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Original Article

Diagnostic performance of GeneXpert and Ziehl-Neelson microscopy in the detection of tuberculosis in Benue State, Nigeria

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ABSTRACT

Background: Accurate and timely diagnosis of tuberculosis (TB) is key to effective treatment and management. This study was designed to compare the diagnostic performance of GeneXpert and Ziehl-Neelson (ZN) microscopy test using culture as the reference.

Methods: Cross-sectional study was conducted in a tertiary hospital to compare the performance of GeneXpert and ZN test among HIV and non-HIV patients. Sputum samples were collected from 261 suspected TB patients and analyzed in the laboratory using GeneXpert, ZN test and culture. Statistical analysis included calculation of sensitivity, specificity, positive predictive value and negative predictive value. χ^2 was used to compare the outcome of diagnostic test and demographic variables. p-value < 0.05 was considered significant.

Results: Comparison of TB prevalence among urban versus rural areas using the three diagnostic tests are: ZN test: 32 (12.3%) vs 16 (6.1%; χ^2 7.63, P = 0.007); GeneXpert: 63 (16.1%) vs 20 (7.7%, χ^2 9.01, P = 0.003) and culture: 22 (8.4%) vs 10 (3.8% χ^2 4.44, P = 0.05). Also, prevalence of TB was significantly (P < 0.05) higher among HIV negative 25 (9.6%) than HIV positive 23 (8.8%) patients. The overall prevalence of rifampicin resistance was 12 (4.60%). Out of 261 sputum samples examined for TB, 48 (18.38%) tested positive by ZN test, 62 (23.76%) by GeneXpert and 32 (12.26%) by culture. Two out of 32 samples that tested positive by culture were negative by GeneXpert. GeneXpert had higher sensitivity (93.75%) than ZN test (50.00%). However, they had equal specificity (86.03%). The sensitivity and specificity of ZN and GeneXpert among HIV patients was 58.33% (95% CI = 29.66–84.83) and 79.31% (95% CI; 69.24–87.26) and 91.67% (95% CI 61.51–99.79) and 80.67 (95% CI; 70.58–88.17) while among HIV negative patients; ZN test and GeneXpert assay had 45.00% (23.05–68.45) and 90.14% (84.04–94.50) and 95.00% (75.13–99.87) and 89.44% (83.13–93.96), respectively.

Conclusion: GeneXpert demonstrated higher level of performance than ZN microscopy. We recommend the use of GeneXpert for diagnosis of TB in Benue State and Nigeria in general for effective treatment and management of tuberculosis.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by members of the genus *Mycobacterium*. Nine species in the genus are referred to as *Mycobacterium* tuberculosis complex (MTBC).[1–3] Among the MTBC, *Mycobacterium* tuberculosis (MTB), *M. africanum* and *M. canetti* are the frequent etiologic agents of TB in humans.

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However, *M. bovis* which has the widest host range and the common cause of TB in animals also cause TB in man, known as zoonotic tuberculosis.[4,5] Tuberculosis is a major global public health challenge and accounts for over 9.0 million new cases and about 1.5 million deaths annually.[6]

Nigeria, the largest black nation in the world has a human population of over 196 million. Nigeria is ranked second in Africa and among 22 high burdened TB countries worldwide.[7] Benue State in Nigeria has high TB prevalence due to poverty and HIV/AIDS.[8–10] Tuberculosis diagnosis and treatment is free in Benue State at all level.[11] However, the diagnosis of TB is fraught with challenges not only in Benue State but in most TB endemic settings worldwide. The conventional methods of TB diagnoses such as acid-fast microscopy (AFB), culture, phenotypic drug susceptibility test (DST) are unable to correctly detect MTBC, time consuming and require special laboratory and expertise.[12] Other diagnostic techniques like X-ray and clinical diagnosis based on symptoms are subjective and expose patients to radiation hazards. Therefore, efficient treatment and management of TB require rapid, accurate and safe methods of diagnosis.[13]

In recent time, molecular diagnostic technique have been developed to address these issues. These include line probe assay (LPAs), PCR based RD-typing, spoligotyping, real time PCR and GeneXpert MTB/RIF (GeneXpert).[14–18]

The GeneXpert is an automated technology that rapidly and simultaneously process sputum specimen and detect MTBC as well as rifampicin resistance mutation in the rpoB gene.[19] This assay was approved and recommended for diagnosis of TB by WHO especially suspected HIV-TB comorbidity globally.[20] However, clinical diagnosis and detection of AFB are the common routine diagnostic test for TB in Benue State, Nigeria. Therefore, the aim of this study was to compare the diagnostic performance of GeneXpert and AFB test using solid culture as reference.

