Nucleic Acid–Based Rapid Detection of Tuberculosis and Rifampicin Resistance in a Resource-Limited Setting

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Abstract

Background

The GeneXpert MTB/RIF assay for the diagnosis of tuberculosis (TB) and its uptake has been limited in most developing countries. This may lead to under-diagnosis, ineffective treatment and high mortality. We determine the prevalence of *Mycobacterium tuberculosis* (MTB) and Rifampicin-resistant TB using a nucleic acid-based test and AFB-smear in a Nigerian population.

Methods

We analyzed a single early morning sputum and extrapulmonary specimen from suspected cases of TB. Smear microscopic and real-time PCR for the detection of MTB and mutation on the *rpoB* gene using a molecular beacon was performed.

Results

The median age of the participants was 40 years (range: 6 weeks to 120 years). The incidence of MTB and RR-TB determined using GeneXpert were 620 (16.6%) and 59(1.6%), respectively. Acid-fast bacilli (AFB) smear identified AFB only in 2 out of 59(3.4%) cases with RR-TB. The agreement between the paired test of GeneXpert and AFB smear was poor (Kappa = 0.009, $p = 0.01$; McNemar's $p < 0.0001$). AFB sensitivity, specificity positive and negative predictive value were 2.4%, 98.2%, 1.9 and 98.1 respectively. The distribution of the RR-TB are more prominent among new TB-AFB-negative cases, those residing in densely populated semi urban communities and migrants at border towns.

Conclusion

The GeneXpert test shows a high detection for MTB in the paediatric population but a lower RR-TB than the national average. There is a need for aggressive escalation of the nuclei acid based test in low middle-income-countries in order to achieve the end TB strategy in a global village.

Introduction

Tuberculosis (TB) is a communicable disease caused by the bacterium, *Mycobacterium tuberculosis* (MTB).^[1] It remains a global public health challenge, posing a growing concern in developing countries, Nigeria inclusive.^[2] The World Health Organization (WHO) 2019 global tuberculosis report indicated that 1.7 billion people are at risk of TB, with an estimated 10 million (range of 9.0 to 11.1 million) new cases every year.^[3] Also, notification of TB cases appears to be improving from 6.4 million new cases of TB notified in 2017 to 7.0 million in 2018. The actual burden of tuberculosis in developing countries remains largely unknown. The suggested reasons could be under-reporting and challenges with diagnosis, as two in every three cases of drug-resistant TB pass unnoticed.^[3] These limitations are more

prominent among high-risk populations like children and immunocompromised individuals, in whom sputum for acid-fast bacilli test (AFB) perform poorly.^[3]

In most developing countries, TB is still diagnosed based on the demonstration of the presence of AFB in clinical samples, such as sputum stained with Ziehl-Neelsen staining.^[3] The microscopic confirmation of MTB in such smears is observer dependent and useful mainly in advanced disease; also, high false negative results are typical with paucibacillary samples^[4] with sensitivity ranging from 6% to 80%.^[4, 5]

In contrast, nucleic acid amplification (NAA) testing of diagnostic specimens reduces the overall turnaround time to as low as 2 hours compared with AFB smear which may last three days and Lowenstein Jensen culture for six weeks. The NAA test had demonstrated a high diagnostic value for TB.^[3, 6] Among the NAA tests recommended by the WHO is the rapid test (Xpert MTB/RIF®), which runs on Cepheid's GeneXpert™ automated polymerase chain reaction (PCR) platform.^[3] The rapid turnover of two hours, lack of need for extensive training for conducting the test and can detect Rifampicin resistance makes the Xpert MTB/RIF® the preferred choice for diagnosing TB in resource limited settings.^[7, 8]

The scourge of increasing multidrug-resistant TB (MDR-TB) and the extensively drug resistant tuberculosis (XDR MTB) can only be curbed with early diagnosis and adequate treatment of TB.^[7, 8] The GeneXpert MTB/RIF system allows prompt diagnosis and early commencement of anti-tuberculosis drugs.^{[9] [10]}

This study determined the diagnostic profile and the incidence of Rifampicin resistance TB using GeneXpert MTB/RIF assay and Ziehl-Neelsen staining technique at a tertiary health facility in Southwestern Nigeria.

