Xpert MTB/RIF performance to diagnose tuberculosis and rifampicin resistance in a reference centre in southern Brazil

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ABSTRACT   Effective treatment of tuberculosis (TB) remains a serious public health problem in many countries, including Brazil, especially when considering drug-resistant disease. Xpert MTB/RIF has been implemented in many countries to reduce the time to TB diagnosis and to rapidly detect rifampicin resistance. The study aimed to describe and evaluate Xpert MTB/RIF performance in diagnosing pulmonary TB and rifampicin resistance in a tertiary healthcare facility in Brazil.

A cross-sectional study was performed, which included all isolates of confirmed pulmonary TB patients from 2015 to 2018. Both Xpert MTB/RIF and GenoType MTBDR*plus* assays were performed to detect rifampicin and isoniazid resistance. In addition, isolates with detected resistance to rifampicin and/or isoniazid were analysed by phenotypic testing using MGIT-960 SIRE kit and whole-genome sequencing (WGS) using Illumina MiSeq Sequencing System.

2148 respiratory specimens tested with Xpert MTB/RIF were included: n=1556 sputum, n=348 bronchoalveolar lavage and n=244 gastric washing. The overall Xpert MTB/RIF sensitivity in sputum was 94% and the overall specificity was 98%. The negative predictive value in sputum of all the patients was 99% with a positive predictive value of 89%. The concordance between Xpert MTB/RIF and phenotypic susceptibility test was 94.1%, while its concordance with WGS was 78.9%.

Xpert MTB/RIF is a rapid and accurate diagnostic strategy for pulmonary TB, which can contribute to improvement in TB control. However, detection of rifampicin resistance might be associated with false-positive results.

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Xpert MTB/RIF has the potential to reduce the time to diagnose TB, with high accuracy, including paucibacillary disease. It is also feasible to detect rifampicin resistance, with a high concordance with phenotypic tests and whole-genome sequencing. http://bit.ly/2WW4jmt

Introduction

Tuberculosis (TB) remains among the top 10 causes of death worldwide and the leading cause from a single infectious agent. An estimated 10 million new cases and 1.6 million TB-related deaths occurred in 2017, according to a World Health Organization (WHO) report [1].

Drug-resistant TB remains a public health threat, since 558 000 rifampicin-resistant cases were estimated in 2017 and of these 82% were classified as multidrug-resistant (MDR) (resistance to rifampicin and isoniazid). Conventional culture-based drug susceptibility testing is time consuming, which negatively impacts on the transmission of drug-resistant TB in the community [2].

Allied to the strategies to control the disease, in 2011 the WHO endorsed the use of Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), which is a rapid, automated and cartridge-based molecular test, as a method for the initial diagnosis test for suspected TB cases. In a timeframe of 2 h, the test detects *Mycobacterium tuberculosis* DNA and the main mutations that cause rifampicin resistance, providing high sensitivity and specificity values for pulmonary specimens [3]. In addition, the WHO has endorsed Xpert MTB/RIF for use in extrapulmonary specimens, with lower and variable sensitivity values [4].

The Brazilian National Program for TB Control (Ministry of Health) implemented this diagnostic technology in 92 high-burden cities between 2014 and 2015. However, there is very little published information about Xpert performance for pulmonary tuberculosis (PTB) and drug-resistant TB diagnosis in Brazil. Clinics Hospital at the University of São Paulo (Ribeirão Preto, Brazil) has been using Xpert MTB/RIF since 2015 to test both pulmonary and extrapulmonary samples [5].

This study aims to describe and evaluate Xpert MTB/RIF performance in respiratory samples (sputum, bronchoalveolar lavage (BAL), gastric washing) to diagnose PTB and rifampicin resistance in a tertiary reference hospital.

Methods

This retrospective cross-sectional study enrolled all samples of confirmed PTB patients from 2015 to 2018 in a tertiary hospital located in São Paulo state, southern Brazil. All the individuals who had at least one pulmonary sample tested with Xpert MTB/RIF in the hospital were included. Xpert MTB/RIF was performed according to manufacturer's instructions. All included cases were classified into one of two categories: confirmed PTB and probable PTB cases. In addition to clinical, epidemiological and radiological evaluation, PTB confirmed cases always had a *M. tuberculosis*-positive culture and/or biopsy showing a typical histopathological pattern characterised as a chronic granulomatous inflammatory reaction with caseous necrosis, and the presence of acid-fast bacilli (AFB). Probable cases included those with clinical, epidemiological and radiological evaluation with clinical response to specific treatment to TB [6].

