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Short Communication **Open Access**

Rifampicin resistant tuberculosis among patients attending General Hospital, Kagarko, Kaduna State, Nigeria

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Abstract:

Background: The National Tuberculosis and Leprosy Control Program (NTBLCP) in collaboration with Koninklijke Nederlandse Centrale Vereniging tot bestrijding der tuberculose (KNCV) (Dutch Tuberculosis Foundation) and National Agency for Control of AIDS (NACA) installed and equipped many health centres in Kaduna State, Nigeria with modern diagnostic tools (GeneXpert) to offer molecular services for the rapid detection of *Mycobacterium tuberculosis* complex (MTBC) and rifampin resistance.
Methodology: This study analyzed routine samples from patients attending General Hospital Kagarko, from September 2016 to March 2019 with total samples of 1056 from 1056 patients. The GeneXpert machine was used for the rapid detection of *M. tuberculosis* and rifampin resistance (RIF) from all the sputum samples received in the clinical laboratory department of the hospital.
Results: A total of 182 (17.2%) samples tested positive for *M. tuberculosis* out of which 5 (2.7%) were resistant to rifampicin. Males were more frequently affected with a prevalence of 23% than females with a rate of 10.7% (X^2=27.801, p=0.0001). RIF was detected in 3 male and 2 female patients (p=1.000). The prevalence of MTB was highest in the age group 36-45 years (23%) and age group 26-35 years (20.3%) and lowest in age group 5-15 years with 10.9% (t=0.599, p=0.55).
Conclusion: There is need for the GeneXpert technology to be replicated in other health centers across the state and the country at large to reduce the burden of multi-drug resistant tuberculosis (MDR-TB) in Nigeria.

Keywords: RIF resistance, MDR-TB, GeneXpert, *Mycobacterium tuberculosis*

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Tuberculose résistante à la rifampicine chez les patients de l'hôpital général de Kagarko, État de Kaduna, Nigéria

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Abstrait:

Contexte: Le programme national de lutte contre la tuberculose et la lèpre (NTBLCP) en collaboration avec Koninklijke Nederlandse Centrale Vereniging tot bestrijding der tuberculose (KNCV) (Fondation néerlandaise pour la lutte contre la tuberculose) et l'Agence nationale de lutte contre le sida (NACA) ont installé et équipé de nombreux

centres de santé dans l'État de Kaduna , Nigeria, avec des outils de diagnostic modernes (GeneXpert) pour offrir des services moléculaires pour la détection rapide du complexe *Mycobacterium tuberculosis* (MTBC) et la résistance à la rifampicine.
Méthodologie: Cette étude a analysé des échantillons de routine de patients fréquentant l'hôpital général de Kagarko, de septembre 2016 à mars 2019, avec des échantillons totaux de 1056 sur 1056 patients. L'appareil GeneXpert a été utilisé pour la détection rapide de *M. tuberculosis* et de la résistance à la rifampicine (RIF) pour tous les échantillons d'expectorations reçus dans le service de laboratoire clinique de l'hôpital.
Résultats: Un total de 182 échantillons (17,2%) ont été testés positifs pour *M. tuberculosis*, dont 5 (2,7%) étaient résistants à la rifampicine. Les hommes étaient plus fréquemment touchés avec une prévalence de 23% que les femmes avec un taux de 10,7% (X^2=27,801, *p*=0,0001). Le RIF a été détecté chez 3 hommes et 2 femmes (*p*=1.000). La prévalence du VTT était la plus élevée dans le groupe d'âge 36-45 ans (23%) et le groupe d'âge 26-35 ans (20,3%) et la plus faible dans le groupe d'âge 5-15 ans avec 10,9% (t=0,599, *p*=0,55).
Conclusion: Il est nécessaire que la technologie GeneXpert soit reproduite dans d'autres centres de santé de l'État et du pays dans son ensemble pour réduire le fardeau de la tuberculose multirésistante (TB-MR) au Nigéria.

Mots-clés: résistance au RIF, TB-MR, GeneXpert, *Mycobacterium tuberculosis*

Introduction:

Drug-resistant tuberculosis (DR-TB) is a tuberculous disease resulting from *Mycobacterium tuberculosis* resistant to at least one first-line anti-TB drug; isoniazid (INH) and rifampicin (RIF). Drug resistant TB could be mono-resistance in which there is resistance to only one first-line anti-TB drug or poly-resistance in which there is resistance to more than one first-line anti-TB drugs other than isoniazid and rifampicin (1,2). Multidrug resistance (MDR) is defined as resistance to at least both isoniazid and rifampicin, and extensively drug resistance (XDR) as resistance to any fluoroquinolone, and at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin) in addition to multidrug resistance (2,3). Rifampicin resistance (RR) is resistance to rifampicin, detected by phenotypic or genotypic methods, with or without resistance to other anti-TB drugs, and includes mono-resistance to rifampicin (2,4).

