COMPARISON OF LIQUID BACTEC MGIT 960 AND SOLID LOWENSTEIN-JENSEN CULTURE FOR DETECTION OF *Mycobacterium tuberculosis* IN CLINICAL SAMPLES AT ANAMBRA STATE, NIGERIA.

Authors:
Uzoewulu, G. N[1]., Goyal, M.[2]., Ibeh, N. I.[3], Lawson, J. O. [4]

Author Affiliations:
[1] Medical Microbiology, Department of Medical Microbiology and Parasitology, FBCS Nnamdi Azikiwe University Awka. Nigeria
[2] Micobiology University of Hertfordshire, Hatfield, England, UK .
[3] Medical Microbiology, Department of Microbiology, University of Benin Edo state.
[4]MBBS. Zankli Medical Centre ,Utako Abuja.

Corresponding author:
Uzoewulu, Gertrude. N[1]
Department of Medical Microbiology and Parasitology,
Nnamdi Azikiwe University Awka
gertrudeuzor@gmail.com

xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

ABSTRACT

Background: Pulmonary tuberculosis is a major public health problem. Rapid and accurate detection of *Mycobacterium tuberculosis* (*M.tb*) and its drug resistance is fundamental for effective control.

Aim: This study evaluated the diagnostic performance, agreement and drug susceptibility testing (DST) using MGIT 960 compared with LJ culture of 530 clinical samples for detection of *Mycobacterium tuberculosis*.

Materials and Methods: A cross-sectional study was conducted at NAUTH, GHO and COOUTH in Anambra state. Sputum samples of suspected TB participants were collected and cultured using liquid MGIT-960 and solid LJ media. Drug sensitivity testing of positive *Mycobacterium tuberculosis* isolates against Streptomycin, Isoniazid, Rifampicin and Ethambutol, was performed. Data was analyzed using Epi-info.

Results: MGIT-960 detected 428/530 (80.7%) positive *M.tb*, 62 (11.7%) negatives, 36 (6.8%) contaminated, and 4 (0.8%) NTM with detection time 11 (6) days compared to LJ that detected

411/530 (77.5%) positive *M.tb*, 92 (17.4%) negatives, 22 (4.2%) contaminated and 5 (0.9%) NTM with detection time 30 (11) days, 456(86.0%)were concordant positive *M.tb* isolates. Assuming LJ as the gold standard, MGIT 960 showed a sensitivity of 94.6%, specificity of 63.2%, positive predictive value of 90.9%, negative predictive value of 75.3%, and accuracy of 86.0%. DST was performed on 389 culture-positive samples. Concordance of resistance between MGIT and LJ varied by drug, Streptomycin 73.8%, Isoniazid 81.8%, Rifampicin 71.4%, and Ethambutol 71.1%. Total prevalence of MDR-TB 20.8%, Concordance MDR-TB for both method 35.8% and discordant MDR-TB 64.2%, MDR-TB 6.4% by MGIT-960 and 6.9% by LJ while Susceptibility was high for all drugs.

Conclusion: MGIT 960 demonstrated high diagnostic yield for *M.tb*, and identified additional resistant isolates not detected by solid LJ medium. Combined use of both methods improves accurate diagnosis of TB, drug resistance, reduce contamination and guide effective treatment strategies.

Keywords*: Mycobacterium tuberculosis*, Liquid BACTEC MGIT 960 system, Solid Lowenstein-Jensen medium, Accuracy, Drug susceptibility testing.

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (*M.tb*) remains a significant global health burden, particularly in low- and middle-income countries causing morbidity and mortality, it is estimated to have caused disease in 10.6 million new cases and 1.3 million deaths were reported in 2022[1]. Despite global efforts toward TB control, case finding, HIV, multidrug-resistant TB (MDR-TB) remains a significant challenge in diagnosis, treatment and public health surveillance[2]. The main problem is that, clinical specimen often contains very few mycobacterium bacilli, in which the slow growth rate limits detection using conventional method. Multidrug-resistant TB is the most challenging forms of resistance because treatment with the second-line drugs is more toxic, more expensive and must be administered for a longer period of time than standard first-line drug therapy[1,3] . World Health Organization**,** reported Nigeria among one of the 30 high TB, multidrug-resistant TB (MDR-TB) and TB/HIV co-infection burden countries[1]. The estimated prevalence rate of multidrug-resistant TB in Nigeria is 4.3% of new tuberculosis cases and 25% of previously treated TB cases, though the rate varies according to countries or regions, based on various susceptibility testing methods available especially for first-line anti-TB drugs[2,3]. A study within Southeast Nigeria, reported 8% MDR-TB in cultured sputum of 180 patients[4]. In developed countries, TB diagnosis depends on culture of sputum in liquid and solid

media while in developing countries diagnosis depends only on microscopic examination of sputum stained for acid-fast bacilli (AFB), though used as international standard for TB diagnosis but the emergence of the HIV and multidrug-resistant TB epidemics uncovered its limitations since HIV-infected TB patients frequently have negative sputum smears[5].

