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Performance of GeneXpert Assay in Detecting Pulmonary Tuberculosis and Rifampicin Resistance in Patients Attending Kitui County Hospital, Kenya

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Abstract

Background: To combat the challenges of TB epidemic in Kenya there has been massive scale up of both treatment and diagnostic facilities.The challenges encountered in these centers is failure to accurately detect *Mycobacterium tuberculosis*. In this work, we aimed at evaluating the performance of GeneXpert MTB/RIF assay in detection of pulmonary TB and drug resistant testing.

Methods: Smear microscopy, niacin test, culture and GeneXpert MTB/RIF assay were used to test for the presence of *Mycobacterium tuberculosis* in 400 patient's sputum samples. Drug susceptibility testing was done using the culture method and GeneXpert MTB/RIFassay.

Results: Out of the 400 samples analysed 37.5% were smear positive of which 60% (p<0.05) were male. For the culture and GeneXpert assays the positive samples were 33% and 32.25% respectively. Smear microscopy had the highest number of false positives (28%) and false negatives (9.6%). For bacilli identification the sensitivity, specificity, positive predictive value and negative predictive values for smear microscopy were 81.8%, 84.3%, 72% and 90.4% while for GeneXpert they were 97.7%, 100%, 100% and 98.9% respectively. This implies that GeneXpert was a better method.

Drug susceptibility testing using the culture method showed that 23 isolate were rifampicin resistant and with GeneXpert they were 26, implying 3 false positives. The sensitivity, specificity, positive predictive value and negative predictive value for GeneXpert assay in drug susceptibility testing was 100%, 97%, 89% and 100%. Cost of testing samples with GeneXpert assay was higher than culture but it offers rapid detection in that on average it took between 2 hours against up to 8 weeks for the culture method.

Conclusion: GeneXpert MTB/RIF assay offers high potential for rapid diagnosis of TB and drug susceptibility testing in an austere Kenyan environment.

Introduction

Tuberculosis (TB) is a communicable disease of global concern that is caused by the acid-fast bacillus *Mycobacterium tuberculosis.* The disease is mainly pulmonary but on average 15% of the cases affects other parts of the body [1]. About 5%-10% of the people infected by *Mycobacterium tuberculosis* will go to develop the disease while the rest will remain asymptomatic and non-infectious [2]. In 2012, about 8.6 million people were for the first time infected with tuberculosis and in the same year there were about 1.3 million deaths [1]. In Kenya, the prevalence rate of the disease was 291 per 100,000 populations [3].

Conventionally over the past century, smear microscopy for acid fast bacilli has been the initial diagnostic tool. Its simplicity and low cost made it ideal especially in resource poor settings and most national TB control programs continue to rely on it despite its low sensitivity. In many developing countries, including Kenya, co-infection with human immunodeficiency (HIV) infection causes reduction of the sensitivity of microscopy which makes it unable to detect most smear positive patients except in cases where the diseases is advanced [4]. It has been reported that the number of expected cases of TB that tests positive by microscopy is as low as 28% [4]. Culture is the gold standard for final determination, but it is slow and it delays time to treatment which may take up to 2 to 8 weeks. The culture method is also prone to contamination and it requires specialized laboratories and highly skilled staff to execute [5].

Failure to accurately and rapidly detect tuberculosis and its drug resistant forms leads to increased mortality, nosocomial outbreaks, secondary resistance or resistance to additional anti-tuberculosis drugs and ongoing transmission. This has therefore impeded the efforts to lower the global burdenof tuberculosis disease [6,7]. There is therefore increased need for rapid and accurate methods for diagnosis of tuberculosis, especially in developing countries where smear microscopy is the method of choice.

Early and rapid identification of the bacilli and the drug resistance strains is essential for improving patient health and also for controlling the spread of TB. Methods based mostly on nucleic acid amplification for direct organism detection have reduced the diagnostic time while increasing sensitivity [7-9]. In early 2011, WHO endorsed a novel, rapid, automated, cartridge based nucleic acid amplification test, the GeneXpert MTB/RIF assay also known as GeneXpert or Xpert that can simultaneously detect TB and rifampicin resistance [10]. The assay uses hemi nested real-time polymerase-chain-reaction assay to amplify a MTB-specific sequence of the *ropB* gene, which is probed with molecular beacons for mutations within the rifampicin-resistance determining region [11]. GeneXpert has the potential of revolutionizing the testing and treatment of tuberculosis in developing countries. Currently there is limited published information on the effectiveness of GeneXpert assay for identification of *Mycobacterium tuberculosis* in an austere Kenyan environment. Confirmation of its sensitivity and quick turnaround time will provide an additional tool that will help in improving the diagnosis and management of tuberculosis.

Materials and Methods

Setting and patients

The prospective analytical study was carried out at Kitui District County Hospital which is the focal health facility in Kitui County, Kenya. The County has an area of about 30,496.5 km^2 and a population of 1,012,709 comprising of 48% male and 52% Female. A total of 400 patients were recruited for the study. The respondents were 18 years of age and older, with characteristics of TB infection. All patients included in this study provided informed consent. This research received ethical approval by Kitui County hospital.

Sputum collection and work flow

Patients provide three sputum specimens over a 2-day period. One was a spot sample and the second and third samples were obtained in the morning. The two samples were decontamination and analysed by smear microscopy and were cultured on Lowenstein-Jensen (L-J) and mycobacteria growth indicator tube (MGIT) 960 media. Samples that grew in culture were subjected to drug sensitivity testing on Middle-Brook-Cohn (7H-10) media. The third sample of sputum was collected in specialized containers and was tested directly using the GeneXpert assay.