The World Health Organization (WHO) estimates that TB kills approximately 2 million people worldwide each year, and MDR-TB is becoming an increasing public health challenge in many parts of the world, basically due to poor patient adherence to the six-month tuberculosis treatment regimen. About 5% of all TB cases are reported to be MDR-TB (2). Resistance to rifampin is one of the primary reasons for treatment failure and fatal clinical outcome in TB patients (4,5).

Treatment of drug-resistant TB is difficult and inappropriate management can lead to life-threatening outcomes (1,6). The traditional method for diagnosis of drug resistant TB is usually by susceptibility testing involving the growth of *M. tuberculosis* on liquid or solid culture medium, which takes about 2 months. Culture methods are expensive and time consuming, thus delay opportunity for prompt treatment of patients. With the recent development of nucleic acid amplification technologies, which are rapid, sensitive, and specific, opportunity of timely, accurate and precise diagnosis of drug resistant TB is broadened (3,7). The GeneXpert assay uses real-time polymerase chain reaction (PCR) technology to simultaneously amplify and detect *M. tuberculosis*-specific sequence of the *rpoB* gene (1,6).

Rifampicin has significant early bactericidal effect on metabolically active *M. tuberculosis* and excellent terminal removal activity on low inactive micro-organisms passing through short bursts of metabolic activity. The mechanism of action of rifampicin is inhibition of mycobacterial transcription by targeting DNA dependent RNA polymerase Resistance to rifampicin develops from mutations within the 27 codons in the well-defined 81 base pair (bp) central region of the gene (*rpoB*) that encodes the β-subunit of RNA polymerase. More than 96% of the rifampicin-resistant strains contain a mutation in this region thus facilitating a straightforward rapid approach for detecting rifampicin resistance and/or MDR (3,8,9). Therefore, the aim of this study was to determine the prevalence of rifampicin resistance in TB patients attending General Hospital, Kagarko using the GeneXpert MTB/RIF assay.

Materials and methods:

Study setting

The study was conducted in General Hospital, Kagarko in Kagarko Local Government Area (LGA) of Kaduna State, Nigeria. The laboratory department of the hospital receives samples within the hospital covering the entire LGAs and from other health facilities in neighboring LGAs.

Study population

This study population was made of patients who presented with cough of two or more weeks duration and referred to the clinical laboratory department of the hospital for sputum examination by the GeneXpert assay between November 2016 and March 2019. One sputum sample was collected per patient according to the GeneXpert sample collection guidelines, and the total number of patients was 1056.

Ethical approval

The study was approved by the Research and Ethics Committee of the Kaduna State Ministry of Health and Human services.

Laboratory analysis by GeneXpert assay

Sample reagent was added to sputum sample in a 2:1 ratio and in a 3:1 ratio to suspended sediment of decontaminated or digested sputum specimen. The lid of the sputum container was replaced, and manually shaken vigorously 10-20 times (one back and forth movement in single shake), and incubated at room temperature for 10 minutes. This was again shaken vigorously 10-20 times (or vortex) and further incubated for 5 minutes at room temperature with samples perfectly fluidics with no visible clumps of sputum at the end of incubation.

The cartridges were labeled appropriately on the left or right side with sample ID. Two ml of the prepared sputum samples were transferred to the cartridges using transfer pipette with avoidance of solid particles and aerosols or bubbles. The lids of the cartridges were firmly closed and then inserted into the test platform located in the clean room. The test platform (Cepheid) is the integrated diagnostic device that performs sample processing and simultaneous amplification and detection of fluorescence (real-time polymerase chain reaction; rt-PCR) in a single hands-free step for the identification of *M. tuberculosis* and simultaneous rapid detection of rifampicin (RIF) resistance in the sputum specimens. The electronic results were sent directly from the MTB/RIF test system to the central database in about 120 minutes.

Statistical analysis

Data were entered into Microsoft Access (Microsoft Corp., Redmond, WA, USA) and analyzed with Microsoft Excel. Comparison of the prevalence of MTB and rifampin resistance with respect to age group and gender of patients was done with Chi square test and $p<0.05$ was taken as significant value.

Results:

This study included 1056 patients (568 males, 488 females) with presumptive TB whose sputum samples (one sample per patient) were received and tested at the clinical laboratory department of General Hospital Kagarko, Kaduna State between September 2016 and March 2019. A total of 182 (17.2%) sputum specimens were positive for *M. tuberculosis* with the GeneXpert MTB/RIF assay including 5 (0.5%) that were rifampicin resistant, while 874 (82.7%) were negative (Table 1).

The prevalence of TB was significantly higher in the male patients with 23% (130 of 568) than the female patients with 10.7% (52/488) (p=0.0001) but the rifampicin resistance rate of 0.5% (3/568) in the male was not significantly different from 0.4% (2/488) in the female patients (p=1.000) (Table 1).