Hence, timely diagnosis and reliable drug susceptibility testing (DST) is crucial for guiding appropriate treatment and limit the spread of resistant strains particularly in region like Southeast Nigeria where diagnostic delays and treatment failures contribute to ongoing transmission[4,6]. Rapid molecular assays like Gene Xpert MTB/RIF have improved early detection but the recommended method for DST remain the gold standard for definitive diagnosis of *M. tuberculosis* performed on solid Lowenstein-Jensen (LJ) culture, an egg-based medium that supports *M. tuberculosis* growth, allows for visual observation of colony morphology, isolation of viable organisms, phenotypic drug testing and ease of preparation[7]. However, limitation due to slow growth of colonies for result interpretation makes the agar proportion LJ method take long incubation time 4–8 weeks, thus causes delay DST results that can hinder timely initiation of appropriate therapy, its lower sensitivity especially in paucibacillary or early-stage cases and contamination risks if media are improperly prepared or stored, influence their utility in resource-limited settings[7,8]. While solid culture methods are more common due to cost constraints, there is growing interest in transitioning to liquid culture for faster and more accurate diagnosis. WHO recommended the use of liquid culture systems like Mycobacteria Growth Indicator Tube 960 (MGIT 960 system) media for TB diagnosis and DST where feasible, especially in reference or tertiary laboratories, though most secondary and tertiary hospitals rely on ZN microscopy [9,10]. The BACTEC MGIT 960 system has been evaluated for the detection of resistance to first-line anti-TB drugs against the standard proportion solid LJ method and has shown good concordance, a sensitivity of 100% for Rifampicin (RIF) and Isoniazid (INH), specificity ranging from 89% to 100% [11,12].

Nevertheless, comparing liquid MGIT 960 and solid LJ culture methods for DST of *Mycobacterium tuberculosis* is crucial to guide diagnostic optimization, inform national TB control strategies on whether to replace one method for the other or to run the two in parallel to improve patient outcomes especially in resource-limited settings like Anambra state, Nigeria. This study compared diagnostic yield, time to detection, contamination rate, and DST accuracy using liquid MGIT 960 and solid LJ culture media for detection of *Mycobacterium tuberculosis* from sputum specimen at secondary and tertiary health care facilities in Anambra state.

MATERIAL AND METHODS

Study design: This was a cross-sectional study, conducted at Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, General Hospital Onitsha (GHO) and Chukwuemeka Odimegwu Ojukwu University Teaching Hospital (COOUTH)

Awka in Anambra state, Nigeria. The aim was to compare the isolation rate of *Mycobacterium tuberculosis* from sputum and the level of agreement of DST to first-line anti-TB drugs using BACTEC-MGIT-960 and solid LJ culture methods

Ethical approval: The study was ethically cleared by Institutional Ethics Committee of Nnamdi Azikiwe University Teaching Hospital Nnewi (reference number: NAUTH/CS/66/VOL.3/35). Information on the purpose of the study was provided to all the participants. The confidentiality of data and freedom to withdraw from the study at any time were assured to participants. All individual participants provided written consent to participate, following the procedures approved by the ethics committees. Specimen were collected after getting written informed consent from all the study participants.

Specimen collection: A total of 530 sputum specimen of 318 males and 212 females within the age of 2 to 80 years old were collected from both new suspected TB cases and those who had failed first-line anti-TB treatment were enrolled. Patients unable to produce sputum or did not provide informed consent to participate, smear-negative cases and specimen with poor quality sputum or saliva or insufficient quantity for both cultures were excluded.

Based on the assumption that solid Lowenstein-Jensen culture is the gold standard allows calculation of diagnostic accuracy, the sample sizes were calculated to assess the prevalence of drug resistance in new and retreatment patients using Lawson method, assuming that 10% of newly diagnosed patients and 40% of the patients failing first-line treatment would be resistant to one or more of the first-line drugs tested[13]. Concordance, discordance, and diagnostic accuracy of MGIT 960 were evaluated, accounting for contaminated and non-tuberculosis mycobacteria (NTM) results.

Sample processing: Three sputum specimen were collected from each patient with their bio-data and submitted for direct Ziehl Neelsen (ZN) smear microscopy[4] . All muco-purulent sputum specimen were sent for culture and DST at Zankli Medical Research Laboratory (ZMRL) at Abuja within seven days of collection. Samples were refrigerated prior to shipment and maintained in cold-chain during shipment with ice packs. At ZMRL, each specimen was decontaminated using Petroff's method (4% NAOH) and inoculated onto Lowenstein-Jensen medium and on an automated MGIT-960 system[13]. Two hundred microliters of decontaminated sputum were inoculated on each of two glycerol enriched solid LJ slopes and 500µl in MGIT-960 7H9 bottles. The MGIT 960 was enriched with supplement (OADC) and antibiotics (PANTA) prior to inoculation. Cultures were incubated at 37°C for up to 8 weeks on LJ and 42 days in MGIT 960 media. Solid LJ cultures were checked weekly and MGIT 960 cultures were monitored continuously through the automated system as direction from manufacturers[12]. Isolates were confirmed as *M.tb* by ZN smear, also isolates were observed by serpentine cords typical of *M.tb*, with the nitrate reduction test and temperature liability of the catalase test[4,13]. These were the only available tests in the

laboratory at the time of the study. The time for *M.tb* detection on solid LJ and MGIT 960 media were recorded. MGIT time to detection was defined as the interval between inoculation and the bottle being flagged as positive by the machine while LJ time to detection was defined as the time between inoculation and the culture being considered positive by naked eye reading[8].

