TLA and MGIT or 7H11 medium were compared to the CRS (Table 3). One discrepant DST result for INH and RMP was removed from the analysis, as spoligotyping was discordant between the sediment used for TLA and the isolate tested using MGIT.

INH DST results were initially discordant for six samples, all resistant on MGIT and susceptible on TLA. Five isolates that carried mutations in the *Kat*G gene and showed an MIC above the cut-off were considered as false-susceptible on TLA. The sixth discrepancy was resolved in favour of TLA as the sediment was wild type (wt) by MDRTB*plus* and sequencing, the MIC was 0.1 μg/ml and the paired sample confirmed INH susceptibility in both TLA and MGIT. All INH-resistant results on TLA were concordant with MGIT. While smear positivity was similar ($P = 0.2$), time to TLA GC positivity was significantly longer for discrepant DST results than

Table 1 TLA vs. MGIT™ and 7H11 for provision of valid DST results

	MGIT DST		7H11 DST		
	DST valid	Others*	DST valid	Others*	Total
TLA	*n*	*n*	*n*	*n*	*n* (%)
Valid direct DST	279	6	280	5	285 (89)
Others*	33	2	31	4	35 (11)
Total, *n* (%)	312 (97.5)	8 (2.5)	311 (97.2)	9 (2.8)	320

* Culture-negative, contaminated or invalid DST.
TLA = thin-layer agar; MGIT = Mycobacteria Growth Indicator Tube; DST = drug susceptibility testing.

concordant INH results (14 days, standard deviation (SD) 3.14 vs. 7 days, SD 3.51; $P = 0.04$).

In the case of RMP, one sample was TLA-susceptible but MGIT-resistant. MTBDR*plus* detected an *rpo*B gene mutation, confirmed by sequencing as Leu531, while the MIC was >160 μg/ml. This sample was considered as false-susceptible on TLA. Of the eight OFX-resistant results in either technique, two were discrepant. One isolate that was TLA-susceptible but MGIT-resistant had a wt *gyr*A and *gyr*B profile on LPA and sequencing, with an MIC of 4 μg/ml, one dilution above the critical concentration. The paired isolate was susceptible on both techniques, confirming OFX susceptibility in favour of TLA. The sediment from the remaining isolate that was resistant on TLA and susceptible on indirect DST was not viable, and as the isolate was contaminated, it was not available for sequencing; however, the paired isolate was resistant by both techniques, confirming OFX resistance in TLA.

Of the eight discordant KM results, all TLA-susceptible and 7H11 agar-resistant, four were wt on sequencing, with MIC at 2.5 μg/ml. Two of these wt isolates belonged to the same patient. Another patient yielded KM-susceptible results by both techniques for the paired isolate, whereas for the remaining patient paired DST results were not available. These four discrepant results were resolved in favour of TLA. The remaining four samples showed an MIC $\geqslant 5$ μg/ml, three were wt and one had an *rrs* A 1401 G mutation. All four samples were considered false-susceptible on TLA. To further investigate the reasons for the KM discrepancy, we established the MIC for all 11 concordant resistant results. All strains had an MIC of $\geqslant 5$ μg/ml on REMA. Distribution of smear positivity grade and time to TLA GC positivity did not differ significantly in concordant and discordant results. After resolving the discrepancies, the corrected sensitivity of the TLA DST was respectively 94.7%, 98.2% 100% and 78.9% for INH, RMP, OFX and KM, with a specificity of 100% for all drugs (Table 4).

Of the 115 patients who submitted two samples, 112 (97.4%) showed concordant TLA results on the paired samples, while three (2.6%) were discordant

Table 2 Comparison of TLA, MGIT and 7H11 agar medium DST results prior to resolution of discordances ($n = 279$)

	TLA-resistant *n*	TLA-susceptible *n*	TLA total *n*	Sensitivity % (95%CI)	Specificity % (95%CI)
MGIT					
INH-resistant	90	7	97	92.7 (85.7–97.0)	100
INH-susceptible	0	182	182		
Total	90	189	279		
RMP-resistant	54	2	56	96.4 (87.7–99.6)	100
RMP-susceptible	0	223	223		
Total	54	225	279		
7H11					
OFX-resistant	5	1	6	83.3 (35.9–99.6)	99.6 (98.0–100)
OFX-susceptible	1	273	274		
Total	6	274	280		
KM-resistant	11	8	19	57.9 (33.5–79.7)	100
KM-susceptible	0	261	261		
Total	11	269	280		

