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Copyright AJCEM 2022: <https://dx.doi.org/10.4314/ajcem.v23i2.8>**Original Article****Open Access****Detection of multi-drug resistant tuberculosis (MDR TB) using microscopic observation drug susceptibility (MODS) assay in Lagos State, southwest Nigeria***¹George, O. T., and ²Oduyebo, O. O.¹Lagos State Biobank, Mainland Hospital Yaba, 1 Mainland Drive, Yaba, Lagos, Nigeria²Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos and Lagos University Teaching Hospital, Lagos, Nigeria*Correspondence to: olamilekangeorge@gmail.com**Abstract:**

Background: Nigeria has the second highest case of multi-drug resistant tuberculosis (MDR TB) in Africa, estimated at 29,000 in 2015. The laboratory diagnosis of MDR TB in Nigeria is currently done using GeneXpert assay that generates results in less than two hours but can detect resistance to only rifampicin and has high-technology requirements. The objective of this study is to detect MDR TB using Microscopic Observation Drug Susceptibility (MODS) assay in Lagos State, Nigeria.

Methodology: A total of 80 patients who were positive for TB by GeneXpert in three Directly Observed Treatment Short-course (DOTS) centres in Lagos were studied. Spot sputum samples were collected from each patient and transported on ice-packs to Lagos University Teaching Hospital (LUTH) DOTS laboratory for decontamination. Culture and drug sensitivity test (DST) were performed on the pellets obtained by MODS assay in 24-well plates and examined with an inverted light microscope within 6 to 21 days of incubation at 35°C.

Results: Of the 80 patients, males were 43 (53.8%) while females were 37 (46.2%), with mean age of 36.2±11.6 years. Seventy-six (95.0%) of the patients had cough at presentation, 60 (75.0%) had not commenced anti-TB treatment, 15 (18.8%) were previously treated (PT) TB cases, and 14 (17.5%) were HIV positive. MODS assay detected *Mycobacterium tuberculosis* (MTB) in 52 (65.0%) patients across all the age groups but association between age groups and MTB detection by MODS assay was not significant ($p=0.447$). MODS assay detected MTB in 50 (83.3%) of 60 patients who had not commenced anti-TB drugs compared to 2 (10.0%) of 20 who had commenced anti-TB drugs at the time of sample collection ($p<0.0001$). Nine (60.0%) of 15 PT TB cases had MTB detected compared to 43 (66.2%) of 65 new cases of TB ($p=0.7657$). Nine of the 14 (64.3%) HIV positive patients were co-infected with MTB detected by MODS assay compared to 43 (65.2%) of 66 HIV negative patients ($p=1.000$). MDR TB was detected by MODS assay in 2 (2.5%) of the 80 patients (aged 30 and 38 years) who were previously identified as rifampicin resistant by GeneXpert assay ($p=0.0003$). The 2 MDR-TB cases were seen in the 15 PT (13.3%) cases, and in 1 of the 14 HIV (7.1%) positive patients.

Conclusion: MODS assay detected MDR-TB among PT TB patients in Lagos, Nigeria at the rate of 2.5%. Hence, MODS assay is an effective, low-tech, liquid culture technique to accurately detect TB and MDR-TB simultaneously.

Keywords: microscopic observation drug susceptibility; multi-drug resistant tuberculosis; directly observed treatment short-course; GeneXpert; Lagos

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Détection de la tuberculose multirésistante (TB-MDR) à l'aide d'un test d'observation microscopique de sensibilité aux médicaments (MODS) dans l'État de Lagos, au sud-ouest du Nigeria

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Résumé:

Contexte: Le Nigéria compte le deuxième cas le plus élevé de tuberculose multirésistante (TB-MDR) en Afrique, estimé à 29 000 en 2015. Le diagnostic en laboratoire de la TB-MDR au Nigeria est actuellement effectué à l'aide du test GeneXpert qui génère des résultats en moins de deux heures, mais peut détecter la résistance à la seule rifampicine et a des exigences de haute technologie. L'objectif de cette étude est de détecter la tuberculose multirésistante à l'aide du test d'observation microscopique de la sensibilité aux médicaments (MODS) dans l'État de Lagos, au Nigeria.

