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REVIEW

Validation of GeneXpert using extrapulmonary samples at the National Tuberculosis Reference Laboratory in Lesotho

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Background: Tuberculosis (TB) is a potentially life-threatening disease that primarily affects the lungs. *Mycobacterium tuberculosis* (*M. tuberculosis*) can also infect other body sites like the pleura, kidney, and skin, causing extrapulmonary tuberculosis (EPTB). The number of EPTB deaths can be reduced significantly if rapid and effective technologies such as Xpert Ultra are implemented for early diagnosis of this fatal disease. This study aimed to validate the GeneXpert utilising non-respiratory specimens for early EPTB detection at Lesotho's National Tuberculosis Reference Laboratory (NTRL).

Methods: Although this was primarily an experimental study, it did incorporate data from the NTRL database. A variety of extrapulmonary clinical specimens were tested using the GeneXpert (index test) and culture (gold standard) for the presence of *M. tuberculosis*. All ages and sex were included. IBM Statistical Package for the Social Sciences (SPSS) version 26 was used for statistical analysis.

Results: From 2016 to 2019, a total of 176 specimens were tested for EPTB. Pleural fluid was the most routinely obtained specimen type, representing 39.2%, followed by 14.2% ascitic fluid, and 13.6% cerebrospinal fluid (CSF). From the specimens analysed, 34 were positive for EPTB and were detected using both GeneXpert and culture techniques. The GeneXpert's overall sensitivity was 92.3% and specificity was 87.1%.

Conclusion: Results from this study suggest that the GeneXpert can be trusted as a critical tool for the early detection of various forms of EPTB and early treatment initiation. The GeneXpert can be successfully used as an adjunct diagnostic tool at the NTRL in Lesotho.

Keywords: culture, non-respiratory specimens, *Mycobacterium tuberculosis*, Xpert Ultra

Introduction

Tuberculosis (TB) remains a major public health concern around the world. It is confirmed to be amongst the top 10 killer diseases.[1] Despite this, there has been a significant shift in the overall diagnosis of TB during the previous decades. This shift is the result of the emergence of several technologies that use polymerase chain reactions (PCR) which directly detect the nucleic acids of *Mycobacterium tuberculosis (M. tuberculosis)* in clinical specimens.[2,3] *M. tuberculosis* is notorious for causing pulmonary TB. Furthermore, *M. tuberculosis* can infect other anatomical sites such as the central nervous system, pleura, lymphatics, skin, and bones/joints; giving rise to a lethal form of TB called extrapulmonary tuberculosis (EPTB).[1,3,4]

The culture method is still considered the gold standard for diagnosing TB in clinical samples.[1] Since microorganisms are not only identified at the species level but also evaluated for drug sensitivity, culture results provide a conclusive diagnosis of TB.[5-7] In addition, the approach can also be used to monitor patients who are currently receiving treatment. Culture has a higher sensitivity than microscopy since it only requires a sputum sample with an estimated 10 live bacilli/ml, whereas a positive microscopy result requires a sputum sample with 5 000 bacilli/ml.[8] Most extrapulmonary samples require decontamination procedures that, if performed incorrectly, could be harmful to mycobacteria, making the culture method not 100% effective.[3] Mycobacterial culture yields have been reported to range from 30% to 80%, but findings typically take two to eight weeks to be released, which is too long to assist with treatment decisions.[9]

Lesotho is reported to be one of the countries severely hit by TB and human immune deficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) and the country's TB/HIV status remains amongst the top in the world rankings.[10,11] According to estimates, the incidence rate of TB in Lesotho was 611/100 000 population in 2018.[11] This figure was comparable to the incidence of about 654/100 000 population in 2019, which ultimately resulted in Lesotho's TB status being ranked number one around the globe for the year 2019.[1] A slight decrease of -0.61% was reported for 2020 as the incidence of TB was 650/100 000 population.[12] Lesotho's estimated TB detection rate is just 51%, which means that around half of TB cases go undiagnosed and untreated. A significant percentage of TB patients, who also have HIV (62%), frequently have a paucibacillary or extrapulmonary disease, which is less likely to be discovered by sputum testing and further presents a problem for TB diagnosis.[10]

Approximately 30% of TB patients worldwide do not receive a diagnosis or treatment.[10] To prevent death, lessen morbidity from the disease, and stop transmission, it is crucial to identify

