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Original Article

FEASIBILITY AND COST ANALYSIS OF PROGRAMMATIC IMPLEMENTATION OF MICROSCOPIC-OBSERVATION DRUG SUSCEPTIBILITY (MODS) ASSAY IN NIGERIA.

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ABSTRACT

OBJECTIVES
Detection of Multi-drug resistant tuberculosis in Nigeria still remains a challenge. We evaluated the feasibility of programmatic implementation of the Microscopic-Observation Drug Susceptibility (MODS) assay, a rapid culture and drug susceptibility testing technique for drug susceptibility testing in a low resource setting.

METHOD
In a novel laboratory setting in Nigeria, we obtained data from the market on the cost of materials necessary for MODS assay. Three routinely collected sputum specimens from 160 tuberculosis suspects were evaluated by smear microscopy while only the early morning specimen was used for MODS culture.

RESULTS
MODS assay detected *M. tuberculosis* in 97.7% (42/43) of smear positive and 6.0% (7/117) of smear negative TB suspects. There was a statistically significant advantage of a single MODS culture over 3 smear microscopies (P=0.019). The modal time from culture of specimen to detection of *M. tuberculosis* and availability of drug susceptibility result for MODS was 7days with a mean of 8.4 days (Range= 5-13 days). Culture and susceptibility result was available in 18.4% (9/49) of patients within 5days of culture. Turnaround time for smear microscopy in the centers was 3 days. Cost of processing one specimen by MODS assay in the study was USD2.65. Multi-Drug resistant tuberculosis (MDR-TB) was detected in 4.1% (2/49) while Isoniazid mono-resistance was detected in 2.0% (1/49) of the culture positive cases. All the drug resistant isolates were from re-treatment cases with a statistically significant association (P=0.005).

CONCLUSION
The MODS technique is simple, and its implementation in this novel setting was feasible. MODS can be scaled up to meet the demand for MDR-TB confirmation in XpertMTB/Rif deployed sites in Nigeria.

KEYWORDS: Tuberculosis, MDR-TB, Mycobacterium tuberculosis

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INTRODUCTION

In the last three years, tuberculosis has resulted in the death of more than 4.6 million people globally.[1] It is the second leading infectious cause of death in the world. The highest burden of disease is borne by developing countries which are also saddled with the challenges of scarcity of resources. In Nigeria, a high tuberculosis burden country with an incidence rate of 204/100,000 population, Multi-Drug Resistant tuberculosis (MDR-TB) prevalence rose from 9.5% to 13.1% from 2010 to 2012 (Average 1.8%/year).[1,2] Without concerted efforts at combating these MDR-TB strains, a consistent upsurge is likely since an untreated case of tuberculosis has the capacity of infecting 10-15 people annually.[3]

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Due to lack of facilities, tuberculosis culture and drug susceptibility testing is not routinely done in Nigeria. Current attempts to scale-up genotypic diagnosis and rifampicin mono-resistance detection with XpertMTB/RIF (Cepheid Inc., California USA) still falls short of expectations as the need for MDR-TB detection has not been met despite the high cost of equipment.[4,5] Furthermore, with the XpertMTB/RIF algorithm[6,7] several rifampicin mono-resistant patients will be inappropriately commenced on MDR-TB treatment before the results of a second test using an MDR-TB detecting platform is available. This will substantially contribute to health system cost. In a resource limited environment, the concurrent deployment of scientifically validated new simpler and cheaper non-commercial techniques may reduce cost and time before commencement of appropriate therapy. One such technique is the Microscopic Observation Drug Susceptibility (MODS) assay[8,9,10] which was endorsed by the World Health Organization (WHO) in 2010 for use in the field in resource poor settings.[11]

The objective of the study was to evaluate the feasibility and cost implication of the programmatic scale-up of the Microscopic-Observation Drug Susceptibility (MODS) assay in Nigeria as a tool for improving MDR-TB detection.

