MEMORANDUM
OF
UNDERSTANDING

NAAC RE-ACCREDITED WITH 'A' GRADE

Sevadal Mahila Mahavidyalaya

Place for Higher Learning and Research (Research Academy)
Sakkardara Square, Umrer Road,
Nagpur - 440024 (India)
E-mail: sevamahilamv@gmail.com
Website: https://sevadalmahilamahavidyalaya.ac.in/

AND

Vishakha Clinical Microbiology Laboratory

612, A-Wing, 6th Floor, Lokmat Bhawan,
Wardha Road,
Nagpur, India (MH)
Website: www.vcml.in

Academic Session

2021 - 2022

SEVADAL MAHILA MAHAVIDYALAYA NAGPUR

Principal
Sevadal Mahila Mahavidyalaya
Umrer Road, Nagpur-9.

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Nagpur, India (MH)
E-mail: vcmlnagpur@gmail.com
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MEMORANDUM OF UNDERSTANDING

This **Memorandum of Understanding** is signed between:

Sevadal Mahila Mahavidyalaya, Sakkardara Square, Umrer Road, Nagpur-440024 (M.S.), which was established in the year 1992 and affiliated to Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur and acting through the

Prof. Pravin Charde
Principal,
Sevadal Mahila Mahavidyalaya,
Nagpur - 440024

AND

Dr. Yagnesh Thakar
Director,
Vishakha Clinical Microbiology Laboratory,
612, A-wing, Lokmat Bhawan, Wardha Road,
Nagpur - 440012

The purpose of this MoU is to develop academic, educational co-operation and to promote mutual understanding in research between **R.T.M. Nagpur University affiliated B.Voc. Course in "Medical Laboratory & Molecular Diagnostic Technology", Sevadal Mahila Mahavidyalaya, Nagpur and Vishakha Clinical Microbiology Laboratory, Nagpur.**

Both of these organizations agree to develop the following collaborative activities in the academic and corporate areas of mutual interest, on a basis of equality and reciprocity.

a) Exchange of Faculty and administrative staff
b) Conducting guest lectures and organizing symposia.
c) Exchange of academic information and materials.

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d) Promoting collaboration in fields of mutual interest

e) Providing internship and final placements to students in various disciplines.

f) Permitting students for research in clinical microbiology.

1) FIELD OF CO-OPERATION

A) Both the institution and the laboratory shall evolve a mutually acceptable schedule to develop programs, hold seminars and exchange visits and also for research activities.

B) The said academic interaction and intellectual assimilation may include

1) Faculty / staff development and exchange;
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5) Final placements.

2) EXCHANGE OF STUDENTS/TEACHERS/INDUSTRY PERSONNEL

a) Reciprocal arrangements based on mutually acceptable terms shall be accomplished to give an impetus to collaborative research and joint projects. Teachers, researchers, guides and students of both the institutions shall be encouraged to work in tandem in the laboratories, workshops, faculties and departments of both the institutions.

3) MISCELLANEOUS

a) The details for the efficacious implementation of this Memorandum of Understanding shall be jointly worked out on mutually acceptable terms within the parameters of the policies, rules and regulations of both the institutions.

b) The parties to this memorandum may by mutual consent, add modify, amend, delete, review or revise any term(s) and condition(s) of this agreement.

c) The intent and implementation of this memorandum is subject to the policies of the respective states (in case of inter-state agreements) and the laws of the land.

d) The MoU shall remain in force for a period of **5** years from the date of its signature and seal, and may be terminated by either side by giving

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Umrer Road, Nagpur-9.

a six months notice to that effect in writing. However, notwithstanding the notice of the intent terminate the memorandum, all rights obligations and subsisting therein shall be respected and mandated till the finalization and accomplishment thereof.

e) The parties to this MoU undertake to treat as CONFIDENTIAL AND PRIVILEGED information of the other institution, which is so classified in advance. The terms and confidentiality and mode of disclosure shall be as per mutually acceptable terms.

f) This MoU shall require the ratification of the competent academic/executive body of both the institutions.

Signed at Nagpur on this the _____ day of _____ 2021

(Authorized Signature on behalf of **Vishakha Clinical Microbiology Laboratory, Nagpur**

(Dr. Yagnesh Thakar)
Director, Vishakha Clinical Microbiology Laboratory, Nagpur

VISHAKHA CLINICAL
MICROBIOLOGY LABORATORY
612-A, A-Wing, Lokmat Bhawan
Lokmat Square, Wardha Road
NAGPUR-440012.