Concentrated smears were stained using Ziehl–Neelsen staining for detection of AFB, and culture was performed in liquid media (BACTEC MGIT 960). *M. tuberculosis* complex strains were identified in culture using the rapid immunochromatographic test Sd Bioline TBAgMPT64 (Standard Diagnostics, Seoul, South Korea).

In addition, all the isolates were analysed according to manufacturer's instructions using the genotypic test GenoType® MTBDR*plus* (Hain Lifescience, Nehren, Germany), which provides information about mutations associated with rifampicin and isoniazid resistance. All the *M. tuberculosis* with resistance detected using Xpert®MTB/RIF underwent routine phenotypic drug susceptibility tests (DST) on liquid media MGIT-960 SIRE kit (MGIT-960; Becton Dickinson Diagnostic Systems, Sparks, MD, USA). The critical concentration used was 1.0 μg·mL^{-1} for rifampicin and 0.1 μg·mL^{-1} for isoniazid.

Isolates with at least one commercial molecular test indicating rifampicin drug resistance were evaluated using a whole-genome sequencing (WGS) technique. The WGS was performed using an Illumina MiSeq Sequencing System MiSeqV2-500 cycles (Illumina, San Diego, CA, USA). DNA libraries were prepared using the Nextera XT library preparation kit. Sequencing was performed using the MiSeq Reagent Kit v2 (500 cycles) per the manufacturer's protocol, producing 250 bp paired-end reads. TB profiler was used to identify mutations known to cause drug resistance [7]. In addition, sequences were analysed with a pipeline composed of open source software as described previously [8]. Briefly, the trimmomatic tool was used for trimming of adapters and low-quality bases (Phred quality score <20) and filtering for a minimum read length of 36 [9]. Reads were aligned to the *M. tuberculosis* H37Rv (Genbank: AL123456) genome using three different alignment algorithms: the Burrows–Wheeler alignment tool [10], Novoalign and SMALT [11]. For all sequenced isolates, >98% of the reference genome was covered by at least one read and an average depth of coverage of 44 (min 20, max 80) was achieved. The alignment files were subjected to local realignment and de-duplication using the Genome Analysis Toolkit (GATK) [12] and Picard tools [13]. Variants (single nucleotide polymorphisms) and insertion/deletions in coding and

noncoding regions were identified from each alignment file using GATK [12] and SamTools, and the variants identified in all three alignments were used for further analysis. Variants were annotated and drug resistance was inferred from a combination of drug-resistance mutation libraries [7].

We further evaluated patient records with discrepant results on DST to better define the most likely diagnoses. Xpert MTB/RIF sensitivity, specificity, positive and negative predictive values were calculated using culture results, clinical data and response to specific therapy as the standard for a confirmed TB case.

This study was approved by the Clinics Hospital ethics committee (protocol number: 17471/2014).

Results

During the study period 2241 respiratory specimens were tested using Xpert MTB/RIF: n=1625 sputum, n=354 BAL and n=262 gastric washing. 2148 valid Xpert MTB/RIF results were obtained.

Sputum

Of the 1625 sputum samples, 69 (4.2%) were excluded due to invalid results and 207 (13.3%) had a positive Xpert MTB/RIF result. 185 (89.3%) patients with a positive Xpert MTB/RIF had confirmed TB, of which 178 (96.2%) were culture-positive. Among three patients with negative culture and positive Xpert MTB/RIF and AFB there were two cases with *M. tuberculosis* detected in culture of nonrespiratory specimens in the same period. One patient had symptoms and chest radiological images suggested TB and cured after TB treatment only.

There were 26 patients with negative culture and AFB with a positive Xpert MTB/RIF result. Four showed symptoms and chest radiological findings and were treated for TB with complete recovery after 6 months. Among the remaining 22 patients, 19 had confirmed previous TB (1–3 years prior), but without detection of active TB during the study period, and three patients had an alternative diagnosis (fungal disease).