MATERIALS AND METHODS

Study setting and **Subjects selection**

The study was carried out from July to August 2012 in the Cross River State Microbiological Reference Laboratory situated at Dr. Lawrence Henshaw Memorial Hospital, Calabar, Nigeria. The Facility has a Level 3 laboratory which was newly constructed by Family Health International 360 (FHI360). Ethical Approval for the study was obtained from the Health Research Ethics Committee of the Cross River State Ministry of Health.

Patients presenting at Dr. Lawrence Henshaw Memorial Hospital, Calabar and University of Calabar Teaching hospital with symptoms of cough ⩾ 2 weeks, fever, night sweat and weight loss (WHO tuberculosis symptom screening tool) were enrolled in the study. Specimens from patients who had commenced anti-tuberculosis medication were excluded as they might be excreting dead bacilli which would not grow on culture. Critically ill patients and children less than 3 years who were not able to follow instructions to produce sputum by coughing were also excluded.

Specimen collection

Tuberculosis risk evaluation was done by the attending clinician using a structured questionnaire. Information requested included previous tuberculosis treatment, history of cough ⩾2weeks, haemoptysis, fever, weight loss, excessive night sweats, contact with a tuberculosis patient, BCG vaccination and HIV status. When available, chest X-rays and full blood counts suggestive of tuberculosis were also used to enroll patients. Three sputum specimens were collected for smear microscopy from tuberculosis suspects. The first specimen was collected at first visit and supervised (spot specimen), the second was collected at home unsupervised (early morning specimen) while a third was collected at time of submission of the early morning specimen (spot specimen). Only the early morning specimen was used for MODS assay. The specimens were transported in closed carrier boxes to the laboratory. The carrier boxes were clearly marked as biohazards and only opened in a biological safety cabinet. Where immediate processing was not feasible, specimens were preserved at 2-8°C but for not longer than 3 days from the date of expectoration.

Cost data collection and analysis

All the reagents required for MODS processing were obtained from the open market and the cost systematically calculated down to the cost of processing a single specimen. The cost of decontamination of specimen was excluded as this is common for all tuberculosis culture techniques.

Specimens processing

Microscopy

Smear microscopy of the three sputum specimens was performed by trained personnel in the laboratory. These were later retrieved from the sputum registers after the MODS results were obtained in order to avoid bias.

Specimen decontamination for culture

The specimens were decontaminated using the Sodium Hydroxide- Sodium Citrate-N-Acetyl Cysteine (NaoH-NALC) method. Briefly, 2ml of decontamination solution was added to 2ml of specimen and mixed using a vortex mixer. Samples less than 2ml were made up to 2ml with buffer solution. This was allowed to stand for 15 minutes before opening and decontamination stopped by adding 10ml of phosphate buffer. The mixture was then centrifuged at 3000g for 15 minutes, the supernatant was decanted into a jar containing 10% sodium hypochlorite disinfectant solution and the deposit retained.

Culture and susceptibility testing

Culture and sensitivity testing were carried out as described in the Microscopic Observation Drug Susceptibility (MODS) protocol.[12] This involved re-suspending the decontaminated deposit in 5.1mls of Middlebrook 7H9 medium supplemented with casitone, glycerol, OADC (Oleic acid, Albumin, Dextrose and Catalase) and antibiotics, Polymyxin B,

Amphotericin B, Nalidixic acid, Trimethoprim and Azlocillin (PANTA) to kill contaminants that may have survived the initial decontamination step. One milliliter of the re-suspension was removed and preserved at 2-8°c as backup for reprocessing if initial culture becomes contaminated. Nine hundred microlitres (900µl) of the mixture was then added to separate wells in a 24wells plate containing 100µl of middlebrook 7H9+OADC, Middlebrook7H9+OADC+p-Nitrobenzoic acid, middlebrook7H9+OADC+Isoniazid and midlebrook7H9+OADC+Rifampicin. The concentrations of the final mixture of *p*-Nitrobenzoic acid, Isoniazid and Rifampicin were 500µg/ml, 0.4µg/ml and 1µg/ml respectively. Each row was used for one specimen (Figure1). The plates were placed in a zip lock bag, incubated at 37°C and examined every alternate day from day 5 to 21 using an inverted microscope. *M. tuberculosis* was detected by its characteristic serpentine cord like growth and lack of growth in the *p*-Nitrobenzoic acid containing wells.