Decontamination of sputum using N-acetyl-L-cysteine and sodium hydroxide

Equal volume of sputum and 0.1 M N-acetyl-L-cysteine (NALC) sodium hydroxide (NaOH) solution were mixed and incubated at 37ºC for 30 minutes with shaking after every 5 minutes. Five hundred microliters of phosphate buffer solution was added into the mixture and centrifuged at 3000 rpm for 15 minutes. The sediment was used to prepare the smear for microscopy.

Acid fast bacillus microscopy

The smear was air-dried, flame fixed, stained with basic carbol-fuschin and decolourized using 3% acid alcohol. The smear was counter stained with methylene blue and examined under light microscopy.

Lowenstein-Jensen media

Lowenstein-Jensen slant media was inoculated with 100 µl suspension of inoculum and incubated aerobically at 37ºC for 2-8 weeks. Culture positive colonies were subjected to Ziehl-Neelsen staining to establish their acid fast status.

Mycobacteria growth indicator tube (MGIT 960) media

A 100 µl aliquot of the suspension was inoculated into 0.8 mL MGIT 960 media supplemented with PANTA antibiotics and growth supplements. The samples were incubated in the MGIT 960 instrument at 37ºC for a maximum of 14 days. An instrument-positive control tube containing approximately 10^5 to 10^6 colony forming units per millilitre (CFU/mL) was incorporated. The tubes were monitored for increasing fluorescence to determine if the test samples contained viable organisms and the results were read automatically. Positive samples were subjected to smear microscopy and also drug susceptibility testing using Middle-Brook-Cohn 7H-10 agar.

Niacin test

The test organism was inoculated in L-J medium culture slant and incubated at 37ºC for up to 10 weeks. The slope was removed and 0.5 ml of cyanogen bromide added followed by 0.5 ml aniline solution and after 5 minute interval the colour change was monitored.

Drug susceptibility testing

Growth from primary pure cultures were used to make the inoculum where 1 part of bacterial suspension was mixed with 9 parts of modified Middlebrook 7H9 broth. The bacterial broth suspension was used to innoculate Middle-Brook-Cohn 7H-10 agar plates. Single drug disc were placed on theplatesthat were incubated at 37ºC for 18-24 hours in the absence of CO2. Results were read by observing the diameter of the zone of inhibition by the organism or clearance by the drugs rifampicin, isoniazid, ethambutol and pyrazinamide.

GeneXpert assay

The GeneXpert procedure was performed as per the manufacturer's recommendation. The sample reagent buffers (two volumes of 0.1 M NaOH and 0.1 M isopropanol) were mixed with the specimen in a 3:1 ratio and incubated at ambient temperature for 15 minute. A 2-3 ml aliquot of the digested sputum mixture was loaded into the GeneXpert system. The resulted were generated in 90 minutes and the read outs were either TB positive rifampicin resistant or TB positive non rifampicin resistant or no TB detected.

Data analysis

Continuous variables were described using mean ± standard deviation or median. Categorical variables were reported as frequencies. Pearson's chi-square test was used to compare categorical variables and the means were compared using Student's t-test.

Results

Patient characterization

Of the 400 patients who presented to Kitui District County Hospital laboratory with suspected cases of *Mycobacteria tuberculosis* during the period of study, the proportion of male to female was 46% vs. 54%.

The age of respondents ranged from 18 to 96 years with a mean of 42 ±15.9 years and a median of 40 years. The modal age group was 38-47 years (23.75%) while the age group with the lowest frequency (16.25%) was that of >57 years.

Smear microscopy

Smear microscopy showed that 37.5% (150/400) of the samples were positive indicating exposure to *Mycobacterium tuberculosis*. The bacilli in the positive slides were scored and 67% (101/150) of the samples had between 1-10 acid fast bacilli per field (scanty) while the rest (33%; 49/150) had between 10-99 acid fast bacilli per field (+1). When the smear positive samples were grouped according to gender 60% (90/150) were male and 40% (60/150) were female indicating a significant association between gender and *Mycobacterium tuberculosis* infection (p<0.05). For males only 48.9% (90/184) were smear positive while for females only 27.8% (60/216) were positive. When the respondents were grouped according to age it was noted that there was an increase in frequency of smear positive respondent with age. The peak frequency was in the 28-37 years group (11%) after which there was a gradual decrease in frequency to a low of 4% in the oldest (>57 years) age group (Table 1). The relationship between smear result and age group was tested using Pearson chi-square and there was no significant association (p=0.062).

Age group (years)	Smear results		
	Smear positive	Smear negative	Total
18-27	30 (7.5%)	44 (11%)	74 (18.5%)
28-37	44 (11%)	49 (12.25%)	93 (23.25%)
38-47	34 (8.5%)	61 (15.25%)	95 (23.75%)
48-57	26 (6.5%)	47 (11.75%)	73 (18.25%)
>57 yrs	16 (4%)	49 (12.25%)	65 (16.25%)
Total	150 (37.5%)	250 (62.5%)	400 (100%)

Table 1: Smear positive respondents according to age groups.