2. Materials and methods

2.1. Study design and criteria

A cross-sectional study was conducted to evaluate the diagnostic performance of AFB test and GeneXpert assay in a tertiary hospital in Benue State, Nigeria. Study participants were recruited based on the following criteria.

2.2. Inclusion criteria

- Availability of test results for AFB test, GeneXpert and culture.
- All ages
- Ability to produce sputum without inducement
- Suspected TB patient based on clinical symptoms

2.3. Exclusion criteria

- Unknown HIV status
- Extrapulmonary TB
- Induced sputum sample collection
- Non-respiratory specimen
- Sputum sample less than 3 ml
- Invalid and error results from GeneXpert

2.4. Study participants and sample collection

Individuals of all ages who were clinically diagnosed as suspected TB patients were included in the study. All patients who consented after counseling about HIV were tested for HIV infection. Patient's information was collated from hospital records following approval from appropriate authority. Portion of sputum sampled from routine AFB test and GeneXpert were collected and stored at −20 °C for an average of 4 weeks. Thereafter, it was transported from Federal Medical Centre, Makurdi to Tuberculosis Laboratory, Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for microbiological culture.

2.5. Microbiological analyses

2.5.1. Acid-fast microscopy

Using a sterile application stick, a drop of raw sputum was placed on a grease free glass slide and evenly spread, dried, and heat fixed. Thereafter, Ziehl-Neelson staining was done according to previously described methods.[21]

2.5.2. GeneXpert

GeneXpert test was performed according to manufacturer's instruction. Briefly, with the aid of a sterile pipette, 2 ml of GeneXpert reagent was added to 1 ml of sputum sample and incubated at room temperature for 15 min. The mixture was agitated twice at 5 min interval. The liquefied mixture was transferred into the GeneXpert cartridge using a sterile pipette and loaded into GeneXpert instrument. Results was available within 2 h.

2.5.3. Culture and identification

The remaining sputum sample was processed using N-acetyl-L-cysteine-NaOH (NALC-NaOH) method as earlier described.[14] Briefly, equal volume of freshly prepared NALC-NaOH was added to the remaining sputum sample (variable volume of sputum) vortexed and then incubated for 15 min at room temperature. The mixture was neutralized by adding phosphate buffer (pH 6.8) two times it's volume. It was then centrifuged at 3000*g* for 20 min. The supernatant was discarded, and the residue was re-suspended in 2 ml phosphate buffer, 0.5 ml of the concentrated cells was inoculated on to Lowenstein-Jensen (LJ) media and incubated for 8–12 weeks at 37 °C. Colony suspected of MTBC was tested using AFB microscopy and confirmed with the aid of SD Bioline assay.

2.5.4. SD Bioline assay

The isolates were subjected to SD Bioline® TB AgMPT64 analysis. The test was done according to the manufacturer's instructions. This test consisted of test cassette which consists of a sample pad, a gold conjugate, a nitrocellulose membrane, and an absorbent pad. Mouse monoclonal anti-MPT64 was immobilized on the nitrocellular membrane as the capture material (test line). Another antibody, which recognized another epitope of MPT64, conjugated with colloidal gold particles were used for antigen capture and detection in a sandwich type assay. The cassette has a letter T and C as test line and control line, respectively on the surface of the case. Positive results produced red to purple band. Absence of band on the control line was regarded as invalid.