TB and start effective treatment as soon as possible. Actively screening relevant individuals for TB is a crucial step for bridging the TB diagnostic gap in countries with high TB rates.[10] However, there is still a significant hurdle in the diagnosis of EPTB all over the world. This is due to the disease's vague symptoms, non-respiratory samples with poor bacterial load, which reduces the diagnosis technique's sensitivity, and the challenge of acquiring quality specimens for testing. All these factors may impede prompt EPTB diagnosis, ultimately resulting in late therapy or misdiagnosis, as well as other serious consequences. The GeneXpert is one of the most important diagnostic tools to assist in stopping the spread of TB.[5,6,13-15]

In Lesotho, there are 290 healthcare facilities, of which 265 are primary health centres/clinics, and 25 are hospitals. There are approximately 20 medical laboratories found in the 25 hospitals in Lesotho.[16] Unfortunately, there is only one TB reference laboratory in the country, namely the National Tuberculosis Reference Laboratory (NTRL), which serves as a referral laboratory for the diagnosis of TB for patient care and management countrywide.[17] EPTB diagnosis is a significant and frustrating health problem in developing countries due to a lack of advanced technologies and methods that are faster and more effective than traditional methods used to detect EPTB, such as culture and Ziehl-Neelsen (ZN) staining. Traditional methods such as culture led to unsatisfactory turn-around time (TAT) and have low sensitivity (microscopy).[18]

A significant improvement in TB and rifampicin-resistant TB (RR-TB) diagnosis was made on a global scale with the advent of the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, USA).[1] The Xpert® MTB/RIF (GeneXpert) assay is an in vitro, partially quantitative molecular method that utilises real-time polymerase to detect *M. tuberculosis* complex (MTBC) deoxyribonucleic acid (DNA) content from sputum specimens. Mutations in the *rpoB* gene linked to rifampicin resistance can be detected simultaneously using GeneXpert.[1,19] This diagnostic tool was endorsed by the World Health Organization (WHO) in 2010 for rapid diagnosis of pulmonary TB owing to its high sensitivity and specificity in sputa, and results are available in less than two hours. In 2013, the WHO suggested that the assay be used to diagnose other forms of EPTB using specimen types such as lymph nodes, cerebrospinal fluid (CSF) and other tissues.[20-22] It nonetheless demonstrated poor specificity and sensitivity (especially regarding smear-negative TB and HIV-positive individuals) when compared to the reference standard of culture. A new generation assay with enhanced sensitivity, Xpert® MTB/RIF Ultra (also known as Xpert Ultra), was assessed and recommended by the WHO in 2017 to diagnose pulmonary TB and various forms of EPTB. Like Xpert MTB/RIF, this novel assay runs on the GeneXpert® platform.[1]

In Lesotho, the GeneXpert has only been validated and verified for diagnosis of TB using sputum, but not yet approved for diagnosis of TB using extrapulmonary samples. As a result, this technique must be validated utilising extrapulmonary samples prior to its use as a reliable diagnosing tool for EPTB. This study aimed to evaluate the performance efficiency and reliability of the GeneXpert test for rapid EPTB diagnosis using non-respiratory specimens at Lesotho's NTRL.

