

Utilization of GeneXpert for Point of Care Testing of Tuberculosis: A Bench Mark Approach Over Ziehl-Nelson Smear Microscopy

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Abstract

Tuberculosis (TB) is a wasting disease caused by *Mycobacterium tuberculosis*. It is an ancient disease that has claimed several lives which was initially diagnosed with the smear microscopy Ziehl-Nelson staining technique especially in poor resource setting. The smear microscopy technique is simple and inexpensive but less sensitive, less specific and requires at least 10,000/ml of sputum sample to detect TB. It has a poor track record in detecting TB from extra pulmonary samples hence unable to readily diagnose pediatric TB and patients co-infected with HIV. Although it has been an integral part of global strategy for tuberculosis control, two to three samples will be needed to confirm the presence of TB infection which is where 95% of TB cases and 98% of deaths occur. However, modern diagnostic technology using GeneXpert has indicated outstanding results for point of care diagnosis. It has a shorter turnaround time of two hours against 24 to 48 hours for smear microscopy. It is specific and sensitive in the detection of *Mycobacterium tuberculosis* and rifampicin resistance. It has a minimal biohazard risk and requires less expertise. One sample is enough for diagnosis with 131/ml of sputum sample for pulmonary tuberculosis. Prompt patient tracking and care is therefore enhanced because loss to treatment failure and follow up as a result of repeated sputum examination with prolonged turnaround time is a thing of the past.

Keywords: Tuberculosis; Ziehl-Nelson Smear Microscopy; GeneXpert; Rifampicin Resistance; *Mycobacterium tuberculosis*

Introduction

Tuberculosis (TB) is an air borne disease that has claimed the lives of several people locally and internationally. According to the Global Tuberculosis report 2014 of World Health Organization (WHO), TB remains one of the world's deadliest communicable diseases that is caused by the Bacterium *Mycobacterium tuberculosis* (MTB) [1]. Nigeria is one of the countries included among the 30 high burden countries for TB, TB/HIV and DR-TB [2]. According to the WHO, the estimated incidence of TB in Nigeria is 322 per 100 000 population with only 15% of the total burden of the disease in the country being notified in 2015 [2]. TB culture which is the conventional method of TB diagnosis takes a long time up to eight weeks before commencement of patient treatment. It also requires sophisticated equipment such as the biosafety cabinet, aspirators, and a well-designed biosafety level 2 laboratory with well trained staff hence it is not suitable for a low- income setting. Likewise, the Ziehl-Nelson (ZN) smear microscopy method of diagnosing TB is cumbered with divers limitations although it has been the primary method for diagnosis of pulmonary tuberculosis in low and middle-income countries which is where nearly 95 per cent of TB cases and 98 per cent of deaths due to TB occur. It is a simple, rapid and inexpensive which is highly specific in areas with a very high prevalence of tuberculosis. It also identifies the most infectious patients and is widely applicable in various populations with different socio-economic activities. Therefore, it is an integral part of the global strategy for TB control [3-5].

Nevertheless, it has significant limitations which cannot be overemphasized. The sensitivity is grossly compromised when the bacterial load is less than 10,000 organisms/ml sputum sample. It also has a poor diagnostic record in extra-pulmonary tuberculosis, pediatric tuberculosis and in patients that are co-infected with HIV as a result of compromised immunity [6]. The ZN smear microscopy method requires serial sputum examinations, and in the process some patients who do not come back for repeated sputum examinations for the second and third time as in the Directly Observed Treatment Short Course (DOTS) strategy become defaulters to the program [7]. Some do not come back for results, and are lost to treatment and follow up which is a contributory factor for the emergence of drug resistant TB (DRTB) and Multi drug Resistant (MDR) TB worldwide.

