Microbes and Infectious Diseases 2025; 6(3): 1084-1093

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

GeneXpert MTB/RIF assay of pulmonary tuberculosis and HIV co-infection at a Central TB Referral Centre in Anambra State, Nigeria

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ARTICLE INFO

Article history:
Received 4 January 2025
Received in revised form 15 February 2025
Accepted 17 April 2025

Keywords:
Mycobacterium tuberculosis
GeneXpert MTB/RIF
Rifampicin
HIV

ABSTRACT

Background: The use of GeneXpert MTB/RIF for direct molecular detection of *mycobacterium tuberculosis* (MTB) and rifampicin resistance tuberculosis (TB) has dramatically improved the diagnosis of tuberculosis. This study was aimed at using Gene Xpert to determine the prevalence of MTB/ rifampicin-resistant strains and human immunodeficiency virus (HIV)/MTB co-infections. **Methods*:*** This was a cross-sectional study carried out between 2018 and 2022, using 2,579 patient samples analyzed for *mycobacterium tuberculosis* (*M. tuberculosis)* and rifampicin resistance by GeneXpert MTB/RIF assay. HIV screening was carried out using the National Approved Serial Algorithm for HIV screening with Alere Determine and Uni-Gold kits for screening and confirmation, respectively. **Results**: Among the 207 positive TB patients, 31(4.7%) were co-infected with HIV/AIDS. Highest TB infection (38.6%) was detected among the age group 31-45 while the least infection rate was recorded among 0-15 and 61 > (2.4% and11.1%, respectively). There was no significant difference (p= .500) between gender in the TB prevalence. The prevalence rates of TB positive cases showed increasing rates from 2018 to 2022, with a decline in 2020. Rifampicin resistant strain of MTB and Rif - indeterminate were detected in 5(2.4%) samples each. The prevalence rates of TB and HIV within the years did not vary directly. **Conclusions***:* The prevalence of MTB/Rif was effectively determined using the Gene Xpert MTB/RIF. However, the limitations of this method against the conventional cultural technique involved the use of high-concentration specimens. Further studies would require the inclusion of conventional assay methods to determine the multi-resistant TB strains.

Introduction

Tuberculosis is one of the deadliest bacterial infectious diseases worldwide. *Mycobacterium tuberculosis* is the causative organism of tuberculosis. It is an acid-fast bacillus that affects the lungs to cause pulmonary. It can also affect other organs of the body to cause extrapulmonary TB [1]. The disease had an estimated 10.6 million cases in 2021 with 1.6 million deaths. This was about a 4.5% increase in 2020 TB cases, according to the World Health Organization's Global TB Report [2]. Nigeria ranks as the sixth country with the highest TB burden

DOI: 10.21608/MID.2025.350105.2437

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worldwide and second in the global missed TB burden of 2.9 million cases [3].

Tuberculosis transmission occurs by inhalation of aerosols released from pulmonary TB-infected individuals into the air by coughing, sneezing, and talking. The fact that TB is an airborne disease transmitted through the inhalation of aerosols is a major contributing factor, while smoking, intake of immunosuppressive or hard drugs or steroids, and diabetes are all predisposing factors to TB infection [4]. Other important factors that lead to the rise in TB infections, particularly in most developing countries, are poverty, the movement of displaced people, and the emergence of multi-drug-resistant strains of *mycobacterium tuberculosis*, as well as alcoholism, contact with active TB patients, and malnutrition [5, 6]. The mortality rate associated with TB infection is very high, but treatment is possible if diagnosed early, followed by a proper and completed treatment regimen [7]. Therefore, monitoring tuberculosis disease in a geographic area or population is an important public health program.

The predisposing factors have contributed much to the massive increase in TB cases, coupled with the emergence of multi-drug-resistant strains of *mycobacterium tuberculosis*. All these continue to make tuberculosis a great public health challenge in most developing countries despite all interventions by WHO and other public health bodies [6, 8].