Seal
Date:

(Authorized Signature on behalf of **Sevadal Mahila Mahavidyalaya, Nagpur**)

(Prof. Pravin Charde)
Principal, Sevadal Mahila Mahavidyalaya, Nagpur

Seal

Date :

Witnesses: 1. ---------- 2. ----------

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2017 - 2018

NAGPUR

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d) The MoU shall remain in force for a period of **5** years from the date of its signature and seal, and may be terminated by either side by giving a six months notice to that effect in writing. However, notwithstanding

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f) This MoU shall require the ratification of the competent academic/executive body of both the institutions.

Signed at Nagpur on this the 01st day of July 2017

(Authorized Signature on behalf of **Vishakha Clinical Microbiology Laboratory, Nagpur**

(Dr. Yagnesh Thakar)
Director, Vishakha Clinical Microbiology Laboratory, Nagpur

VISHAKHA CLINICAL
MICROBIOLOGY LABORATORY
612-A, A-Wing, Lokmat Bhawan
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Seal
Date:

(Authorized Signature on behalf of **Sevadal Mahila Mahavidyalaya, Nagpur**)

(Prof. Pravin Charde)
Principal, Sevadal Mahila Mahavidyalaya, Nagpur

Seal

Date :

Witnesses: 1. ---------- 2. ----------

Principal
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Umrer Road, Nagpur-9.

"(Established by Government of Central Provinces Education Department by Notification No. 513 dated the 1st of August, 1923 & presently a State University governed by Maharashtra Public Universities Act, 2016 (Mah. Act No. VI of 2017).)"

Office of the Director, Board of Examination and Evaluation
(Ph.D. Cell)
Examination Bhawan, Laxminarayan Institute of Technology Premises,
Rashtrasant Tukadoji Maharaj Nagpur University,Nagpur-440033

No. RTMNU/Ph.D. Cell/RRC./2017/ 414 Dated : 01 / 11/2017

To

Ms. Anita Y. Kulkarni
C/o Ashwin Kher.
75/8, Park View Dagadi Park.
Ramdaspeth Nagpur-440010

Subject :- Registration for Ph.D. Degree (**Full time**/ Part time) application dated 18/01/2017

Reference :- 1) Office Letter No. RTMNU/Ph.D Cell/RRC/2017/2395, Dt/-04/03/2017
2) Your Letter No. 256, Dated 27/10/2017

Sir/Madam.

I am to inform you that the Research and Recognition Committee in the subject **Microbiology** under the faculty of **Science & Technology** held on **03/03/2017** has Considered and Approved your application for Ph.D. Registration subject to Submission of P.G. Approved Letter of Guide.

Since the said document (s) has/have been submitted by you on 27/10/2017. Hence your Ph.D. Registration in the said subject will be operative with effect from 27/10/2017. The Approved topic, Supervisor, Place of Research and date of Registration are as under.

Topic of Research	:	**Prevalence of Pulmonary Tuberculosis in Central India and Detection of Multidrug Resistant (MDR) Isolates with Special Emphasis on Efficacy of Molecular of Molecular Methods for Rapid and Accurate Diangnosis**
Supervisor	:	**Dr. Kirti V. Dubey**
Place of Research	:	**Sevadal Mahila Mahavidyalaya, Umred Road, Nagpur.**
Date of Registration	:	**27/10/2017**
Suggestion if any	:	

Your date of registration will be considered subject to the fulfillment of the following requirements within 30 days from the receipt of this letter.

1) Your application has been considered as per the provisions of the Direction No. 1/2016. Direction No. 81/2016 and Direction No. 11/2017. Direction No. 11/2017 issued as per the provisions of the Public Notice issued by the University Grants Commission No. F.No.2014/(PS). dated 10th March, 2017 regarding Regular Mode with reference to the Ph.D. Degree. According the this notice Ph.D. Degree which is pursued either full time or part time will be treated as degree awarded through regular mode provided this is in conformity with the existing Statutes/bylaws/ordinances etc. of the degree awarding University.

2) Full time Ph.D. Degree program shall be for the minimum duration of three years and part time degree programe shall be for the minimum duration of four years and six months including course work and a maximum duration will be of 6 years for full time as well as for Part time.

3) Date of Registration will be granted from the date of application received by the University.

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VISHAKHA CLINICAL MICROBIOLOGY LABORATORY

Dr. Yagnesh Thakar
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Tel. 0712-2425984
Mob. : 9822868753
Email : vcmlnagpur@gmail.com
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Date: 11/12/2017.

To,
The Principal
Sevadal Mahila Mahavidyalaya,
Nagpur

Subject: Permission to avail laboratory facility at Vishakha Clinical Microbiology Laboratory for Ph. D. Program in microbiology.