As a way forward to proffer solutions to these limitations highlighted above, the GeneXpert technology was developed which was endorsed by World Health Organization (WHO) in December 2010 as an accurate, feasible, rapid, affordable, and point-of-care TB diagnostic test for use in resource-limited settings [8]. The GeneXpert assay works by detecting MTB and RIF resistance by polymerase chain reaction (PCR) based amplification of the 81-bp *rpoB* gene segment and probing for the mutations that are related to RIF resistance. The assay is automated and completes within 2 hours [9,10]. After the redesign of probe B in December 2011 [11], studies have assessed the performance of GeneXpert in detection of MTB and MDR-TB. We aimed to evaluate as a main objective the diagnostic specificity and sensitivity of Xpert MTB/RIF assay in comparison to conventional Ziehl-Nelson smear microscopy diagnosis of TB in our setting.

Materials and Methods

Study Area

The area that was chosen for this study was Port Harcourt in Rivers State, Nigeria.

Sample Collection

Samples were collected from participants who presented symptoms of TB such as fever, weight loss, pain in the chest, chills, distorted breath, loss of appetite, night sweats and whose cough had lasted for at least two weeks. The samples were then transported in cold chain to the South- South Tuberculosis Zonal Reference Laboratory University of Port Harcourt Teaching Hospital.

Ethical Approval

Ethical approval was obtained from the Rivers State Hospital Management Board Port Harcourt.

Study Design

A Comparative Cross-Sectional design was employed to assay a total of 600 samples collected from mixed population with different age groups. Samples were assayed to determine the specificity and sensitivity of GeneXpert technology over Ziehl-Nelson Smear microscopy.

Data Availability

All relevant data required are contained in this article.

Laboratory Approach

Participants were given sputum cups labelled with their names and counselled to wash their mouth with clean water, breath in and out for three to five times and then expectorate sputum directly into the cups. The cups were labelled with participant's names and a laboratory identification (ID) number was given to each cup containing sputum.

Ziehl-Nelson's Smear Microscopy Method

Upon receipt of the sputum samples in the Laboratory, the macroscopy of the sample was recorded and then smears were made on grease free slides labelled with participants ID numbers. They were left to air dry before staining with the Ziehl-Nelson's staining technique using standard methods.

GeneXpert MTB/RIF Assay

The sputum samples collected from participants were mixed with the GeneXpert reagent that is provided with the assay in 1:2 ratios. A portion of the sample was mixed with two portions of the sample reagent in a biosafety cabinet. It was further vortexed for 5 minutes and left to incubate for 10 minutes under room temperature. A second 5minutes vertexing was done and left to incubate for 10 minutes. Thereafter, 2 ml of the suspension was transferred into the GeneXpert cartridge. The GeneXpert camera was used to scan the cartridge barcode. Biodata of participants including the sample ID were entered manually into the machine and it was commanded to start from the system menu. All GeneXpert results were originated from the GeneXpert machine.

The Gene Xpert Dx System version 4.4a and 4.7b were used for the analysis of the samples.

Results

The results of this study are represented in the table below.

Age group (years)	Mycobacterium tuberculosis (MTB) detected						Total MTB Positive (%)	Total Smear Positive (%)	Total MTB not Detected (%)	Total MTB Detected, Rifampicin Resistance Detected (%)	Invalid Test (%)
	Low (%)	Smear Positive (%)	Medium m (%)	Smear Positive (%)	High (%)	Smear Positive (%)					
16 - 25	2 (11.76)	1 (10.0)	5 (16.67)	5 (17.86)	5 (23.81)	5 (23.81)	12 (17.39)	11 (18.97)	87 (16.54)	0 (0.0)	1 (20.0)
26 - 35	3 (17.65)	1 (10.0)	4 (13.33)	4 (14.29)	6 (28.57)	6 (28.57)	13 (18.84)	10 (17.24)	83 (15.78)	3 (50.0)	1 (20.0)
36 - 45	4 (23.53)	4 (40.0)	6 (20.0)	5 (17.86)	3 (14.29)	3 (14.29)	13 (18.84)	12 (20.69)	87 (16.53)	0 (0.0)	0 (0.0)
46 - 55	3 (17.65)	2 (20.0)	7 (23.33)	6 (21.43)	3 (14.29)	3 (14.29)	13 (18.84)	11 (18.97)	86 (16.34)	1 (16.67)	0 (0.0)
56 - 65	1 (5.88)	1 (10.0)	5 (16.67)	5 (17.86)	4 (19.05)	4 (19.05)	10 (14.84)	10 (17.24)	87 (16.53)	1 (16.67)	2 (40.0)
65 - 75	4 (23.53)	1 (10.0)	3 (10.0)	3 (10.71)	0 (0.0)	0 (0.0)	7 (10.14)	4 (6.89)	91 (17.30)	1 (16.67)	1 (20.0)
	17	10	30	28	21	21	69	58	526	6	5