Co-infection of HIV increases the reactivation of latent or relapse TB infection and also reduces the time between TB infection and development to active TB disease [9]. The mortality rate with HIV/TB co-infection is usually higher than with single HIV or single TB infection [10]. Tuberculosis infection with HIV has been reported to be the deadliest infectious disease globally, with 1.5 million recorded deaths [11]. Another major global issue hindering success in the diagnosis, treatment, and control of TB is the cases of extra-pulmonary tuberculosis. The clinical manifestations in extrapulmonary tuberculosis vary so much among several individuals, thereby making it difficult to suspect extrapulmonary TB in most cases. Not knowing actually, the affected organ and how to access it poses another challenge in collecting the proper sample for laboratory diagnosis. Many people have died of extrapulmonary TB, including health workers (especially those attending to TB patients), due to delay or improper diagnosis [12]. However, gene expert has highly sensitive in diagnosing/detection of rifampicin resistance in extrapulmonary TB cases. Rifampin (RIF) has been one of the major first-line antituberculosis drugs that inhibit DNA-directed RNA synthesis of M. tuberculosis. Rifampicin (RIF) resistance is epidemiologically significant and a valuable surrogate marker for MDR-TB strains. The resistance is a result of mutations in the rpoB gene, encoding the β subunit of RNA polymerase, which have been associated with RIF-resistant phenotypes in multiple study populations [13]. Generally, a rpoB mutation has been discovered 95–97 % of RIF-resistant MTB strains worldwide [14] and globally MDR-TB is big threat to human especially in developing countries.

GeneXpert MTB/RIF assay is a rapid automated molecular test that is employed in early diagnosis of MTB and assessment of rifampicin resistance simultaneously [15-17]. The test records excellent sensitivity and specificity for diagnosis of pulmonary and extrapulmonary *mycobacterium tuberculosis* (MTB) infection [18]. The utility of Xpert MTB/RIF for extra pulmonary tuberculosis is well established especially for Lymph nodes and Cerebrospinal fluid samples [19]. The World Health Organization recommended GeneXpert MTB/RIF for pulmonary tuberculosis and extrapulmonary tuberculosis diagnosis respectively, due to its turnaround time of two hours with high sensitivity and specificity [2]. The molecular point-of-care test (POCT) Xpert MTB/RIF assay does not only detect the presence of *mycobacterium tuberculosis* but also detects resistance to rifampin (RIF-R). Hence, the diagnosis of tuberculosis has been improved with the use of Xpert *mycobacterium tuberculosis*/rifampicin (MTB/RIF) with an added advantage of detecting rifampicin resistant TB (RR-TB). Nigeria adopted Gene Xpert MTB Rif as a primary diagnostic tool for MTB/RIF since 2016, but geographical coverage is still low. This study determined the prevalence of MTB/RIF resistance and HIV co-infections in a TB centre in Southeast Nigeria using Gene Xpert assay.

Materials and methods

Study site and location

This study was carried out in a central TB research laboratory located in the Aguata Local Government Area of Anambra State. This study location was chosen based on the availability of GeneXpert equipment, which was already installed

and functional there. The land density of Aguata LGA is 1,898/km² (4,920/sq mi) with a total area of 195 km² (75 sq mi) and contains fourteen villages/towns. It has a total of 370,172 by 2006 population. The average annual temperature ranges from 17 to 30 degrees Celsius, rarely falling below 13 or rising over 31 [20].

Ethical considerations

Ethical clearance with number COOUTH/HREC/ETH.C/VOL.1/FN:04/385 was obtained from the ethical committee of Chukwuemeka Odumegwu University Teaching Hospital Awka, Anambra State, Nigeria. Informed consents were also obtained.

