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A comparison of the Xpert® MTB/RIF and GenoType® MTBDR*plus* assays in Georgia

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SUMMARY

Few studies have directly compared the performance of rapid molecular diagnostic tests for tuberculosis (TB). We found that the commercially available molecular diagnostic tests Xpert® MTB/RIF and GenoType® MTBDR*plus* both provided timely and accurate results compared to conventional phenotypic tests in detecting TB and rifampicin resistance.

KEY WORDS: tuberculosis; diagnosis; molecular; drug resistance; multidrug resistance

MULTIDRUG-RESISTANT TUBERCULOSIS (MDR-TB), defined as resistance to at least isoniazid (INH) and rifampicin (RMP), presents a major obstacle to achieving global TB control. The World Health Organization (WHO) recently indicated that global efforts are 'off track' in MDR-TB management and that fewer than 25% of patients have their disease and associated drug resistance detected.[1] Promising rapid molecular diagnostic tests have been developed in response to the low MDR-TB detection rates, and the WHO has endorsed the line-probe assay (LPA) (GenoType® MTBDR*plus*; Hain Lifescience, Nehren, Germany) and the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) for clinical use. Comparison of these two molecular technologies is crucial to validate the accuracy and feasibility of the implementation of the tests in local laboratory settings and to help national TB programs optimize allocation of limited resources.[2,3] Within this context, we compared the performance of Xpert and GenoType in a setting with high rates of MDR-TB.

METHODS

The study was conducted at the National Reference Laboratory (NRL) of the National Center for Tuberculosis and Lung Diseases (NCTLD) in Tbilisi, Georgia, and was approved by the Center's Institutional Ethics Committee. Over a 5-month period in 2013, all TB suspects who were acid-fast bacilli (AFB) smear microscopy positive on routine testing underwent sputum culture as well as GenoType and Xpert testing.

AFB-positive sputum specimens were divided into three portions and were tested using AFB culture, Xpert and GenoType (version 2.0) LPA. The processed specimen was inoculated onto Löwenstein-Jensen (LJ) based solid medium and/or the BACTEC™ MGIT™ 960 broth culture system (BD, Sparks, MD, USA), as described elsewhere.[4] First-line drug susceptibility testing (DST) was performed on all cultures positive for *Mycobacterium tuberculosis* using either the absolute concentration method on LJ medium (INH 0.2 mg/ml, RMP 40 mg/ml) or in 7H9 broth using the BACTEC MGIT 960 system (INH 0.1 mg/ml, RMP 1 mg/ml).[4] A 500 μl portion of decontaminated sample was used to perform the GenoType assay according to the manufacturer's instructions (http://www.ipaqt.org/wp-content/uploads/2013/02/MTBDRplusV2_product-insert.pdf). Xpert was performed on the third portion of the clinical sample using G4 cartridges according to the manufacturer's instructions. All sample results data were entered into an online database and analyzed using SAS, version 9.3 (Statistical Analysis System Institute, Cary, NC, USA).

RESULTS

Of 382 AFB smear-positive sputum samples from TB suspects included in the study, 264 (69%) were from new TB cases and 128 (31%) from retreatment cases. Overall, 357 (94%) samples were culture-positive for *M. tuberculosis* on solid or liquid culture, 20 (5%) were culture-negative and 5 (1%) had contaminated cultures. Of the 357 culture-positive samples, respectively 346 (97%) and 336 (94%) were detected by the Xpert

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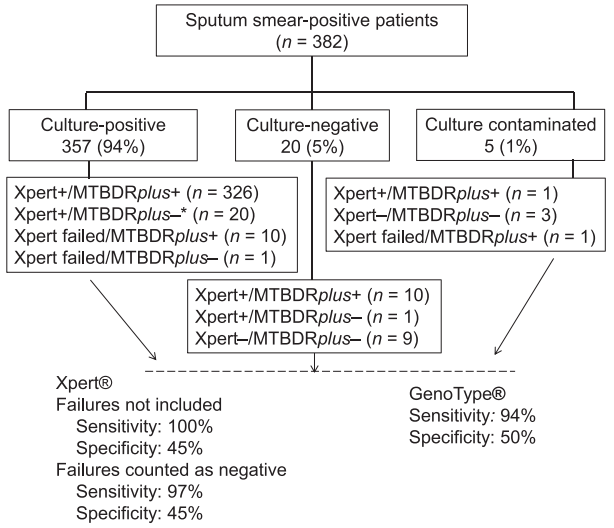

Figure Sputum culture, Xpert® TB/RIF, and GenoType® MTBDR*plus* assay results. AFB smear results: 1+ ($n = 14$); 2+ ($n = 4$); 3+ ($n = 2$). * Quantitative AFB smear results; patients with discrepant results had low bacillary burden. + = positive; − = negative; AFB = acid-fast bacilli.

and GenoType assays. Xpert failed 12 (3.1%) times, and there were no GenoType failures. If Xpert failures were excluded from the analysis, the sensitivity of the assay was 100% when compared to culture (Figure).

Both Xpert and LPA showed good sensitivity (87% and 83%, respectively) and excellent specificity (both 99%) in detecting RMP resistance. The overall agreement between Xpert (κ 0.89) and LPA (κ 0.88) with culture-based DST in the detection of RMP resistance was excellent. The additional advantage of LPA was the detection of INH resistance. The sensitivity and specificity in detecting INH resistance was respectively 83 (95% confidence interval [CI] 76–90) and 100 (95%CI 99–100).

The median time to *M. tuberculosis* detection was substantially shorter with both the GenoType (5 days) and Xpert (2 days) assays than with solid culture (33 days) and liquid culture (9 days) (Table 1).