Quality control (QC)

Positive controls using Isoniazid and Rifampicin sensitive and MDR strains of *M. tuberculosis* were run on separate plates after the test specimen cultures had been secured in the zip lock bag to prevent cross contamination. This was done by harvesting colonies of *M. tuberculosis* from Lowenstein-Jensen (LJ) cultures using a sterile loop into a sterile tube containing 100µl water-tween-80 solution and six sterile glass beads. The tubes were vortexed for 2minutes and allowed to stand for 5minutes then vortexed again for 20seconds after adding 3ml of water-tween-80 and allowed to stand for 30minutes. The supernatant was transferred to another tube and the turbidity adjusted to 0.5 McFarland turbidity equivalents. During culture, 5µl of the 0.5 McFarland equivalent suspensions were added to 5ml of supplemented Middlebrook 7H9 broth. The preparation was cultured in 24 wells tissue culture plates with each column containing two drug free wells and one well containing 0.4µg /ml Isoniazid and the other, 1µg/ml Rifampicin. Due to the unavailability of standard QC strains, the strains used were obtained from external quality assessment(EQA) isolates whose susceptibility pattern had previously been validated by HAIN GenoType MTBDRplus assay (HAIN Lifescience Nerhen, Germany). The isolates were subsequently passaged in LJ slants. Culture medium mixtures without specimens were also added to one row of wells in each plate as negative control.

Interpretation of results

Results were considered valid only if the susceptible and MDR controls passed QC. An entire plate was considered cross contaminated and discarded if *M. tuberculosis* was seen in the negative control wells.

Detection

Cultures were regarded as positive for *M. tuberculosis* if $\geqslant$ 2colony forming units (cfu) were seen in the drug free well and negative if none is seen. Wells with only 1cfu were regarded as indeterminate while wells with bacterial or fungal overgrowth were reported as contaminated. The preserved back-up specimen was used to reprocess indeterminate and contaminated specimens.

Susceptibility testing

The drug containing wells were read and reported on the same day as the drug free (detection) wells. Isolates were regarded as susceptible to either Rifampicin or Isoniazid if no cfu was seen in the drug containing well, and resistant if $\geqslant$ 2cfu are seen. Drug containing wells with 1cfu were regarded as indeterminate and reprocessed using the 1ml back-up aliquot of specimen following a re-decontamination step. Contaminated specimens were also reprocessed. Any growth observed in the drug containing wells after the drug free wells have been read was disregarded as described in the MODS protocol[12]. These breakthrough growths may occur due to deterioration of the drug overtime.

Data analysis

All categorical data are presented as % (n/sample size). Chi square (χ^2) and Pearson's correlation were used to evaluate statistical significance. Confidence interval was set 95% with *p*-values < 0.05 considered significant. All data were analyzed using SPSS statistical software version 19.0(SPSS Inc. Chicago, USA).

RESULTS

One hundred and sixty patients were enrolled into the study. This comprised 53.1% (85/160) males and 46.9% (75/160) females. The Median age was 35years (IQR 27-48). Fourteen patients were retreatment cases (failure of treatment, relapse of disease or return after default) while 146 were new tuberculosis suspects. Out of this 160 patients enrolled, *M. tuberculosis* was detected in 50(31.2%) by either smear microscopy or MODS assay. Smear microscopy from the combined 3 specimens detected tuberculosis in 26.9% (43/160) of the patients while *M. tuberculosis* was cultured in 30.6% (49/160) patients by the MODS assay. These comprised of 97.7% (42/43) of smear positive specimens and 6.0% (7/117) of smear negative patients (Table1). One smear positive specimen was culture negative. Also, one smear negative specimen was culture contaminated even after reprocessing the back-up specimen. There was a statistically significant advantage of culture of a single early morning specimen by MODS over smear microscopy using 3 specimens (P=0.019). The appearance of the *M. tuberculosis* as seen with a 100xmagnification in successive days is as shown in figure 1.