Methods

This was a retrospective review of a single early morning sputum and extrapulmonary specimen from suspected TB cases between November 2015 and December 2019 at Bowen University Teaching Hospital Ogbomoso. This hospital serves as a regional diagnostic centre for all suspected TB cases seen in the hospital or referred from Primary Health Centres, State General Hospitals and tertiary health facilities in the nearby states and municipalities. Ogbomoso community is made up of 5 local government councils. The occupants are predominantly Yorubas but the farmland are occupied by migrants from the North east and north central part of the country with higher HIV sero-prevalence rate.^[11] The new TB cases, patients with TB treatment relapse, treatment failure and treatment defaults for rifampicin sensitivity testing provided sputum for AFB and NAA test. The indications for NAA test included failed six months regimen, symptomatic contacts of drug resistant TB cases, people living with HIV with symptoms of TB, extra-pulmonary tuberculosis (EPTB), re-treatment outside relapse failure, children aged less than 15 years, Health Workers with TB, symptomatic presumptive TB cases that were smear-negative. Each suspected TB case provided a single early morning sputum into a 25-ml leak-proofed, wide-mouthed screw-cap cup.

The sputum and an aspirate of an extrapulmonary sites such as cerebrospinal fluid, gastric lavage and ascitic fluids were subjected to both smear microscopy test and NAAT using GeneXpert. For PCR, the sample were diluted with carbonic acid buffer at a ratio 1: 2 (v/v) mixed vigorously and incubated for 10 minutes at room temperature for complete liquefaction. A 2ml aliquot of the diluted specimen was dispensed into the supplied cartridge port compatible with the GeneXpert machine (GeneXpert® Sunnyvale, CA, USA) and loaded into the machine. The ultrasonic lysis of the mycobacterium released the DNA. The GeneXpert uses an integrated micro-fluidic based system to extract, purify, amplify and quantify the DNA using real-time PCR technology. It also detects mutation in the rpoB gene for RR-TB using a molecular beacon. The results can be read off at two hours. Quality control is established by first standardizing the instrument through a check control on the probe with the standard. The sample processing control mechanism further enhanced internal quality control. Acid fast stained microscopy was performed using the Ziehl-Neelsen staining methods previously described.¹² We excluded presumed TB patients who failed to produce a specimen for the test, those with an invalid test result and those with incomplete data from the study. Subjects with a positive result were subsequently referred to the Damien Foundation TB treatment centre in the hospital for appropriate management. All patients with presumed TB were screened for HIV1 and HIV2 by parallel testing using Determine® Kits (Alere Medical, Matsuhidai, Matsudo-shi, Chiba-ken Japan), and Uni-Gold® HIV (Trinity Biotech, Wicklow, Ireland), and a tie-break with STAT-PAK® (Chembio Diagnostic System, Medford NY,USA). We also used Geographic information system (GIS) software (ArcGIS 10.8) for mapping (Appendix 1). Data were entered into a Microsoft Access file and analyzed using the Statistical Package for Social Sciences (S.P.S.S) version 23. The level of significance was set at $P < 0.05$. The study was conducted in line with the Declaration of Helsinki and approved by the Bowen University Health Research Ethics Committee (HREC) with approval number BUTH-REC - 047

Results

Figure 1 & Appendix 1 shows the flow diagram of patients' recruitment and the geographic distribution of MTB and RR- TB cases. Of the 8,743 presumed cases evaluated for TB, we excluded 99 (1.1%) because of incomplete data, giving a study response rate of 98.9%. There were no significant differences in demographic and clinical characteristics between the 8,624 patients and the 99 excluded. The median age was 40 years (range 6weeks -120 years). Children age ≤ 15 years constituted 877 (10.2 %) of the study population. Four thousand five hundred and seventy-four (53.0%) were male giving a male to female ratio of 1.1:1. Out of the 8,624 samples analysed, 3744 were eligible for NAA test. The overall incidence of MTB by GeneXpert assay was 16.6% (95% CI 15.4-17.8%). The incidence in adults was 15.0%; (95% CI 13.9 - 16.2%) while children accounted for 1.5%; (95% CI 1.1 to 1.9%) of the MTB detected. The incidence of RR TB was 1.6%, (95%CI: 0.8 to 2.9%). The turnaround time for GeneXpert MTB/RIF was 2hrs (IQR 2-24hrs) and smear microscopy was 51.6 ± 23.2 hrs, $p = < 0.001$.

Sampling and screening test for Tuberculosis

Table 1 shows the sampling and screening test for TB. We screened 8,598 (99.7%) subjects via sputum sample. A total of 3744(43.4) of the recruited subjects had their samples analysed using GeneXpert. Other details are shown in Table 1.

