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Original article

The diagnostic impact of implementing a molecular-based algorithm to standard mycobacterial screening at a reference laboratory with an intermediate prevalence for non-respiratory samples

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ABSTRACT

Rapid, reliable results can be given by molecular, direct detection and identification of the *Mycobacterium tuberculosis* (MTB/Mtb) complex from clinical samples. The Xpert MTB/RIF assay is an assay that has been availablefor more than a decade for identification of Mycobacterium tuberculosis and resistance to rifampicin. However, there is minimal evidence on its clinical usefulness in paucibacillary, non-respiratory samples. The Xpert MTB/RIF assay clinical utility index, its diagnostic characteristics and the number required to diagnose 2935 non-respiratory specimens submitted for routine mycobacterial work-up in a reference laboratory in an intermediate prevalence setting per specimen form were evaluated. The Xpert MTB/RIF assay showed a variable clinical utility index and number required to diagnose (NND) depending on the type of specimen, which was moderate in tissue biopsies (NND = 1.8) and excellent in pus and urine samples, compared to acid-fast microscopy and culture as a gold standard technique (NND = 1.1 and 1.2). Microscopy, on the other hand, consistently showed a weak to fair index of clinical usefulness in all specimen forms, with in NND of 2.3–12.5. The NND for detecting tuberculous infection in the cerebrospinal fluid by the Xpert MTB/RIF assay was noted to be 1.2, with a moderate clinical utility index of 0.8. The evidence presented indicates that the overall appropriate diagnostic utility of the Xpert MTB/RIF assay is clinically successful in most non-respiratory samples. To check the cost-effectiveness and prognostic effect of integrating this completely automated molecular-based assay into the routine testing algorithm for non-respiratory mycobacterial specimens, further data must be collected.

1. Introduction

Tuberculosis (TB) is a bacterial infection triggered by acid-fast aerobic bacteria containing the complex *Mycobacterium tuberculosis* (Mtb), which normally affects thelungs (Goud et al., 2020). TB is the world's largest infectious assassin with an estimated 10 million new cases and 1.5 million deaths (Tiberi et al., 2020). Usually, TB affects the lungs (pulmonary TB), but 15% of the total cases of extrapulmonary TB (EPTB) may also affect other organs (Harding, 2020). Despite the importance of an effective microbiological diagnosis of mycobacterial disease, there are significant limitations to the current traditional methods. The gold standard of TB diagnosis, phenotypic techniques such as mycobacterial culture, is considered to be labor-intensive and time consuming. While smear microscopy is an easy and fast method of detecting Mtb bacilli, its low sensitivity and negative predictive value (NPV) as well as the number of samples necessary limit its clinical effects (Hillemann et al., 2011, Ryan et al., 2014). Evidence from previously published research indicated that traditional mycobacterial experiments such as microscopy and culture for pulmonary diagnosis and EPTB could not be substituted by nucleic acid amplification technologies (NAAT) (Pai and Ling, 2008). A few years later, however, "Xpert MTB/RIF (Cepheid Inc., Sunnyvale, California,

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USA)" modified this trend, showing 88% and 98% pooled sensitivity and specificity for the diagnosis of pulmonary TB specimens, respectively (Boehme et al., 2010, Steingart et al., 2013). However, data for the diagnosis of EPTB using Xpert MTB/RIF is still controversial and variable according to specimen types (Lawn and Zumla, 2012). Furthermore, some studies reported low sensitivity and specificity of Xpert MTB/RIF with EPTB samples (McNerney and Zumla, 2015, Nataraj et al., 2016). For example, higher sensitivities to culture and composite reference standards have been recorded for cerebrospinal (79.5% and 55.5%) compared to pleural and other sterile body fluids (17–43.7%) (30148542., Kohli et al., 2018).

Further research focusing on the diagnosis of EPTB have found that the detection rate of Xpert In the different EPTB tissues MTB/RIF varied, with overall sensibility lesser than that of pulmonary tuberculosis; lymph nodes (83% sensitivity), cerebrospinal fluid (CSF) (80% sensitiveness) and pleural fluid (46% sensitivity) (Kohli et al., 2018, Seo et al., 2020, Somily et al., 2016, Denkinger et al., 2014). These research studies suggest that Xpert MTB/RIF perform best with respiratory specimens because of the low paucibacillary number of EPTB specimens.