TLA = thin-layer agar; MGIT™ = Mycobacteria Growth Indicator Tube; DST = drug susceptibility testing; CI = confidence interval; INH = isoniazid; RMP = rifampicin; OFX = ofloxacin; KM = kanamycin.

for INH. The delay between sample collection and processing was significantly longer (median 14 days, IQR 11–18) for samples that yielded invalid TLA DST results relative to those with valid results (median 12 days, IQR 9–14, $P = 0.009$), while microscopy positivity was significantly higher ($P < 0.001$) in samples yielding valid TLA DST results. Valid TLA plates turned positive after a median of 7 days (IQR 6–10) vs. 20 days (IQR 13–30) for those with invalid DST results. With regard to conventional DST methods, MGIT results were available after a median of 23 days (IQR 20–26), including primary isolation, whereas an additional 49 days was required for 7H11, including 21 days on average for LJ subcultures, for a total delay of 72 days (IQR 69–75).

Excluding equipment and infrastructure costs, consumables for TLA DST were estimated at 2€ and 1.6€ for a smear,[11] vs. 7.5€ for MRDTB*plus*, 8.89€ for Xpert at FIND-negotiated prices, and 15€ for culture, identification and DST on MGIT and 7H11 agar, with MGIT consumables purchased at FIND-negotiated prices. The total time for one TLA plate, including medium preparation, test processing and plate readings, is on average 30 min vs. 50 min for conventional DST methods, 2 h for Xpert or 5 h for MRDTB*plus*, according to the manufacturer's instructions.

DISCUSSION

The study shows that direct TLA DST detects INH and RMP resistance reliably compared to the CRS, and represents the first attempt to apply TLA as a direct method to detect OFX and KM resistance, reducing the time to DST results from 72 to 7 days. TLA correctly detected OFX resistance when com-

Table 3 Confirmatory tests for discordant results

Identification	ITM smear result	Drug tested	Indirect DST	TLA	MTBDR*plus*	MIC μg/ml	Sequencing	Result from paired sample: TLA	Result from paired sample: MGIT	Final result
77B	3+	INH	R	S	*inh*A WT; *Kat*G delWT1, MUT1	>16	—	Not available		R
51A	8 AFB	INH	R	S	*inh*A WT; *Kat*G delWT1, MUT1	>16	—	Not available		R
52B	3+	INH	R	S	*inh*A WT; *Kat*G delWT1, MUT1	8	—	R	R	R
2B	1+	INH	R	S	*inh*A WT; *Kat*G delWT1,MUT1	>16	—	R	R	R
104B	2+	INH	R	S	*inh*A WT; *Kat*G delWT1, MUT1	>16	—	R	R	R
19B	2+	INH	R	S	*inh*A WT; *Kat*G WT	0.1	*inhA* WT, *Kat*G WT	S	S	S
51A	8 AFB	RMP	R	S	*rpo*B delWT8; MUT3	>160	*rpo*B Leu 531	not available		R
28A	4+	OFX	R	S	*Gyr*A WT	4	*Gyr*AB WT	S	S	S
31A	3+	OFX	S	R	Sediment and isolate not available			R	R	R
83A	2+	KM	R	S	—	2.5	WT	DST invalid		S
96A	3+	KM	R	S	—	2.5	WT		Sample A	S
96B	4+	KM	R	S	—	2.5	WT		Sample B	S
4B	3+	KM	R	S	—	2.5	WT	S	S	S
20A	4+	KM	R	S	—	5	WT		Sample A	R
20B	4+	KM	R	S	—	5	WT		Sample B	R
15A	3+	KM	R	S	—	>5	WT	R	R	R
38A	2+	KM	R	S	—	>5	A 1401 G	N/A	NA	R

ITM = Institute of Tropical Medicine; DST = drug susceptibility testing; TLA = thin-layer agar; MIC = minimum inhibitory concentration; MGIT™ = Mycobacteria Growth Indicator Tube; INH = isoniazid; R = resistant; S = susceptible; WT = wild type; AFB = acid-fast bacilli; RMP = rifampicin; OFX = ofloxacin; KM = kanamycin.