Méthodologie: Un total de 80 patients positifs pour la tuberculose par GeneXpert dans trois centres de traitement de courte durée sous surveillance directe (DOTS) à Lagos ont été étudiés. Des échantillons ponctuels d'expectorations ont été prélevés sur chaque patient et transportés sur des sacs de glace au laboratoire DOTS de l'hôpital universitaire de Lagos (LUTH) pour décontamination. La culture et le test de sensibilité aux médicaments (DST) ont été effectués sur les culots obtenus par dosage MODS dans des plaques à 24 puits et examinés au microscope optique inversé dans les 6 à 21 jours d'incubation à 35°C.

Résultats: Sur les 80 patients, les hommes étaient au nombre de 43 (53,8%) tandis que les femmes étaient au nombre de 37 (46,2 %), avec un âge moyen de 36,2±11,6 ans. Soixante-seize (95,0%) des patients avaient toussé au moment de la présentation, 60 (75,0%) n'avaient pas commencé de traitement antituberculeux, 15 (18,8%) étaient des cas de tuberculose précédemment traités (PT) et 14 (17,5%) étaient infectés par le VIH. positif. Le test MODS a détecté *Mycobacterium tuberculosis* (MTB) chez 52 (65,0%) patients dans tous les groupes d'âge, mais l'association entre les groupes d'âge et la détection de MTB par le test MODS n'était pas significative ($p=0,447$). Le test MODS a détecté MTB chez 50 (83,3%) des 60 patients qui n'avaient pas commencé les médicaments antituberculeux par rapport à 2 (10,0%) des 20 qui avaient commencé les médicaments antituberculeux au moment du prélèvement de l'échantillon ($p<0,0001$). Neuf (60,0%) des 15 cas de TB PT avaient une MTB détectée contre 43 (66,2%) des 65 nouveaux cas de TB ($p=0,7657$). Neuf des 14 (64,3%) patients séropositifs pour le VIH étaient co-infectés par le MTB détecté par le test MODS, contre 43 (65,2%) des 66 patients séronégatifs pour le VIH ($p=1 000$). La tuberculose multirésistante a été détectée par le test MODS chez 2 (2,5%) des 80 patients (âgés de 30 et 38 ans) précédemment identifiés comme résistants à la rifampicine par le test GeneXpert ($p=0,0003$). Les 2 cas de TB-MR ont été observés chez les 15 cas PT (13,3%) et chez 1 des 14 patients séropositifs (7,1%).

Conclusion: Le test MODS a détecté la TB-MR chez les patients PT TB à Lagos, au Nigeria, à un taux de 2,5 %. Par conséquent, le test MODS est une technique de culture liquide efficace et de faible technologie pour détecter simultanément avec précision la tuberculose et la tuberculose multirésistante.

Mots-clés: observation microscopique de la sensibilité aux médicaments; tuberculose multirésistante; traitement de courte durée sous observation directe; GeneXpert; Lagos

Introduction:

Tuberculosis (TB) is an infectious disease caused by members of the *Mycobacterium tuberculosis* complex (MTBc) including *Mycobacterium bovis* (zoonotic tuberculosis) (1). TB is one of the biggest killers amongst infectious diseases despite the worldwide use of the live-attenuated Bacillus Calmette-Guérin (BCG) vaccine and several available antibiotics (2,3). Overall, 5–15% of estimated 2–3 billion people infected with *M. tuberculosis* will develop TB disease during their lifetime. However, immuno compromised individuals are more likely to develop TB (4). The emergence of various types of drug-resistant tuberculosis (DR-TB) has long been reported and this has complicated the control of TB worldwide (4,5,6).