Because of the paucibacillary nature and the invasiveness of specimen collection procedures, EPTB infection remains a diagnostic challenge. The world health organization (WHO) officially endorses Xpert MTB/RIF as the only rapid molecular test for the rapid diagnosis of TB (mondiale de la Santé, 2014, Organization, 2018). WHO suggested the first use of Xpert MTB/RIF testing in 2010 for pulmonary TB diagnosis, then added EPTB cases when needed in 2013? Food Drug Administration (FDA) has also cleared this assay for the Mtb diagnosis (Control and Prevention, 2015). Xpert MTB/RIF assay is a quick, automated, semi-quantitativereal-time polymerase chain reaction (RT-PCR) cartridge-based assay that can detect both the presence of a complex genome. of Mycobacterium tuberculosis in a patient's sample and also detect the presence of genomic sequences of the major Rifampicin resistance mutations; rpoB gene mutation with assay turnaround time (Guillet-Caruba et al., 2014).This study examines the diagnostic impact of the addition of Xpert MTB/RIF as a routine test in the diagnosis of EPTB in the Reference Saudi Arabian Laboratory compared to the gold standard phenotypic process and the old microscopy. Specifically, the goal was to measure the "number needed to diagnose" (NND) and the "clinical utility index" (CUI +) of the assay in different non-respiratory specimen forms.

2. Materials and methods

2.1. Research settings and participants

This study was conducted in Mycobacteriology Reference Laboratory at Regional Laboratory in Riyadh. Between November 2012 and February 2019, a total of 2935 EPTB specimens enrolled in this study (405 cerebrospinal fluids, 280 pus samples, 614 tissue biopsies, 217 gastric aspirations, 1059 pleural fluids, 47 urine samples and 313 other miscellaneous samples). Samples were collected from each suspected EPTB, all samples were processed for the Kinyoun staining procedure, the Mtb culture and drug susceptibility test (DST) by the BACTECTM MGITTM 960 Mycobacterial Detection System (Becton, Dickinson, and Company [BD], New Jersey, U. S.), and the Xpert MTB/RIF (Cepheid Inc.). All procedures were conducted at the Mycobacteriology Reference Laboratory in Riyadh (the ISO15189 and CAP certified TB reference laboratory in Saudi Arabia). Mtb H37Rv has been used as a normal strain for quality control. The MGIT culture and DST as well as the Xpert MTB/RIF experiments were carried out in compliance with the manufacturing instructions. EPTB specimens of both children and adults were included in the study.

2.2. Sample processing & techniques of MTB detection

EPTB specimens initially divided into two major classes according to the sterility of the specimen. Aseptically-extracted-tissues-and infected specimens-were-first-concentrated-at 3000 g for-15-min, and sediments-were-resuspended-in-2–5-ml-of-sterile-phosphate buffer saline for 15 min (PBS). Biopsy-was cut-into-small-pieces-with-a-sterile-scalpel or blade. They were then homogenized into a sterile saline. For sterile liquid specimens, a part (500 µl) without-decontamination-was-inoculated-into-the MGIT liquid-medium-using the-BACTEC MGIT 960 method inoculation, 1 ml was used for the Xpert MTB/RIF assay and 1 drop (~50 µl) was used for the frottis microscopy. Contaminated samples were decontaminated with N-acetyl-Cysteine sodium hydroxide (NALC-NaOH). After centrifugation, the sediments were resuspended in 1.0 to 1.5 ml of sterile phosphate buffer (pH 6.8). This suspension has been used for inoculation of culture media.

2.3. AFB smears

Following the treatment of the specimens, the test samples were prepared and analyzed at reference mycobacteriology laboratory for the existence of AFB. All smears were stained by Kinyoun AFB staining technique and tested using light microscope.