Culture method

Following culture in Lowenstein-Jensen solid media 33% (n=132) of the isolates showed growth and the colonies appeared dry, yellow-coloured, raised and wrinkled which is consistent with *Mycobacterium tuberculosis*. The 132 isolates were identified as acid fast bacilli following Ziehl-Neelsen staining. For niacin test all the 132 specimens displayed a yellow compound which was an indication of the production of niacin which is a metabolic by product of *Mycobacterium tuberculosis*. These results indicated a frequency rate of 33% for *Mycobacterium tuberculosis* amongst patient presented to Kitui District County hospital with suspected cases of tuberculosis.

Mycobacterium growth indicator tube media

This liquid media confirmed the findings of the LJ solid media in that 33% (132/400) of the isolates displayed viable organisms that appeared granular, while the rest were non-fluoresce implying no growth. In total 41% of the positive samples showed a signal of 10^6 CFU/ml while the rest recorded a signal of 10^6 CFU/ml.

Drug susceptibility testing on Middle-Brook-Cohn agar

Drug susceptibility testing was performed on the 132 samples that had been confirmed to be true positives of *Mycobacterium tuberculosis* using LJ media, MGIT 960 media, Acid fast microscopy and Niacin test. Out of the 132 samples tested for drug susceptibility 82.6% (109/132), were rifampicin sensitive and 17.4% (23/132) were rifampicin resistant. When susceptibility testing was done using isoniazid, ethambutol and pyrazinamide all 132 (100%) isolates were susceptible to the three drugs.

GeneXpert assay

The samples were also characterized using GeneXpert assay and 32.25% (129/400) of the isolates were found to contain the sequence of the RNA polymerase β subunit (rpoB) gene which is specific to the mycobacterium complex and this is a diagnostic feature for *Mycobacterium tuberculosis*. When the isolates were tested for rifampicin resistance using the same equipment by hybridizing the rpoB specific molecular beacons and the rpoB amplicon it was found that 20.2% (26/129) had mutations within the 81-bp rifampicin resistance determining region of the rpoB gene indicating that they were rifampicin resistance.

Comparison of GeneXpert assay, culture method and smear microscopy

When all 400 clinical specimens were tested using the culture method as the reference standard it found that 33% of the samples had growth of acid fast bacilli indicating presence of *Mycobacterium tuberculosis* (Table 2). When the culture results were compared to smear microscopy it was found that out of the 150 smear positive samples only 72% grew in culture and were true positive, while 28% had no growth indicating they were false positives. As for the 250 smear negative samples 90.4% showed no growth in culture and were true negatives, while 9.6% recorded growth and were false negatives (Table 2).

			Culture result		
			MTB growth	No MTB growth	Total
Smear positive	GeneXpert MTB	MTB detected	105	0	105
		MTB not detected	3	42	45
		Total	**108**	**42**	**150**

Smear negative	GeneXpert MTB	MTB detected	24	0	24
		MTB not detected	0	226	226
		Total	**24**	**226**	**250**
Xpert	Total for GeneXpert	MTB detected	129	0	129
		MTB not detected	3	268	271
Culture	**Total for Culture**		**132**	**268**	**400**

Table 2: Comparison of identification results of *Mycobacterium tuberculosis* between the culture, GeneXpert and smear microscopy methods.

The sensitivity, specificity, positive predictive value and negative predictive values for AFB smear microscopy for identification of *Mycobacterium tuberculosis* were 81.8%, 84.3%, 72% and 90.4%, respectively. Results of culture were also compared those of GeneXpert assay. Out of the 132 isolates that grew in culture 129 (97.7%) were identified by GeneXpert as *Mycobacterium tuberculosis* and were true positives. For the GeneXpert assay the sensitivity, specificity, positive predictive value and negative predictive value were 97.7%, 100%, 100% and 98.9%, respectively.

Drug susceptibility testing using conventional Middle-Brook-Cohn culture method showed that 17.4% (23/132) of the isolates were rifampicin resistant. When the samples were tested using GeneXpert assay the results showed that 26 isolates had genotype coding for rifampicin resistance indicating that there were 3 false positives. The sensitivity, specificity, positive predictive value and negative predictive value for GeneXpert assay in drug susceptibility testing was 100%, 97%, 89% and 100%.

Feasibility and cost effectiveness of GeneXpert test in routine laboratory use

The feasibility and cost effectiveness of GeneXpert test for routine laboratory use for the identification and susceptibility testing of *Mycobacterium tuberculosis* was compared to the gold standard culture method and results are presented in Table 3. The GeneXpert test is automated and the total hands-on time for processing each sample was only 5 minutes. On the other hand all steps of the culture methods were manual and the hand on time for processing a sample was higher of about 20 minutes per sample. The time it took GeneXpert assay to generate results for reporting was computed and on average this took between 2 hours. This compares to up to 8 weeks that it took for the culture method to generate results. Another difference was the reporting procedures between the two methods and for GeneXpert assay it was semi-quantitative while for conventional culture method it was qualitative.

Characterstic	GeneXpert MTB/RIF assay	Culture method
Work flow	On demand batch mode is not required	On demand batch mode is required
Sample format	Cartilage	Culture plate, tube
Sample preparation	Liquefaction and inactivation of sample then injection into	Inactivation of sample then inoculation on LJ and MGIT 960
Dna extraction	Integrated in catridge	None
Operation	Automated	Manual
Time to results	2 hrs	Upto 8 weeks
Hands on time	5 min/sample	20 min/sample
Reporting	Semi-quantitative report	Qualitative
Training	Low	High
Laboratory facilities	Ordinary	Enhanced
Cost	Ksh 3000/sample	Ksh 500/sample

Table 3: Comparison of the feasibility and operation cost of GeneXpert assay and the culture method.