Of the 1349 Xpert MTB/RIF negative patients, 10 were culture-positive for *M. tuberculosis*, 129 were culture-positive for nontuberculous mycobacteria (NTM) and all the others had negative culture for mycobacteria. All six samples with positive AFB and negative Xpert MTB/RIF were identified as NTM. Among the three cases with negative culture and a negative Xpert MTB/RIF but AFB-positive, two had symptoms and chest radiological images suggestive of TB. These patients received treatment for TB and recovered from the symptoms completely. The symptoms of the third patient could be explained by other diagnoses (figure 1).

The overall sensitivity in sputum was 94%: 98.3% among AFB-positive samples and 87.2% for paucibacillary PTB. Overall specificity was 98%: 100% among AFB-positive samples and 98.4% among AFB-negative samples (table 1).

BAL

Among the 354 BAL samples, six (1.7%) were excluded due to invalid Xpert MTB/RIF results and 27 (7.8%) of the 348 valid tests had Xpert-positive results. 22 (81.4%) patients with Xpert-positive results had confirmed TB; 19 out of 22 had positive culture. Among the three cases with negative culture and positive Xpert MTB/RIF and AFB, one patient had *M. tuberculosis* culture-positive sputum in the same period. The other two patients had confirmed previous TB (1 year prior), but without active disease during the study period.

Five patients were culture-negative, AFB-negative and had a positive Xpert result. Among these, two had *M. tuberculosis* detected in sputum culture in the same period. Two patients had confirmed previous TB (1–2 years prior) without active disease, and the one patient had an alternative diagnosis (false-positive). Overall, among the confirmed TB cases and Xpert-positive, 11 (50%) were AFB-positive.

Among the 321 negative Xpert cases, four had positive culture results. Three of these were identified as NTM (figure 2).

The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for BAL are described in table 1.

Gastric washing

Among the 262 gastric washing samples, 18 (6.9%) were excluded due to invalid results. 29 (11.9%) of the 244 valid tests had an Xpert-positive result and all of these had confirmed TB, 24 with positive culture. Among the Xpert-positive results, 18 (62%) were AFB-positive. All of the five culture-negative patients had *M. tuberculosis* detected in a sputum culture taken during the same period.
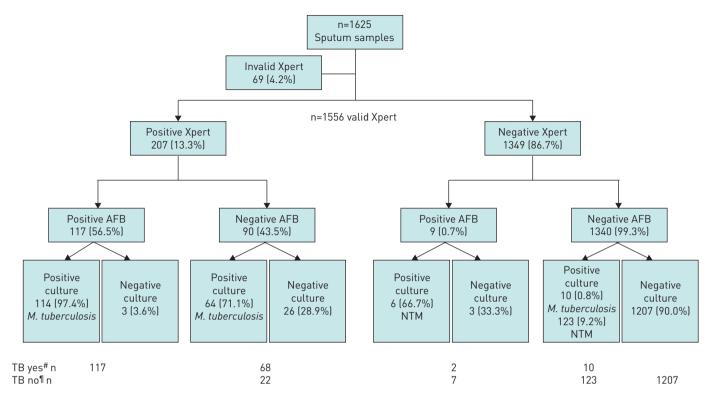
FIGURE 1 Xpert MTB/RIF results for 1625 sputum samples from suspected pulmonary tuberculosis (TB) patients. Xpert: Xpert MTB/RIF; AFB: acid-fast bacilli; *M. tuberculosis*: *Mycobacterium tuberculosis*; NTM: nontuberculous *Mycobacteria*. #: confirmed pulmonary TB; ¶: TB not confirmed.

Among the 215 negative Xpert MTB/RIF cases, 11 had positive culture results. 10 out of 11 were identified as NTM and one as *M. tuberculosis* (figure 3).

The sensitivity, specificity, NPV and PPV for gastric washing are described in table 1.

Table 1 shows Xpert MTB/RIF assay sensitivity, specificity, PPV and NPV for the diagnosis of 2148 respiratory specimens from patients suspected of pulmonary tuberculosis.