Antibiotic susceptibility testing: Drug sensitivity testing for Streptomycin (STR), Isoniazid (INH), Rifampicin (RIF) and Ethambutol (EMB) was performed on positive *M.tb* isolates using the proportion method on solid LJ medium according to the Clinical and Laboratory Standard Institute (CLSI) procedures and recommended critical concentrations[14] and in MGIT 960 bottles according to the manufacturers recommendations[4]. Drug concentrations in LJ were 8.0 µg/ml for STR, 0.2 µg/ml for INH, 40 µg/ml for RIF and 2.0 µg/ml for EMB. While MGIT 960 were 1.0 µg/ml for STR, 0.1 µg/ml for INH, 1.0 µg/ml for RIF and 1.0 µg/ml for EMB[4,13]. Concisely, 1.0 McFarland standard isolate suspension was serially diluted 10-fold, from 10^{-1} to 10^{-4}, in sterile distilled water, inoculated onto L-J slants with and without drugs, and incubated at 37°C[15]. Results were read at 28 days and up to 42 days, depending on control growth. An isolate was considered resistant to a given drug when growth of 1% or more above the control was observed in drug-containing medium[8]. A susceptible *M. tuberculosis* H37Rv reference strain and a known ZMRL determined MDR -*M. tuberculosis* isolates (resistant to STR, INH, RIF, and EMB), were used for quality control[13]. Zankli Medical Research Laboratory undergoes external quality assurance of DST for first-line anti-TB drugs through the WHO Supranational reference laboratory network (SRLN) for MGIT 960 and solid LJ media. The external quality assurance (EQA) of the laboratory results was 100% for STR, INH, RIF and EMB when using MGIT 960; 100% for STR, RIF and EMB and 80% for INH when using solid LJ media[4,13].

Data analysis: All data were analyzed using Epi-info, proportions were compared using Chi squared tests. The degree agreement of the DST results obtained using both methods were compared using Cohen's Kappa (K) statistics [8,16]

RESULTS

Socio-demographic characteristics of the study participants:

A total of 530 sputum samples were collected from suspected TB participants, aged 2 to 80 years with a mean age of 34 ± 15 years or a median of 32 years. They were examined using two cultured methods, Liquid BACTEC MGIT 960 and solid Lowenstein-Jensen media. The majority of TB cases were within the age group 25–44years (52.8%), indicating that tuberculosis predominantly affected the economically active population. The male-to-female ratio was 1·5:1, with 318 (60%) males and 212 (40%) females, suggesting that males were more affected than females, possibly due to greater exposure risks and health-seeking behavior differences (Table 1).

Culture results on liquid MGIT-960 and Solid LJ Media

Of the 530 sputum samples cultured MGIT-960 system detected 428 (80.7%) positive *M.tb*, 62 (11.7%) negatives, 36 (6.8%) contaminated, and 4 (0.8%) NTM with detection time of 11 (6) days. While the solid LJ medium detected 411 (77.5%) positive *M.tb*, 92 (17.4%) negatives, 22 (4.2%) contaminated, and 5 (0.9%) NTM, with detection time of 30 (11) days. MGIT 960 detected 17 (3.2%) more positive *M.tb* cases and 14(2.6%) more contaminants compared to solid LJ alone. Solid LJ detected 30 (5.7%) more negative *M.tb* cases and less contaminated compared to MGIT 960 alone. The culture diagnostic yield of both methods showed that, of 530 sputum samples, 456 (86.0%) were positive for *M. tuberculosis*, 57 (10.8%) negatives, 14 (2.6%) contaminated and 3 (0.6%) NTM. Using both methods together increased detection of 28 (5.3%) *M.tb* by MGIT-960 alone and detection of 45(8.5%) *M.tb* by solid LJ alone. Also combined culture detected 57(10.8%) negative *M.tb,* minimizing false negative results, 14(2.6%) Contaminants restraining Contamination rate (Table 2a).

.**Table 1: AGE AND SEX DISTRIBUTION OF STUDIED PARTICIPANTS**

AGE GROUP	**GENDER**		
Years	**Male (%)**	**Female (%)**	**Total (%)**
0- 24	22 (4.1%)	18(3.4%)	40(7.5%)
25- 44	190 (35.8%)	90(17.0%)	280(52.8%)
45-64	60 (11.3%)	60(11.3%)	120(22.6%)
65- 80	46(8.8%)	44 (8.3%)	90(17.0%)
Total	318 (60%)	212 (40%)	530(100%)

Table 2a: **FREQENCY OF ISOLATION OF *Mycobacterium tuberculosis*** ON **LIQUID MGIT-960 AND SOLID LJ**

Culture result	MGIT 960 %	LJ %	MGIT960/LJ%
Positive *M.tb*	428 (80.7%)	411(77.5%)	456 (86.0%)
Negative *M.tb*	62(11.7%)	92(17.4%)	57(10.8%)
Contaminant	36(6.8%)	22(4.2%)	14(2.6%)
NTM	4(0.8%)	5 (0.9%)	3(0.6%)
Total	530	530	530 (100%)

Comparison of BACTEC-MGIT-960 and LJ, ** p = 0.057. *($\chi^2 = 3.63$, $p = 0.057$)*

M.tb-Mycobacterium tuberculosis. NTM-Non tuberculosis mycobacteria. MGIT-960-Mycobacteria Growth Indicator Tube-960. LJ- Lowenstein–Jensen. %-Percentage.