2.4. MGIT960

The 500 µl treated specimen was inoculated into MGIT tubes and incubated at 37 °C in MGIT 960 instrument, the tubes were subsequently incubated. For tubes that were positive, an acid-fast bacilli (AFB) test of a tube sample was prepared and further mycobacterial distinction was carried out by molecular techniques.

2.5. Drug susceptibility testing

The drug susceptibility testing (DST) for SIRE and PZA (streptomycin, isoniazid, rifampicin, ethambutol, and pyrazinamide) was performed using the Bactec MGIT 960 process (MGIT 960; Becton Dickinson Diagnostic Systems) following the manufacturer's guidelines, (Kim, 2005).

2.6. Xpert MTB/RIF assay

The Xpert MTB/RIF protocol was performed as per Banada et al. (2010) protocol.

2.7. Statistical analyses

Data collection was carried out using the Microsoft® Excel® 2016 spreadsheet and statistical data analysis was conducted using the GraphPad Prism 8 program. The "amount required to diagnose" (NND) and "clinical utility index" (CUI +) were determined and interpreted as follows: NND = 1/[Sensitivity-1-Speciality)], where the smaller the NND, the more useful the assay is. The utility index has been listed as a diagnostic method (Khan et al., 2019).

3. Results

3.1. Routine processing of specimens received and tested using Xpert MTB/RIF, MGIT culture and Kinyoun staining.

A total of 2935 mycobacterial specimens have been examined. The demographic data of the samples and the characteristics of the patients to whom they belonged are summarized in Table 1. Samples contained 405 cerebrospinal fluids, 280 pus samples, 614 tissue biopsies, 217 gastric aspirations, 1059 pleural fluids, 47 urine samples and 313 other miscellaneous samples. Of the 2935 EPTB samples tested by Xpert MTB/RIF, 200 (7%) were found to be positive. The positive EPTB samples identified by Xpert MTB/RIF are 86 (31%) pus samples, 8 (17%) urine samples, 60 (6%) pleural fluids, 10 (5%) gastric aspirations, 19 (3%) tissue biopsies, 9 (2%) cerebrospinal fluids and 8 (2%) other miscellaneous samples (Fig. 1). The total number of EPTB samples tested positive for culture was 259 (9%) while 2676 (91%) samples were considered negative. The positive EPTB samples found by culture are 96 (34%) pus samples, 9 (19%) urine samples, 82 (8%) pleural fluids, 16 (7%) gastric aspirations, 33 (5%) tissue biopsies, 12 (4%) other miscellaneous samples and 11 (3%) cerebrospinal fluids. Both EPTB specimens obtained for Xpert MTB/RIF and culture testing within 7 years.

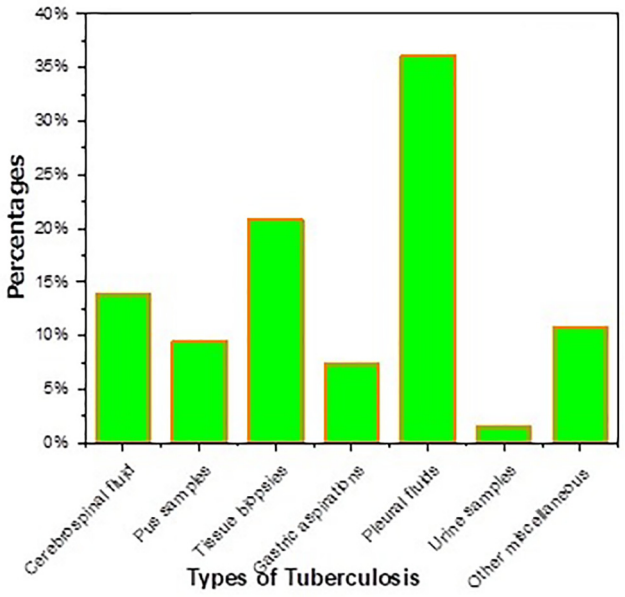

Fig. 1. Various types of tuberculosis diseases enrolled in this study.