Table 4 Comparison of TLA DST results and composite reference standard for INH, RMP, OFX and KM after resolving discrepancies

CRS	TLA-resistant *n*	TLA-susceptible *n*	TLA total *n*	Sensitivity % (95%CI)	Specificity % (95%CI)
INH-resistant	90	5	95	94.7 (88.2–97.7)	100
INH-susceptible	0	183	183		
Total	91	187	278		
RMP-resistant	55	1	56	98.2 (90.4–100)	100
RMP-susceptible	0	222	222		
Total	55	223	278		
OFX-resistant	6	0	6	100	100
OFX-susceptible	0	273	273		
Total	6	273	279		
KM-resistant	15	4	19	78.9 (54.5–93.9)	100
KM-susceptible	0	260	260		
Total	15	264	279		

TLA = thin-layer agar; DST = drug susceptibility testing; INH = isoniazid; RMP = rifampicin; OFX = ofloxacin; KM = kanamycin; CRS = Composite Reference Standard; CI = confidence interval.

pared to the CRS, but performance for KM was poorer than for the other drugs. Several factors may have contributed to the KM discrepancies, such as the MIC close to cut-off or possible drug adsorption by polypropylene tubes in the 7H11 agar test; difficult inoculum standardisation can only partially explain the variations in results between techniques, as for the four KM-resistant strains missed by TLA; the results for other drugs tested were all concordant. While performance is poorer than the other phenotypic methods, TLA sensitivity is higher than that of other rapid methods such as indirect MDRTB*sl* (78.9% vs. 66.9%).[12] Due to the high specificity, TLA results could be used to rapidly identify extensively drug-resistant TB (XDR-TB) cases. Future studies on the optimisation of TLA performance for injectable agents can test the effect of a less diluted inoculum, a lower drug concentration, additional plate readings after prolonged incubation and comparing results between different injectables.

The culture positivity rate for TLA and MGIT was respectively 95.3% and 97.8%, slightly higher than the 91.3% and 96.7% reported by Robledo et al.[6] Twenty TLA-positive cultures showed insufficient growth on GC to allow DST reading, with significantly lower microscopy positivity and longer sample processing delay than those with valid GC growth. Despite the significant impact of delay in sample processing on mycobacterial viability and DST interpretation, also reported by others,[13] contamination did not prevent interpretation of the plates. Robledo et al. found a contamination rate of 4.1%; this difference might be explained by our addition of the antibiotic mixture. One pair of samples yielded different spoligotype patterns, suggesting an administrative error, cross-contamination during MGIT DST inoculation or mixed infection in the patient.

Results for INH and RMP were slightly lower than the 100% sensitivity for both drugs on TLA reported by others;[6,7] however, these studies included a limited number of drug-resistant samples. The study results are also in line with other non-commercial methods: respectively 97% and 98% for INH and RMP using CRI and MODS, and 91% and 97% with NRA,[5] while the specificity was less than 100% for all techniques.[5] Compared to other rapid methods, TLA sensitivity for INH was higher than MDRTB*plus* V.2 when applied to smear-positive samples (89.3%),[14] and comparable to MDRTB*plus* and Xpert (98.1% and 98.9%, respectively) for RMP detection.[15]

All five false INH-susceptible isolates on TLA showed a high MIC, and the missed RMP-resistant isolate showed a 531Leu mutation, usually conferring high resistance levels.[16] False-susceptible results may be due to the slower growth of some strains, a short incubation time or the difficulty in standardising the inoculum for direct tests; however, only one sample was false-susceptible to more than one drug. To reduce the number of false-susceptible results, Schaberg et al. suggest limited dilution of the inoculum to ensure sufficient growth by diluting the sediment according to the sample microscopy grade.[7] In our study, the sediments had different positivity grades (Table 3), but all were diluted 1:5 as resuspension.

TLA provides results considerably more quickly than conventional DST as well as the non-commercial direct DST methods (7 days for TLA vs. 10 days for NRA and 12 days for MODS).[17–19] Moreover, in contrast to conventional DST, TLA reduces the biohazard risk, as there is no need to open the plates; TLA can therefore be implemented in moderate-risk TB laboratories.[20] In addition, TLA is less expensive and presents a lower workload than gold standard techniques.