The prevalence of TB in relation to the age group showed that age group 36–45 years had the highest MTB detected with 23% (43/187), followed by age group 26–35 years with 20.3% (53/261), age group 46–55 years 18.4% (27/147), age group > 55 years 14.8% (27/199), age group 16–25 years 12.8% (23/180) and age group 5-15 years 11% (9/82) (p=0.55) (Table 2).

Table 1: Frequency distribution of MTB detection by GeneXpert with respect to gender of patients

Gender	No tested	MTB positive (%)	MTB Rif Resist (%)	Total (%)	χ^2	*p* value
Male	568	127 (22.4)	3 (0.5)	130 (23)	27.801	0.0001*
Female	488	50 (10.2)	2 (0.4)	52 (10.7)		
Total	1056	177 (16.8)	5 (2.7)	182 (17.2)		

*$p<0.05$ – statistically significant for MTB; X^2 = Chi square

Table 2: Frequency distribution of MTB detection by GeneXpert with respect to age group of patients

Age group (years)	No tested	MTB positive (%)	t	df	CI	*p* value
5 – 15	82	9 (11.0)	0.599	288.9	0.159-0.299	0.55*
16 – 25	180	23 (12.8)				
26 – 35	261	53 (20.3)				
36 – 45	187	43 (23.0)				
46 – 55	147	27 (18.4)				
> 55	199	27 (13.6)				
Total	1056	182 (17.2)				

**p*>0.05 – not statistically significant; CI = Confidence Interval at 95%

Discussion:

MDR TB has become a serious public health challenge globally but especially in developing countries (10). The GeneXpert MTB /RIF assay has opened up opportunity for the rapid detection of MTB and resistance to primary anti-TB drugs (11). In this study, MTB was detected by the GeneXpert in 17.2% of the 1056 sputum specimens obtained from patients with presumptive TB among whom 5 (2.7%) were rifampicin resistant MTB. Majority of the MTB patients were between the age groups 36-45 (23%) and 26-35 years (20.3%), which is generally considered the active and vulnerable age group to MTB infections. Also, the prevalence rate of TB by the GeneXpert assay in males (23%) was significantly higher than in females (10.7%) (p=0.0001). The difference could be related to social attitude of the males which make them more exposed and vulnerable to MTB infection than the females.

Otu et al., (12) in their study reported drug resistance rate of 42% to at least one anti-TB drug on mycobacterial culture in patients with pulmonary TB in Calabar, south-south Nigeria. They found mono-resistance rate of 7% to ethambutol and 7% to strepto-mycin but none to rifampicin. The difference in methods of testing for rifampicin resistance does not allow for direct comparison as we used GeneXpert MTB/RIF assay in our study which is programmed to detect only rifampicin resistance. The rifampicin resistance rate of 2.7% reported in our study is lower than the 14.7% reported by Ikuabe and Ebuenyi (1) in Yenagoa using GeneXpert MTB/RIF assay.

The low rifampicin resistance rate in our study may be related to the new history of use of rifampicin in this part of the country and Africa as a continent (12) but differences in rates may also be related to level of patients' awareness, compliance/adherence to anti-TB drugs and geographical location. Poor manag-ement of active pulmonary TB is a known factor for emergence of drug resistant TB, which could be aided by several factors such as poor prescribing practices with insufficient treat- ment duration and poor drug compliance by patients (13). Other factors include poor public health awareness and financial resources, shortages in drug supplies to directly observed treatment (DOT) centres and lack of proper health education of patients on the medication (11, 13). Rifampicin resistance rate in our study was not associated with gender and age group as the rate of 0.5% in the males was not significantly different from the rate of 0.4% in the females (p=1.000), however, this result should be interpreted with caution because of the small number of patients with RIF in this study.

Conventional laboratory techniques such as microscopy for detection of acid-fast bacilli (AFB) in sputum samples for the diagnosis of tuberculosis are far from being sensitive. Moreover, mycobacterial cultures are time-consuming, require biosafety measures, and need trained laboratory personnel. In our study, the GeneXpert assay used have been reported to be highly sensitive and specific (over 97%) for TB diagnosis from pulmonary specimens, and generate results rapidly, therefore reducing the turn-around time for TB diagnosis (14). The GeneXpert assay also targets the region of rifampicin resistance associated *rpo*B gene by real-time polymerase chain reaction (rt-PCR) with three specific primers that simultaneously detect *M. tubercu-losis* DNA and rifampicin resistance. The processes of bacterial lyses, DNA extraction, real time amplification and amplicon detection are automated, which give room for less interference and contamination.

Conclusion:

The prevalence of MDR-TB as determined by rifampicin resistance rate of 2.7% in our study emphasizes the need to make the GeneXpert MTB/RIF machines widely available to facilitate rapid detection of MDR-TB in Nigeria.

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