The cost for analysis samples using the two methods was determined by adding the cost of specific consumables, personnel salary, utilities and preventive maintenance of equipment. The cost of running the GeneXpert test was Ksh 3000 per sample and this was six times the cost for the culture test which was Ksh 500. This high cost

was mainly contributed by the cartilage which accounted for 30% of the total cost.

However, the initial capital cost for setting up GeneXpert system was high at Ksh 4,000,000 and this included cost of purchase of the main equipment, calibration kit and UPS, shipping and installation

charges. While that of the culture system was 2.5 lower at Ksh 1,600,000 and the single most expensive item was the MGIT 960 machine which accounted for 32% of the total cost.

Skills required to run the two assays were also compared and for GeneXpert the assay is automated so human intervention was minimal and therefore the level of training that was needed to allow expertise in running the test was also minimal. In contrast the culture method was manual and specialized skills were required and more training was needed to enable the worker acquire the skills required to independently perform the assay.

There were also differences in the type of laboratory facilities required to perform the two tests. For GeneXpert assay it is a closed automated system and therefore there was minimal risks of infection so the assay was performed in an ordinary BSL2 laboratory space. However, the culture assay is an open system so manipulations were performed in an enhanced laboratory facilities in order to minimize risk of infection during handling.

Discussion

Gender and age are both traditionally known variables in terms of incidence and prevalence of pulmonary tuberculosis [12-14]. In this study demographic data showed that the ratio of male to female seeking treatment at Kitui County hospital for tuberculosis diagnosiswas 0.9 (46% male *vs.* 54% female) which is statistically similar to 0.85 (p>0.05) which is the general population ratio of male to female (47.5% female *vs.* 52.5% male) in Kitui County [15]. Therefore there was no association between gender and health seeking behavior in the County. This result indicates that in Kitui women have equal access to care like men and that both have similar TB diagnosis health-seeking behavior. This observation discounts the several reports that generally imply that female patients in developing countries have inequitable access to health care facilities [12,16,17]. On the other hand, the data is in agreement with a study carried out in Peruvian shantytowns which reported equal tuberculosis diagnostic and treatment care to men and women [13]. In Peru the TB programme was available to all members at no direct cost and it consisted of diagnostic tests, treatment and a social care component that include food and assistance to HIV testing. In the present study the balance in incidence ratio of male and female respondents seeking treatment for suspected cases of TB infection was most likely due to the fact that diagnosis and treatment services under the TB programme in Kenya are offered free of charge and also that there is high community awareness of the disease.

Acid fast bacilli microscopy showed that there were more smear positive male patients than female (60% vs. 40%; p<0.05). This indicates that males have a high risk of contracting pulmonary tuberculosis. An observation that is in line with several studies that have reported higher risks of contracting pulmonary tuberculosis in male respondents [12,17-20]. These studies suggested that there are genuine gender differences in the biology and epidemiology of TB [12,17,21]. The findings of the present study and the global pattern [22], where male are at higher risk than female are in contrast with observations in Afghanistan, Iran and Pakistan where incidence rates of pulmonary TB are higher in female than male [14,23-25]. The cause of disparity is not well understood but it has been attributed to socio-cultural and economic factors and reporting biases [25-29].

The respondent ages ranged between 18-96 years with mean of 42 ± 15.9. Most of the smear positive (11%) respondents were in the 28-37

years age category while the oldest age group (>57 years) had the fewest (4%) respondents that were smear positive, but there was no association between smear results and age (p>0.05). This contrasts several studies that reported significant association between TB infection and age. For example, it was reported that compared to young adults pulmonary tuberculosis was more prevalent (p<0.001) in young and old people often because of the undeveloped and weaker immune systems, respectively [30,31]. Further investigation are required to determine why age is not a risk factor in tuberculosis infection in Kitui County. Although there was no significant association between age and TB infection in Kitui it is noted that most smear positive were in the 28-37 years followed by those of 38-47 years, an observation that is consistent with other reports [19,23,32,33]. This finding is of concern because the age group most affected by tuberculosis in Kitui is the most economically productive [34], posing a significant economic burden on affected households and the resultant economic cost for society is high.

Drug sensitivity testing on culture showed that all the isolates tested were susceptible to isoniazid, pyrazinamide, and ethambutol and that 17.4% were resistant to rifampicin. Therefore, the overall drug resistance was also 17.4% which correlated well with a recent study in Kenya that reported an overall drug resistance of 18.8% [35], but was significantly lower than an earlier study that reported an overall resistance of 30.2% [36]. A major difference between the present study and the other two studies was that here resistance was only to rifampicin (17.4%) while in one of the study study [35], resistant was to isoniazid (11.6%), rifampicin (6.5%), ethambutol (11%) or streptomycin (5.1%). While in the other [36] resistance to isoniazid (30.2%) streptomycin (11.6%) ethambutol (4.5%) rifampicin (1.4%) or pyrazinamide (10.4%). In another study in Egypt [37], it was reported that 21.9% isolates were mono-resistant to the first line drugs streptomycin (13.5%), isoniazid, (3.9%), rifampicin (1.9%), ethambutol (1.3%) or pyrazinamide (1.3%).