DISCUSSION

The sensitivity of both molecular assays was excellent, and in line with previous reports.[2] The Xpert and GenoType assays identified *M. tuberculosis* complex DNA in respectively 11 (55%) and 10 (50%) of the 20 culture-negative samples. This phenomenon (positive molecular test and negative culture) has been described previously, and has been attributed to the amplification of DNA released from non-viable bacilli, laboratory cross-contamination, or a transcription error that failed to indicate that the sample was a treatment follow-up sample rather than a diagnostic sample.[5] However, in some cases the positive molecular test result may in fact be true-positive, as indicated by two

Table 1 Time to results for sputum culture and the Xpert® MTB/RIF and GenoType® MTBDR*plus* assays ($n = 382$)

	Time to result* Days	
Test	Mean ± SD	Median [IQR]
Solid culture ($n = 155$)	36.7 ± 12.9	33.0 [27–41]
Liquid culture ($n = 227$)	11.6 ± 11.3	9.0 [7–11]
Xpert	3.3 ± 2.3	2.0 [2–4]
GenoType	5.5 ± 2.4	5.0 [3–7]

* Time from sample collection to recorded results
SD = standard deviation; IQR = interquartile range.

study patients with negative baseline cultures and a positive molecular test result who were culture-positive for *M. tuberculosis* at follow-up.

In comparison to other studies, the sensitivity of RMP resistance detection was lower than in South Africa and higher than in India, highlighting the fact that genetic mutations conferring phenotypic resistance, and hence the performance of molecular assays, may vary in different settings.[3,5] One of the most important and obvious reasons for the use of molecular tests is the significantly reduced detection turnaround time. Earlier time to detection can result in earlier time to appropriate treatment and improved clinical outcomes.[6]

The main distinguishing factor between the Xpert and GenoType assays is that the GenoType assay can detect INH resistance. We found that 32% of *M. tuberculosis* isolates were resistant to INH on DST, including 38 with INH monoresistance and 78 with resistance to both INH and RMP. Studies have shown less than optimal treatment outcomes, longer treatment durations, and progression to MDR-TB among patients with INH monoresistance, thus arguing for the evaluation of new regimens.[7]

GenoType targets mutations in the *kat*G and *inh*A genes associated with INH resistance and thus has the ability to detect low vs. high level INH resistance, which may be important if high-dose INH treatment is found to be useful for low-level INH resistance.[8] A barrier to GenoType implementation is the requirement for well-trained staff and suitably equipped laboratories. Some additional factors influencing test implementation are given in Table 2. Further studies comparing the cost-effectiveness of each assay and clinical trials evaluating alternative regimens for INH-monoresistant TB could help decide which assay to use in specific settings.

CONCLUSIONS

We found that both the Xpert and the GenoType assays performed well in detecting TB and RMP resistance and had substantially shorter turnaround time than culture. Implementation of either molecular assay can reduce the time to detection of drug-resistant TB disease, and offers great promise in improving MDR-TB care and prevention.

Table 2 Comparative characterization of the Xpert® MTB/RIF and GenoType® MTBDR*plus* assays

	GenoType	Xpert
Infrastructure		
Space requirements	High (three separate rooms)	Limited (one bench)
Biosafety requirements	Advanced BSL-2	None
Test performance space		
Temperature control	Real time*	15–30°C
Storage space temperature control	Needed (+4°C; −20°C)	Room temperature (2–28°C)
Power supply backup	Required (generator)	Uninterrupted power supply required
Equipment		
Manufacturer	Twincubator (Hain Lifescience)	Xpert machine (Cepheid)
Additional equipment	Thermocycler Water bath Refrigerator (+4°C) Refrigerator (−20°C) Thermoblock	None required
Maintenance		
Annual calibration	Suggested	Required
Software support	None	None
Service guarantee	None	1-year warranty provided
Service available on site	No	No
Human resources		
Staff qualification	MBB or higher	Laboratory technician
Training needs	Advanced	Basic
Test turnaround time		
Hands-on work	3–4 h	15 min
Procedure duration	7–8 h	2 h
Result submission	Second day	Same day
Test interpretation		
M. tuberculosis complex identification	Yes	Yes
M. tuberculosis speciation	No	No
INH resistance detected	Yes	No
RMP resistance detected	Yes	Yes
MDR-TB† detected	Yes	No
Quality control		
Internal	Available	Available
External	Not available	Not available
Interpretation	Manual	Automated

* Only during the hybridization process.
† Resistance to at least INH and RMP.
BSL = biosafety level; MBB = Bachelor of Medicine; INH = isoniazid; RMP = rifampicin; MDR-TB = multidrug-resistant tuberculosis.

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Conflicts of interest: none declared.

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RESUME

Peu d'études ont directement comparé les performances des tests moléculaires rapides de la tuberculose (TB). Nous avons constaté que les tests de diagnostic moléculaires disponibles dans le commerce, Xpert® MTB/RIF et GenoType® MTBDR*plus*, avaient tous deux des résultats rapides et précis par comparaison aux tests phénotypiques conventionnels de détection de la TB et de la résistance à la rifampicine.

RESUMEN

En pocas investigaciones se ha cotejado directamente el rendimiento de las pruebas moleculares rápidas de diagnóstico de la tuberculosis (TB). En el presente estudio se observó que las pruebas moleculares diagnósticas existentes en el mercado, como la prueba Xpert® MTB/RIF y la prueba GenoType® MTBDR*plus*, ofrecen ambas resultados oportunos y exactos, en comparación con las pruebas fenotípicas corrientes de detección de la TB y la resistencia a rifampicina.