2.6. Statistical analyses

Data obtained were cross tabulated, χ^2 was used to compare differences in TB detection rate among demographic variable using SPSS version 17. Results obtained were considered significant at a level of P value < 0.05. Sensitivity, specificity, positive predictive value and negative predictive value was performed at 95% confidence interval using GraphPad InStat version 3.05 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

3. Results

3.1. Demographics and tuberculosis detection rate

Prevalence of TB was not statistically different among sex and age group for all three diagnostic tests (Table 1). We observed significant difference in the prevalence of TB between rural and urban areas. Patients from urban area had higher TB detection rate by AFB 32 (12.3%), $\chi^2 = 7.63$, P = 0.007), GeneXpert 42 (16.1%), $\chi^2 = 9.01$, P = 0.003) and culture 22 (8.4%) $\chi^2 = 4.44$, P = 0.05) compared with patients from rural area. Comparing the results of GeneXpert by ethnicity we found out that there was significant difference (P = 0.007) in the prevalence of TB among different ethnic groups in Benue State. The Idoma ethnic group had the highest TB prevalence 32 (12.3%) (S1). Results from AFB showed that prevalence of TB was significantly (P = 0.032) higher among HIV negative 25 (9.6%) than HIV positive 23 (8.8%) patients. Rifampicin resistance was detected among 12 (4.60%) patients by GeneXpert assay. Patients from rural areas had higher rifampicin resistance (7 (2.7%) than those from urban areas (P = 0.002) (Table 1).

3.2. AFB and GeneXpert diagnostic performance

A total of 261 sputum specimens from suspected TB patients were tested for tuberculosis by AFB test, GeneXpert and microbiological culture. 48 (18.39%) were detected by AFB test, 62 (23.76%) by GeneXpert, 40 (24.70) of the samples were positive by culture and identified by AFB as mycobacteria. 8 (20.00%) samples were positive by culture and either positive or negative by AFB and 4 (10.00%) were positive by culture and AFB but negative by SD-Bioline assay. 32 (12.26%) was confirmed by SD-Boiline assay as MTBC. Among the 32 samples that were positive by culture, 2 (6.25%) were GeneXpert negative. 229 (87.74%) specimens were negative for mycobacteria. The rate of contamination was observed to be very high. 16 (33.33%) of the 48 samples that were positive by AFB also grew MTBC (Table 2).

The overall sensitivity, specificity, positive predictive value and negative predictive value for AFB Test and GeneXpert when culture was used as a reference standard (Table 3). GeneXpert had higher sensitivity (93.75%) than AFB test (50.00%). However, both test had equal specificity (86.03%). The positive predictive value was poor for AFB test and GeneXpert assay (Table 3).

Table 2
Comparison of AFB test and GeneXpert assay using culture as a reference standard.

Tests		Culture		
		Grew Mycobacteria	Contamination or no growth	Total
AFB Test	Positive	16	32	48
	Negative	16	197	213
Total		**32**	**229**	**261**
GeneXpert	Positive	30	32	62
	Negative	2	197	199
Total		**32**	**229**	**261**

Table 3
Comparison of the performance of AFB test and GeneXpert assay among TB patients in Benue state.

Parameters	AFB test	95% C.I.	GeneXpert	95% C.I.
SEN	50.00	31.87–68.13	93.75	79.18–99.23
SPE	86.03	80.89–90.25	86.03	80.89–90.25
PPV	33.33	20.38–48.46	48.39	35.49–61.44
NPV	92.49	88.09–95.65	98.99	96.42–99.88

SEN = Sensitivity, SPE = Specificity, PPV = Positive predictive value, PPN = Negative predictive value.

Furthermore, AFB test was able to detect TB among HIV positive patients than HIV Negative patients. However, we observed that GeneXpert had 91.67% and 95.00% sensitivities among HIV positive and HIV negative patients respectively (Tables 4 and 5).

4. Discussion

The findings of this study revealed high TB prevalence among urban dwellers than patients from rural area using the three diagnostic tests. This finding agreed with study conducted in Enugu State, Nigeria where Oshi et al.[12] reported a frequency of 68.1% TB among urban dwellers compared to 31.9% among patients from rural area. Similarly, TB in the European Union had been reported to be concentrated in big cities. It was also noted that immigrants, homeless people, drug misuse and alcohol intake are risk factors for high TB prevalence in urban area.[22] Globally, WHO also

Table 1
Demographic data and detection rates of AFB Microscopy, GeneXpert and Culture suspected TB Patients