It is not clear why in this study resistance was confined to rifampicin despite the other drugs being part of the combination therapy, and further investigations on the matter is advocated. However, this observation is not unlikely as it has been previously reported that in combination therapy resistance to one drug can occur due to differences in early bactericidal activity of the drugs in the combined pill [38]. It is also noted that rifampicin has a high bactericidal activity on semi-dormant bacilli. Therefore during treatment none of the other drugs has effect on these groups of *Mycobacterium tuberculosis* therefore a high risk of selecting resistant mutants [39].

The risk factors for drug-resistant TB include previous TB treatment, poor adherence to treatment regimens, inadequate regimens and positive smear result at the end of the second and third month of treatment [40]. Additionally, controversial patient characteristics such as HIV co-infection, alcohol abuse and younger age are also believed to influence the drug resistance. In Kenya TB resistance is associated with poor adherence to treatment regimens, inadequate regimens, slow smear conversion and technical issues related to acid fast bacilli smear microscopy [41].

The performance of smear microscopy and GeneXpert assay for pathogen identification were compared to the gold standard culture method. It was found that the sensitivity and specificity for smear microscopy was 81.8% and 84.3%, respectively. These values are within the ranges of contemporary reports from other laboratories that show sensitivity ranging from 20%-80% and specificity from 74.5%-100% [7,42-45]. The huge variations in the sensitivity of smear microscopy

are contributed by various factors including: specimen collection; smear preparation; slide examination; use of fluorescence versus conventional microscopy and differences in the performance depending on the operator [7,45]. Although smear microscopy has the advantages of low cost, rapidity, simplicity of procedure and its relative specificity and simple infrastructure [46], this study shows that smear microscopy is prone to TB misdiagnosis. Misdiagnosis of TB has grave implications such as continued transmission, higher mortality rates and delays in initiating appropriate therapy [47]. Therefore, there is justification in developing alternative strategies to diagnose the condition.

For GeneXpert assay the sensitivity and specificity for identification of *Mycobacterium tuberculosis* was 97.7% and 100%, respectively. This performance is comparable to that reported in other laboratories. The first analytical study to validate the technology observed that GeneXpert assay had 100% sensitivity and specificity [48]. In another study it was reported thatin Vietnam and Uganda sensitivity of 71.1%-100% and specificity of 100%were reported [49]. In a multicenter study involving Peru, Azerbaijan, South Africa and India reported an overall sensitivity of 97.6% with 98.1% specificity [50]. A meta-analysis of 16 GeneXpert assay studies revealed a pooled sensitivity of 90% and specificity of 98% [51]. In a hospital based evaluation study done in Zambia, the GeneXpert had an overall sensitivity of 86.1% and specificity of 95.7% [52]. While in Egypt GeneXpert assay showed a sensitivity and specificity 98.2% and 75% respectively [53].

The findings of this study shows that for diagnosis of *Mycobacterium tuberculosis* GeneXpert assay has high specificity and sensitivity and therefore more diagnostic power than smear microscopy. This is attributed to the fact that the lower limit of detection for GeneXpert assay is 100 CFU/ml whereas that of smear microscopy is around 5,000-10,000 CFU/ml [45,54]. Therefore, in high-volume laboratories with low sensitivity for sputum smear microscopy, GeneXpert is likely to substantially improve the diagnostic confirmation of *Mycobacterium tuberculosis*, since it is less dependent on the skill and time of individual technician [55]. On the other hand GeneXpert was less sensitive than the culture method but with similar specificity. This observation is in agreement with the general view that culture method can detect between 10-100 CFU/ml while for GeneXpert the lower limit of detection of *Mycobacterium tuberculosis* is 100 CFU/ml [56,57]. The findings of the present study therefore supports the use of GeneXpert as the initial diagnostic tool for diagnosis of pulmonary tuberculosis.

The performance of GeneXpert for detection of rifampicin resistance was compared to the gold standard culture method. The sensitivity and specificity of GeneXpert was 100% and 97.2% respectively. This means that GeneXpert has the same sensitivity like culture method. The slightly lower specificity was due to 3 false positive isolates. False-positive results have been associated with mixed infection, administrative errors and indeterminate causes [58]. It was also reported that a specimen that was repeatedly rifampicin resistant on GeneXpert but susceptible on phenotypic culture and *rpo*B sequencing [56]. In another study six rifampicin-resistant cases on GeneXpert were identified, five of which were susceptible on phenotypic culture, although five were genotypically resistant by sequencing and/or MTBDR *plus* [59]. The complexity of these investigations demonstrates the difficulty in confidently distinguishing false-positive from true-positive rifampicin-resistant results, particularly in clinical practice. In response to reports of false-positive

rifampicin-resistant results, the manufacturer performed a root cause analysis, which identified the bead manufacturing scale-up and annealing temperature requirements of probe B as potential causes. Solutions include improved bead reconstitution, a software change and adjustment of probe B to increase robustness; all of which are being evaluated [50].

The performance of GeneXpert assay for drug susceptibility has been evaluated in several studies. In an initial study a sensitivity and specificity for rifampicin resistance of 94.4% and 98.3% [60]. In a 7 study subset of the meta-analysis, the pooled sensitivity of rifampicin resistance detection was 94% and specificity of 97% [52]. Sensitivities of 100% have also been reported in several studies [48,49,55]. In a recent study in a programmatic setting in South Africa a specificity of 99.5% was reported [61]. Generally, these findings are in agreement with the present study and they indicate that GeneXpert assay is a valuable tool for testing rifampicin resistant.