3.2. Performance of Xpert MTB/RIF and Kinyoun microscopy compared to MGIT culture

Table 2 demonstrates the precision of Xpert MTB/RIF and Kinyoun staining using the MGIT mycobacterial culture system as a reference method for the different EPTB sample forms and 2,935 specimens. The overall sensitivity and specificity of Xpert MTB/RIF was 76% (95% CI, 70% to 81%) and 100%, respectively. The PPV and NPV was 98% (95% CI, 95% to 99%) and 98% (95% CI, 97% to 98%), respectively. Whereas, the overall sensitivity and specificity of Kinyoun staining was 15% (95% CI, 11% to 20%) and 100% (95% CI, 99% to 100%), respectively. The PPV and NPV was 85% (95% CI, 72% to 93%) and 92% (95% CI, 92% to 93%), respectively.

3.3. Performance of Xpert MTB/RIF and Kinyoun microscopy with smear positive and negative samples compared to MGIT culture

The overall sensitivity, specificity, PPV and NPV of Xpert MTB/RIF with 49 smear positive samples are (95% [95% CI, 83% to 99%]), (100% [95% CI, 63% to 100%]), 100% and (80% [95% CI, 51% to 94%]) respectively whereas, with 2886 smear negative samples are (72% [95% CI, 66% to 78%]), 100%, (98% [95% CI, 94% to 99%]) and (98% [95% CI, 97% to 98%]) respectively. Among all EPTB samples with smear positive 7 gastric aspiration fluids show sensitivity of (100% [95% CI, 59% to 100%]) and PPV of 100%, 4 tissue samples represent sensitivity of (100% [95% CI, 29% to 100%]), specificity (100% [95% CI, 2% to 100%]), PPV 100% and NPV 100%, 2 other miscellaneous samples show sensitivity of (100% [95% CI, 2% to 100%]), specificity (100% [95% CI, 2% to 100%]), PPV 100% NPV 100%, 22 pleural fluids represent sensitivity of (95% [95% CI, 70% to 100%]), specificity (100% [95% CI, 54% to 100%]), PPV 100% and NPV (86% [95% CI, 47% to 97%]) and14 pus samples show sensitivity of (93% [95% CI, 66% to 100%]) and PPV 100%. Table 2 illustrates the specimen-stratified NND and CUI + for Xpert MTB/RIF and Kinyoun microscopy.

3.4. EPTB distribution according to gender and nationality of over seven year's period

Between 2013 and 2019, 1,783 male and 1,152 female specimens were sent to the mycobacteriology laboratory in Riyadh. EPTB has been found to be prevalent in males over a span of 7 years relative to females. In addition, 1,749 Saudi and 1,186 non-Saudi EPTB specimens were sent for 7 years. It has been shown that non-Saudi cases have the highest EPTB rates in 2013 and 2016 compared to Saudi cases, although in 2014, 2015, 2017, 2018 and 2019 it is clear that Saudi people have more EPTB cases than non-Saudi cases (Fig. 2). Since 2013 data show that total positive EPTB cases have steadily decreased over the 7-year period. In

Table 1
Demographic characteristics of all studied patients.

Parameter	No of tested patients (n = 2935)	%	No of detected TB cases (n = 259)	%
Age (Average years)				
<25	841	28.7	69	26.6
25–44	951	32.4	137	52.9
45–64	719	24.5	43	16.6
≥65	424	14.4	10	3.9
Sex				
Male	1783	60.7	173	66.8
Female	1152	39.3	86	33.2
Nationality				
Saudi	1748	59.5	138	53.3
Non-Saudi	1187	40.5	121	46.7

Table 2
Xpert MTB/RIF and AFB Kinyoun staining sensitivity, specificity, PPV and negative predictive value (NPV) for 2,935 EPTB specimens against MGIT reference method.