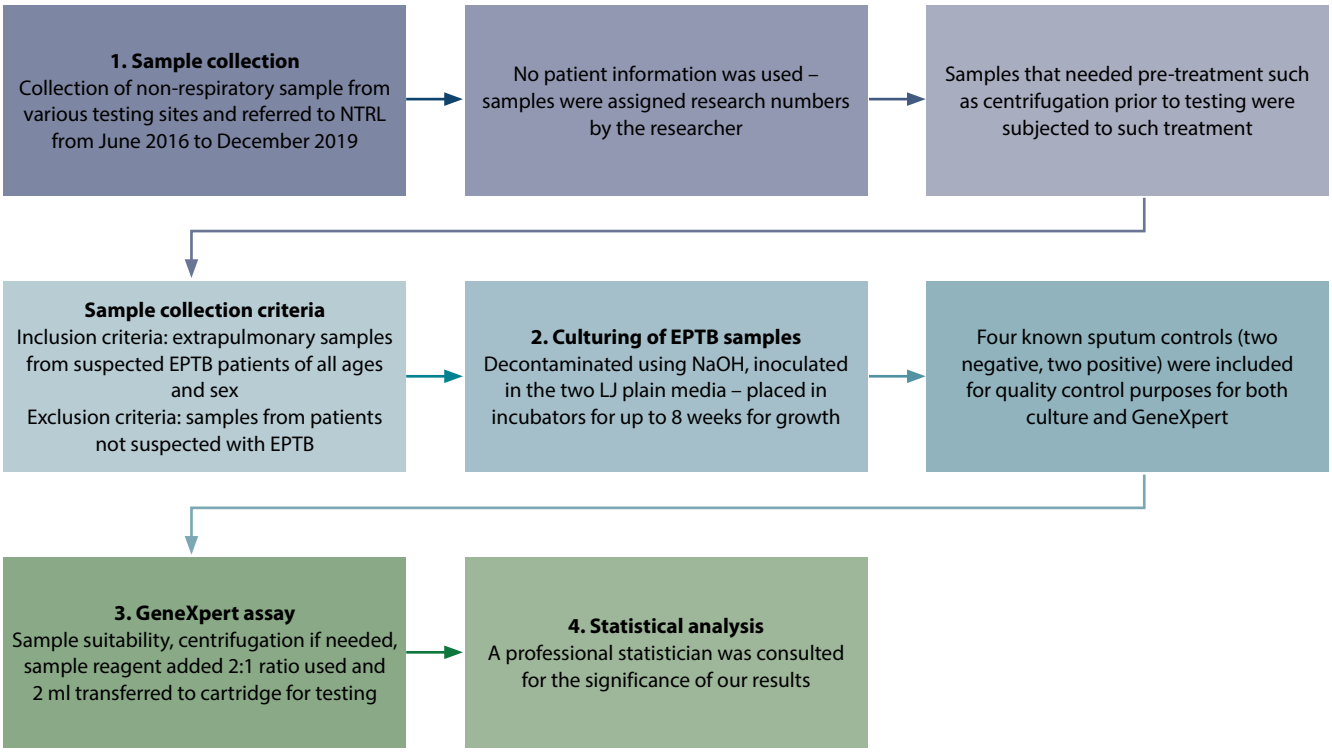

Figure 1 – A flow diagram showing how validation of the GeneXpert using extrapulmonary specimens at NTRL was carried out. Pre-treatment procedures such as centrifugation was done, inclusion and exclusion criteria were included, and additives or preservatives were not added. Löwenstein-Jensen (LJ) culture method was used as the reference method while the GeneXpert was the index test. Known sputum samples were used as quality controls in both culture and GeneXpert testing.

NaOH – sodium hydroxide

Methodology

A flow diagram showing how validation of the GeneXpert using extrapulmonary specimens at NTRL was carried out. Pre-treatment procedures such as centrifugation was done, inclusion and exclusion criteria were included, and additives or preservatives were not added. The Löwenstein-Jensen (LJ) culture method was used as the reference method while the GeneXpert was the index test. Known sputum samples were used as quality controls in both culture and GeneXpert testing.

Specimen collection

The study is comprised of 176 extrapulmonary specimens from patients suspected to have EPTB. During 2016 and 2019, various non-respiratory specimens were collected at Berea Hospital, Maluti Hospital, Mafeteng Regional Hospital, Queen Elizabeth II Hospital, Motebang Hospital, Queen 'Mamohato Memorial Hospital, and Seboche Hospital, among others. Each specimen was aliquoted into two parts (one for GeneXpert testing and the other for culture testing), resulting in 352 aliquots. Before samples were collected, ethical approval was obtained. Samples were collected from patients with suspected EPTB in all age groups and sex. Non-respiratory specimens included in the study were pleural fluid, ascitic fluid, CSF (only collected at the hospital level), pus swab, body fluid, lymph node aspirate, urine, scrotal box, abdominal fluid, synovial fluid, gastric aspirate, throat swab, auxiliary lymph node fine needle aspirate, fine needle aspirate (FNA), and others such as bone marrow aspirates and peritoneal fluid. Patients who had not been suspected of having EPTB were excluded, and insufficient specimens (which were less than 3 ml) were also excluded. Furthermore, as per GeneXpert's manufacturer's guidelines, specimens containing particles that could obstruct the GeneXpert probe, such as faeces and blood, were not accepted.