Respected Sir,

This is to appraise you that your research scholar Miss. Anita Kulkarni, Department of Microbiology, is permitted to use laboratory facilities of Vishakha Clinical Microbiology Laboratory for her Ph. D. Program on, "Prevalence of Pulmonary tuberculosis in central India and detection of multi drug resistance (MDR) isolates with special emphasis on efficacy of molecular methods for rapid detection and accurate diagnosis."

She can also learn latest tools and techniques useful in the detection of *Mycobacterium tuberculosis* in our laboratory.

Thanking you,

Yours sincerely,

Dr. Yagnesh Thakar
Director

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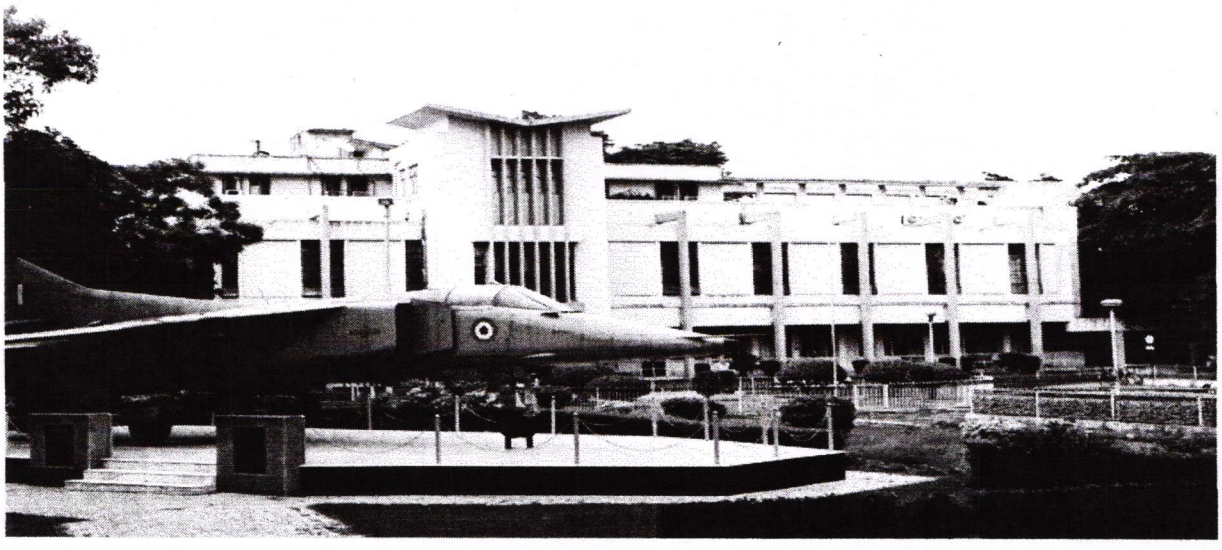
"Intervention of Science and Technology, Social Problems and Sustainable Development"

Government of India
Ministry of Human Resource Development

Interdisciplinary National Conference on "Intervention of Science and Technology, Social Problems and Sustainable Development" 17th June, 2018

Editor
Dr. G. N. Nimbarte



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Department of Humanities and Social Sciences

Visvesvaraya National Institute of Technology, Nagpur

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Interdisciplinary National Conference

On

Intervention of Science and Technology, Social Problems and Sustainable Development

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June -17, 2018

31.Newer methods for better & rapid diagnosis of Tuberculosis and detection of Multi drug resistance.

Anita Kulkarni
KirtiDubey
YagneshThakar
Vishakha Clinical Microbiology Laboratory,Nagpur.
SewadalMahilaMahavidyalaya, Nagpur

Abstract: Tuberculosis is the world wide problem and a major health issue in India, especially due to emergence of Multi Drug Resistance (MDR) strains of Mycobacterium tuberculosis.
A total of 112 respiratory specimens comprising of 54 sputum, 42 bronchial aspirates and 16 pleural fluids were tested for tuberculosis by ZiehlNeelson staining (ZN), culture and Line Probe Assay (LPA), a Polymerase Chain Reaction (PCR) based rapid test. M. tuberculosis was detected in 48 (42.86%) samples in which ZN stain was positive in 28 (25.00%), culture in 39 (34.82%) and LPA in 48 (42.86%) samples. All these tests were simultaneously positive in 27 (56.25%) of 48 positive samples, any two tests positive in 13 (27.08%) and only LPA positive in 8 (16.67%) samples. MDR was observed in 5 out of 39 (12.82%) culture positive samples and 6 out of 48 (12.5%) LPA positive samples. ZN stain sensitivity is low and culture is time consuming. LPA is most sensitive test, it is rapid and can be used for diagnosis of tuberculosis and detection of MDR as well in a single test run.