Specific specimen	No	Xpert						AFB					
		Sensitivity	Specificity	PPV	NPV	NND	CUI +	Sensitivity	Specificity	PPV	NPV	NND	CUI +
CSF	405	82 (48–98)	100 (99–100)	100	99 (98–100)	1.2	0.8	0 (0–28)	100 (99–100)	NA	NA	NA	NA
Pus	280	89 (82–95)	100 (98–100)	100	95 (91–97)	**1.1**	**0.89**	14 (8–23)	100 (98–100)	100	69 (67–71)	7.1	0.14
Tissue	614	57 (39–74)	100 (99–100)	100	98 (96–98)	1.8	0.57	9 (2–24)	100 (99–100)	75 (24–96)	95 (94–95)	11.1	0.07
Gastric Aspirate	217	62 (35–85)	100 (98–100)	100	97 (95–98)	1.6	0.62	44 (20–70)	100 (98–100)	100	96 (93–97)	2.3	0.44
Pleural Fluid	1059	71 (60–80)	100 (99–100)	97 (88–99)	98 (97–98)	1.4	0.69	18 (11–29)	99 (99–100)	71 (50–86)	94 (93–94)	5.6	0.13
Urine	47	89 (52–100)	100 (91–100)	100	97 (86–99)	**1.2**	**0.89**	0 (0–34)	100 (91–100)	81 (81–81)	NA	NA	0
Others	313	58 (28–85)	100 (98–100)	87 (48–98)	98 (97–99)	1.7	0.5	8 (0–38)	100 (99–100)	100	96 (96–97)	12.5	0.08
All specimens	2,935	76 (70–81)	100	98 (95–99)	98 (97–98)	1.3	**0.74**	15 (11–20)	100 (99–100)	85 (72–93)	92 (92–93)	6.7	**0.13**

Data are presented as n or % (95% CI), unless otherwise stated. CSF: Cerebrospinal Fluid.
NND: number needed to diagnose
CUI+: Clinical Utility Index:< 0.2 poor, > 0.2 < 0.4 fair, > 0.4 < 0.6 moderate, > 0.6 < 0.8 good and > 0.8 < 1 excellent.

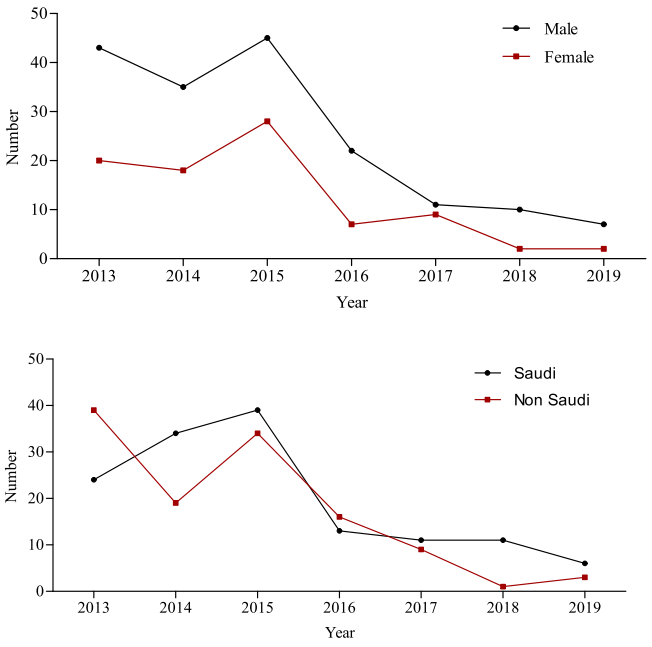

Fig. 2. Distribution of confirmed tuberculosis from non-respiratory samples according to (a) gender and (b) nationality of over a seven-year period.

2015, there was a drastic rise to 73 cases, followed by a strong decrease to 9 cases in 2019.

3.5. Comparison between Xpert MTB/RIF and MGIT drug susceptibility test (DST)

A total of 14 and 13 Mtb isolates from EPTB patients had RIF resistance results from both the Xpert MTB/RIF assay and the MGIT DST respectively. The sensitivity, specificity, PPV and NPV of RIF Xpert MTB/RIF using MGIT DST as reference method were (100% [95% CI, 75% to 100%]), (99% [95% CI, 97% to 100%]), (93% [95% CI, 65% to 99%]) and 100% respectively. MGIT DST found 13 RIF resistant cases, of which 10 were also isoniazid (INH) resistant, i.e., multi-drug resistant (MDR). It has been observed that among 2,935 EPTB isolates, 29 nonresistant and 5 isolates are polyresistant (Table 3).