Study population and sampling

Patients presented for the GeneXpert MTB tests were those that had signs and symptoms suspicious of tuberculosis infections. A convenience sampling method was employed. The sample represented a broader population. There were no biases; the remote populations and high-risk groups were included during the referrals. During the 5-year period, the Central TB Research Laboratory Centre received referrals from some clinics, health centres, hospitals, and medical laboratories without the testing facilities. Others were those that voluntarily visited the central TB research laboratory. The total number of patients tested for TB was 2579. Out of this, 1102 (42.7%) were males and 1477 (57.3%) were females. The distributions of the number of patients tested in the 5-year period are shown in Table 1. Out of the 2,579 samples in this study tested for TB, 2,549 were sputum samples while 30 were stool samples. Amongst these patients, 1959 were tested for HIV, while the HIV status of 620 patients was unknown.

Laboratory analysis

The laboratory analysis was performed following biosafety measures according to the standard laboratory protocol. To avoid contamination, the samples were kept at room temperature when the analyses were to be carried out immediately, but when there was a delay in analysis for ≥ 7 days, they were preserved at 4°C in a refrigerator.

Sputum samples from adults or stool samples from infants were analysed using the GeneXpert MTB/RIF test standard procedures. The sputa were collected and were mixed in the cartridge supplied with the sample reagent buffer in a 1 in 2 volume ratio. The cartridge was sealed and vortexed for 15 seconds; it was thereafter left to stand at room temperature for 10 minutes. The cartridge was vortexed again after 10 minutes and left to stay for 5 minutes. A pasteur pipette was used to place just about 2 ml of the processed sample in the cartridge. This was then loaded into the GeneXpert machine, and the results were collected through a GeneXpert computer after 2 hours [21].

Prior to the TB testing procedures, the patients' HIV status was documented. Patients who did not have evidence of their HIV status who were willing were screened using the National approved serial algorithm for HIV screening with Alere Determine and Uni-Gold kits for screening and confirmation, respectively. An automated pipette was used to collect 50 litre of whole blood sample from the EDTA container to apply to the sample pad of the test strip. One drop of chase buffer was also added, and the result was read after 15 minutes. A positive result is indicated by a double line on the test strip, while a single line indicates a negative result [22].

The testing procedure with the Uni-Gold Kit involved using the included pipette to apply 2 drops of a whole blood sample to the sample pad. Also, 2 drops of the wash solution in a dropper were added to the sample, and the result was read after 10 minutes. A double line indicates a positive result, while a single line indicates a negative result [23].

Data analysis

Data was entered into Microsoft Access file and analysed using IBM SPSS Statistics for Windows, version 21. The statistical values were interpreted using 95% confidence limit or significance probability of $p < 0.05$. Descriptive statistics were presented as a frequency table to summarize demographic data of age/age group and chi-square for gender. Frequency table was also used to present the proportion of people who tested positive for MTB and RR-TB. Students T-test using paired samples tests and correlations were carried out to compare the prevalence of TB and HIV in the various years.

Results

Table 1 displays the population and number of samples tested for the five years. Among 2,579 patients tested, 1,102 were males and 1477 were females. The distributions of the number of samples tested in various years are also seen in the table. The overall rate of TB recorded 207(8.0%), among these 125(11.3%) males were positive while

82(5.6%) females had MTB **(Table 2).** The HIV prevalence (17.4%) was also recorded in **table (2).** The positive males were 160(14.5%) and females that showed up positive were 289 (26.2%). However, the HIV status of 620 patients was not captured.

The highest MTB infections were reported among the age group 31-45 (38.6%). The lowest prevalence was recorded in the age group 0-15 (4.2%). The prevalence in the age group 61 & above was also low at 11.1%. The annual distributions of TB for the years 2018 to 2022 were 18.4, 20.3, 17.4, 21.7, and 22.2 percentages, respectively **(Table 3).** For HIV it was 34.5, 17.8%, 9.8%, 22.3%, and 15.6% for the years 2018, 2019, 2020, 2021, and 2022, respectively. Those in the age ranges of 0-15 and 61+ also had the lowest prevalence of HIV (4.2% and 6.7%, respectively). Age group 31-45 had the highest (39.0%) **(Table 4).** The total rate of co-infection was 4.7%. There was no case of co-infection in 2019 and the lowest (2.5%) was in 2020. In 2018, 2021 and 2022 it was 3.6%, 9.0% and 7.8%, respectively **(Table 5).**