The modal time from culture of specimen to detection of *M. tuberculosis* and availability of drug susceptibility result (time to detection) for MODS was 7days with a mean of 8.4 (SD2.5) days and range of 5 to 13 days (Table 2). Culture and susceptibility result was available in 18.4% (9/49) patients within 5days of culture. Turnaround time for smear microscopy in the centers was 3 days.

Among the 50 tuberculosis positive patients, new suspects accounted for 31.5% (46/146) while 28.6% (4/14) were from retreatment cases. MDR-TB was detected in 4.1% (2/49) while Isoniazid mono-resistance was detected in 2.0% (1/49) of the culture positive cases. All the drug resistant isolates were from re-treatment cases and the association was statistically significant (P=0.005).

Cost analysis of processing one sample using the MODS technique in Calabar gave an average cost of five hundred and twenty nine naira sixty kobo (₦529.60) (Table 3). At an exchange rate of one hundred and sixty naira (₦200.00) to one United States dollar (USD), this is about USD2.65.

Discussion

Mycobacterium tuberculosis culture and drug susceptibility testing have remained challenging to most developing countries due to lack of facilities. In order to surmount this challenge, the introduction of scientifically validated rapid techniques such as MODS is necessary.

Detection of *M. tuberculosis* by MODS assay using a single early morning specimen was significantly higher than the regular smear microscopy using three specimens in this study. Moore et al had earlier shown the superiority of MODS over smear microscopy and the absence of any incremental benefit of multiple MODS cultures.[8] The seven smear negative patients would have continued the spread of the disease if their tuberculosis had not been detected with the MODS technique. Only one smear positive culture negative case was seen in this study. The observation of smear positive culture negative specimens may have been due to infection with non-tuberculosis mycobacteria, laboratory error, contamination of water and stains with environmental mycobacteria or prolonged decontamination of culture specimen. In this study, ingestion of anti-tuberculosis medication which could also be a cause was avoided by exclusion of this category of patients. It was possible to reduce loss of specimens to bacterial and fungal contamination of MODS cultures by reprocessing the preserved backup specimen. In mild bacterial or fungal contamination however, serpentine cords of *M. tuberculosis* complex could still be clearly delineated.

The time to detection and time to availability of susceptibility result of 5-13 days and mean of approximately 8.4(SD2.5) days in this study for culture and drug susceptibility testing clearly confirms that MODS assay is one of the most rapid techniques of drug susceptibility testing (DST). This turnaround time (TAT) compares very well with other studies in Peru,[8] Thailand,[10] and Ethiopia[13] which compared MODS head to head with other culture techniques with MODS also showing better sensitivity and specificity. A head to head evaluation was not feasible in this study due to unavailability of materials and skilled manpower for other culture techniques for a blinded comparison. Although sputum was used in this study, the same quick turnaround time had been reported for cerebrospinal fluid (CSF) and gastric aspirate from children.[10,14,15] The major factors which affect growth in MODS are the quality of the OADC and the incubation temperature. *M. tuberculosis* grows at temperatures of 35±2 ^{O}C.[16] The near absence of electricity in the study facility during weekends may have been responsible for the wide range of the turnaround time in this study. Another new technique with a short turnaround time, the Thin Layer Agar (TLA) microcolony detection technique [17] although had the advantage of using a conventional light microscope, will however per sample consume four times the amount of OADC which is the most expensive consumable in both techniques. Other new *M. tuberculosis* DST techniques such as nitrate reductase assay (NRA)[127] and broth micro-dilution methods (BMM)[18,19] still depend on pure cultures of *M. tuberculosis* on LJ which takes time to achieve. The MODS technique detects *M. tuberculosis* and MDR-TB directly from patient's specimens making it faster to obtain results sometime within 5 days which even in comparison with smear microscopy is relatively short with the added advantage of improved detection and DST.