Indications for nucleic acid amplification test using GeneXpert and the Pattern of Rifampicin Resistance. Table 2a & 2b shows indications for GeneXpert Test. Symptomatic presumptive TB cases with smear negative microscopy results 2777 (73.8%) were the most frequent indication for GeneXpert, followed by diagnosis of TB in presumed HIV/PTB co-infection 599 (15.9%). The sputum AFB was positive in 177 (4.7%) of GeneXpert negative samples. Only 84 (2.2%) of children less than 15 years had the GeneXpert test.

Of the 620 (16.6%) TB cases detected by NAAT using GeneXpert, 562 (90.6%) were adults while 323(52.1) were males. NAAT detection rate in children was 58/84 (69.0%). Seroprevalence of HIV-TB co-infection was 66 (10.6%).

Fifty-nine (1.6%; 95% CI:0.8 - 2.9%) of the 620 with GeneXpert- positive MTB result had RR-TB. Adults 54/59; 91.5% (95% CI 80.7-97.4%) compared with children 5/59; 8.5% (95% CI 2.8-18.7); [$X^2=80.6$, $p<0.001$]. Most were male 36/59; 61.0% (95% CI: 43.4 -76.8%) compared with the females 23/59; 39.0 % (95% CI: -26.5-52.6%); [$X^2=5.673$, $p=0.02$]. One hundred and seventy-four (2.0%; 95% CI:0.5-5.4%) had indeterminate GeneXpert result.

RR-TB cases are more prevalent among the combinations of symptomatic presumptive TB cases and AFB negative result 45/59; 76.3 % (95% CI: 63.4 to 86.4) compared with other indications 14/59 23.7% (95% CI: 13.6-36.6); [$X^2=13.516$, $p=0.0002$]. AFB smear identified AFB in only 2/59 (3.4%) who had RR-TB. New presumptive TB cases accounted for 55/59 (93.3%) of RR-TB cases. Relapse and treatment failure cases accounted for 2/59 (3.4%) and 1/59 (1.7%) respectively.

Appendix I shows the pattern of distribution of MTB and RR-TB. RR-TB appears to be commoner in densely populated semi urban communities than the rural setting. The distribution of the RR-TB also appears to be more prominent around the communities where non indigenous migrant from other states in Nigeria with higher HIV infection rates are settled.

Agreement between methods of testing for MTB

Table 3 shows the agreement between the paired test of smear microscopy and NAAT using GeneXpert. Of the 620 MTB detected by GeneXpert, only 15/620 tested positive with the AFB test. There was a poor inter-rater agreement between the paired test (Kappa=0.009, $p=0.01$; McNemar $p<0.001$). The diagnostic value of the AFB smear microscopy relative to the GeneXpert as standard reference show a sensitivity of 2.4%, a specificity of 98.2 %, a positive predictive value of 1.9% and negative predictive value of 98.1%.

Discussion

Tuberculosis remains a major public health challenge particularly in the developing countries, Nigeria inclusive. In this study, the prevalence of tuberculosis using the nucleic acid-based rapid detection test (GeneXpert) was 7.2%. The 6.7 to 7.8% incidence of TB observed in the present study is similar to the reported incidence in the literature.^[1, 13] Ukwaja *et al*^[13] in Southeastern Nigeria recorded an incidence rate of 5.9% to 11.4% between 1999-2006. Our finding is also comparable to the prevalence of 6.9% reported by Kehinde *et al*^[1] in the same geopolitical zone, South-west Nigeria. The similarity in these prevalence reports may be due to the homogeneity of the socio-cultural, demographic, economic and similarity of health indices as these are known determinant of health and diseases outcomes in this setting.^[14, 15] However, the present rate is far lower than 23.6% reported by Khadka *et al*^[2] in Nepal, India and 24.8% recorded by kooffreh *et al*^[9] in Calabar, Nigeria. The reason for this disparity may be due to the difference in population characteristics as the present study encompasses both adult and children, relatively lower presence of the male gender. TB has been reported to be more prevalent in adults, particularly in the male population in agreement with previous studies.^[1, 7, 8, 11] This gender difference has been linked to biological characteristics, cultural factors and different access to health care.^[7]

It is also obvious that the study period by kooffreh *et al*^[9] and Khadka *et al*^[2] has limited access to diagnosis using nucleic acid based testing; GeneXpert, thus the use of Ziehl-Neelson smear for diagnosis has a propensity for false positive.^[16]