Rifampicin-resistance evaluation

Xpert MTB/RIF detected rifampicin resistance in 23 patients (18 in sputum samples, three in BAL and two in gastric washing). 14 (60.9%) patients had results from at least two Xpert tests, the second one

TABLE 1 Xpert MTB/RIF and acid-fast bacilli (AFB) sensitivity, specificity, predictive positive value (PPV) and negative predictive value (NPV) for 2148 respiratory specimens for pulmonary tuberculosis diagnosis

	Sputum		BAL		Gastric washing	
Subjects n	1556		348		244	
Xpert						
Sensitivity	185/197	94 (0.90–0.97)	22/23	96 (0.78–1.00)	29/30	97 (0.83–1.00)
Specificity	1337/1359	98 (0.98–0.99)	320/325	98 (0.96–0.99)	214/214	100 (0.98–1.00)
PPV	185/207	89 (0.84–0.93)	22/27	81 (0.62–0.94)	29/29	100 (0.88–1.00)
NPV	1337/1349	99 (0.98–1.00)	320/321	99 (0.98–1.00)	214/215	99 (0.97–1.00)
AFB						
Sensitivity	119/197	60 (0.53–0.67)	9/23	39 (0.20–0.61)	18/30	60 (0.41–0.77)
Specificity	1352/1359	99 (0.99–1.00)	323/325	99 (0.98–1.00)	214/214	100 (0.98–1.00)
PPV	119/126	94 (0.89–0.98)	9/11	82 (0.48–0.98)	18/18	100 (0.81–1.00)
NPV	1352/1430	95 (0.93–0.96)	323/337	96 (0.93–0.98)	214/226	95(0.91–0.97)

Data are presented as n/N or % (95% CI), unless otherwise stated. BAL: bronchoalveolar lavage.
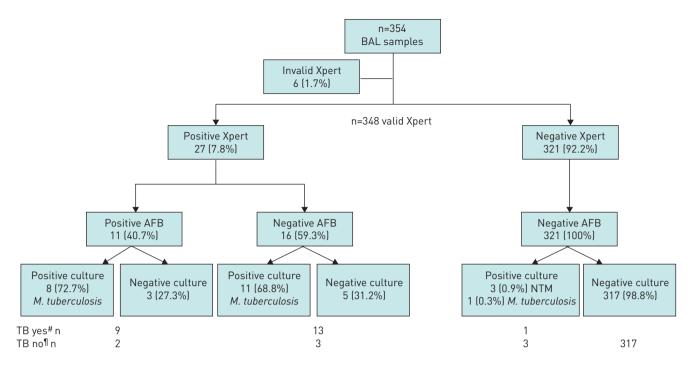
FIGURE 2 Xpert MTB/RIF results for 354 bronchoalveolar specimens from patients suspected of pulmonary tuberculosis (TB). BAL: bronchoalveolar lavage; Xpert: Xpert MTB/RIF; AFB: acid-fast bacilli; *M. tuberculosis*: *Mycobacterium tuberculosis*; NTM: nontuberculous *Mycobacteria*. [#]: confirmed pulmonary TB; [¶]: TB not confirmed.

performed after the first result had shown rifampicin resistance. The isolates were tested using Genotype MTBDR*plus* and only the first sample was submitted to phenotypic DST and WGS. 12 (85.7%) out of 14 patients had concordant Xpert rifampicin-resistance results and two (14.3%) had discordant tests (one showing rifampicin resistance and the other with susceptibility to this drug). One of these came from BAL and the other from gastric washing. Nine patients had only one Xpert test with a rifampicin-resistant
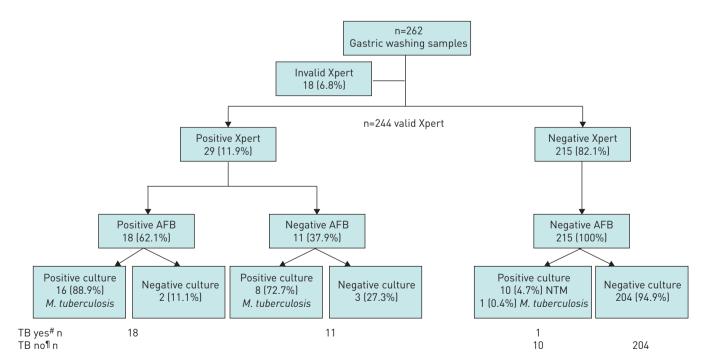

FIGURE 3 Xpert MTB/RIF results for 262 gastric washing specimens from patients suspected of pulmonary tuberculosis (TB). Xpert: Xpert MTB/RIF; AFB: acid-fast bacilli; *M. tuberculosis*: *Mycobacterium tuberculosis*; NTM: nontuberculous *Mycobacteria*. [#]: confirmed pulmonary TB; [¶]: TB not confirmed.

profile detected. No additional Xpert tests were positive for *M. tuberculosis*, so it was not possible to confirm rifampicin resistance by Xpert.