Culture results of BACTEC MGIT 960 and solid LJ methods by individual hospitals:

Out of 240 (45.3%) sputum samples from NAUTH, MGIT 960 detected 183 (34.5%) positive *M.tb* ,37 (7.0%) negatives, 2(0.4%)NTM and 18(3.4%) contaminants while solid LJ detected 176 (33.2%) positive *M.tb* , 50(9.4%) negative, 3(0.6%) NTM and 11(2.1%) contaminants. GHO identified 200 (37.7%) sputum samples cultured, MGIT 960 detected 160(30.2%) positive *M.tb*, 23(4.3%) negatives, 2(0.4%) NTM and 15(2.8%) contaminants while solid LJ detected 156(29.4%) positive *M.tb*, 35 (6.6%) negatives, 2(0.4%) NTM and 7 (1.3%) contaminants. COOUTH identified 90(17.0%) sputum samples, MGIT 960 detected 85(16.0%) positive *M.tb*, 2(0.4%) negatives, 0(0%) NTM and 3(0.6%) contaminants, whereas solid LJ detected 79(14.9*%)* positive *M.tb* , 7(1.3%) negatives, 0(0%) NTM and 4 (0.8%) contaminants, (Table 2b).

Concordant and discordant positive *Mycobacterium tuberculosis* isolates by MGIT960 and LJ method

In comparison of culture results, only patients with positive results for both culture methods were included. The BACTEC-MGIT-960 and solid LJ culture results were concordant in 456 (86.0%) samples, with substantial agreement between the methods (Kappa = 0.70, Standard error = 0.046)[16], confirming that both methods perform consistently under standardized laboratory conditions. The distribution of agreement included both cultures being positive for *M. tuberculosis* in 389 samples, 50 negatives, 13 contaminated and 4 NTM samples. The high proportion of concordant positive cultures reflects the overall reliability and comparable efficiency of both MGIT-960 systems and LJ media in detecting *M. tuberculosis*. In comparison, discordant or discrepant results showed 74 (14.0%) samples for both two cultures method.

Among these, 12 samples were positive on LJ but negative on MGIT-960, while 30samples were negative on LJ but positive on MGIT-960. 10 samples were positive on LJ but contaminated on MGIT 960 and 12 samples were negative on LJ but contaminated on MGIT 960. Also 9 were contaminated on LJ but positive on MGIT 960 and one NTM sample on LJ but contaminated on MGIT 960. The largest proportion of additional 30 cases were LJ culture negative and BACTEC-MGIT-960 culture positive. This high difference in LJ-negative/ MGIT- 960 positive cases demonstrated the greater sensitivity of the MGIT-960 system and both methods resulted in an additional yield of cultures, likely due to the enhanced nutrient composition and automated growth detection in the liquid medium. Conversely, the few 12 LJ-positive/MGIT-negative samples may reflect, slow-growing or low bacterial load specimens not detected within the MGIT incubation period. Similarly, a total of 58 cultures were contaminated, 22 samples by LJ method and 36 samples by MGIT-960 method while only 13 samples were contaminated by both methods. (Table 3).

Table 2b. CULTURE RESULT OF BACTEC MGIT 960 AND LJ MEDIA BY STUDIED SITES

	Hospitals							
	NAUTH		GHO		COOUTH		Total %	
Result	MGIT960 %	LJ %	MGIT960%	LJ %	MGIT960%	LJ%	TMGIT960%	TLJ%
Positive	183(34.5%),	176(33.2%)	160(30.2%),	156(29.4%)	85(16.0%),	79(14.9%)	428(80.7%)	411(77.5%).
Negative	37 (7.0%),	50(9.4%)	23(4.3%)	35(6.6%)	2 (0.4%)	7(1.3%)	62(11.7%)	92(17.4%).
NTM	2 (0.4%)	3(0.6%)	2(0.4%)	2 (0.4%)	0 (0%)	0(0%)	4(0.8%)	5 (0.9%).
Cont	18(3.4%)	11(2.1%)	15(2.8%)	7(1.3%)	3 (0.6%)	4(0.8%)	36 (6.8%)	22(4.2%).
Total	240(45.3%)	240 (45.3%)	200(37.7%)	200(37.7%)	90(17.0%)	90(17.0%)	530 (100%)	530(100%)

NTM=Non-tuberculosis mycobacteria. Cont=contaminant. T=Total *

Table 3a: CONCORDANT AND DISCORDANT POSITIVE *Mycobacterium tuberculosis* ISOLATES BY MGIT 960 AND SOLID LJ CULTURE METHODS

	LJ				
MGIT 960	**Positive**	**Negative**	**Contaminated**	**NTM**	**Total**
Positive.	389	30	9	0	428
Negative.	12	50	0	0	62
Contaminated.	10	12	13	1	36
NTM.	0	0	0	4	4
Total	411	92	22	5	530

Concordant results (agreement)**:** 456/530 (86.0%). K = 0.70. Discordant results (disagreement)**:** 74/530 (14.0%)

Table 3b: COMPARISON OF CONCORDANT AND DISCORDAND RESULTS

Pattern of Concordant	**Results**	**%**	**Pattern of discordant**	**Result**	**%**	**Total sample**
Both positive *M.tb*	389	73.4	MGIT positive/LJ negative	30	5.7	
Both negative *M.tb*	50	9.4	MGIT negative/LJ positive	12	2.3	
Both contaminated	13	2.9	MGIT positive/LJ contaminated	9	1.7	
Both NTM	4	0.7	MGIT contaminated/LJ positive	10	1.9	
--	--	--	MGIT contaminated/LJ negative	12	2.3	
--	--	--	MGIT contaminated/LJ NTM	1	0.1	
Total concordant	456	86.0%	Total discordant	74	14.0%	530

Diagnostic performance of liquid MGIT 960 and solid LJ culture:

The diagnostic accuracy of liquid BACTEC MGIT 960 culture was compared to solid Löwenstein–Jensen (LJ) culture as the gold standard, BACTEC MGIT 960 demonstrated a sensitivity of 94.6% *(*95% CI: 91.7–96.3*),* but the two methods were complementary rather than interchangeable *($\chi^2 = 3.63$, $p = 0.057$)*. The specificity was 63.2% *(*95% CI: 58.3–75.1*)* and overall diagnostic accuracy of 86.0% *(*95% CI: 85.4–91.0*)*. The positive predictive value was 90.9% (95%CL:87.8-93.3) and negative predictive value 75.3% (95%CL:68.6-84.7) (Table 4).