Specimen storage and transportation

After collection, specimens were transported immediately to the NTRL whenever possible or kept at 2–8 °C in remote health facilities if the specimens could not reach the NTRL on the same day they were collected. Specimens were transported to the NTRL within three days using triple packaging systems for optimal results. The CSF specimens were treated as exceptions as they were not refrigerated but transported immediately to the NTRL. Specimens that could dry out, such as swabs and tissues, were kept moist during transportation by adding 1 ml sterile 0.9% saline. No additives or preservatives were added to the extrapulmonary samples. Delays exceeding seven days and improper storage were not processed as they could interfere with the final culture results: thus, causing an increased contamination rate in the specimens, according to the NTRL laboratory handbook.[17] In this study, retrospective results found on the NTRL database were also used as part of the analysis.

Sample processing

GeneXpert

One aliquot of the specimens was processed using the GeneXpert Ultra following manufacturer instructions. Working in a bio-safety cabinet level three, the Xpert MTB/RIF sample reagent (buffer solution) was added to each specimen at a 2:1 ratio. After 15 seconds of vigorous stirring, the mixture was incubated for 15 minutes at room temperature (17–25 °C), then shaken again before being incubated for another 10 minutes at room temperature.

After the mixture was liquefied, a sterile pipette was used to slowly transfer 2 ml of the specimen and buffer mixture into a labelled GeneXpert cartridge via a port, which was then put into the GeneXpert instrument for testing. Results were captured on the monitor within two hours. The remaining specimen and buffer mixture in the specimen container were kept refrigerated at 2–8 °C in case a repeat test was required. The cartridges were discarded in a biohazard bag that was fastened and kept in the biohazard waste bin once the results were displayed on the GeneXpert monitor and considered satisfactory. Whenever the biohazard bag was 3/4 full, it was sealed tightly and immediately removed from the biohazard waste bin to be incinerated.

Table I: The acceptance criteria of results according to the ability of the GeneXpert to detect MTB in EPTB samples

MTB detection in EPTB samples	Results interpretation
MTB detected (high, medium, low or very low)	Positive
MTB not detected	Negative

It should be noted that positive GeneXpert results were released immediately as preliminary results which physicians used together with the clinical diagnosis to make prompt treatment decisions for EPTB patients.

If any of the following test outcomes occurred during the GeneXpert run, the test had to be repeated with a fresh cartridge:

- An "invalid" result signified that the sample processing control was unsuccessful. The sample was improperly handled, or the PCR was interrupted. Possible causes of this flag include an incorrectly filled reaction tube, a reagent probe integrity fault being discovered, an exceeded pressure limit, or even a GeneXpert module failing.
- A "no result" indicated that insufficient data was gathered. For instance, the operator halted a test that was running.

Quality control for GeneXpert

The GeneXpert assays have inbuilt quality control features that were checked with every cartridge use. Internal quality checks were also conducted every week using two known controls (positive and negative sputum samples) per good laboratory practice. Manufacturer's kit controls (lot-to-lot verification of reagents) were carried out with every newly shipped cartridge (to check the integrity of the cartridges for quality results) or every new lot change to ensure the quality and consistency

of lots before their use, and records were documented in the quality control logbook.

Culture

The LJ medium was prepared according to the instructions in the Mycobacteriology Laboratory Manual.[23] The second aliquot of each specimen was processed for culture purposes. Briefly, a 10 µl inoculating loop was utilised to tap and streak the sediment on two slopes of LJ media, with two to three drops inoculated on each. In an incubator set at 35–37 °C aerobically, the inoculated culture medium was then placed in a slanted position in a rack. For a week, the media tube was loosely closed to allow the inoculum to be evenly distributed and absorbed. The culture tubes were screw-capped tightly after a week. This was done to reduce the evaporation and drying of the media. The tubes were then placed in an upright position. Up to the eighth week, growth was checked weekly (twice in the first week to observe contamination early and request repeat specimens promptly if necessary), and results were reported and documented in the relevant register and worksheet. After eight weeks of incubation, the results were then declared negative if there was no growth on the LJ slant and the negative slants were discarded in the biohazard bin. For each positive LJ slant detected, the results were reported immediately as preliminary, and further testing was done to confirm the microorganism detected.