Out of the 207 samples that were positive for MTB, 5 (2.4%) were each detected to be rifampicin resistant and indeterminate strains. Among them, 2 (1.6%) of the male participants and 3 (3.7%) of the female counterparts accounted for the rifampicin resistance **(Table 6).** The prevalence rates of rifampicin resistance in 2018, 2021, and 2022 were 2.6%, 4.4%, and 4.3%, respectively. There was no record of the rifampicin resistance in 2019 and 2020. Intermediate resistances were only discovered in 2020 and 2022 (5.6% and 6.5%, respectively) **(Table 7).**

Table 1. Distributions of populations and number of samples tested from 2018-2022.

Year	No. of males	No. of females	Total (%)
2018	220	257	477
2019	179	243	422
2020	235	304	539
2021	281	383	664
2022	187	290	477
Total	1102(42.7%)	1477(57.3%)	2579(100)

Table 2. Prevalence of MTB and HIV by gender.

MTB prevalence			
	Males	**Females**	**Total**
Positives	125(11.3)	82(5.6)	207(8.0)
Negatives	977(88.7)	1395(94.4)	1477(57.3)
Total	1102(42.7)	1477(57.3)	2579(100)
HIV prevalence			
Positives (%)	160(14.5)	289(26.2)	449(17.4)
Negatives (%)	683(62.0)	827(56.0)	1510(58.5)
Unknown (%)	259(23.5)	361(24.4)	620(24.0)
Total (%)	1102(42.7)	1477(57.3)	2579(100)

Table 3. Annual prevalence of *mycobacterium tuberculosis* infection by age.

	----------Years of study----------					
Age range	**2018**	**2019**	**2020**	**2021**	**2022**	**Total prevalence**
0-15	1(2.6)	2(4.8)	2(5.6)	0(0.0)	0(0.0)	5(2.4)
16-30	6(15.8)	14(33.3)	10(27.8)	11(24.4)	17(37.0)	58(28.0)
31-45	20(52.6)	19(45.2)	12(33.3)	19(42.2)	10(21.7)	80(38.6)
46-60	8(21.1)	3(7.1)	8(22.2)	11(24.4)	11(23.9)	41(19.8)
61>	3(7.9)	4(9.5)	4(11.1)	4(8.9)	8(17.4)	23(11.1)
Total	**38(18.4)**	**42(20.3)**	**36(17.4)**	**45(21.7)**	**46(22.2)**	**207(100.0)**
P value	.084	.070	.180	.051	.007*	
SD	7.4364	7.63544	4.14729	7.31437	4.43847	
S E M	3.32566	3.41467	1.85472	3.27109	1.98494	

Table 4. Annual prevalence of HIV infection by age.

Age range	----------Years of study----------					Total prevalence
	2018	2019	2020	2021	2022	
0-15	9(5.8)	191.3)	2(4.5)	3(3.0)	4(5.7)	19(4.2)
16-30	33(21.3)	19(23.8)	10(22.7)	23(23.0)	18(25.7)	103(22.9)
31-45	68(43.9)	38(47.5)	20(45.5)	25(25.0)	24(34.3)	175(39.0)
46-60	40(25.8)	15(18.8	11(25.0)	37(37.0	19(27.1)	12(27.2)
61 >	5(3.2)	7(8.8))	1(2.3)	12(12.0)	5(7.1)	30(6.7)
Total	155(34.5)	80(17.8)	44(9.8)	100(22.30	70(15.6)	449(100.0)

Table 5. Prevalence rates of MTB/HIV and co-infections.

Years	Total number of TB & HIV positives	HIV pos (%)	TB pos (%)	Co-infection (%)
2018	193	155(34.5)	38(18.4)	7(3.6)
2019	122	80(17.8)	42(20.3)	0(0.0)
2020	80	44(9.8)	36(17.4)	2(2.5)
2021	145	100(22.3)	45(21.7)	13(9.0)
2022	116	70(15.6)	46(22.2)	9(7.8)
Total	656	449(68.4)	207(31.6)	31(4.7)

Table 6. Prevalence of rifampicin resistance among the participants by gender.