The MODS technique is relatively safer than all other tuberculosis cultures techniques since the culture plates are encased in a zip-lock bag and never opened throughout the procedure. The low culture cross contamination in MODS reported elsewhere[20] may reflect a low aerosolisation rate of the liquid culture preparation during sample inoculation. The inherent danger of infection during MODS processing may therefore not be different from that of microscopy smear preparation. A study had shown that although tuberculosis laboratory workers are at higher risk of infection than the general population, this risk is least during microscopy smear preparation and highest during cultures manipulation towards *M. tuberculosis* identification and DST.[21]

A cost of USD3.3 makes MODS relatively cheap in Nigeria. A cost of USD2 was recorded in Peru where

USD6 and USD52 were also reported for LJ and automated MGIT respectively.[10] MODS is therefore relatively cheap. The slightly higher cost in Nigeria was mainly due to the cost of importation of consumables which will likely reduce when materials are available in-country with MODS scale-up. Recently, a TB MODS kit (Hardy Diagnostics Inc, USA) which is more convenient and contains all the reagents and 24 well plates required for MODS has been produced. Centers already doing MGIT culture will only require to purchase an inverted microscope and 24 well plates while those doing LJ culture will require an inverted microscope, 24 well plates and OADC MGIT supplement. The cost of a Euromax inverted light microscope (Euromax, Holland) as purchased from a supplier in Nigeria was about Six Hundred and Fifty Thousand Naira (₦650,000.00).

Conflict of Interest: We declare that we have no conflict of interest.

Acknowledgment: We wish to acknowledge all the staff of the Cross River state Microbiological Reference Laboratory, Dr. Lawrence Henshaw Memorial Hospital for assistance in specimen transportation and microscopy smear preparation.

Table1. Comparison of Sensitivity of a single MODS assay with smear microscopy.

	Smear Microscopy n=160		
	Negative	Positive	Total
MODS culture			
Negative	109(93.1)	1(2.3)	110(68.8)
Positive	7(6.0)	42(97.7)	49(30.6)
Contaminated	1(0.9)	0(0.0)	1(0.6)
Total	117(73.1)	43(26.9)	160(100.0)

X^2=124.4; df=2; *P*=0.019

Table 2. Time of detection of M. tuberculosis and availability of susceptibility result after MODS culture of specimen.

Day after culture	Culture positive No. (%) n=49
5	9(18.4)
7	17(34.7)
9	4(8.2)
10	6(12.3)
11	6(12.3)
12	4(8.2)
13	3(6.1)

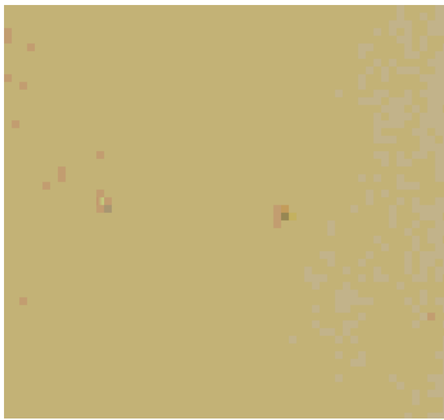
Mean= 8.4(SD2.5) Days

Table 3. Cost estimate per sample of running MODS in Nigeria for centers with tuberculosis culture facilities