4. Discussion

Prompt tuberculosis diagnosis enables prompt clinical intervention and decreases spread. The research presents cumulative seven-year data on routine molecular testing of non-respiratory samples submitted for mycobacterial testing in a reference national laboratory in Saudi Arabia, the third largest Arab country in the Arabian Peninsula. As of 2019, the total population is 34.21 million, of which 23.47 million are Saudi nationals and 10.74 are non-Saudi nationals. A total of 821 cases of EPTB cases in Saudi Arabia were registered in 2018 (Yahya R). In the capital city of Saudi Arabia, which has the highest population density, our study shows that the EPTB trend is decreasing over 7 years. In 2013, the total confirmed EPTB cases were 63, followed by a steady decline to 53,73, 29,20, 12 and 9 for the 2014–2019 period. Although there are a number of studies evaluating the Xpert MTB/RIF assay in EPTB in gulf countries (Somily et al., 2016, Jing et al., 2017). The size of a large sample of non-respiratory specimens increases the reliability of our findings. Traditional laboratory techniques such as microscopy for the diagnosis of EPTB are struggling from being sensitive as shown in this study. Our findings indicate that the overall sensitivity of Kinyoun staining compared to culture method is 15%, 0% for CSF and urine samples, 8% for other simultaneous samples, 9% for tissue, 14% for pus, 18% for pleural fluids and 44% for gastric aspirations. A previous study was conducted in China with EPTB specimens with fluorescent staining smear showed that the overall sensitivity was 61.5% and 11.1% for urine, 1.2% for CSF, 3.2% for pleural fluids and 33.3% for tissue samples (Cheng et al., 2004). Although, the low sensitivity of smear microscopy, this finding shows higher sensitivity of EPTB samples with fluorescent staining than Kinyoun stain using culture method as reference method.

Numerous studies evaluated the performance of the PCR technique for the diagnosis of EPTB (Hasaneen et al., 2003, Hofman et al., 2003, Scott et al., 2014, Hillemann et al., 2011, Maynard-Smith et al., 2014). This study shows that Xpert has a high specificity in all non-respiratory samples, although their sensitivity varies depending on the type of specimen. This indicates that Xpert MTB/RIF is used as a valuable diagnostic method to diagnose some forms of EPTBs, i.e. tuberculous meningitis, cold abscess, urinary TB and tuberculous pleurisy with a combined NND of 1.2, 1.1, 1.2 and 1.4 respectively (Table 2). The average sensitivity of Xpert MTB/RIF is 76%, although it varies significantly between different types of samples (89% for pus, 98% for urine, 82% for CSF, 71% for pleural fluids, 62% for gastric aspirations, 58% for other simultaneous samples and 57% for tissue samples). Data in this study show that pus has the highest sensitivity among EPTB samples, while tissue has the lowest sensitivity of 57% compared to gold culture. In the previous analysis, the overall sensitivity and specificity of Xpert MTB/RIF was shown to be 77.3% and 98.2% respectively (Maynard-Smith et al., 2014). It was concluded that tissue samples had the lowest sensitivity of 57% of all EPTB specimens. One of the reasons for this may be that the extraction of Mtb bacilli from tissue mass is not

Table 3
Comparison between Xpert MTB/RIF and MGIT drug susceptibility test (DST).

Xpert category and *M. tuberculosis* test-specific subcategory	No. of specimens MGIT positive and testedby					
	Xpert MTB/RIF	MGIT DST	Sensitivity	Specificity	PPV	NPV
RIF resistant	14	13	100 (75–100)	99 (97–100)	93 (65–99)	100
MDR	–	10			–	
Mono-resistant	–	29			–	
Polyresistant	–	5			–	

easy. However, the lower sensitivity of tissue samples, Xpert MTB/RIF shows a much better sensitivity than direct microscopy, which shows 9% sensitivity compared to culture. Previous systematic analysis research found that the pooled tissue sensitivity of all samples was 88% (95% CI, 76% to 94%) compared to culture (Woods, 2001). This finding suggests that the sensitivity of tissue specimens can differ depending on the geographical location and the laboratory technique.