Years	Rifampicin resistance (%)	Rifampicin intermediate (%)	Rifampicin sensitive (%)	Total TB pos
2018	1(2.6)	0(0.0)	37(97.4)	38
2019	0(0.0)	0(0.0)	42(100)	42
2020	0(0.0)	2(5.6)	34(94.4)	36
2021	2(4.4)	0(0.0)	43(95.5)	45
2022	2(4.3)	3(6.5)	41(89.1)	46
Total	5(0.2)	5(0.2)	197(7.6)	207

Table 7. Rates of rifampicin susceptibility/resistance annually.

Gender	Rifampicin resistance (%)	Rifampicin intermediate (%)	Rifampicin sensitive (%)	Total (%)
Male	2(1.6)	3(2.4)	120(96)	125
Female	3(3.7)	2(2.4)	77(93.9)	82
Total	5(2.4)	5(2.4)	197(95.2)	207

Discussion

The use of GeneXpert MTB/RIF assay in the diagnosis of MTB has really revolutionized TB diagnosis and surveillance. The major advantages of this assay lie in its ability to detect not only MTB but also rifampicin-resistant strains of MTB and its reduced turnaround time.

In this study, MTB prevalence recorded was 8.03%, which is a little bit higher than the 7.8% MTB prevalence reported by **Dahal *et al.*** [24] in Jos, but lower than the 21.3% reported at Enugu State of Southeast [25]. A value of 24.8% was recorded in Calabar, cross River State within the Southern region of Nigeria [26] and 11.9% recorded by **Egah *et al.*** [27]. The variations in these reported studies, even from the same region, may be attributed to the level of awareness, accessibility to healthcare facilities, and effectiveness of TB control programs in the various regions. The reduced prevalence recorded in this study reveals that an increase in awareness, accessibility, and improvement in the TB control programs in the study population

The Nigeria National Tuberculosis Control Programme and the donor partners have improved their community-based case finding by engaging non-medical personnel trained to go into the very remote villages and collect samples from suspected ignorant villagers. The availability and accessibility

of improved TB diagnostic methods, coupled with effective TB treatment regimens and follow-up monitoring, have really reduced the prevalence of tuberculosis in Nigeria [28]. However, the high cost of GeneXpert technology could limit the implementation and availability of molecular techniques, especially in the rural areas. The decentralizing of the services may not be achievable in the near future.

That the HIV statuses of some of the participants were unknown can be attributed to the fact that most of the participants did not visit the TB centre for the diagnosis of HIV; rather, their samples were collected by the trained personnel who visited them in their homes to send to the TB diagnostic centre. These personnel were not trained or equipped with HIV kits for screening. The total rate of co-infection in this study was low. Low rates were also recorded in the years of study. Infection with HIV invariably reduces the time between TB infection and development to active TB disease [9]; however, in the absence of HIV, the rates of TB are low, resulting in low rates of co-infection. **Corbett *et al.*** [29] reported that the increase in HIV/AIDS is a major factor responsible for the rise in TB [29]. However, the value obtained in this study was higher than those reported by **Hassan Dirie *et al.*** [30] in Somalia, who obtained the rate of 1.5% HIV/TB co-infection. This may be a pointer to the low prevalence of HIV in these regions and shows that the HIV subjects were compliant in taking their medications. The report of no co-infection in 2019 and a low rate in 2020 is a suggestion that during COVID-19, new cases of TB did not visit the centre and old patients who were adherent to medications might have shown up with negative results in the MTB status. Generally, the number of new patients visiting the centre has been reduced. This low rate could also be indicative of effective HIV treatment and prevention strategies in the study population.