1	ITEM	DESCRIPTION	COST/ PACK ₦	COST/ SAMPLE ₦	COMMENTS
2	OADC 6x15ml/pack	15 ml =20samples+4neg contros+2positive controls +working drug solution preparation	25,000	208.3	BD MGIT supplement contains OADC/PANTA at ₦50,000
3	PANTA 6x15ml/pack	1 vial diluted to 3ml=30 samples	25,000	138.8	
4	24 well plates 50/pack	5 samples +1negative control/ plate + allowance for drugs preparation and positive control per run	30,000	150.0	
5	1000µl Aerosol barrier tips 96/pack	1per sample	2000	20.0	
6	Middlebrook 7H9 500g	5.9g=900ml=200samples 500g=16949samples	35,000	2.1	
7	Casitone 500g	1.25=900ml=200samples 500g=80,000sampls	15,000	0.2	
8	Glycerol 1 litre	3.1ml=900ml=200samples 1000ml=64516 samples	10,000	0.2	
9	200ul aerosol barrier tips 100/pack	About 10/per run including controls and wastage. Assuming 20 samples/run	2,000	10.0	Cost will reduce if larger number are run at once
10	Antibiotics	BD SIRE pack contains 1g each of SIRE and 6 vials OADC assuming half the price is SIRE 1g = 625000samples	25,000	0.04	Price will be less if sigma powder can be obtained or RIF/INH alone can be purchased
	TOTAL			**529.6**	

Note: all figures rounded upwards to the nearest decimal

Figure 1.Inverted microscopy appearance of M. tuberculosis in MODS (100x magnification)

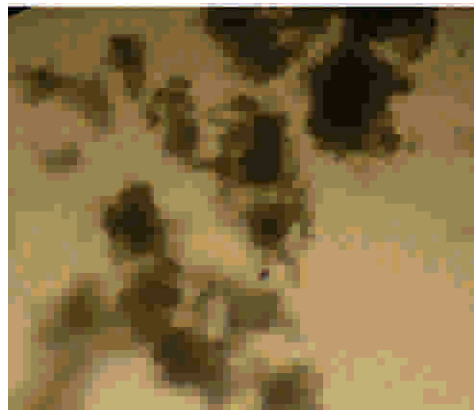


Day 5

Day 7

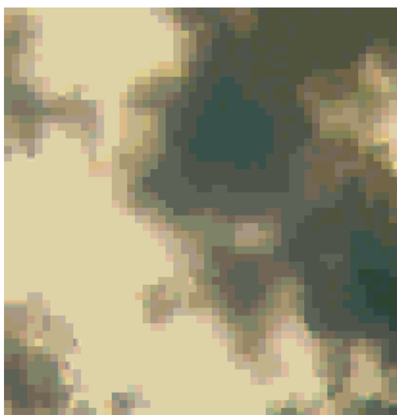
Day 9

Day 11

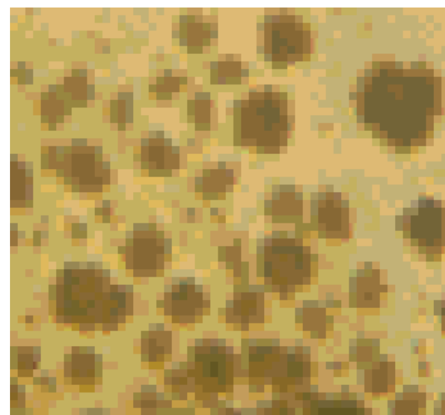


Day 13

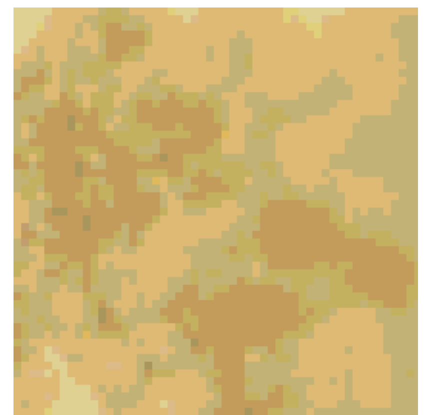
Day 15



Day 21



Fungal contamination



M.TB +fungal contamination

M.TB =Mycobacterium tuberculosis

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