Introduction: Tuberculosis is the ninth leading cause of deaths worldwide and the leading cause due to single infectious agent. India is contributing nearly one third of the world's tuberculosis cases andhas the highest rate of new TB cases. The estimated prevalence of bacteriologically positive TB (Smear and/or culture) is 211 per 100,000 for India[1].Prevalence of multi drug resistant TB (MDR TB) cases is on the rise in India and proportions of new cases of MDR TB have been observed to vary from 1.1% to 5.3% in most of the reported studies. The proportion of previously treated patients with MDR TB varied from 8% to 67%[2].

MDRTB has been a cause of concern in both developed and developing countries. Worldwide emergence of multidrug resistant TB has been reported. These isolates resistant to both Isoniazid(INH) and rifampicin with or without resistance to other drugs have caused concern worldwide due to high mortality particularly in persons co-infected with HIV. Continuous surveillance of the primary and acquired drug resistance pattern of M.TB is important in assessing the efficacy of chemotherapy which will help in designing TB control programme[3]. Development of efficient laboratory strategies for rapid and reliable antimicrobial susceptibility testing of M.tuberculosis is paramount for proper management of patients, particularly those with multi drug resistant tuberculosis. Susceptibility testing has to be performed for each new patient.If resistance to Isoniazid,Rifampicin or ethambutol is detected, an extended spectrum of anti tuberculosis drugs should be tested immediately. Also, further emergence of resistance has to be carefully monitored by repeating susceptibility testing with subsequent isolates within a short time frame.[4]

Conventional diagnostic method for tuberculosis is based on microscopy and culture. The microscope based techniques are less sensitive. Though culture is a gold standard for diagnosis,it takes about 4 weeks with additional weeks for diagnosis of drug resistance[5].

Such delayed diagnosis facilitates further transmission of TB.A quick and reliable diagnosis can permit early chemotherapeutic intervention and interrupt further transmission.Molecular methods consisting of Nucleic acid amplification targeting certain genes which are fairly sensitive, specific and very rapid are now available. Hence this study is planned to detect multi drug resistant tuberculosis rapidly by molecular methods. The data presented in this article is our preliminary experience with Line Probe Assay (LPA), the GenoType MTBDR plus Ver2 Kit for detection of M. tuberculosis in clinical samples and simultaneous assessment of their sensitivity to INH and Rifampicin, which is based of Polymerase chain reaction followed by Reverse Hybridization.

Material & Methods: The study data comprises of respiratory samples received in the laboratory from patients suspected of having pulmonary tuberculosis. A total of 112 samples were received which comprised of 54 sputum samples, 42 bronchial aspirates and 16 Pleural fluids.

The samples were subjected for decontamination with NALC-NaOH[6].Smears were prepared and stained by Ziehl Nelson method[6]. The cultures were done in the liquid media and processed in BacT Alert 3D Automated culture system.

Molecular genetic assay was carried out by Geno Type MTBDR plus Ver2 kits (by HAIN Lifesciences,Germany) for identification of resistance to Rifampicin and Isoniazid of the Mycobacterium tuberculosis complex. The whole procedure is divided into three steps.1) DNA extraction from decontaminated specimen 2)amplification with biotinylated primers 3) reverse hybridization. The results are obtained in the form of bands on the strips. (Figure 1).

Results: Out of all 112 samples M. tuberculosis was detected in 48(42.86%) samples either in individual test or in combination.The ZN stain was positive in 28 (25.00%) samples, culture was positive in 39 (34.82%) samples and LPA test was positive in 48 (42.86%) samples. Their distribution is shown in Table 1.

Table 1: Positivity of different tests in clinical samples.

	NO.	ZN TEST	CULTURE	LPA
SPUTUM	54	12 (22.22%)	16(29.63%)	24(44.44%)
BRONCH. ASP.	42	16(38.10%)	23(54.76%)	23(54.76%)
PLEURAL FLUID	16	0(0.00%)	0(0.00%)	1(6.25%)
TOTAL	112	28(25.00%)	39(34.82%)	48(42.86%)

Out of 48 samples all the three tests were positive in 27(56.25%) cases, any two in 13(27.08%) cases and only single test was positive in 8(16.67%) cases as shown in Table 2.

Table 2: Simultaneous positivity of Staining, Culture and LPA in different samples.