Phenotypic DST was available in 17 out of these 23 patients while GenoType MTBDR*plus* testing was performed in 20 out of 23 patients and WGS in 19 out of 23 patients.

Considering phenotypic DST and/or WGS as the standard reference for detection of rifampicin resistance, we compared the results from Xpert MTB/RIF in one or two clinical specimens, depending on availability. Regarding the overall concordance between Xpert and WGS results, 15 (78.9%) out of 19 showed rifampicin resistance detected by both tests. Among 17 isolates with phenotypic DST results available, 16 (94.1%) were concordant with Xpert results. Among nine isolates with one Xpert test detecting rifampicin resistance, one was a false-positive TB case, due to a probable contamination of the bronchoscope. Seven patients with one Xpert test also presented with phenotypic DST and/or WGS rifampicin resistance, while in one patient WGS failed to identify rifampicin resistance-causing mutations.

The individual results for Xpert, GenoType MTBDR*plus*, WGS and phenotypic DST are presented in table 2.

Discussion

There is still a lack of information about Xpert performance under programmatic conditions in Brazil despite its regular use in many high-burden cities since 2014 [5]. Recent studies in Brazil showed that the use of the Xpert MTB/RIF increased detection of TB cases among paucibacillary patients [14], adolescents [15] and the HIV-positive population [16].

The high diagnostic performance observed in this study has been reported in other studies by STEINGART *et al.* [17], CHANG *et al.* [18], LI *et al.* [19] and THERON *et al.* [20]. Xpert MTB/RIF outperformed smear microscopy and established diagnosis in a significant proportion of patients who were smear-negative, detected additional culture-negative patients and has excellent rule-out value for MDR-TB. The pooled sensitivity and specificity obtained by CHANG *et al.* [18] in smear-negative specimens (75.0% and 98.2%, respectively) were lower than the rates detected in smear-positive specimens (98.7% sensitivity and 98.2%

TABLE 2 Rifampicin susceptibility profiles for rifampicin for 23 pulmonary *Mycobacterium tuberculosis* isolates comparing Xpert MTB/RIF, GenoType MTBDR*plus*, phenotypic drug susceptibility tests (pDST) and whole-genome sequencing (WGS) looking for mutations that confers resistance to rifampicin

Sample	Xpert tests performed n	Sample	Xpert result	GenoType plus	pDST	WGS: rpoB mutation	Concordance Xpert and pDST or WGS[#]
1	1	Sputum	1 R	R	R	NA	Yes
2	1	Sputum	1 R	R	NA	H445N	Yes
3	1	Sputum	1 R	NA	NA	No mutation	No
4	1	Sputum	1 R	R	R	S450L	Yes
5	1	Sputum	1 R	NA	R	NA	Yes
6	1	Sputum	1 R	R	R	S450L	Yes
7	1	BAL	1 R	NA	NA	NA	Not TB[¶]
8	1	BAL	1 R	R	R	S450L	Yes
9	1	GW	1 R	R	R	D435 V/H445D	Yes
10	2	Sputum	2 R	R	R	S450L	Yes
11	2	Sputum	2 R	R	R	S450L	Yes
12	2	Sputum	2 R	S	NA	L430P/H445N	Yes
13	2	Sputum	2 R	R	R	S450L	Yes
14	2	Sputum	2 R	R	R	S450L	Yes
15	2	Sputum	2 R	R	S	No mutation	No
16	2	Sputum	2 R	R	R	S450L	Yes
17	2	Sputum	2 R	R	R	S450L	Yes
18	2	Sputum	2 R	R	R	S450L	Yes
19	2	Sputum	2 R	R	R	S450L	Yes
20	2	Sputum	2 R	R	R	No mutation	Yes
21	2	Sputum	2 R	R	R	S450L	Yes
22	2	BAL	1 R; 1 S	S	NA	No mutation	Yes
23	2	GW	1 R; 1 S	S	NA	NA	NA

R: resistant; NA: not available; BAL: bronchoalveolar lavage; GW: gastric washing; S: susceptible. [#]: concordance between RR-Xpert compared with WGS and pDST; [¶]: Xpert-positive with resistance to rifampicin in a BAL sample collected after a bronchoscopy in a patient not confirmed with pulmonary tuberculosis (contamination).

specificity). Li *et al.* [19] also reported a higher sensitivity in patients with positive pulmonary smears (97–99%) than in patients with negative smear results (68–73%).