Drug resistant tuberculosis remains a health challenge, particularly where its preponderance is among the newly diagnosed. This may result in late diagnosis, increased spread and treatment failure especially where smear for AFB is the primary tool for diagnosis of TB. The rate of RR-TB among the subjects in this study is 1.6%. The reported rates are quite variable ranging from 0.8% to as high as 56.7% as shown in Table 4. The varying rate may be consequent on the difference in methods as some are secondary survey from initial screening with smear microscopy, while other reasons may include the spectrum of drug resistance ranging from mono-resistant to extensively drug resistant, the intensity of HIV-TB co-infection and the sample sizes for different study to mention a few.^[16-22]

The 1.6% of RR-TB incidence obtained in the present study is higher than the 0.8% reported by Khadka *et al*^[2] in Nepal, India but similar to findings the of 2.0% reported by Idigbe *et al*^[19] among prison inmates in Nigeria.^[19] Our incidence rate for RR-TB is however lower than the 7.2% reported by Rasaki *et al*,^[17] the 12.1% reported by Audu *et al*^[6] in Nasarawa, 23.4% recorded by Adejumo *et al*^[23] all in Nigeria and the WHO reported incidence in India, China and Russian Federation.^[3, 24] The reported incidence may be reassuring to reaching the third sustainable development goal (SDG3) target of ending the TB epidemic by 2030 and improved quality of life.^[3] This may be a reflection of an effective on-going TB and HIV control program through contact tracing and an effective adherence counselling coupled with a holistic approach to addressing a tripartite problem that has physical, mental and spiritual components.^[25, 26] It however worth mentioning that GeneXpert is limited in identifying some mutant forms of drug RR-TB and

variants of organism in the HIV-TB co-infection individuals, for example, the Ile491Phe strain reported in Switzerland and other strains reported in the literature, hence our lower incidence.^[27-29]

Although mixed reports have been established in the relationship between HIV- TB co-infection and the risk of RR-TB,^[17, 21, 22, 30] the current study found that more HIV negative individuals had RR-TB. The reason for the above finding is not very clear. However, this suggests that anyone with HIV are very much at an increased risk for developing TB. It is also possible that the gains from enhanced adherence counselling for HIV-TB co-infecting are yielding dividend, perhaps a negative fall back of overemphasis and support for TB-HIV co-infection at the expense of the HIV uninfected.

One of the alarming findings from the present study is the high incidence of RR-TB arising from the new cases in contradiction to the usual higher rates from previously treated cohorts. This is in line with the mathematically modelled pathway for developing MDR/RR-TB resistant in Africans, American and South-East Asians as shown by Ragonnet *et al.*^[31] This has a major implication for the TB control program and portends a grim prospect for most low-income countries where smear microscopy remains the first line for TB diagnosis. It is, therefore not surprising that TB treatment outcome in this region of the world remains abysmally low.^[32]

As with a retrospective study, the analysis has to deal with the exclusion of incomplete data which may not always be inconsequential although the clinical and socio-demographics of those included were not different from the excluded. Consequent to the varying indication for GeneXpert over the study period, only 43% of the study population had a GeneXpert test for their samples. Comparison of MTB Xpert MTB/RIF with composite reference standard culture would have further enhance the findings from this study.

As we look forward to a new century of improved TB diagnosis and near-perfect drug sensitivity testing, nucleic acid amplification test using GeneXpert servers as a bridge between the past of sputum smear microscopy, though penny cheap but misses 96.7% of TB cases and the future of whole-genome sequencing for diagnosis and treatment of TB. The Xpert holds great potential, particularly in low resource settings as a newer generation of these devices (GeneXpert) are produced with the capability to subverting the challenges posed by strains of *rpoB* mutations.

Conclusion

The acid fast bacilli test for detection of MTB grossly under diagnosed tuberculosis, including those with RR-TB, compared with the nucleic acid based test. The distribution of the RR-TB are more prominent around, new TB-AFB-negative cases, densely populated semi urban communities and borders of communities where non indigenous migrant from other states in Nigeria with higher HIV infection rates are settled. There is a need for aggressive escalation of the nuclei acid based test in low middle income countries in order to achieve the end TB strategy in a global village.

Declarations

Competing interests: The authors declare no competing interests.

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Tables

Due to technical limitations, the tables are only available as downloads in the supplementary files section.

Figures

Figure 1: Flow chart for screening of patients with presumed tuberculosis and rifampicin resistance

Figure 1

See image above for figure legend.

Supplementary Files

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