All the 26 samples that were reported to be rifampicin resistance using GeneXpert assay were able to grow in culture meaning that they were viable organism. This means that the assay is a suitable implement for monitoring patients including those on treatment. This finding differs with other observations that that suggests that molecular tests, including GeneXpert MTB/RIF, are not suitable for patient monitoring as these tests detect DNA from both viable and non-viable bacilli [62]. They also stated that conventional laboratory capacity is, therefore, required to monitor treatment response of patients detected by GeneXpert and to conduct additional drug susceptibility testing in patients with rifampicin resistance.

The feasibility and cost effectiveness of GeneXpert test for routine laboratory use in the identification and susceptibility testing of *Mycobacterium tuberculosis* was compared to the gold standard culture method. The hands on time for processing each sample was only 5 minutes for GeneXpert assay while for culture method it was higher at 20 minutes. The difference is because the former is automated and the only manual step was the liquefaction and inactivation of the sputum while for the latter all the process are manual. There were also significant differences on the time it took to generate results for GeneXpert assay on average it took between 2 hours while culture method took up to 8 weeks to generate results. The short turnaround time is essential for it substantially faster initiation of appropriate tuberculosis therapy, particularly for patients with smear negative disease. Noting the short turn around time for GenXpert, Recent Studies [63], reported that scale-up of laboratory capacity for detection of TB and drug resistance is urgently needed, but may be costly.

In this study the running costs for GeneXpert were six times more than the culture method. In a review [7], it was reported differences in running costs of between 2 and 7 times. Here most of the costs for molecular testing were influenced by consumable costs, especially the cartilage which accounted for 30% of the total cost. Studies [64], reported that GeneXpert and MTBDRplus are the two WHO recommended platforms for rapid detection of TB and drug-resistant TB and many low and middle-income countries qualify for negotiated discounts on these assays. This has implications in that the costs reported in this study are likely to come down after discount thereby increasing affordability. Overall, the present suggest that decentralized test implementation is feasible and could lead to an improvement in tuberculosis care and control. Similar findings were reported in a study that suggested that decentralized test implementation is feasible and could lead to an improvement in tuberculosis care and control [60].

The current reference standard approaches involving culture for identification and susceptibility testing are slow, have biosafety hazard and are resource intensive for laboratories to perform. GeneXpert assay necessitated rapid diagnosis of tuberculosis and detection of rifampicin resistance at once because it was giving results within 2 hrs. This assay was found to be essential for early disease diagnosis and management in clinical specimens. The early availability of results for positive pulmonary tuberculosis offers numerous advantages which include bringing down transmission rates and saving of costs through shortening of isolation and hospitalization time for patients.

Conclusion

This study shows that women have equal access to care like men and that both have similar TB diagnosis health-seeking behaviour. It also indicates that gender play a more important role than age as a risk factor for contracting pulmonary tuberculosis in Kitui. For detection of *Mycobacterium tuberculosis* GeneXpert assay has diagnostic power that is comparable to the gold standard culture method but it has more power than acid fast bacilli smear microscopy. Therefore, in high-volume laboratories with low sensitivity for sputum smear microscopy, GeneXpert is likely to substantially improve the diagnostic confirmation of the bacilli. For drug susceptibility testing GeneXpert assay has a sensitivity that is similar to the culture method. It is therefore a valuable tool for determining rifampicin resistance in patients infected with *Mycobacterium tuberculosis*. Although the cost of testing samples with GeneXpert assay is higher than culture it offers rapid detection. Overall, GeneXpert assay was found to be essential for early disease diagnosis and management in clinical specimens in the Kenyan environment.

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Competing Interests

The authors declare that they have no competing interests.

References

1. WHO (2013) Global tuberculosis report. WHO, Geneva.

2. Ullah I, Ullah N, Khan K, Hayat MK, Kamal R, et al. (2015) Prevalence of tuberculosis in the population of district kohat and lower orakzai agency. MRJMMS 3: 462-466.

3. WHO (2012) Global tuberculosis surveillance report. Bull World Health Organ. WHO, Geneva.

4. WHO (2009) Global tuberculosis control: A short update to the 2009 Report. WHO, Geneva.

5. WHO (2010) Treatment of tuberculosis: Guidelines.WHO, Geneva.

6. Moore DA, Evans CA, Gilman RH, Caviedes L, Coronel J, et al. (2006) Microscopic-observation drug-susceptibility assay for the diagnosis of TB. MTB/RIF system. Policy statement. N Engl J Med 355: 1539-1550.

7. Molicotti P, Alessandra B, Stefania Z (2014) Cost-effectiveness in the diagnosis of tuberculosis. Choices in developing countries. J Infect Dev Ctries.

8. Sechi LA, Acetia A, Zanettib S, Muraa M, Turrinic F, et al. (1997) Identification of HIV patients with active pulmonary tuberculosis using urine based polymerase chain reaction assay. Thorax.

9. Dixit P, Sigh U, Sharma P, Jain A (2012) Evaluation of nitrate reduction assay, resazurin microtiter assay and microscopic observation drug susceptibility assay for first line antitubercular drug susceptibility testing of clinical isolates of M. tuberculosis. J Microbiol Meth 88(1): 122-126.

10. WHO (2011) Published evidence and commentary on the Xpert MTB/RIF assay. Technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance.

11. El-Hajj HH, Marras SA, Tyagi FR, Kramer L, Alland D, et al. (2001) Detection of rifampicin resistance in Mycobacterium tuberculosis in a single tube with molecular beacons. J Clin Microbiol 39: 4131-4137.