TESTS	SPUTUM n=54	BRONCHIALWASH n=42	PLEURAL FLUID n=16	TOTAL n=112
(A)ONLY ONE TEST				
ZN TEST	0	0	0	0
CULTURE	0	0	0	0
LPA	7	0	1	8
SUB TOTAL (A)	7	0	1	8
(B)TWO TESTS				
ZN + CULTURE	0	0	0	0
ZN + LPA	1	0	0	1
CULTURE + LPA	4	8	0	12
SUB TOTAL (B)	5	8	0	13
(C)ALL THREE	12	15	0	27
TOTAL (A+B+C)	24	23	1	48

Of all the 48 positive samples 5 (10.42%) showed resistance to INH only and 6 (12.5%) were resistant to both INH and Rifampicin (MDR). Rests of the 37 (77.08%) positive patients were sensitive to both INH and Rifampicin. The results of the resistance detection by culture as well as LPA tests were comparable. (Table 3)

Table 3: Detection of Drug Resistance by Culture and LPA.

	MDR	RESISTANT TO INH	RESISTANT TO RIFAMPICIN	SENSITIVE
CULTURE (n=39)	5 (12.82%)	5 (12.82%)	0	29 (74.36%)
LPA (n=48)	6 (12.5%)	5 (10.42%)	0	37 (77.08%)

Discussion:The present data indicates that the tests positivityfor suspected cases of pulmonary Tuberculosis is to the tune of 42.86%. It does not reflect the correct prevalence as only suspected cases of Tuberculosis and suspected MDR cases were referred to the laboratory. Of all the positive cases 12.5% were MDR which is again within the expected range reported[2].

Of the three types of respiratory samples, Bronchial aspirate is the most appropriate sample, followed by sputum sample. Positivity of all the tests performed in the study is very less in pleural fluids.

Of the three tests, it is well known that the ZN smear positivity has the least sensitivity. But sometimes it is preferred for its speed, simplicity and economy. The culture is considered to be the most specific diagnostic tool but it is very much time consuming. However, with the advent of automated systems and liquid cultures the time duration has been cut down substantially; but it may still take couple of weeks before we get the results.

A large number of Nucleic Acid Amplification techniques like PCR, TMA and LPA have been employed for rapid diagnosis of tuberculosis. Of these Genotype MTBDR plus Ver2 kits have the advantage of simultaneous detection as well as determination of INH and Rifampicin resistance in the same run[7,8].

The preliminary data obtained in the present study clearly indicates the superiority of LPA over culture and smear examination for rapid diagnosis of tuberculosis. It has detected 9 more cases of tuberculosis which could have been missed by culture alone. As far as detection of drug resistance is concerned, both culture and LPA were equally consistent with each other. However, the major advantage of LPA is its speed and if positive it will give the results of INH and Rifampicin sensitivity in the same test run without any extra cost. This test fails to detect Atypical mycobacteria and in some doubtful cases confirmation by culture and phenotypic tests resistance tests become essential.

Although early results for use of automated cultures and Molecular methods are very encouraging for diagnosis of tuberculosis. The performance of the tests will better be evaluated and better inferences will be drawn when more number of samples will be tested over a period of time.

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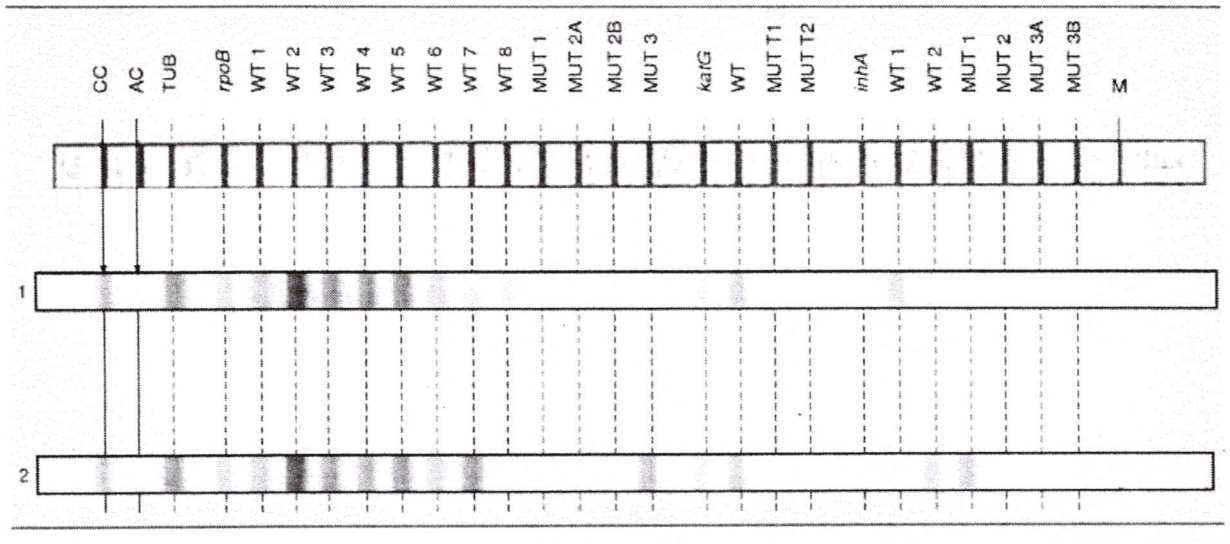
Figure 1: Strip 1: INH-Sensitive, Rifampicin-Sensitive. Strip 2: MDR (MUT bands positive)