Two to three colonies were picked from each positive growth and checked for acid-fast bacilli by smear microscopy. This is where respective slides were labelled with the unique laboratory number assigned to each specimen, stained with the ZN stain, and examined microscopically. If the acid-fast bacilli were detected by microscopy, then a further identification test was performed by using several colonies scraped from the positive LJ slants. The scraped colonies were added to 200 µl of extraction buffer of the MPT/MPB 64 antigen test kit for the identification of *M. tuberculosis* at species level according to the method by Stinson et al.[23] The results were declared culture positive after two lines formed in the cartridge of the test kit. The *M. tuberculosis* isolates were then stored at -80 °C in sterile 10% skim milk.

Quality control for LJ culture

Quality control for LJ culture was carried out by a trained laboratory technologist on duty or by the study's primary investigator under the direction of a more experienced laboratory technologist. Positive controls were prepared by using H37RV strains and cultured for two to eight weeks before being examined for growth. Negative controls were made using distilled water/buffer.

Statistical analysis

The IBM SPSS version 26 was used to conduct descriptive and inferential statistical analyses. All laboratory requisition forms generated during the research were kept totally confidential by gathering them monthly and keeping them in a secure, locked cabinet. The computerised DISA system can keep logged information safely and securely since information is password protected.

Results

From 2016 to 2019, a total of 176 extrapulmonary specimens were analysed using the GeneXpert and culturing on LJ medium in Lesotho; NTRL. From the results, pleural fluid accounted for 39.2% of all specimens collected in health facilities, followed by 14.2% ascitic fluid, and 13.6% CSF. EPTB samples such as gastric aspirates, throat swabs, auxiliary lymph node FNA, and synovial fluids were rarely examined (Figure 2).

The detection rate of *M. tuberculosis* when two techniques, namely GeneXpert and culture, were used are reported and compared in Table II. The results showed that GeneXpert detected *M. tuberculosis* from more specimens than the culture technique.

Between 2016 and 2019, a total of 34 EPTB cases were determined to be positive when both GeneXpert and culture tests were used at the NTRL in Lesotho. The GeneXpert detected 33 cases, whereas only 13 cases were found using the culture technique. Both methodologies were successful in finding

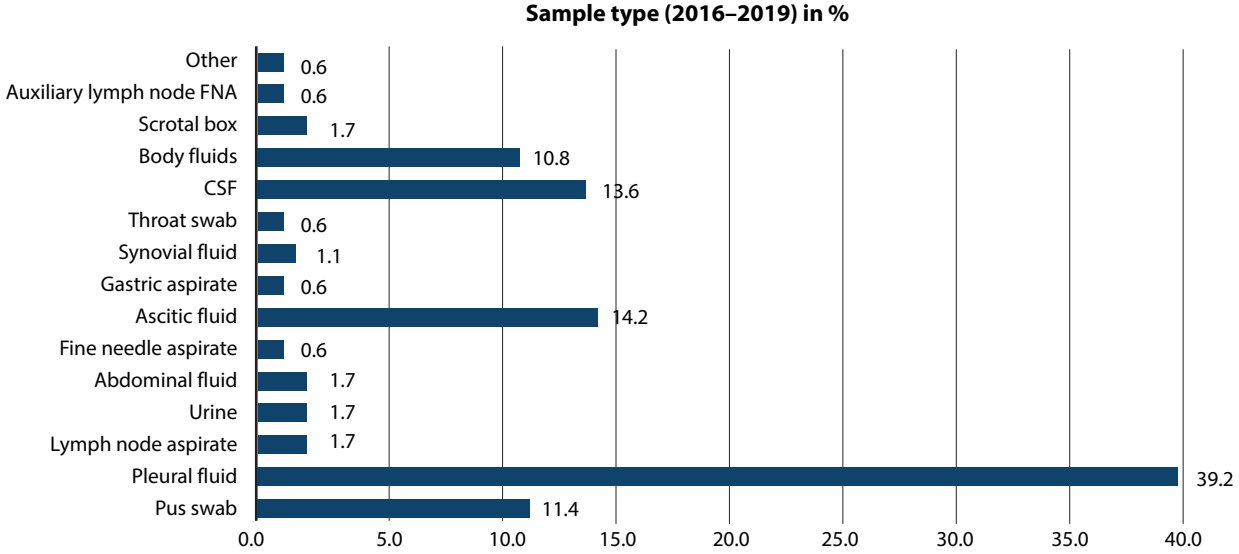

Figure 2: Different sample types submitted to the NTRL for EPTB testing from 2016 to 2019