Table 4: **DIAGNOSTIC PERFORMANCE OF LIQUID MGIT 960 AND SOLID LJ CULTURE WITH 95% CONFIDENCE INTERVAL (CL)**

Measure	Formula	Calculation	Estimate	%	95%CL
Sensitivity	TP/TP + FN	389/411	0.946	94.6%	91.7-96.3
Specificity	TN/TN + FP	67/106	0.632	63.2%	58.3-75.1
PPV	TP/TP + FP	389/428	0.909	90.9%	87.8-93.3
NPV	TN/TN + FN	67/89	0.753	75.3%	68.6-84.7
Accuracy	TP + TN/TP + FP + FN + TN	456 /530	0.860	86.0%	85.4-91.0

($\chi^2 = 3.63,, p = 0.057$) .PPV- positive predictive value; NPV- negative predictive value; CI, confidence interval., TP- True positive, TN- True negative, FP- False positive. FN- False negative

Drug Susceptibilty Test

DST was successfully performed on 389 concordant culture-positive *M. tuberculosis* isolates recovered from both MGIT-960 and LJ medium. The DST results demonstrated variable levels of resistance to first-line anti-tuberculosis drugs, including Streptomycin, Isoniazid, Rifampicin, and Ethambutol. Patterns of drug resistance showed that, resistance was most frequently observed in Ethambutol (32.9%), followed by Isoniazid (28.3%), Streptomycin (27.5%), and Rifampicin (14.4%). The highest proportion of resistance detected in both methods was in Ethambutol (23.4%). Mono-resistance detected only by one method varied slightly, and MGIT-960 detected more unique resistant isolates for Streptomycin 20(5.1%) and Rifampicin 11(2.8%) than LJ culture while LJ detected more unique resistant isolates for Isoniazid 15(3.9%) and Ethambutol 20 (5.1%) than MGIT 960. Among the 389 concordant *M. tuberculosis* isolates, 81 isolates were classified multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least Isoniazid and Rifampicin, highlighted a total prevalence rate of 20.8%. Of these, 29 (7.5%) were MDR-TB by both MGIT-960 and LJ methods, 27 (6.9%) MDR-TB by LJ culture and 25 (6.4%) by MGIT-960 only. Specific concordance for individual drugs showed, 81.8% Isoniazid, 73.8% Streptomycin, 71.4% Rifampicin and 71.1% Ethambutol resistant isolates detected by both MGIT960 and LJ cultures. Ethambutol showed slightly lower concordance compared with Isoniazid and concordance varies by drug. Concordance of MDR-TB detected 29/81 (35.8%), while discordant of MDR-TB detected the remaining 52/81(64.2%) by only one method (27 LJ, or 25 MGIT 960 only). The remaining 308 (79.2%) isolates were fully susceptible concordance to all four first-line drugs and Cohen's Kappa indicated good agreement (K = 0.70) as summarized on (Table 5).

Table 5. **DST AGREEMENT OF CONCORDANT POSITIVE SAMPLES ON LIQUID MGIT 960 AND SOLID LJ METHODS.**

	RESISTANT PATTERN						
Drugs	RLJ (%)	RMGIT960 (%)	RLJ/MGIT960 (%)	TRLJ/MGIT960 (%)	CRLJMGIT (%)	TSLJMGIT (%)	TTESTED
STR	8(2.1%)	20(5.1%)	79 (20.3%)	107(27.5%)	73.8	282 (72.5%)	389
INH	15(3.9%)	5(1.3%)	90 (23.1%)	110(28.3%)	81.8	279(71.7%)	389
RIF	5 (1.3%)	11(2.8%)	40 (10.3%)	56(14.4%)	71.4	333(85.6%)	389
EMB	20 (5.1%)	17(4.4%)	91(23.4%)	128(32.9%)	71.1	261(67.1%)	389
MDR-TB	27(6.9%)	25(6.4%)	29(7.5%)	81(20.8%)	35.8	308(79.2%)	389

NB; Total MDR-TB prevalence = 81/389 = 20.8%, Concordance of MDR-TB resistance = 29/81 = 35.8%, Discordant MDR-TB = 27 (LJ-only) + 25 (MGIT-only) = 52/81 = 64.2%.
RLJ =Resistant LJ, RMGIT=Resistant MGIT, RLJ/MGIT=Resistant LJ/MGIT, TRLJMGIT=Total resistant LJ/MGIT, CRLJ/MGIT- Concordance resistance LJ/MGIT, TSLG/MGIT, Total susceptible LJ/MGIT, TTested =Total tested, STR=Streptomycin, INH=Isoniazid, RIF=Rifampicin, EMB= Ethambutol.