Age group	No tested	MTB Detected (Gene Xpert)	Ziehl- Nelson Smear Positive	MTB NOT Detected (Gene Xpert)	Ziehl- Nelson smear Negative
16 - 25	100	12	11	88	89
26 - 35	100	13	10	87	90
36 - 45	100	13	12	87	82
46 - 55	100	13	11	87	89
56 - 65	100	10	10	90	90
66 - 75	100	7	4	93	96
Total	600	69	58	531	542

Table 2: Comparison of GeneXpert and Ziehl-Nelson Smear Microscopy.

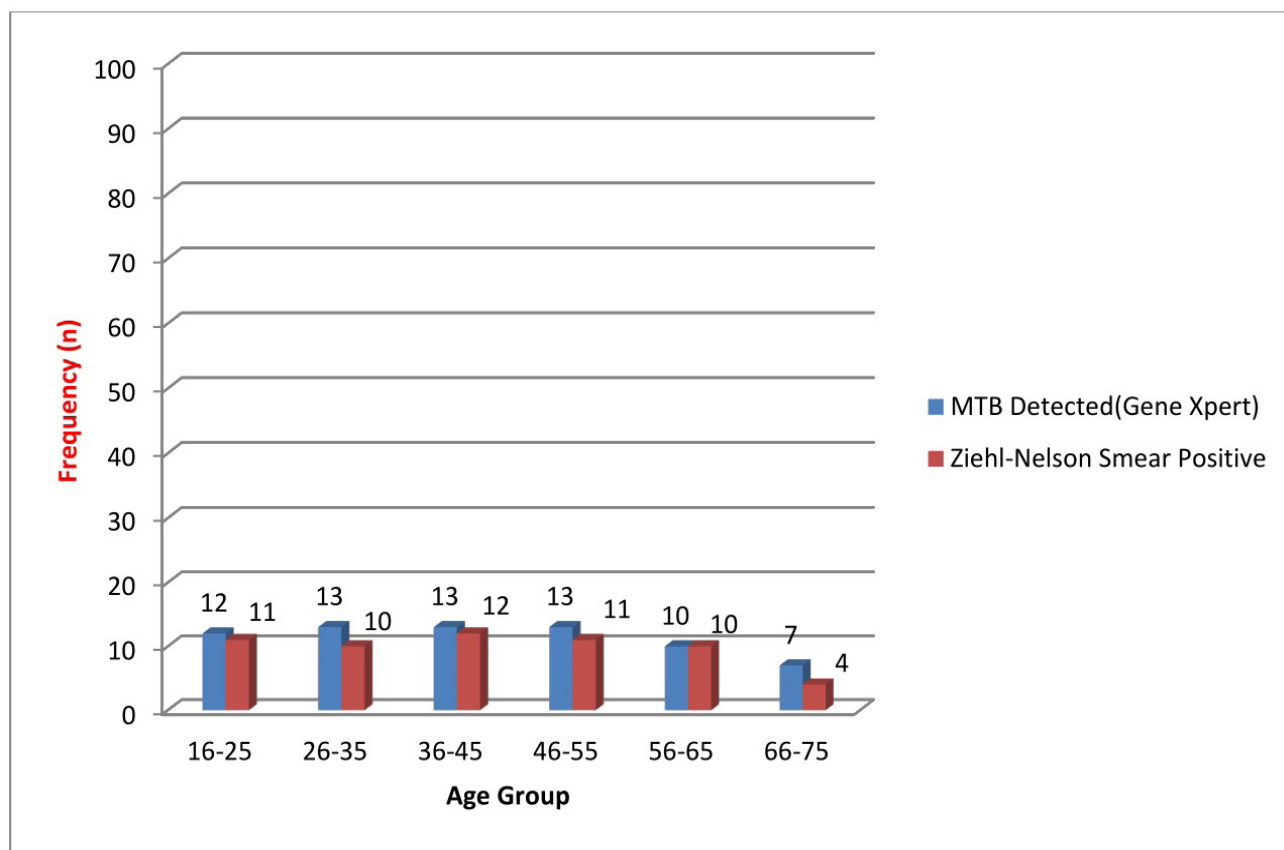


Figure 1: Comparison of Gene Xpert and Ziehl-Nelson Smear Microscopy

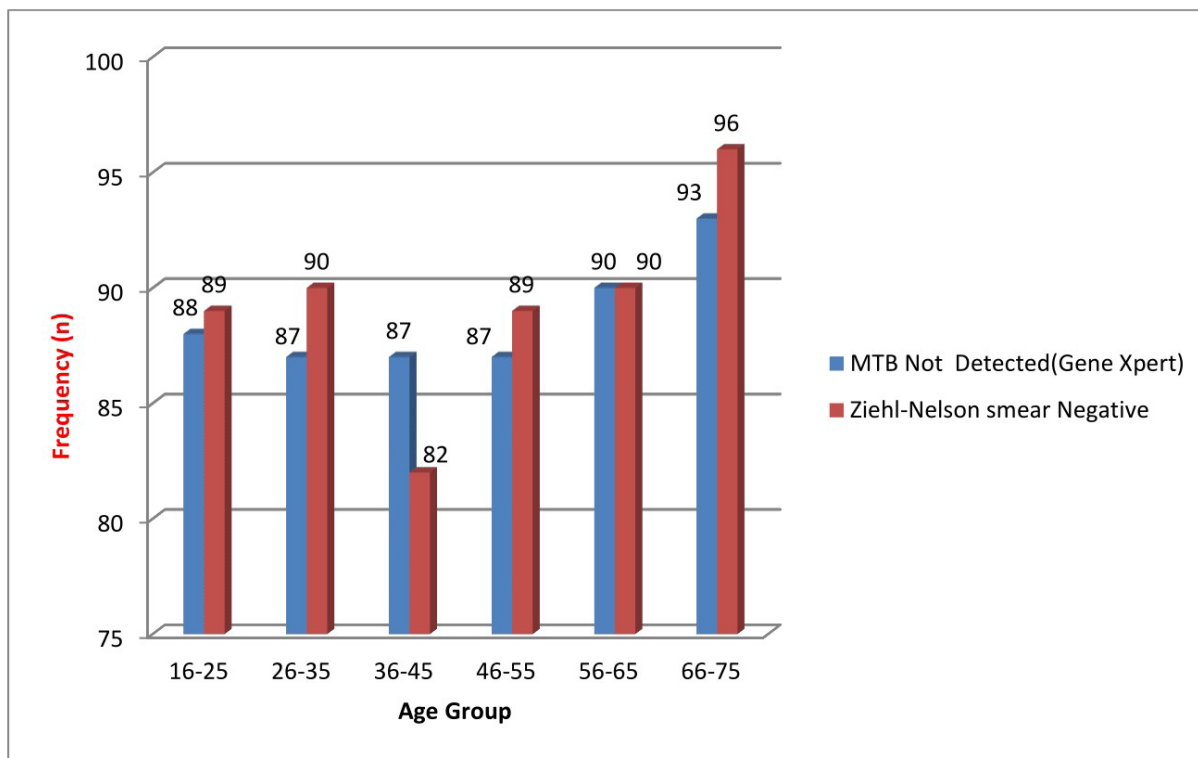


Figure 2: Comparison of Gene Xpert MTB Not Detected and Ziehl-Nelson Smear Negative.