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International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Drug Resistance Detection in Mycobacterium Tuberculosis

Anita Kulkarni[1], Kirty Dubey[2], Pallavi Wanjari[3], Yagnesh Thakar[4]

[1, 3, 4]Vishakha Clinical Microbiology Laboratory, Nagpur, Maharashtra, India

[2]Sewadal Mahila Mahavidyalaya, Nagpur, Maharashtra, India

Abstract: ***MDR cases of tuberculosis are increasing and they are difficult to treat. This study was conducted to assess prevalence of drug resistance in Mycobacterium tuberculosis. Study comprises of 67 sputum specimen received for Mycobacterium tuberculosis (MTB) culture. Culture was done in Middlebrook broth using BacT Alert 3D automated culture system. The study period was from Dec 2018 to Nov 2019. Out of these MTB was grown in 31 (46.27%) cases. The first line sensitivity testing was done in liquid culture media (BacT Alert3D) against Isonizid, Rifampicin, Ethambutol, Streptomycin. Isonizid and Rifampicin resistance was seen in 3 (9.68%) cases. Only Isonizid resistance was seen in 9 (29.03%) cases. This indicates that multidrug resistance is present to the tune of 9.68 % while monoresistance to isonizid is more common in 29.03%. Monoresistance to rifampicin was not observed. Resistance to ethambutol and streptomycin was not observed in the present study. The advantage of doing Drug susceptibility testing in liquid media over solid media is speed and the results can be obtained within 15 days as compared to over 4 weeks when tested in solid media. Rifampicin resistance can be considered as a marker for labeling the strain as MDR.***

Keywords: Mycobacterium tuberculosis, Multi drug resistance (MDR), Bact-alert 3D, Rifampicin, Isoniazid.

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, remains one of India's biggest health problems. Every year, India reports over 2 million TB cases. With the emergence of severe forms of drug-resistant TB and concerns about TB drug shortages, there is much work to be done to control the epidemic(1). The worldwide prevalence of TB was 9.6 million in 2014, with 1.5 million deaths(2). Pulmonary infection is the most prevalent contagious clinical form of tuberculosis, and is therefore of prime public health importance(3).Fast and effective microbiological diagnosis is essential to control the spread of TB.

The BacT/Alert 3D system is a fully automated liquid culture system which allows the growth and detection of mycobacteria. It is a rapid and sensitive method for recovery of mycobacteria from clinical specimens using an N-acetyl-L-cysteine-NaOH decontamination method (4). However, the emergence of resistant strains, including multi-drug resistant (MDR) and extremely drug resistant (XDR) strains has posed a significant challenge. Though advances in drug therapy have been limited, TB control has greatly benefited from the advent of newer diagnostic tests including use of liquid culture media. Culturing *Mycobacterium tuberculosis* remains the gold standard for the laboratory diagnosis of pulmonary tuberculosis.

Multidrug-resistant tuberculosis (MDR-TB) is defined as disease due to *Mycobacterium tuberculosis* that is resistant to isoniazid (H) and rifampicin (R) with or without resistance to other drug(5). The emergence of multidrug-resistant tuberculosis (MDR-TB) and, more recently, extensively drug-resistant TB (XDR-TB) is widely considered a serious threat to global TB control. Detection of drug resistance in Tuberculosis by conventional disc diffusion is not possible due to slow speed of growth of Mycobacteria. Therefore dilution methods have been used(6).

In the present study the drug susceptibility testing was standardized in liquid culture media using the automated system of BacT Alert 3D (bioMerieux) and its utility was assessed for clinical use.

2. Literature and Review

Multidrug-resistant TB (MDR TB), i.e. resistance to at least isoniazid (Inh) and rifampin (Rif), and extensively drug-resistant TB (XDR TB), i.e. MDR plus resistance to amikacin, kanamycin or capreomycin and a fluoroquinolone, are the most problematic forms of resistance because treatment options are limited and the second-line drugs used for therapy are more toxic, less effective, more expensive, and must be administered for a longer period of time than standard first-line drug therapy(7). Conventional culture and DST on solid media is a slow process, and in high income, low-incidence countries these systems have been supplemented (or replaced) by automated liquid culture systems. The World Health Organization now recommends expanded use of liquid culture systems in resource constrained settings.