DISCUSSION

Proper diagnosis and early treatment of tuberculosis provide easy cure for patients, thus reduce the spread of TB. This study compared automated Liquid BACTEC MGIT 960 system to solid LJ culture for isolation of *Mycobacterium tuberculosis* in clinically suspected pulmonary tuberculosis cases and the results demonstrated a substantial correlation between the two culture systems in detecting *Mycobacterium tuberculosis*. Out of 530 sputum samples cultured, 86.0% samples showed concordant results, indicated substantial agreement using both MGIT-960 and solid LJ culture methods (Kappa = 0.70, SE = 0.046), suggested that both methods are dependable for routine mycobacterial culture. The majority of TB cases 52,8% were within 25–44 years age group, showed that tuberculosis predominantly affects the economically active population. The male-to-female ratio was 1.5:1, suggested that males were more affected than females, possibly due to greater exposure risks and health-seeking behavior differences. The MGIT-960 method demonstrated a slightly higher positive detection rate reflecting its enhanced sensitivity 80.7% and faster detection time 11 (6) days, compared to 77.5% detection rate by solid LJ culture with longer detecting time 30 (11) days. The mean time to detection was shorter with liquid culture and its monitoring can be automated to facilitate large number of specimens, but implementation challenges in

resource-constrained settings require a stable and sustainable diagnostic approach. This is consistent with findings from similar studies that highlighted the superior recovery, 76%, 81.8%, 94% of *Mycobacterium tuberculosis* and shorter detection time of liquid MGIT 960 culture systems, demonstrating its advantage in rapid diagnosis [10,11,24]. The slightly higher contamination rate 6.8% was observed in MGIT-960 compared to LJ culture with lower contamination rate of 4.2%. A finding reported in previous study, emphasizes the need for improved specimen processing, strict adherence to decontamination protocols when using liquid media, though the contamination rate in MGIT 960 system was within acceptable limits and may reflect its increased nutrient-rich environment which can support growth of contaminants but regular quality assurance measures are recommended to minimize contamination in liquid culture systems. Thus, MGIT 960 detected 3.2% more positive *M.tb* cases and 2.6% more contaminants cases while solid LJ detected 5.7% more negative *M.tb* cases compared to MGIT 960 alone. However, false positives or negative due to poor sample quality and technical errors can affect the sensitivity, thus the need for dual testing in high-priority cases. However, LJ culture remains valuable due to its lower cost, reduced maintenance demands, and is more accessible for peripheral laboratories particularly in hospital or research settings lacking automated equipment[19]. The culture diagnostic yield of both methods showed that, 86.0% were positive for *M. tuberculosis*, and using both methods together increased detection of 5.3% *M.tb* by MGIT-960 alone and detection of 8.5% *M.tb* by solid LJ alone. Comparing individual TB detection rate by hospitals, the rate was observed slightly higher in NAUTH 33.2%, followed by GHO 29.4% compared to COOTH 14.9% TB cases, these were similar with reported lower rates, 10.6%, 24% and 41% *M. tuberculosis* isolated in MGIT 960 cultures system[20,22.23.]. These variations could be due to differences in population and mycobacterial characteristics, sampling techniques and microbiological methods applied. The inclusion of samples from three different hospitals increases the generalized findings and highlights variability in sample handling, contamination, and processing infrastructure. The diagnostic accuracy of liquid BACTEC MGIT 960 culture was compared to solid Löwenstein–Jensen (LJ) culture as the gold standard, BACTEC MGIT 960 demonstrated a sensitivity of 94.6%, but there was no statistically significant difference $(\chi^2 = 3.63, p = 0.057)$, between MGIT and LJ results after accounting for concordant contaminated and NTM samples, emphasizing that the two methods are complementary rather than interchangeable. For MGIT960 detecting significantly more TB cases than LJ culture, highlighted its superior sensitivity, although at the expense of a higher contamination rate and NTM samples. These findings support the use of MGIT 960 as a highly sensitive diagnostic tool, ideally in combination with solid culture to optimize accuracy[8]. The specificity was moderate 63.2% reflecting additional TB detection by MGIT 960 not identified by solid LJ culture and overall diagnostic accuracy of 86.0%, for detection of *Mycobacterium tuberculosis*, reflecting

good agreement with LJ culture results. The positive predictive value was 90.9% and negative predictive value 75.3%, indicated that MGIT-negative results were reliable.