Multi-drug resistant tuberculosis (MDR) is defined as resistance to isoniazid (INH) and rifampicin (RIF). It could result from either primary infection with a drug-resistant TB strain (primary resistance) or may develop during a patient's treatment due to inadequate therapy that enables the selection of drug resistance (7). High burden of TB and MDR-TB has been reported in countries with high populations.

Nigeria is estimated to have a population of 182 million with more than half its people currently below 30 years of age (8). Lagos, a port city is the most populous city in Nigeria with an estimated population of 21 million in 2016 (9). In 2016, Nigeria ranked 10th among the 30-high burden MDR-TB countries in the world and second among high burden MDR-TB countries in Africa, with 1,241 laboratory-confirmed MDR-TB cases of the esti-

mated 3,700-5,700 cases notified to National Tuberculosis and Leprosy Control Programme (4). Since the emergence of DR-TB, the conventional method was used for its diagnosis. However, with the advancement in technology, many new methods and techniques have been developed.

GeneXpert test, microscopic observation drug susceptibility (MODS) assay, liquid-based automated assay (MGIT) and line probe assay (LPA) have been endorsed by the World Health Organization (WHO) for detecting MDR-TB (10,11). However, in lower-middle-income settings such as Nigeria, the GeneXpert test is the diagnostic tool used for detecting MDR-TB. This test gives results in less than 2 hours, detects simultaneously both MTB and rifampicin resistance, and is free of cost for patients, although it is funded by international partners with counterpart-funding from the government of Nigeria. However, the GeneXpert test cannot detect INH resistance, which requires high technology. Moreover, in Lagos, the diagnosis of MDR TB still poses a challenge as unpublished data revealed that the operationalization of GeneXpert machines for testing MDR-TB is below optimization due to issues of interrupted electricity supply and inadequacy of sustainable air-conditioned ambience. In addition, as at the period of this study, there is a dearth of published data on the actual case detection rates of MDR-TB in Lagos State, Nigeria. Finally, the WHO recommends that GeneXpert test results should be confirmed with conventional drug susceptibility test (DST) (4), but this is rarely practiced in Lagos probably due to the long turn-around-time of conventional indirect DST.

The objective of this study therefore is to detect MDR-TB using microscopic-observation-drug-susceptibility assay among pulmonary TB patients previously positive for TB by the GeneXpert test in Lagos State, southwest Nigeria. The results from this study will provide baseline information to guide the tuberculosis control programme in the effective diagnosis and control of MDR-TB.

Materials and method:

Study settings and population

This study focused on pulmonary TB patients attending the three DOTS centres in Lagos for TB diagnosis, treatment and care between March and June 2017. The centres were Lagos University Teaching Hospital (LUTH) Idi-Araba, Mainland Hospital Yaba, and Randle

General Hospital, Randle Surulere, Lagos, Nigeria. The Mainland Hospital Yaba specializes in infectious disease care and control, with isolation wards for different forms of TB cases including MDR-TB. LUTH is a tertiary level hospital while Randle General Hospital is a secondary level hospital.

Ethical clearance

Ethical approval was obtained from the Health & Ethics Research Committees of Lagos University Teaching Hospital and Lagos Health Service Commission. All institutional guidelines of ethics involving human experimentation in research were strictly complied with. Written informed consent and confidentiality were obtained from each subject participant.

Study design and sample size

This was a cross sectional study carried out among pulmonary TB patients who were previously positive by GeneXpert test and were attending the Directly Observed Treatment Short-course (DOTS) centres in Lagos State. The sample size for this study was calculated using the formula; $n = (Z^2_{(1-\alpha)/2} \times pq) / d^2$ where 'n' is the expected sample size, ' $Z^2_{(1-\alpha)/2}$ ' is the standard normal score at 95% confidence interval with type 1 error (=1.96), 'p' is the prevalence of MDR TB from a previous study of 4.1% (12), d is the absolute error set at 0.05. This gives the calculated minimum acceptable sample size of 60, but an addition of 40% was made to allow for attrition, bringing the calculated sample size to 84.