Variables	No Sampled (%)	AFB Test (%)	χ^2	P value	GeneXpert (%)	χ^2	P value	RIF (%)	χ^2	P value	Culture (%)	χ^2	P value
Sex													
Male	132 (50.6)	23 (8.8)	0.166	0.402	37 (14.7)	2.695	0.067	8 (3.1)	1.303	0.200	20 (7.7)	2.075	0.105
Female	129 (49.4)	25 (9.6)			25 (9.6)			4 (1.5)			12 (4.6)		
Age group													
0 – 14	15 (5.7)	1 (0.4)	3.076	0.380	3 (1.1)	0.524	0.914	0 (0.0)	1.911	0.591	2 (0.8)	0.485	0.922
15 – 34	104 (39.8)	20 (7.7)			24 (9.2)			6 (2.3)			14 (5.4)		
35 – 54	96 (36.8)	21 (8.9)			25 (9.6)			3 (1.1)			10 (3.8)		
≥ 55	46 (17.6)	6 (2.3)			10 (3.8)			3 (1.1)			6 (2.3)		
Location													
Urban	211 (80.8)	32 (12.3)	7.632	**0.007**	42 (16.1)	9.012	**0.003**	5 (1.9)	12.465	**0.002**	22 (8.4)	4.444	**0.058**
Rural	50 (19.2)	16 (6.1)			20 (7.7)			7 (2.7)			10 (3.8)		
Ethnicity													
Tiv	144 (55.2)	21 (8.0)		0.201	24 (9.2)	9.843	**0.007**	6 (2.3)	0.200	0.905	13 (5.0)	5.275	0.072
Idoma	93 (35.6)	22 (8.4)			32 (12.3)			5 (1.9)			13 (5.0)		
others	24 (9.2)	5 (1.9)			6 (2.3)			1 (0.4)			6 (2.3)		
HIV Status													
HIV Positive	99 (37.9)	25 (9.6)	5.004	**0.032**	28 (10.3)	1.806	0.117	5 (1.9)	0.075	0.503	12 (4.6)	0.003	0.561
HIV Negative	162 (62.1)	23 (8.8)			34 (13.0)			7 (2.7)			20 (7.7)		

Table 4
Comparison of the performance of AFB test and GeneXpert assay among HIV patients in Benue state.

Parameters	AFB test	95% C.I.	GeneXpert	95% C.I.
SEN	58. 33	29.66–84.83	91.67	61.51–99.79
SPE	79.31	69.24–87.26	80.67	70.58–88.17
PPV	28.00	12.07–49.43	39.29	21.51–59.42
NPV	93.24	84.91–97.77	98.59	92.40–99.96

SEN = Sensitivity, SPE = Specificity, PPV = Positive predictive value, PPN = Negative predictive value.

Table 5
Comparison of the performance of AFB test and GeneXpert assay among non-HIV patients in Benue state.

Parameters	AFB test	95% C.I.	GeneXpert	95% C.I.
SEN	45.00	23.05–68.45	95.00	75.13–99.87
SPE	90.14	84.04–94.50	89.44	83.18–93.96
PPV	39.13	19.73–61.44	55.88	37.86–72.84
NPV	92.09	86.26–95.98	99.22	95.72–99.98

SEN = Sensitivity, SPE = Specificity, PPV = Positive predictive value, PPN = Negative predictive value.

reported that TB burden was higher in urban area than in rural area.[7] However, in countries where most of the people live in rural area, it was noted that the prevalence of TB was higher among rural dwellers than urban residents and this was attributed to lack of adequate diagnosis and treatment facilities in rural areas.[7] Conversely, we noticed a significantly higher rifampicin resistant among TB patients from rural area than patients from urban area. This observation may be due to failure to adhere to treatment regime, non-availability and accessibility of diagnostic facilities to correctly and timely diagnose rifampicin resistance among rural dwellers.

Ethnicity is not regarded as an important risk factor for TB. Though, other factors that relate with ethnicity such as believe, social education, social behavior, family size, economic activities, social status, housing and food (nutrition) may influence the contribution of ethnicity to prevalence of TB in the community. In this study we observed that the Idoma ethnic group had high TB prevalence with respect to AFB test only. There was no significant difference between Idoma ethnic group and the prevalence of TB when GeneXpert and culture were considered. In a similar study in Taiwan, the Han Chines were found to have less TB incidence than the aborigines in Taiwan tribes.[23] Also, low prevalence of TB was reported among the Afar ethnic group in Ethiopia.[24] The high prevalence of TB among the Idoma ethnic group in this study could have resulted from the inability of AFB to differentiate between MTBC and NTM.[25]