Aslam *et al.* [21] showed that gastric washing is an interesting alternative to investigate PTB in adults unable to expectorate good-quality sputum, with a high Xpert MTB/RIF sensitivity compared to smear and culture. In this study, *M. tuberculosis* was detected on Xpert testing in 91.7% of smear-positive samples (excluding scanty), 88.2% of scanty positives and 11.4% with negative smears. Khalil and Butt [22] and Lu *et al.* [23] evaluated the Xpert performance to diagnose PTB in BAL, showing high sensitivity in this clinical specimen. Lu *et al.* found 33.8% of smear-positive samples among the cases with Xpert-positive results and confirmed TB. The good performance of Xpert MTB/RIF on BAL and gastric washing might be particularly useful for populations such as children or patients living with HIV infection.

Our study detected Xpert MTB/RIF-positive results in 23 clinical specimens collected from patients who had previous confirmed TB (1–3 years prior), but without any evidence of active TB. Molecular techniques with high sensitivity are able to detect DNA from both live and dead *M. tuberculosis*. Thus, a positive Xpert MTB/RIF result does not always imply viable bacilli and should not be used to monitor response to treatment, treatment failure or relapse [24–26]. This is a very important information for the clinician who orders and makes decisions based on Xpert MTB/RIF results.

The sample quality may influence Xpert performance, as described by Acuña-Villaorduña *et al.* [26], who found that mucopurulent sputum samples were associated with increased yield of Xpert in Uganda.

In this study, the specificity of the Xpert MTB/RIF assay was not influenced by the presence of NTMs. During the study period, 136 (6.3%) NTMs were cultivated among 2148 pulmonary specimens tested without a single positive Xpert MTB/RIF result. This is concordant with a recent systematic review, which only identified one Xpert-positive specimen among 180 specimens with NTM. This information is relevant and has the potential to guide clinicians, especially in HIV patients with pulmonary disease plus AFB-positive and Xpert MTB/RIF-negative results [27].

As described earlier, several studies have shown good diagnostic accuracy of Xpert for diagnosing TB, but data on long-term clinical outcomes have been disappointing, as shown by Monedero-Recuero [28] and Hanrahan *et al.* [29], who showed high rates of nondiagnostic results and lack on improvement in the number of patients starting TB therapy. Cost-effectiveness analysis of Xpert implementation in South Africa showed neither decreasing cost nor improvement in the number of patients starting treatment [30]. However, our data represent the performance of Xpert MTB/RIF in a tertiary hospital with 1000 beds where the results are immediately available (real-time) to the clinicians based on the electronic system, which make the connections between the Xpert machine and the electronic patient record.

Pinto *et al.* [31] estimated the incremental cost-effectiveness ratio of Xpert as a substitute for two AFB tests in the diagnosis of drug-susceptible TB and provided support for decision-makers in Brazil as well as other countries with a low prevalence of TB – financial benefit can potentially be expected if physicians rely more on a negative Xpert result. Consequently, the empirical treatment might be reduced.

Importantly, the findings from the current study correlate with previous work that demonstrated the ability of Xpert to support decisions to shorten airborne isolation for hospitalised patients with presumptive TB due to its high NPV. Cowan *et al.* [32] showed that Xpert MTB/RIF may reduce airborne infection isolation in hospitalised patients with presumptive PTB and is comparably sensitive, more specific and more cost-effective than smear microscopy. The sensitivity of one Xpert, two Xperts, two smears or three smears compared to culture in this work was 0.85, 0.95, 0.70 and 0.80, respectively. The cost-effectiveness analysis demonstrated that an Xpert test is the most cost-effective strategy.

Many studies found variable sensitivity and specificity values for the detection of rifampicin resistance. Moreover, other studies have described false-positive resistant cases, which is cumbersome for clinical decision-making, mainly in settings where the prevalence of rifampicin resistance is low [33–35].

Steingart *et al.* [17] obtained 95% of pooled sensitivity and 98% of pooled specificity for detection of rifampicin resistance. The sensitivity and specificity for rifampicin resistance detection in Chang *et al.*'s [18] meta-analysis were 94.1% and 97%, respectively, while Boehme *et al.* [34] found sensitivity of 94.4% and specificity of 98.3%.