The DST results further revealed considerable resistance to first-line anti-TB drugs, particularly Ethambutol (EMB) and Isoniazid, this showed emerging resistance patterns in the population and both methods identified similar proportions of ethambutol mono-resistance with 5.1% LJ and 4.4% MGIT 960 respectively. MGIT 960 has previously been reported to detect ethambutol resistance more accurately due to liquid medium penetration and consistent drug concentration[24]. However, the variation seen with STR and EMB suggested method-specific differences in drug concentrations or interpretation protocols[24]. This is consistent with findings that, MGIT 960 is more sensitive for EMB detection, while LJ may overestimate STR resistance due to inactivation of the drug during media preparation[23,24]. MGIT 960 offer better sensitivity in mono-resistant streptomycin 5.1% cases, confirming its highly resistance prevalent. The total MDR-TB prevalence of 20.8% is concerning and highlights the need for continuous surveillance and effective infection control measures. The close similarity in MDR -TB 7.5% for Rifampicin and Isoniazid detection between the two methods indicated good agreement and reliability of both systems for DST performance but lower for streptomycin and ethambutol. In MDR-TB detection, concordance was lower, 35.8% of MDR-TB resistant isolates were detected by both MGIT and LJ, this means there is substantial discordance and the remaining 64.2% were resistant in only one of the two methods, 27 resistant showed 6.9% MDR-TB cases was found in LJ only, and 25 resistant indicated 6.4% MDR-TB in MGIT 960 only. The slightly higher number of MDR- TB detections by LJ suggests that some resistant strains may grow more slowly or show delayed detection in liquid culture. This highlighted important discrepancies which may have arisen from borderline minimum inhibitory concentrations (MICs), inoculum size variations, susceptibility testing method used, as well as the geographic setting, the prevalence of these strains or differences in critical concentration interpretations between solid and liquid systems. Ethambutol shows slightly lower concordance compared with Isoniazid; this is consistent with known variability in phenotypic DST for this drug[24,25]**.** This shows that using only one method would miss a majority of MDR-TB cases, highlighting the importance of dual-method testing, emphasizing the complementary role of MGIT and LJ in programmatic surveillance. It has been reported that the susceptibility of *M. tuberculosis* to ethambutol and streptomycin is less reliable and reproducible using solid medium[25,26,27]. Advance training in DST interpretation and harmonization of critical concentrations can reduce discrepancies in clinical decision making[27]. The discordance in MDR-TB detection emphasizes the need for dual-method confirmation, particularly in surveillance and treatment of resistant TB management. For all drugs, the majority of samples were susceptible in both methods especially rifampicin 85.6%. This strong

concordance supports the validity of DST results and builds confidence in using either method for ruling out resistance. The problem is that, Nigeria has a growing burden of drug-resistant TB and faces several TB diagnostic challenges especially in Southeast region, particularly Anambra state, this includes limited laboratory capacity and access to rapid diagnostics, inconsistent power supply, high reagent costs, lack of trained personnel and poor access to second-line DST. The National TB and Public health programs should help to implement these lacking resources in particular, tertiary hospital and peripheral health centers in Anambra state. Adoption of MGIT 960 system as the primary culture system due to its faster turnaround and higher sensitivity especially for smear-negative and paucibacillary cases and use of LJ culture simultaneously is very fundamental to maximize TB detection, improve diagnostic accuracy, and reduce false-negative results. Also, LJ culture should be maintained as a complementary method where MGIT960 is unavailable and ensuring access to culture-based diagnosis particularly where contamination is problematic or verification of results is vital, since WHO has endorsed the use of both culture system based on its advantages though with some limitations[8]. Public health programs should also respond to the high MDR-TB prevalence with appropriate treatment regimens and conduct periodic performance evaluation to monitor diagnostic efficiency, especially in MDR-TB surveillance, strengthen decontamination protocols to reduce MGIT960 contamination rates and **e**nhance laboratory capacity, including biosafety, quality assurance, and technician training.

CONCLUSION

This study established that, Clinical laboratories should employ routine use of combined MGIT 960 and LJ culture methods for reliable isolation and drug susceptibility testing of *Mycobacterium tuberculosis,* due to its significant improved TB detection, reduced false-negative results, improved turnaround times, early detection of drug-resistant TB to enhance treatment outcomes and ensure a more complete diagnosis. The substantial agreement between the two methods indicated comparable diagnostic accuracy. Liquid BACTEC MGIT 960 demonstrated a higher diagnostic yield when compared to solid Löwenstein–Jensen culture alone, though with moderate specificity but detected additional positive *Mycobacterium tuberculosis* and mono-resistant cases, with a higher contamination rate 6.8%. The MDR-TB rate of 20.8% highlights the ongoing challenge of drug-resistant TB in Anambra state. In Nigeria, solid culture remains relevant, especially in peripheral laboratories or resource-limited settings for optimizing laboratory performance and strengthen contamination control but is very essential to implement standardized DST protocols and cross-validation to minimize discrepancies in resistance profiles.

Acknowledgement
We appreciate Zankli Medical Centre Utako, Abuja, Nigeria for being part of sponsors of this research.

Competing Interests.
The authors declare that; there exists no conflict of interests.

REFERENCES

1. World Health Organization. (2023). *Global Tuberculosis Report 2023*. Geneva: *WHO*. *Organization* (WHO).

2.WHO Information Fact Sheets/Tuberculosis/2020. Available at https://www.who.int/news-room/fact-sheets/detail/tuberculosis (Last accessed on July 2020).

3. O'Grady J, Maeurer M, Mwaba P, Kapata N, Bates M, Hoelscher M, Zumla A:
New and improved diagnostics for detection of drug resistant
pulmonary tuberculosis. *Curr Opin Pulm Med* 2011, 17:134–141.

4. Uzoewulu NG[1], Ibeh IN[2], Lawson L[3], Goyal M[4], Umenyonu N[3], Ofiaeli RO[4] and Okonkwo R[5] (2014). Drug Resistant *Mycobacterium tuberculosis* in Tertiary Hospital South East, Nigeria. Journal of Medical Microbiology & Diagnosis.ISSN:2161-0703 *Jmmd, an Open Access* Volume 3 • Issue 2 • 1000141.

5. K.R. Steingart *et al.* (2006). Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review . *Lancet Infect. Dis*.

6. Boehme, C. C., Nabeta, P., Hillemann, D., Nicol, M. P., Shenai, S., Krapp, F. & Perkins, M. D. (2010). Rapid molecular detection of tuberculosis and rifampin resistance. *New England Journal of Medicine*, 363(11), 1005-1015.