12. Hamid SMA, Declercq E, Van Deun A, Saki KA (2004) Gender differences in tuberculosis. A prevalence survey done in Bangladesh. Int J Tuberc Lung 8: 952-957.

13. Onifade DA, Bayer MA, Montoya R, Haro M, Alva J, et al. (2010) Gender-related factors influencing tuberculosis control in shantytowns. A qualitative study. BMC Public Health.

14. Noori T, Odone A, Tillmann T, Sandgren A, Williams G, et al. (2015) Tuberculosis among migrant populations in the European Union and the European Economic Area. Eur J Pub Health 4: 506-512.

15. Kenya National Bureau of Statistics (2012) Facts and figures.

16. Diwan VK, Long NH, Johansson E, Lönnroth K, Eriksson B, et al. (1999) Longer delays in tuberculosis diagnosis among women in Vietnam. Inter J Tubercul Lung Dis 3: 388-393.

17. Borgdorff MW, Nagelkerke NJ, Dye C, Nunn P (2000) Gender and tuberculosis. A comparison of prevalence surveys with notification data to explore sex differences in case detection. Int J Tuberc Lung Dis 4: 123-132.

18. Zaman K, Yunus M, Arifeen SE, Baqui AH, Sack DA, et al. (2006) Prevalence of sputum smear-positive tuberculosis in a rural area in Bangladesh. Epidem Infec 134: 1052-1059.

19. Gajbhare DM, Bedre RC, Solanki HM (2014) A study of socio-demographic profile and treatment outcome of tuberculosis patients in an urban slum of Mumbai, Maharashtra. Ind J Med Res 4: 50-57.

20. Heemanshu A, Satwanti K (2016) Determinants of lost to follow up during treatment among tuberculosis Patients in Delhi. Int J Med Res Health Sci 5: 145-152.

21. Radhakrishna S, Frieden TR, Subramani R (2003) Association of initial tuberculin sensitivity, age and sex with incidence of tuberculosis in south India. A 15-year follow-up. Int J Tuberc Lung Dis 7: 1083-1091.

22. WHO (2015) Global tuberculosis control: A short update to the 2009 Report. WHO, Geneva.

23. Kherad O, Hermann FR, Zellweger JP, Rochat T, Jansen JP, et al. (2009) Clinical presentation, demographics and Outcome of Tuberculosis (TB) in a low incidence area: A 4-year study in Geneva, Switzerland. BMC Infect Dis 9: 217-229.

24. Codlin AJ, Khowaja S, Chen Z, Rahbar MH, Qadeer E, et al. (2011) Short report: Gender differences in tuberculosis notification in Pakistan. Am J Trop Med Hyg.

25. Sabawoon LW, Sato H (2012) Sex difference in tuberculosis in Afghanistan: A national cohort study. Mycobacterial Diseases.

26. Cassels A, Heineman E, LeClerq S, Gurung PK, Rahut CB, et al. (1982) Tuberculosis case-finding in Eastern Nepal. Tubercle 63: 175-185.

27. Hudelson P (1996) Gender differentials in tuberculosis: the role of socio-economic and cultural factors. Tuber Lung Dis 77: 391-400.

28. Thorson A, Diwan VK (2001) Gender inequalities in tuberculosis. Aspects of infection, notification rates, and compliance. Curr Opin Pulm Med 7: 165-169.

29. Qureshi SA, Morkve O, Mustafa T (2008) Patient and health system delays. Health-care seeking behaviour among pulmonary tuberculosis patients in Pakistan. J Pak Med Assoc 58: 318-321.

30. Hussein A, Ahmed Z, Abiyu M, Solomon A (2012) Smear positive pulmonary tuberculosis (PTB) prevalence amongst patients at agaro teaching health center, South West Ethiopia. Ethiop J Health Sci 22: 71-76.

31. Grange J (2003) Immunophysiology and Immunopathology. In: Davies PDO (ed). Clinical Tuberculosis (3rd edn.). Arnold, London.

32. Vijay S, Kumar P, Chauhan LS, Vollepore BH, Kizhakkethil UP, et al. (2010) Risk factors associated with default among new smear positive TB patients treated under DOTS in India. PLoS One 5: 100-143.

33. Iqbal T, Raziq MA, Hussain Z, Anium N, Atia S, et al. (2011) Gender differences among suspected pulmonary tuberculosis patients undergoing sputum smear microscopy. Med Sci 7: 14-17.

34. Aubert P, Crépon B (2003) Age, wage and productivity: firm-level evidence. Economie et Statistique 363: 95-119.

35. Nyang'au OL, Amukoye E, Ng'ang'a Z (2015) Determining first line anti-tuberculosis drug resistance among new and re-treatment tuberculosis/human immunodeficiency virus infected patients, Nairobi Kenya. IJSBAR 19: 426-437.

36. Ndungu PW, Revathi G, Kariuki S, Ng'ang'a Z (2013) Risk factors in the transmission of tuberculosis in Nairobi: A descriptive epidemiological study. Scient Res.

37. Abbadi SH (2009) Molecular identification of mutations associated with anti-tuberculosis drug resistance among strains of Mycobacterium tuberculosis. Intern J Infect Dis 13: 673-678.

38. Jindani AJ, Aber VR, Edwards EA, Mitchison DA (1980) The early bactericidal activity of drugs in patients with pulmonary tuberculosis. Amer Rev Resp Dis 121: 939-949.