The highest TB and HIV/AIDS prevalence were recorded among the ages 31-45 years each. This agrees with the report of **Dahal *et al.*** [24] that age group 15-47 had the highest TB prevalence. Holland et al., (31), also reported the highest TB and HIV infection among this age group. Age group 15-47 is the most active group that engages in various activities that expose them to higher risk of getting HIV complicated by TB. **Lee *et al.*** [32] stated that MTB disease progression, symptoms and prognosis varies among individuals depending on age with corresponding changes in immune response. Statistical analysis shows that there was no correlation of the age range in each year (p=.207).

The prevalence rates of TB within the years did not vary directly. However, the prevalence rates of TB positive cases showed an increase from 2018 to 2022. There was a decline in 2020 and there was also a sharp decrease in the rate of HIV in the same year. However, in the year 2020, large population was tested for the TB and HIV infection but inversely, new patients with apparent signs and symptoms that could show up positives probably absented from the program for fear and stigma of being diagnosed of corona virus during the COVID 19 Pandemic. This was probably so being that MTB/HIV and corona infections had some overlapping signs and symptoms. As a result of this, the prevalence rate of *mycobacterium tuberculosis*, HIV and MTB/HIV infections declined as seen in tables 3, 4 and 5. Remote data collection was not applicable because of technological limitations hence the equipment for the analysis was only available at the TB Referral Centre in the region selected this study.

There were significant differences (p= .007) in the rates of TB infection among the age groups in 2022. For 2018, 2019, 2020 and 2021 there were no significant differences (.084, .070, .180 and .051, respectively). The rate of co-infection recorded in this study (4.7%) was a contrast to that recorded in other regions in studies conducted in the past: 16.7% in Enugu [25], 24.8% in Calabar [26] and 24.1% [34]. The disparity/low rate of co-infection is probably due to the fact that some HIV patients were not referred by clinicians to the centre but visited there on their own to carry out check-up tests. With this they did not undergo clinical examinations for TB. Their results showed negative for MTB. It may also mean that the HIV patients in this study were Anti-retroviral Therapy (ART) compliance and did not have AIDS or TB secondary infections.

The result of this study also recorded higher MTB prevalence among the male participants (11.3%) than in the female counterparts (5.6%). This study showed that the male-female ratio rate was comparable to that obtained in Zimbabwe on age-specific risk of TB [33]. However, there was no significant difference (p = .500) between genders in the rate of TB. The result data is close to that obtained by **Dahal *et al.*** [23] recently, among the male and female participants, respectively. The

result does not agree with those obtained in Ghana [34] and Ethiopia [35], where higher MTB prevalence among the females was reported. This discrepancy might be attributed to reduced access to health care centres for TB diagnosis among the females, which will eventually lead to many cases of unreported TB [36]. The decline noticed in the rifampicin resistance in 2020 was possibly as a result of the decline in new patients visiting the centre for testing during the pandemic year.

The rifampicin-resistant and indeterminate prevalence rates reported in this study were low. These strains that show intermediate resistance are termed low-level RIF resistant, and they exhibit elevated RIF MICs compared to fully susceptible strains but remain phenotypically susceptible by mycobacterial growth indicator tube testing. This type of response has been associated with poor patient treatment outcomes [37]. Some higher prevalence of both rifampicin-resistant and indeterminate was recorded in the North Central, Southwest, and Northwest regions of Nigeria, respectively [24, 38]. The regional variations in the Rifampicin-resistant and indeterminate results may be due to non-adherence to the MTB treatment regimen as recommended by the Nigeria National Tuberculosis Control Programme, as well as other predisposing diseases like HIV, diabetes, and cancer [39].

Conclusion

GeneXpert MTB/RIF effectively detected *mycobacterium tuberculosis* (MTB) and rifampicin-resistant tuberculosis, including co-infection rates. The prevalence rates of TB and HIV within the years did not vary directly. While more efforts are being geared towards the reduction of both MTB prevalence and rifampicin resistance in the region, this molecular diagnostic method should be adopted. It is recommended that more comprehensive studies be conducted in this area to explore risk factors with possible policy implementation that can enhance more TB research and control measures.

Competing interests

None.

Funding

None.

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