Table II: Comparison of detection rate of GeneXpert and culture

Specimen type	GeneXpert		Culture	
	Number of positive	Positive %	Number of positive	Positive %
Pleural fluid	13/69	19	4/69	5
Pus swab	12/20	60	6/20	30
Fine needle aspirate	2/2	100	1/2	50
Throat swab	1/1	100	1/1	100
CSF	3/28	11	1/28	4
Ascitic fluid	1/25	4	0/25	0
Lymph node aspirate	1/3	33	0/3	0
Urine	1/3	33	0/3	0
Total	33/176	19	13/176	7

Table III: GeneXpert diagnosis test vs culture diagnosis test cross-tabulation

			Culture test		Total
			Positive	Negative	
GeneXpert test	Positive	Count	12	21	33
		% within culture diagnosis	92.3	12.9	18.8
	Negative	Count	1	142	143
		% within culture diagnosis	7.7	87.1	81.3
	Total	Count	13	163	176
		% within culture diagnosis	100	100	100

Table IV: Age distribution of positive EPTB individuals detected using the GeneXpert

Age of patients diagnosed with EPTB using the GeneXpert	Sex			Total number of patients
	Male	Female	Unknown	
Below 20	0	1		1
21–40	9	5		14
41–60	8	4		12
Above 60	2	1		3
Unknown age	2		1	3
Total				33

12 cases, as shown in Table III. Compared to the GeneXpert, which missed only one pus case, the culture technique missed 21 positive cases. In addition, the results show that the GeneXpert had 92.3% sensitivity and 87.1% specificity (Table III).

Young adults between the ages of 21 and 40 were the most common age group diagnosed with EPTB, whereas the elderly over the age of 60 were few, i.e. only three. It is also important to note that the ages of three patients were unfortunately not recorded on the patient records. Table IV shows the age distribution of patients diagnosed with EPTB utilising the GeneXpert.

Discussion

The diagnostic performance of the GeneXpert technique for *M. tuberculosis* detection in extrapulmonary specimens was compared with the reference method culture. As shown in Figure 2, the findings of this study demonstrate a broader range of clinical sample types that were investigated (lymph node aspirates, CSF, synovial fluids, gastric aspirates, ascites, pleural fluids, throat swabs, pus, etc.). Pleural fluid was the most frequently collected specimen type (39.2%), followed by ascites (14.2%), and CSF (13.6%). Less frequently collected specimen types were gastric aspirate (0.6%), throat swabs (0.6%), and auxiliary lymph node FNA (0.6%).

The GeneXpert was sensitive enough to detect MTBC from most specimen types listed in Table II. From a total of 69 pleural fluid samples analysed with the GeneXpert, 13 (19%) were found to be *M. tuberculosis* positive, but only four (5%) were found to be *M. tuberculosis* positive utilising culture. In a recent study done in Dhaka by Islam et al., the pleura was found to be the most common site of collection for extrapulmonary specimens tested for EPTB, accounting for 21.2%, urine followed representing 18.6%, and CSF representing 15.6%.[24] In another study by Ravikumar and Priyadarshini, data revealed that the most usually collected non-respiratory specimen was pleural fluid (29.9%), followed by meninges (22.5%), and then abdomen (19.6%).[25] The presence of more pleural fluids than other specimens could be because most people suspected of having TB in high-TB-burden

regions present with pleural effusion as an early symptom of primary TB infection.[26]

Different studies from literature report on the low detection rate of *M. tuberculosis* in pleural fluids ranging from 11.9% to 49.5% when utilising the GeneXpert.[26-29] The paucibacillary nature of EPTB illness may explain the lower identification of MTBC in pleural fluids. Moreover, the Xpert's low sensitivity could potentially be caused by PCR inhibitors found in the pleural fluid, which might have contaminated the sample during invasive specimen collection.[6] Indeed, further research is needed to improve the Xpert assay's sample processing, which could lead to enhanced sensitivity in specimens containing PCR inhibitors.