Seoung-Cheol Kim and co authurs evaluated the performance of the BacT Alert 3D system, a liquid culture system, for mycobacterial culture and drug susceptibility testing by comparison with mycobacterial culture using LJ medium and drug susceptibility testing with M-KIT plates in the situation of a high MDR-TB and XDR-TB burden. In our study population, the prevalence of MDR-TB and XDR-TB in our pool of isolates were 6.0% and 2.3%, respectively (8).

Increase in multidrug resistance from 4.7 to 19.8 per cent in the past 13 years, as observed by Recna Raveendranand freinds, needs to be noticed. In the present scenario of increasing prevalence of MDR-TB and lack of availability of many second line drugs, screening with culture and drug susceptibility testing should be recommended for all smear

positive pulmonary patients. The WHO policy guidelines[3] for the use of new rapid molecular based techniques for early detection of MDR-TB may help the high-burden countries in identifying and treating patients of MDR-TB quickly.(9)

3. Material & Methods

The study period was from May 2018 to Nov 2019. Study comprises of 67 sputum specimen received for Mycobacterium tuberculosis culture. After decontamination Culture was done in Middle brook broth using Bac T Alert 3D automated culture system.

Decontamination using Sodium hydroxide (modified Petroff) method:(10)
Sputum (at least 2 ml, not more than 5 ml) was transferred into a centrifuge tube. Equal volume of 4% NaOH and citrate was added. Cap was tightened cap and solution was vortexed and pinch of NALC (*N*-acetyl-*L*-cysteine) powder was added, mixed and allowed to stand for 15 minutes at room temperature. The tube was filled to within 2 cm of the top (e.g. to the 40-ml mark on the tube) with phosphate buffer, vortexed, mixed and centrifuged at 5000*g* for 15 minutes. The supernatant was carefully poured off into a discard can containing 5% phenol or other disinfectant. The deposit was resuspended into MP bottle of BacTalert.

In the BacT/Alert MB® (bioMérieux, Craponne, France), growth is inferred from increasing CO2 tension in a bottle. Once the bottle is flagged positive by the system, the bottle is removed, small amount of fluid is drawn using the syringe, ZN smear is prepared and Mycobacterium species is confirmed. *M. tuberculosis* was confirmed by TB Antigen MPT64 Rapid test. (SD Bio Line, Standard Diagnostics Inc.).

Drug susceptibility testing was done on the isolated strains of *M. tuberculosis*.

The bottle when flashes positive has 10^6 – 10^7 CFU/mL which is equivalent to 1 McFarland.

Drug Susceptibility Testing: Drug susceptibility was done as follows (11)

Inoculum
To perform the sensitivity for the first line drugs, 6 bottles were used as follows:
1) Growth Control 2) 1% of Growth Control 3) Streptomycin 4) Isoniazid 5) Rifampicin 6)Ethambutol Inoculum of 0.5 ml of 1McF of Mycobacterium growth was added to Growth control bottle. (Bottle no 1)

From separate bottle 0.1 ml of media was discarded and replaced with equal volume of 1 McFarland growth. Now this bottle became 1% (diluted as 100 times) and from this bottle 0.5 ml of inoculum was added to the 1% GC bottle. (Bottle no. 2)

Steps to perform DST
The following concentrations of drugs were prepared:
Streptomycin (S): 20 µg/ml (Solvent: DW)
Isonizid (I): 2 µg/ml (Solvent: DW)
Rifampicin (R): 20 µg/ml (Solvent: DMSO)
Ethambutol (E): 100 µg/ml (Solvent: DW)

The drugs are added to the respective bottles with a volume of 0.5 mL so that the final concentrations in the bottles are as follows:
Streptomycin (S): 1 µg/ml
Isonizid (I): 0.1 µg/ml
Rifampicin (R): 1 µg/ml
Ethambutol (E): 5 µg/ml

Reconstitution Fluid (0.5 ml)was added to the GC bottle also.

The 1 McF primary growth in oculum was added to all drug containing bottles. The rubber septum was cleaned with sterilium and kept in BacT/ALERT 3D equipment for at least 12 days.

Interpretation
The bottle flashing positive before 1% GC bottle with pure growth (confirmed by ZN) should be considered as Resistant and rest as Sensitive which comes positive after 1% GC bottle. The 1% bottle should flash positive in 12 days or else the test should be repeated.

4. Results

Out of 67 sputum liquid cultures, M. tuberculosis was grown in 31 cases with the culture positivity of 46.27 per cent. All the strains were confirmed to be M. tuberculosis by TB Ag MPT64 Rapid test.