Inclusion and exclusion criteria

Patients of both genders aged 15 years and above were considered in this study. Such patients have been previously confirmed to have pulmonary TB by the GeneXpert test (Cepheid, Sunnyvale, USA). Patients with extra-pulmonary TB and children were excluded from the study.

Collection of demographic and clinical data

Interviewer-administered questionnaire was used to obtain information from each patient on demography, site of enrolment, prior history of TB treatment to differentiate 'new' case from 'previously treated' case, clinical symptoms (such as deep productive cough lasting more than 3 months, chest pain, unexplainable weight loss, night sweat, fever, loss of appetite, hemoptysis and shortness of breath) present during the time of sample collection, number of co-inhabitants, HIV status, use of

anti-retroviral drugs, duration on TB treatment (if any) and close contact with persons known or suspected of having MDR-TB.

Sample collection and transport

Each patient was instructed to produce a spot sputum sample at a designated area into a properly labeled, wide-mouth, sterile, leak-proof container with a tight-fitting lid. Thereafter, the samples were transported on ice packs to the DOTS laboratory, LUTH, Idi-Araba for processing. However, when processing was delayed, the samples were refrigerated at 2-8°C to limit the growth of oral normal flora (13).

Sample processing and MODS assay

The samples were processed in a batch of 15 each in class II biosafety cabinet (BSC II) and level two biosafety (BSL-2) practices were followed to standard. The two major stages of processing were decontamination of samples, and culture and drug susceptibility testing by MODS assay. Two milliliters of sputum sample were placed in a 15 ml centrifuge tube (but when less than 2 ml, it was made up to 2 ml with phosphate buffer). Then, 2 ml NaOH-NALC solution was added and the tube was tightly capped, vortexed for 20 seconds and inverted for NaOH-NALC solution to make contact with the entire interior surface of the tube and lid. The tube could stand for at least 15 minutes but prolonged by a few minutes for particularly viscous samples. Thereafter, the tube was filled to 14ml with phosphate buffer (pH 6.8) to neutralize alkali and terminate the decontamination process and then mixed well by inverting 4 times. The resultant mixture was centrifuged at 3000g for 15 minutes and the supernatant was carefully poured off into a liquid waste container with 10% sodium hypochlorite while the pellet was retained (14).

Each 24-well plate was properly labeled with patients' identifiers and the date of inoculation. Using micropipettes, 4 tips were carefully filled with 100 µl of 7H9-OADC (enriched culture medium) and antibiotic working solutions (Column 2 in antibiotic dilution plate). Then 100 µl aliquots were added to the respective wells of column 1 in the 24-well plates. This was repeated until all columns have received 100 µl of medium (drug-free wells) or antibiotic working solutions (including negative control column 3). Thereafter, 900 µl of each final sample suspension (7H9-OADC-PANTA-sample pellet) was placed into the respective wells of a single column. This was

repeated with additional samples until all columns of the plate, except Column 3, were filled (or until all samples were plated). 900 µl of 7H9-OADC-PANTA medium without sample was placed in each of the 4 wells of Column 3 of each sample plate (negative internal controls). The plate was closed with a tight-fitting rubber cover but when the rubber cover was not available, it was closed with its lid and placed in a sealable zip lock polythene bag, sealed (the bag was not opened again from this point onwards) and incubated at 35°C. To minimize the risk of cross-contamination, the positive controls were set up in a separate plate after all plates with samples had been sealed and incubated.

Each of the two properly labelled control strains (drug susceptible MTB, and RIF and INH resistant strains) was adjusted to 1.0 McFarland standards. Using micropipettes with tips, 5 µl of each 1.0 McFarland-control strain suspension was mixed with 5ml of 7H9-OADC medium to make the positive control suspension. 900 µl of each positive control suspension was placed in the 4 wells of a column on the separate positive control plate and the four 100 µl aliquots of medium and antibiotic working solutions were added as was done for samples. The plate was capped with lid and sealed in a zip-lock bag, incubated at 37°C with other plates processed the same day.