In a study by Mechal et al., a higher sensitivity of 100% was achieved on pleural biopsies when using the GeneXpert, compared to 50% sensitivity obtained when using microscopy.[30] This could imply that a pleural biopsy, rather than pleural fluid, is considered the better specimen for diagnosing pleural TB.[28] Previous research by Vadwai et al. and Kim et al. have discovered that the GeneXpert assay has poorer sensitivity for a fluid specimen, like pleura, than for thicker specimens containing solid components, such as tissue biopsies or pus.[31,32]

Findings from the current study show that the GeneXpert detected 12 (60%) positives from all pus samples, while six (30%) proved positive utilising culture. When compared to pleural fluid samples, which had a low sensitivity of 47%, Scott et al. found that Xpert MTB/RIF functioned best on fluid samples with a thick consistency, notably pus, which had the highest sensitivity of 91%.[33] The most plausible explanation is that pus and thick fluids are more viscous like sputum samples, making SR buffer, designed to liquefy sputum, a good fit. Some studies all observed high sensitivity and specificity of Xpert MTB/RIF on pus samples, with varying sensitivity and specificity ranging from 80% to 100%.[6,27,34,35] In other research, a modest sensitivity of 64.3% but a high specificity of 96.9% was reported.[30]

FNA and biopsy are the two main procedures for obtaining specimens used for detecting lymph node tuberculosis (LNTB), and the sensitivity using these two methods differs.[28,36] Moreover, in the present study, the GeneXpert detected MTBC from the two FNA, but only one of the specimens was MTBC-positive when using culture. The lymph node aspirates results showed that 33% of positive cases were detected using GeneXpert, while culture could not detect the MTBC on all these samples. This lack of detection of *M. tuberculosis* by the culture method might have resulted from the low bacillary load in those specimens or the harsh decontamination process employed in the culture method, which could have destroyed the *M. tuberculosis*. Moreover, there could have been dead tubercle bacilli that harboured in the caseous lesion of the lymph node tissue, which GeneXpert detected but culture could not as it detects live bacilli.[22,28]

According to Uppe et al., the sensitivity of the GeneXpert in detecting MTBC in FNA was 57.14%, while it was lower in lymph node biopsies, with a sensitivity of 46.87%.[28] The reduced sensitivity of Xpert MTB/RIF in bodily fluids and tissue is thought to be due to PCR inhibitors.[37,38] Because of the presence of haemoglobin in erythrocytes, lactoferrin in leucocytes, and immunoglobulin G (a protein found in plasma), blood is one of the most common PCR inhibitors found in various specimen types.[39,40] Salts, proteins, and cellular debris (primarily found in tissues after being ground) are all reported as PCR inhibitors of fluids following sample treatment, including centrifugation in the absence of resuspension using a buffer solution.[35]

Both the GeneXpert and culture methods detected MTBC in the sample from the throat swab. Three (11%) of the 28 CSF specimens analysed with the GeneXpert were positive, but only one (4%) of the CSF specimens were positive by culture. Given the low bacillary load in such non-respiratory specimens, culture has been found to have limited sensitivity for several EPTB samples, particularly CSF and pleural fluid.[14,15] When employing CSF specimens for TB meningitis diagnosis, Uppe et al. and Seo et al. found GeneXpert sensitivities of 30.0% and 41.7% respectively.[28,41] This could be related to smaller specimen volume (owing to difficult and intrusive sample collection) affecting the quantity of bacteria, which is critical for the GeneXpert assay's sensitivity. Another possible explanation could be that CSF specimens with a xanthochromic appearance as a result of subarachnoid haemorrhage or jaundice may have contributed to false negative results due to the presence of bilirubin, which is a known PCR inhibitor.[37] According to Denkinger et al. and Bahr et al., centrifugation and the use of a larger volume of CSF leads to an enhanced yield of tubercle bacilli for diagnosis of TB meningitis.[42,43]

MTBC was found in 4% of ascitic fluids in the current study. Low MTBC detection rates were reported when using the GeneXpert to diagnose TB found in ascitic fluids.[44-46] In the present study, 33% of positive TB cases found from urine specimens were identified using the GeneXpert, however none of the cases were found using culture. Urine specimens have low viscosity compared to sputum specimens. The sample reagent buffer of the GeneXpert, which is developed solely for sputum samples, may have further diluted the urine specimens, hampering the detection rate of the GeneXpert.[33] According to Pang et al., the decontamination of non-sterile specimens in culture may have been extremely harsh when applied to urine specimens.[47]