In our study, 19 (90.5%) out of 21 patients showed concordance between rifampicin resistance detection in Xpert with phenotypic DST and/or WGS. The concordance between Xpert and Genotype MTBDR*plus* was high. Genotype MTBDR*plus* had high concordance with phenotypic DST and WGS.

There were two discordant patients. The first showed one rifampicin-resistant Xpert nonconfirmed with another test and WGS showed a pan-susceptible profile. The growth of the bacilli for DNA extraction

could have selected for the rifampicin-susceptible clones. This result might be a false rifampicin-resistance Xpert not confirmed with a second test, as observed in isolates 22 and 23 (table 2). The second patient had two rifampicin-resistance Xpert results, but the phenotypic DST and WGS showed a pan-susceptible profile. For this discrepancy, possible explanations include erroneous phenotypic testing result, resistant minority bacterial populations that were not detected by sequencing, mechanisms of resistance unknown or laboratory labelling error [36].

Regarding false-positive resistance detection, WILLIAMSON *et al.* [33] found that the Xpert test incorrectly detected rifampicin resistance in 31% of the evaluated cases. Possible explanations proposed by this group were the presence of mutation in the analysed genomic region associated with low-level rifampicin resistance and the presence of silent mutations, which results in failure of the corresponding probe to hybridise. For example, a silent CAG (Gln) to CAA (Gln) mutation at codon 510 was found in one isolate evaluated in this study and resulted in failure of the corresponding Xpert probe A to hybridise. Consequently, false-positive rifampicin resistance was confirmed. The same phenomenon was described by other authors in relation to a TTC (Phe) to TTT (Phe) mutation at codon 514 [37, 38].

Incidence of silent mutations varies geographically; they might be very frequent in some regions [39]. However, VALIM *et al.* [40] studied 82 rifampicin-resistant *M. tuberculosis* isolates in Brazil and did not find silent mutation in the *rpoB* gene.

In contrast, there are mutations in the *rpoB* gene which confer resistance to rifampicin, but are not detected by Xpert because they are located outside the 81-bp region, called rifampicin-resistance-determining region, which is the region evaluated by the commercial assays. In a previous study, we found one of these mutations (*rpoB* Val170Phe) in two isolates using WGS without rifampicin resistance detected by Xpert. The drug resistance was confirmed by phenotypic DST [41]. Similarly, ANDRE *et al.* [42] identified the *rpoB* Ile491Phe mutation in up to 30% of MDR-TB isolates in Swaziland.

The confidence of WGS in predicting phenotypic drug resistance is dependent on our knowledge of the association between phenotype and genotype. The accuracy of predicting resistance varies among different classes of drugs as well as different drugs from the same class. *M. tuberculosis* strains with a minimum inhibitory concentration very close to the critical concentration will flip-flop between resistant and susceptible, thereby impacting the predictive value of the mutation causing resistance [43, 44].

This study corroborates WHO requirement for at least two resistance Xpert results to define rifampicin resistance [3] and we add to that the need to verify epidemiological information and the patient's background. The history of previous TB, treatment failure and known contact with drug-resistant TB patients should also be considered in those cases where it was not possible to test the second clinical specimen after the first resistant Xpert result, because clinicians still need to make an informed decision about the best treatment option for each patient.

The main limitation of this study lie in the phenotypic DST and WGS gaps and missing information among the Xpert-resistant and -susceptible isolates. Moreover, PTB cases included in this study were not classified according to disease severity, which could influence Xpert MTB/RIF performance.

The new generation of Xpert MTB-RIF, the Xpert Ultra, increases sensitivity in paucibacillary samples (smear-negative specimens) with a mild decrease in specificity [45, 46], and might be an improvement in PTB diagnosis in the near future in Brazil.

In conclusion, Xpert MTB/RIF is a quick and accurate diagnostic assay to diagnose PTB and can help clinicians to make better and informed therapeutic decisions for patients suspected of PTB in the context of a tertiary hospital and outpatient clinic. Regarding evaluation of rifampicin resistance, it is critical to follow the requirements to repeat resistant Xpert tests and perform confirmatory Genotype MTBDR*plus*, mainly in paucibacillary samples. Another reasonable approach is to consider epidemiological information before changing the patient's treatment.

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