39. Mitchison DA, Davies GR (2008) Assessment of the efficacy of new anti-tuberculosis drugs. J Clin Microbiol 2: 59-76.

40. Gomez JR, Souto A, Maneiro JR, Salgado E, Carmona L, et al. (2014) Risk of tuberculosis in patients with chronic immune-mediated inflammatory diseases treated with biologics and tofacitinib. A systematic review and meta-analysis of randomized controlled trials and long-term extension studies. Rheumatology 10: 1093.

41. Keter LK, Cherogony SK, Korir RK, Mutai C (2015) Factors contributing to drug resistant tuberculosis. A case study of tuberculosis patients attending Rift Valley provincial general hospital Nakuru, Kenya. Science Research.

42. Shea B, Nava AE, Andersson N, Harris E, Mitchell S, et al. (2009) Risk factors associated with recent transmission of tuberculosis.Systematic review and meta-analysis. Intern J Tuber Lung Dis 13: 17-26.

43. Monkongdee P, McCarthy KD, Cain KP, Tasaneeyapan T, Dung NH, et al. (2009) Yield of acid-fast smear and mycobacterial culture for tuberculosis diagnosis in people with human immunodeficiency virus. Am J Respir CritCare Med 180: 903-908.

44. Swai HF, Mugusi FM, Mbwambo JK (2011) Sputum smear negative pulmonary tuberculosis: sensitivity and specificity of diagnostic algorithm. BMC 4: 475.

45. Parsons ML, Somoskovi A, Gutierrez C, Evan L, Paramasivan CN, et al. (2011) Laboratory diagnosis of tuberculosis in resource-poor countries. Challenges and opportunities. Clin Microbiol 24: 314-350.

46. Singhal R, Myneedu VP (2015) Microscopy as a diagnostic tool in pulmonary tuberculosis. Int J Mycobacteriol 12: 6-18.

47. Lawn SD (1997) Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using Xpert MTB/RIF assay: A prospective study. PloS Med 8: e1001067.

48. Blakemore R, Story E, Helb D, Kop J, Banada P, et al. (2010) Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Clin Microbiol 48: 2495-2501.

49. Helb D, Jones M, Story E, Boehme C, Wallace E, et al. (2010) Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 48: 229-237.

50. Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, et al. (2010) Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363: 1005-1015.

51. Chang K, Lu W, Wang J, Zhang K, Jia S, et al. (2012) Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: A meta-analysis. J Infect 64: 580-588.

52. Elliott AM, Namaambo K, Allen BW (2013) Negative sputum smear results in HIV-positive patients with pulmonary tuberculosis in Lusaka, Zambia. Tuber Lung Dis 74: 191-194.

53. Meawed TE, Shaker A (2016) Assessment of diagnostic accuracy of Gene Xpert MTB/RIF in diagnosis of suspected re-treatment pulmonary tuberculosis patients. Egyp J Chest Dis.

54. Pfyffer GE (2012) Mycobacterium tuberculosis Transmission in a country with low tuberculosis incidence: Role of immigration and HIV infection for the Swiss HIV cohort and molecular epidemiology of tuberculosis study groups. J Clin Microbiol 50: 388-395.

55. Arzu NZ, Sezai T, Cengiz C (2011) Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampicin resistance in pulmonary and extra-pulmonary specimens. J Clin Microbiol 49: 4138-4141.

56. Marlowe EM, Novak-Weekley SM, Cumpio J, Sharp SE, Momeny MA, et al. (2011) Evaluation of the Cepheid Xpert MTB/RIF assay for direct detection of Mycobacterium tuberculosis complex in respiratory specimens. J Clin Microbiol 49: 1621-1623.

57. Nhu NTQ, Heemskerk D, Thu DDA, Chau TTH, Mai NTH, et al. (2014) Evaluation of GeneXpert MTB/RIF for diagnosis of tuberculous. J Clin Microbiol 52: 226-233.

58. VanRie A, Page-Ship L, Scott L, Reider HL, Saldiva PH, et al. (2012) Xpert MTB/RIF for point-of-care diagnosis of Tb in high-HIV burden, resource-limited countries. Hype or hope. Expert Rev Mol Diag 10: 937-996.

59. Theron G, Peter J, Van ZSR, Mishra H, Streicher E, et al. (2011) Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. Am J Resp Crit Care Med 184: 132-140.

60. Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, et al. (2011) Feasibility, diagnostic accuracy, and effectiveness of de-centralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance. A multi-centre implementation study. Lancet 377: 1495-1505.

61. Osman M, Seddon JA, Dunbar R, Draper HR, Lombard C, et al. (2014) The complex relationship between human immunodeficiency virus infection and death in adults being treated for tuberculosis in Cape Town, South Africa. BMC Pub Health 15: 556.

62. Weyer K, Mirzayev F, Migliori GB, Van Gemert W, D'Ambrosio L, et al. (2013) Rapid molecular TB diagnosis. Evidence, policy making and global implementation of Xpert MTB/RIF. Eur Resp 42: 252-271.

63. Maunank S, Violet C, Gerrit C, Gavin C, Susan G, et al. (2013) Comparison of laboratory costs of rapid molecular tests and conventional diagnostics for detection of tuberculosis and drug-resistant tuberculosis in South Africa. BMC Public Health.

64. Huyen MNT, Tiemersma EW, Lan NTN, Cobelens FGJ, Dung NH, et al. (2010) Validation of the GenoType®MTBDR plus assay for diagnosis of multidrug resistant tuberculosis in South Vietnam. Infec Dis 10: 149.