Table II also shows that the prevalence of EPTB was 19% when using the GeneXpert and 7% when using culture. The findings of this study differed from those of Fanosie et al. who conducted a study in Ethiopia, whereby the prevalence of EPTB using culture method was 29.8%, but 26.2% when using GeneXpert MTB/RIF.[48] These were higher than the findings of the current study. This variation could be because Ethiopia is one of the countries with a high TB burden, and the high incidence of TB could be linked to socio-demographics, HIV co-infection, TB management factors, and limited TB awareness programmes.[48,49]

The number of EPTB cases found using each method, as well as shared cases, are shown in Table III. GeneXpert detected a total number of 33 cases, whereas culture detected a total of 13 cases. Both methods successfully detected 12 cases. In the current study, there was a lower detection of MTBC from most samples

when using culture when compared to the GeneXpert. This could be attributed to the dead bacilli recognised by the GeneXpert because it can detect both dead and viable bacilli. Another factor could have been the specimens' paucibacillary nature.

A pus swab from a 34-year-old male was the only case found using culture. The most probable explanation for this positive culture but negative GeneXpert result could be that the GeneXpert can only detect mycobacterium species, whereas culture may detect both MTBC and non-tuberculous mycobacterium (NTM), also known as mycobacteria other than tuberculosis (MOTT). Thus, these findings suggest that the positive culture was due to NTM growth rather than MTBC.[50,51] Another reason could be that the number of live bacilli was less than 10, which is much lower than the detection limit of the GeneXpert.[52] According to Table IV, EPTB was prevalent in the age group 21–40 but rare in ages below 20 and above 60. This showed that young adults were more likely to have pleural EPTB than the elderly, children, or infants. According to the study's findings, active males are most likely to develop EPTB. The majority of young men in this age group might work in mines where they may be exposed to silica dust, which is known to have a significant role in the global TB epidemic in low-income countries. Lesotho is one of the countries severely hit by poverty and HIV infection in the world. Another contributing factor may be the high HIV prevalence in males (between the ages of 15 and 59), as well as the lifestyle of males in this age range, such as smoking.[53]

Conclusion

This paper forms an important addition to the growing literature demonstrating the utility of the GeneXpert for EPTB diagnosis when applied to various types of clinical specimens. The results of this study suggest that the GeneXpert can be used as an essential add-on test for EPTB diagnosis in Lesotho. Because the GeneXpert results are produced in less than two hours, clinicians can utilise these results in conjunction with the patient's clinical presentation to assist them in making a faster diagnosis of EPTB based on the presence or absence of MTBC. This will help with the early detection of EPTB, which is one of the most important strategies for a good treatment outcome of this deadly disease that is difficult to diagnose.

Recommendations

The current study had some limitations, which included the low specimen numbers available for the duration of this research study. For higher precision and accuracy, further research of this nature should be performed in various locations across the country with an increased number of samples and sample types. The integrity of the specimens may have been compromised due to sample handling, transportation, and storage prior to analysis. As per the manufacturer recommendations, some specimens, such as blood and stool, were excluded in the current study. There is a need for further research into the use of suitable decontaminants for extrapulmonary specimens apart from sodium hydroxide (NaOH) and N-acetyl-l-cysteine-sodium hydroxide (NALC-NaOH), as they may create harsh conditions that kill tubercle bacilli in certain EPTB samples, which ultimately might result in false negative results. According to Campelo et al., some authors showed that liquid culture, such as mycobacteria growth indicator tube (MGIT), could be used as reference method for TB detection in extrapulmonary specimens as mycobacterium can be detected much faster compared to LJ culture and the yield of positivity rates is better.[54] However, a fundamental disadvantage of liquid culture techniques is that they are more susceptible to contamination. This implies that additional bacteria are more likely to infiltrate the system, which may lead to erroneous results.

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Conflict of interest

The authors declare no conflict of interest.

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Ethical approval

Ethical clearance was obtained from Ministry of Health Lesotho Research and Ethics Committee (clearance ID 174-2019).

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