The low sensitivity of Xpert MTB/RIF in body fluids and tissue is believed to be due to PCR inhibitors. For example, blood is considered to be one of the well-known PCR inhibitors (Pai et al., 2004, Theron et al., 2014). Other PCR inhibitors identified in fluids include salts, proteins or cellular debris (mostly contained in grinded tissues) that are enriched after treatment of specimens, such as centrifugation without subsequent resuspension in buffer solution. These debris interact with the amplification enzyme and thus inhibit the PCR (Theron et al., 2014). Xpert MTB/RIF has an internal processing and amplification control that reports a 'error' due to the existence of inhibitors. Another interpretation is that the Xpert MTB/RIF relays the capture of intact bacilli from the specimen within the cartridge. With the recorded detection limits of the Xpert MTB/RIF, it is not necessary to store and lyse the bacilli for such paucibacillary samples (Nhu et al., 2014). Previous study of Xpert MTB/RIF indicated that the overall sensitivities of the test were 100 percent and 37%, respectively, of the positive and negative EPTB specimens (Zeka et al., 2011). Previous Xpert MTB/RIF studies have demonstrated average test sensitivities of 100% and 37% respectively for smear-positive and smear-negative EPTB specimen (Vadwai et al., 2011). Our findings show that the average sensitivity of the test-positive and test-negative EPTB specimens is 95% and 72%, respectively. Our results also showed that the sensitivity of the smear-positive is higher for all EPTB specimens tested > 90% compared to the smear-negative EPTB samples where the sensitivity is variable depending on the type of sample.

Xpert MTB/RIF not only senses Mtb but also easily decides the MDR status of the patient is at the top of the list in putting an end to the spread of MDR-TB and reducing mortality. The findings of the MGIT DST take at least 1–2 months from the time the culture is inoculated. Rapid methods that enable early start of MDR schemes are urgently needed. In this study, the sensitivity and specificity for RR detection by Xpert MTB/RIF assay was 100% (95% CI, 75% to 100%) and specificity of 99% (95% CI, 97% to 100%) when compared to DST by MGIT. This study result is consistent with the previous analysis, which documented a high sensitivity and specificity of Xpert MTB/RIF to detect RR of 98% and 98% respectively (Hillemann et al., 2007). This study also found that there was 1 patient whose RIF phenotypic DST was in discordance with the Xpert result. RIF vulnerable to phenotypic DST but RIF resistant to Xpert MTB/RIF. This discrepancy in RIF results can be resolved by using bi-directional sequencing as further study is required. The explanation behind this may be due to mutation in certain sequences which may be identified by Xpert but not by phenotypic process (Hillemann et al., 2007), https://unhabitat.org/sites/default/files/2020/05/saudi_city_report.english.pdf.

In conclusion, the results of this study provide evidence that the Xpert MTB/RIF assay has variable sensitivity depending on the type of specimen, while maintaining high specificity and predictive values for the diagnosis of tuberculosis in non-respiratory samples and the detection of RIF resistance. In addition to accuracy and quick turnaround times, the Xpert MTB/RIF test also showed an acceptable number of tests needed for diagnosis and clinical utility index. However, variables that can influence these parameters, such as pre-tested disease probability and MDR-TB, need to be considered when interpreting these rapid diagnostics (Cochrane Database Syst Rev 2013;(1):CD009593). Our work has extended the diagnostic evaluation of Xpert MTB/RIF assay in non-respiratory samples and is the first assessment of Xpert MTB/RIF performance in both adult and pediatric patients in KSA (Al-Ateah et al., 2012). Future, prospective and large-scale research needs to evaluate the predictive role of these molecular-based assays in the clinical outcome and their cost-effectiveness in the current healthcare system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Compliance with ethics guidelines

No need for patients consents. To ensure confidentiality, data were collected from patient's files without name and were saved in electronic version for research purpose under supervision of investigators. Furthermore, the study presents no risks to the participants and ultimately the research does not involve a therapeutic intervention.

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