Plates were examined under an inverted light microscope initially with 10x objective and subsequently with 4x objective lens. Drug-free wells were examined from day 5 through day 21. Two or more colony forming units (>2 CFU) with characteristics serpentine structures in both drug-free wells indicated MTB detection while resistance was defined as mycobacterial growth of >2 CFU in drug-containing wells on the same day that both drug-free wells were positive. Hence, growth in both isoniazid- and rifampicin-containing wells indicated MDR. The result was defined as "indeterminate" if only 1 CFU appears in either drug-free well or in both while results were considered "valid" when the internal negative and positive control wells were examined and interpreted in like manner as the sample wells (13).

Statistical analysis

The Statistical Package for the Social Sciences (IBM SPSS For Windows version 23) was used to analyze data. Qualitative variables, proportions and associations were compared using Chi-square test. All reported confidence intervals were two-sided 95% confidence inter-

vals and $p < 0.05$ was regarded as statistically significant.

Results:

Of the 80 patients previously diagnosed with pulmonary TB by GeneXpert assay whose spot sputum samples were collected, 37 (46.2%) and 43 (53.8%) were females and males respectively, with a mean age of 36.2 ± 11.6 years. Of these patients, 78 (97.5%) were positive for MTB and 2 (2.5%) for rifampicin-resistant MTB. At the time of sample collection, 20 (25.0%) patients had commenced anti-TB drugs for less than four weeks while 60 (75.0%) had not commenced anti-TB drugs. Fifteen (18.8%) were PT TB cases while 65 (81.2%) were 'new' cases of TB. Five (6.3%) had previous contact with MDR-TB patients while 72 (90%) did not have any contact with MDR-TB patients, and 3 (3.7%) were unsure of previous contact with any MDR-TB patient. Fourteen (17.5%) patients were HIV positive while 66 (82.5%) were HIV negative (Table 1).

MODS assay detected MTB in 52

(65.0%) of the 80 patients across all the age groups, and 9 (17.3%) of these were co-infected with HIV but the association between MTB detection and age groups ($p=0.447$), and co-infection of MTB with HIV ($p=1.000$) were not statistically significant (Fig 1 and Table 2). MODS assay detected MTB in 50 of 60 (83.3%) patients who were not on anti-TB drugs compared to 2 (10.0%) of 20 who were on anti-TB drugs at the time of sample collection ($p < 0.0001$) (Table 2). MODS assay detected MTB in 9 of 15 (60.0%) PT TB cases compared to 43 (66.2%) of 65 'new' cases of TB ($p=0.7657$).

MODS assay detected MDR-MTB (i. e. resistant to both rifampicin and isoniazid) in only 2 (2.5%) of the 80 patients (aged 30 and 38 years) and these occurred in 2 of the 15 (13.3%) PT cases, and in 1 of the 14 (7.1%) HIV positive patients. The 2 MDR TB cases detected by the MODS assay were the 2 cases of rifampicin resistance (RR) previously detected by GeneXpert assay, while 78 patients that were not previously RR were not MDR TB positive by the MODS assay ($p=0.0003$) (Table 2).