7. Lawn, S. D., & Nicol, M. P. (2011). Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiology*, 6(9), 1067–1082.

8. Lovett Lawson1*, Nanmdi Emenyonu1, Saddiq T Abdurrahman2, Juliana O Lawson1, Gertrude N Uzoewulu3, Olumide M Sogaolu4, Juliana N Ebisike5, Christopher M Parry6, Mohammed A Yassin7 and Luis E Cuevas7 (2013). Comparison of Mycobacterium tuberculosis drug susceptibility using solid and liquid culture in Nigeria. *BMC Research Notes* 2013, 6:215. http://www.biomedcentral.com/1756-0500/6/215.

9. Hanna, B. A., Ebrahimzadeh, A., Elliott, L. B., Morgan, M. A., Novak, S. M., Rusch-Gerdes, S., ... & Savic, M. (2015). Multicenter evaluation of the BACTEC MGIT 960 system for recovery of mycobacteria. *Journal of Clinical Microbiology*, 37(3), 748–752.

10. Somoskovi, A., Kodmon, C., Lantos, A., Bartfai, Z., & Magyar, P. (2006). Comparison of recoveries of *Mycobacterium tuberculosis* using the automated BACTEC MGIT 960 system, the BACTEC 460TB system, and Löwenstein-Jensen medium.

Journal of Clinical Microbiology, 38(6), 2395-2397.

11. Cruciani, M., Scarparo, C., Malena, M., Bosco, O., Serpelloni, G., & Mengoli, C. (2004). Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of *Mycobacteria*. *Journal of Clinical Microbiology*, 42(5), 2321-2325.

12. Pfyffer, G. E., Kissling, P., Welscher, H. M., Cieslak, C., Casal, M. J., Gutierrez, J., ... & Vincent, V. (1997). Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of mycobacteria from clinical specimens. *Journal of Clinical Microbiology*, 35(2), 364-368.

13. Lawson L, et al 2011 Resistance to first-line tuberculosis drugs in three cities of Nigeria. *Trop Med Int Health 2011*, 16(8):974–980 .

14. National Committee for Clinical Laboratory Standards: Susceptibility testing for Mycobacteria, Nocardiae, and other aerobic Actinomycetes; approved standard. Volume 23: (18), NCCLS document, pM-24A. *NCCLS: Wayne,PA*; 2003.

15. Migliori GB, Lange C, Girardi E, Centis R, Besozzi G, et al. (2008) Extensively drug-resistant tuberculosis is worse than multidrug-resistant tuberculosis: different methodology and settings, same results. *Clin Infect* Dis 46: 958–959.

16.McHugh, Mary L. (2012). "Interrater reliability: The kappa statistic". *Biochemia Medica*. **22** (3): 276–282 doi:10.11613/bm.2012.031. PMC 3900052. PMID 23092060

17. Chihota VN, et al (2010). Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. *Int J Tuberc Lung Dis*. 14(8):1024–1031.

18. Srisuwanvilai LO, et al: Performance of the BACTEC-MGIT-960 compared with solid media for detection of Mycobacterium in Bangkok Thailand. *Diagn Microbiol Infect Dis* 2008, 61(4):402–407.

19. Ani, A. E., et al. (2020). Evaluation of the MGIT system for TB culture in Nigerian TB reference laboratories. *African Journal of Clinical and Experimental Microbiology*, 21(4), 302–309.

20. Somoskovi A, et al: Comparison of recoveries of mycobacterium tuberculosis using the automated BACTEC-MGIT-960 system, the BACTEC460 TB system, and Lowenstein-Jensen medium. *J Clin Microbiol* 2000,38(6):2395–2397.

21. J J Lee[1], J Suo, C B Lin, J D Wang, T Y Lin, Y C Tsai (2003) Comparative evaluation of the BACTEC MGIT 960 system with solid medium for isolation of mycobacteria *Int J Tuberc Lung Dis* .;7(6):569-74..

22. Yagbou, F., et al. (2020). Evaluation of two culture systems for MDR-TB detection in Cameroon. *BMC Infectious Diseases*, 20, 531.

23. Okonkwo, R. C., et al. (2019). Performance of Löwenstein-Jensen culture in TB diagnosis in southeastern Nigeria. *Nigerian Journal of Medical Microbiology*, 17(2), 45–50.

24. Chien, H. P., et al. (2017). Comparative evaluation of the BACTEC MGIT 960 system and Löwenstein–Jensen culture for isolation of *M. tuberculosis*. *Journal of Clinical Microbiology*, 55(2), 453–459.

25. Rivera AB[1], T E Tupasi, E R Grimaldo, R C Cardano, V M Co (1997) Rapid and improved recovery rate of Mycobacterium tuberculosis in Mycobacteria Growth Indicator Tube combined with solid Löwenstein Jensen medium. *Int J Tuberc Lung Dis*;1(5):454-9.

26. E A Macondo[1], F Ba, N C Toure-Kane, O Kaire, A Gueye-Ndiaye, A Gaye-Diallo, C S Boye, S Mboup (2000) Improvement of tuberculosis diagnosis by the Mycobacteria Growth Indicator Tube (MGIT) in a developing country laboratory. *Bull Soc Pathol Exot* ;93(2):97-100.

27. Tortoli E, et al (1999) Use of BACTEC-MGIT-960 for recovery of mycobacteria from clinical specimens: multicenter study. *J Clin Microbiol* , 37(11):3578–3582.