Discussion

A total of 600 sputum samples were tested with the GeneXpert MTB/RIF Assay which was compared with the conventional Ziehl-Nelson Smear Microscopy. Sixty-nine (11.50%) were *Mycobacterium tuberculosis* detected while 531 (88.50%) were MTB not detected. Also, fifty-eight (9.6%) were positive for Ziehl-Nelson (ZN) smear microscopy while 542 (90.33%) were ZN smear microscopy negative and five (0.83%) were invalid. In this study, Fisher’s exact test with $P < 0.05$ was considered to be significant [9]. The significance in sensitivity disparity of GeneXpert compared to ZN smear microscopy was not wide using Fisher’s exact test, $P < 0.05$ although there may be differences with respect to numbers. A proportional difference in sensitivity was only observed among MTB detected low (23.53%) against Smear positivity (10.0%). This is a remarkable advantage of GeneXpert that uses as low as 131 ml of sputum but a limitation for smear microscopy requiring a minimum of 10,000 ml of sputum to diagnose TB. In a similar study conducted in Nigeria, 48 patients (34.3%) had smear positive TB, while 44 patients (31.4%) were multidrug resistant TB. Ten (7.2%) were rifampicin resistant [14]. In a case study conducted in Infectious Diseases Hospital Kano in Northern Nigeria, out of 80 patients sampled, 52 were diagnosed to be acid fast bacilli positive and 28 were acid fast bacilli negative with age group 31 - 40 years having the highest prevalence of 28.8% [19].

The Xpert MTB/RIF Assay has a high specificity for MTB detection and Rifampicin resistance. In this study, six (1%) of the population tested were Rifampicin resistant. This proportion small as it can be is a major public health challenge and a threat to global TB control especially in a low income setting like Nigeria. A prevalence of 355 (18.9%) were positive for MTB out of which 43 (12.1%) were Rifampicin resistant from a study conducted in Nigeria [12]. Other studies that have been conducted in Nigeria reported the prevalence to be

between 7.1% and 18.8% [12-14]. Studies on culture isolates in Nigeria showed that 16% and 31% of MTB isolates are resistant to at least one first line drug while approximately 3.6% were multidrug resistant [16,17]. Rifampicin is a surrogate marker for MDR-TB hence it's prevalence despite the proportion is an alarm. The age group in this study that has the highest MTB prevalence (23.53%) was between 36 - 45 years and 65 - 75 years. The earlier age group are those that are actively involved in home keeping, child bearing, work force and bread winners. Therefore, mortality and morbidity among this age group resulting from MDR-TB is a great economic loss. The later are those whose immunity probably had been compromised as a result of advancement in age. Highest prevalence of rifampicin resistance in this study was seen to be within age group 26 - 35 years. It is not a surprise as people in this age group are those that are within the active sexual age. Human immunodeficiency Virus is a risk factor for active tuberculosis. Although this study was limited with the HIV status of the study participants. This report conforms to the report given in South Africa where patients between the age of 21 and 25 years had higher prevalence of rifampicin resistance [18]. In another study reported in Kwara and Benue, North Central Nigeria over 80% of those who are MTB positive and rifampicin resistant were aged between 11 and 40years [12,13].

The results of this study represent an index to scale up Tb control in Nigeria and an evidence that expansion of GeneXpert to new regions may be a wide means of improving case finding which will guide treatment of drug resistant TB in this setting. Rapid diagnosis of rifampicin resistance potentially allows TB patients to start on effective treatment much sooner than waiting for results from other types of drug susceptibility testing. Availability of quick test results lead to improved patient care and outcomes. The use of fully automated system that requires minimal technical training is a booster for rapid diagnosis and treatment.

Nevertheless, GeneXpert cannot eliminate the need for conventional microscopy, culture and sensitivity testing, as they are still required to monitor treatment progress and to detect other types of drug resistance. The ZN smear microscopy requires absolute proficient technical expertise. It is unable to detect MTB from NTM (Non-Tuberculous *Mycobacterium*) and cannot differentiate viable from non-viable organisms. It also cannot distinguish drug susceptible from drug resistant strains.

Conclusion

In conclusion, the GeneXpert test represents a major milestone for global TB diagnosis and patient care. It also gives new hope for the millions of people who are at the highest risk of TB and drug resistant disease.

Conflict of Interest

There was no conflict of interest.

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