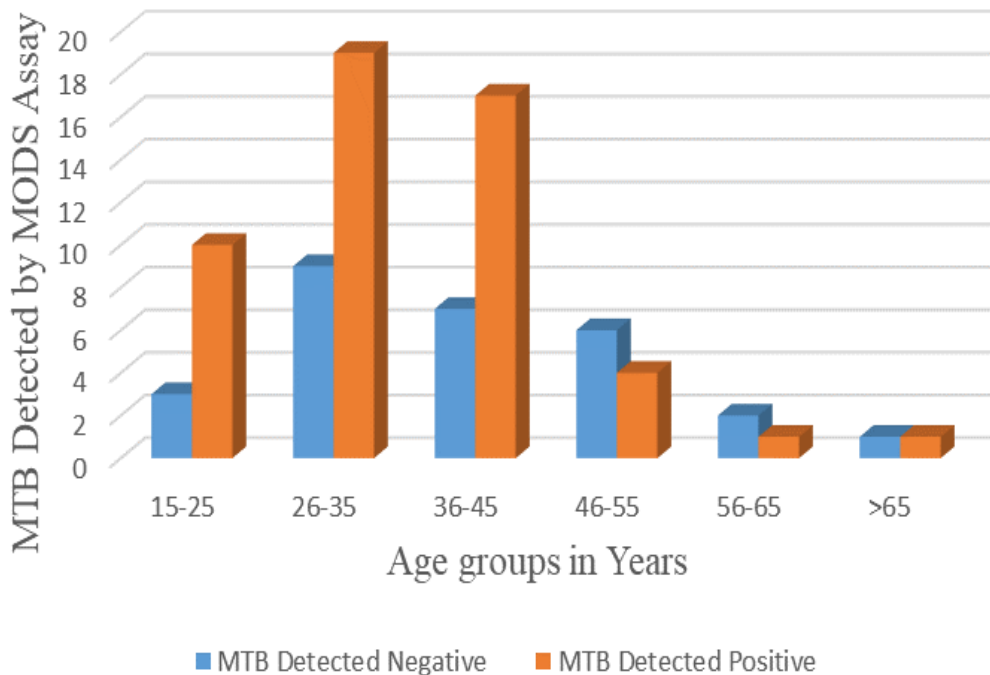


Fig 1: Frequency distribution of *Mycobacterium tuberculosis* (MTB) cases detected by MODS assay with respect to age groups ($\chi^2 = 5.793, p = 0.447$)

Table 1: Demographic and clinical characteristics of the patients from three DOTS centers in Lagos, Nigeria

Characteristics	Frequency (%)
Gender	
Female	37 (46.2)
Male	43 (53.8)
Age group (years)	
15-25	13 (16.3)
26-35	28 (35.0)
36-45	24 (30.0)
46-55	10 (12.5)
56-65	3 (3.8)
> 65	2 (2.5)
Mean age (years)	36.2 ± 11.6
Occupation	
Self-employed	58 (72.5)
Student	9 (11.3)
Civil servant	6 (7.5)
Others	7 (8.7)
DOTS Centre	
LUTH	12 (15.0)
Randle General Hospital	31 (38.8)
Mainland Hospital Yaba	37 (46.2)
HIV status	
Positive	14 (17.5)
Negative	66 (82.5)
Previous history of TB	
Yes	15 (18.8)
No	65 (81.2)
Previous contact with MDR-TB patients	
Yes	5 (6.3)
No	72 (90.0)
Not sure	3 (3.7)
TB symptoms present at screening	
Cough	76 (95.0)
Fever	29 (36.3)
Night sweats	48 (60.0)
Weight loss	66 (82.5)
Chest pain	29 (36.3)
Hemoptysis	11 (13.8)
Currently on TB treatment	
Yes	20 (25.0)
No	60 (75.0)
Previously treated TB cases	
Yes	15 (18.8)
No	65 (81.2)

LUTH = Lagos University Teaching Hospital; DOTS=Directly Observed Therapy Short-course; MDRTB = Multi-Drug Resistant Tuberculosis; TB = Tuberculosis; HIV=Human Immunodeficiency Virus

Table 2: Bivariate analysis of variables associated with MTB detection by MODS assay

Variables	Outcome	MTB detected	MTB not detected	OR	95% CI	p value
HIV status	Negative	43 (65.2)	23 (34.8)	1.039	0.3113-3.466	1.000
	Positive	9 (64.3)	5 (35.7)			
TB treatment at time of sample collection	No	50 (83.3)	10 (16.7)	45.000	8.983-225.43	<0.0001
	Yes	2 (10.0)	18 (90.0)			
Previously treated TB	No	43 (66.2)	22 (33.8)	1.303	0.4109-4.132	0.7657
	Yes	9 (60.0)	6 (40.0)			
Rifampicin resistant	No	0	78 (100)	0.001274	2.073x10 ⁻⁵ -7.828x10 ⁻²	0.0003
	Yes	2 (100)	0			