In conclusion, in low-resource settings where Xpert is not available, TLA could present an affordable alternative to LPA for the rapid detection of MDR- and XDR-TB in high MDR-TB risk groups; in

combination with Xpert, TLA could be used for smear-positive samples to rapidly detect resistance to INH and identify XDR-TB. In appropriate biosafety settings, TLA GC would furthermore provide isolates for extensive DST. TLA would also be of added value in patient monitoring, where NAATs are of little use, to detect drug resistance amplification in smear-positive cases and to provide information on culture conversion in smear-negative cases. Further studies are needed to improve KM performance, to extend OFX testing to include different concentrations for the detection of high-level fluoroquinolone resistance, and to evaluate its application at field level.

Acknowledgements

The authors would like to thank M Eddyani for helpful comments, the Tbilisi TB National Reference Laboratory and Health Centre staff and all patients who participated in the study.

Conflicts of interest: none declared.

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RESUME

CONTEXTE : Les techniques moléculaires détectent rapidement une résistance à la rifampicine (RMP) et l'isoniazide (INH), mais n'éliminent pas le besoin de tests de susceptibilité à d'autres médicaments (DST) basés sur la culture. L'agar en couche fine (TLA), une méthode directe de DST non-commerciale, a montré de bonnes performances pour l'INH et la RMP ; cependant les preuves sont encore limitées, tandis que son applicabilité à l'ofloxacine (OFX) et kanamycine (KM) est inconnue.

DESIGN : Nous avons comparé 279 résultats DST de TLA à MGIT pour l'INH et la RMP, et 280 résultats DST de TLA pour OFX et KM à la méthode des proportions sur agar 7H11, obtenu à partir d'un échantillon de 320 frottis positifs de 165 patients Géorgiens tuberculeux. Les résultats en désaccord ont été résolus en comparaison à un standard de référence composite. La prévalence de la tuberculose multirésistante était de 30/164 patients (18,3%) et parmi eux, 2 cas (6,7%) avaient une tuberculose ultrarésistante.

RÉSULTATS : TLA a montré une sensibilité de 94,7%, 98,2%, 100% et 78,9% à l'INH, RMP, OFX et KM, respectivement, avec une spécificité de 100%. Le temps moyen avant obtention des résultats était de 7 jours pour le TLA, et 23 et 49 jours pour MGIT et 7H11 agar, respectivement.

CONCLUSIONS : Dans les milieux à faibles ressources, TLA peut être utilisé pour la détection rapide de la résistance à l'INH, à la RMP et aux fluoroquinolones. D'autres études sont nécessaires pour améliorer la sensibilité à KM, et évaluer sa performance pour l'OFX et d'autres médicaments, et son applicabilité dans des conditions de terrain.

RESUMEN

MARCO DE REFERENCIA: Las técnicas moleculares permiten un diagnóstico rápido de la resistencia a rifampicina (RMP) e isoniazida (INH), pero no eliminan la necesidad de practicar pruebas de sensibilidad (DST) a otros medicamentos a partir de los cultivos. La prueba en agar de capa delgada (TLA) es un método directo no comercial de DST, que ha demostrado un buen rendimiento diagnóstico con respecto a la INH y la RMP aunque los datos existentes son limitados y se desconoce su aplicabilidad al ofloxacino (OFX) y la kanamicina (KM).

MÉTODO: Se compararon 279 DST a INH y RMP en TLA con el método MGIT y 280 DST en TLA para OFX y KM, con el método de las proporciones en agar 7H11 usando 320 muestras de esputo con baciloscopia positiva, provenientes de 165 pacientes con diagnóstico de tuberculosis (TB) en Georgia. Se resolvieron las discordancias mediante la comparación con un patrón referencia compuesto. La prevalencia de multidrogorresistencia (MDR) fue de 30 en 164 pacientes (18,3%) y dos de ellos exhibieron TB extremadamente drogorresistente (6,7%).

RESULTADOS: La sensibilidad del método TLA con respecto a INH fue 94,7%, a RMP 98,2%, a OFX 100% y 78,9% a KM, con una especificidad de 100%. El lapso promedio hasta obtener los resultados fue 7 días con el método TLA, 23 días con el sistema MGIT y 49 días con el agar 7H11.

CONCLUSIÓN: En los medios con escasos recursos es posible utilizar el método en TLA con el fin de detectar rápidamente la resistencia a INH, RMP y las fluoroquinolonas. Se precisan nuevos estudios que permitan aumentar la sensibilidad en el diagnóstico de la resistencia a KM, evaluar mejor el rendimiento con respecto al OFX y otros medicamentos y estudiar la aplicabilidad de la prueba sobre el terreno.