Our finding indicated a substantially high TB among HIV patients than non-HIV patients considering AFB test alone. Though, there was no significant difference between the occurrence of TB among HIV and non-HIV when GeneXpert and culture were used. This may be because AFB unlike GeneXpert and culture is unable to differentiate between MTBC and NTM. More so, it is a known fact that HIV patients are susceptible to NTM infections due to their immunodeficiency status. Furthermore, smear microscopy (Acid-fast bacilli, AFB) test is the most common diagnostic tool for detection of TB in developing countries. However, AFB test has poor sensitivity and limited specificity; since AFB test often tend to miss TB detection among patients with very low mycobacterial load in their sputum. Hence, this test cannot differentiate between MTBC and NTM. Despite the introduction and recommendation of molecular based diagnostic devices such as line probe assay and GeneXpert for diagnosis of TB, AFB test is still used in poor resource countries like Nigeria due to inadequate infrastructure and access to basic amenities like regular power supply which are necessary for sustainable use of these devices.

Our findings revealed that GeneXpert was able to detect 14 cases that were missed by AFB and two cases that were culture positive but negative by GeneXpert. This finding was consistent with previous studies that evaluated the performance of GeneXpert and AFB test.[26,27] The reason for the false positive result by GeneXpert may be due to presence of PCR inhibitors and low bacterial load.[29] The overall sensitivity and specificity of AFB test in this study were 50.0% and 80.89% which were lower than results obtained in a previous study in Thailand, where sensitivity and specificity of AFB test was reported to be 60.5% and 98.5%.[28] However, Zaka et al.[29] reported a lower sensitivity (43.5%) and higher specificity (99.5%) of AFB test among pulmonary TB patients in Turkey. Also, the sensitivity and of GeneXpert was found to be 93.75% and the specificity was the same as that of AFB test in this study. Data from previous report showed that GeneXpert had higher sensitivity and specificity than AFB test.[25,28,29] However, Bajrami et al.[30] reported higher sensitivity and specificity of AFB than GeneXpert in a comparative study in Kosovo. The reason for the variation of the sensitivity and specificity of GeneXpert and AFB test in various studies could be due to differences in study design, sample processing, storage and transportation. The performance of GeneXpert assay in detecting TB among HIV patients was higher compared with AFB test. This finding was consistent with previous results.[31] Nevertheless, in a similar study among HIV infected prisoners in Malaysia, Al-Darraji et al.[32] reported a low GeneXpert sensitivity (53.3%, 95% CI 30.12–75.2%) and further observed that GeneXpert-negative active TB patients had less advanced HIV infection. Al-Darraji et al.[32] explained that the low sensitivity reported in the study may be due to low bacillary load given that GeneXpert requires a minimum of 131 bacilli/ml compare to 10–100 bacilli/ml for culture. Also, Cavanaugh et al.[27] reported that in patients living with HIV, GeneXpert significantly increased diagnostic yield compared to AFB test and that the highest yield was obtained when GeneXpert was used to test specimens from patients with CD4 count <100 cells/μl. In another study conducted among HIV patient to evaluate the effect of sputum concentration without NALC-NaOH, concentration of sputum treated with NALC-NaOH and filtration through small-membrane filter on the performance of smear microscopy (AFB test). It was observed that small-membrane filter method significantly increases the sensitivity and specificity of AFB test.[33] However, in this study AFB test was performed on direct smear without concentration, treatment with NALC-NaOH or filtration through small-membrane filter and may be the reason for the poor performance of AFB test among HIV patients and the general performance of AFB test in this study.

5. Conclusions

In conclusion, we found that the performance of GeneXpert was superior to AFB test. However, GeneXpert assay had significantly higher sensitivity but lower specificity among non-HIV patients compared to AFB test. Importantly, we also found the prevalence of TB to be higher among urban patients. Conversely, we confirmed a significantly higher rifampicin resistant tuberculosis among patients from rural areas.

Overall, we recommend the use of GeneXpert for rapid and accurate diagnosis of TB in Benue State and other TB endemic settings of the world. This will ultimately help to detect cases that may have been missed by AFB test and since GeneXpert could detect RR-TB, improve diagnosis and enhance patient treatment and management.

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Competing interest

None declared.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajme.2018.09.002.

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