MTB=*Mycobacterium tuberculosis*; HIV=Human Immunodeficiency Virus; TB=Tuberculosis; OR=Odds Ratio; CI = Confidence Interval

Discussion:

The findings of this study revealed that MTB was detected by MODS assay in 52 of 80 (65.0%) patients' samples previously diagnosed positive by GeneXpert assay. Of the 28 samples negative by MODS assay, 18 (64.3%) were already on anti-TB drugs while the remaining 10 (35.7%) could have been negative probably due to poor quality of samples, which has been reported to affect culture yield in previous studies (15-18). Unfortunately, in this study, the quality of samples was not assessed. Of the 20 patients who were on anti-TB drugs at the time of sample collection, 2 (10.0%) were positive for MTB by MODS assay and simultaneously resistant to isoniazid and rifampicin, indicating failure of treatment. This agrees with 10.0% treatment failure rates by MODS assay reported in Zimbabwe, although these were among patients who had been on treatment for more than one month (19), but higher than the failure rate of 2.5% reported by Alobu et al., (20) by smear microscopy among patients who have been on treatment for five months.

Interestingly, the 2 MDR-TB cases detected by MODS in this study were previously diagnosed to be rifampicin-resistant by GeneXpert test. The findings of our study show that all RR MTB are MDR, which is consistent with the WHO report (4) that RR in the absence of INH resistance is very uncommon. Furthermore, our study reveals that the 2.5% detection rate of MDR-TB by MODS assay is lower compared to 18.0% in South Africa (21) with high HIV-prevalent settings, 12.3% of DR-TB suspects in Harare, Zimbabwe (22), 8.8% of smear-positive TB patients in Addis Ababa, Ethiopia (23), and 4.1% of smear-positive PTB patients in Nigeria (12). The higher case detection in these other studies may be attributed to the larger sample size involved. Also, a study conducted in India reported 20% MDR-TB case detection by MODS assay among smear-positive samples. This is higher than 2.5% reported in our study as India is known for its large population having the second highest prevalence of MDR-TB in the world (4). Another study by Minh Ha et al., (24) which evaluated MODS assay among new TB suspects in Vietnam reported case detection of MDR-TB to be 2.7%, which is similar to the findings of 2.5% rate reported in our study.

Among the previously treated cases, there was a 13.3% case detection rate of MDR-

TB by MODS assay in our study, which is higher than the estimated 2.5% MDR-TB rates among previously treated TB cases in Nigeria (4). The higher detection rate may mean the prevalence of MDR-TB has indeed increased in this study population and can be attributed to non-compliance to and inadequate treatment. Such patients pose a great threat to public health as they may transmit these MDR strains to other people and thus, hamper the control of TB and MDR-TB. In addition, this high rate may indicate that there is a drastic increase in MDR-TB among previously treated TB cases in Nigeria between 2016 and 2017.

Lastly, in relation to HIV status, one of the two MDR-TB cases reported was HIV positive, which constituted 7.1% (1/14) of the HIV positive patients, while the other MDR-TB case was HIV negative, which formed 1.5% (1/66) of HIV-negative patients in the study, but there was no significant difference in the finding (OR = 5.00, 95% CI = 0.2934 - 85.216, $p = 0.3212$), indicating no association between HIV and MDR-TB. Similar findings have been reported in Jos and Kwara in north-central Nigeria (25,26), and Calabar in south-south Nigeria (27). However, the findings of our study should be interpreted with caution due to the small sample size because, in contrast, other studies have found a significant association between HIV status and DR-TB (28,29).

Conclusion:

MODS assay detected MDR-TB among previously treated TB patients in Lagos State at the rate of 2.5%. Hence, MODS assay is an effective, low-tech, liquid culture technique to accurately detect tuberculosis and multidrug-resistant tuberculosis simultaneously in Lagos, Nigeria.

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Conflict of interest:

Authors declare no conflict of interest

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