The Drug Resistance testing was consistent in all 31 samples. The bottles became positive from 7 to 12 days. The average duration of resistance detection was 9 days. There were no inconsistent results. Isoniazid and Rifampicin resistance was seen in 3 (9.68%) cases. Only Isoniazid resistance was seen in 9 (29.03%) cases. This indicates that multidrug resistance is present to the tune of 9.68 per cent while monoresistance to Isonizid is more common in 29.03%. (Table 1).

Table 1: Showing Resistance pattern of M. tuberculosis

INH + RIFAMPICIN Resistance	3	9.68%
ONLY INH Resistance	9	29.03%
ONLY RIFAMPICIN Resistance	0	0.00%

In any of the 31 cases resistance was not detected to Ethambutol and Streptomycin.

5. Discussion

Multi Drug resistance is a problem in managing tuberculosis cases. Detection of drug resistance in tuberculosis is an important step in control of tuberculosis. In India, the prevalence of multi drug resistance as reported by WHO is 9.6%(12) and detection of drug resistance is important in order to detect these cases and treat them properly so as to have effective control of spread of disease. In the present study also MDR *M. tuberculosis* was detected in 9.68% cases. Resistance to ethambutol and streptomycin was not

observed in the present study. The agreement was lower for STR and EMB than for RIF and INH. (13) Isolated INH resistance was observed in 29.03% strains in the present study. Resistance to INH has also been reported to be high in other studies.(14), (11).

The drug susceptibility testing by conventional solid media like Lowenstein Jensen Medium will take about three to four weeks. While susceptibility testing using liquid culture media in automated system is fairly rapid, easy to perform and feasible in every set up where mycobacterial culture is done.

6. Future Scope

The technique being rapid and standardized, the resistance testing to second line drugs used for treatment of MDR strains can be performed and the data in this regard can be generated. Once standardized, this method can be used for routine testing of resistance to first as well as second line drugs also. If any potential new drug has to be tested, the same technique may be used with due modifications as required.

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Author Profile

Ms. Anita Kulkarni is Working as a research student for Ph.D. with Vishakha Microbiology Clinical Laboratory at Nagpur, under the supervision of Dr. Kirti Dubey from Sevadal Mahila Mahavidyalaya, Nagpur. She has Presented a paper in 'International Conference on Future Tech of Life Sciences' in Feb 2020. Worked at ZBTC Govt medical college Nagpur, as ZBTC HIV project Technical head. Served in Rainbowmedinova diagnostics Blood bank, as supervisor. She is contributing her services to CIIHO as a blood bank in-charge in technical from 2010.

Dr. Mrs. Kirti V. Dubey is Assistant Professor in Microbiology at Sewa Dal Mahavidyalaya, Nagpur. Ph. D. from RTM Nagpur University. M.Sc. in Microbiology from RTM Nagpur University. She has research experience of over 20 years, teaching experience of over 12 years. She received best poster presentation award at National Symposium on *Microbial Technologies for Environment Management and Resource Recovery*held at New

Delhi, 1-2 October 1997. National Patent entitled "*A process for recovery of biosurfactant from distillery waste*" has been Included in Big Patents India. Received award of '*Distinguished Women Scientist*' was conferred during World Congress WCMANU-2012 and Recipient of *Sandvik India Gender Awards -2018*under Academia category. Deputed to Lund University, Center for Chemistry and Chemical Engineering, Department of Biotechnology, Sweden from NEERI, Nagpur during 21st March 2000 - 1st April 2000. She has published three books as co-author and contributed to chapters in six books. Published 19 research papers in International and national journals. "Member Board of Studies of Microbiology" by RashtrasantTukadojiMaharaj Nagpur University (RTMNU), Nagpur. In Advisory Board of BioInfo Publications 'World Research Journal of Biotechnology Category: International Journal. Served as Reviewer in Indian Journal of Microbiology and also in Environmental Science and Pollution Research

Mrs. Pallavi Shital Wanjari Works as Research Associate in Vishakha Clinical Microbiology Laboratory Nagpur. Submitted my Ph.D. thesis entitled "Study of Biological Virulence Factors of Uropathogenic *Escherichia Coli* and Detection of the Virulence Genes." In RTM Nagpur University, January 2019. Under guidance of Dr. Vinay Tule, Eugeniks laboratory, Nagpur. M.Sc in Biotechnology from RTM Nagpur University. Ad-Hoc lecturer for UG and PG in Biotechnology and also institute for laboratory medicine. Two papers published in international journal. Three poster presented in National and International conferences.

Dr. Yagnesh Shirish Thakar owns Vishakha Clinical Microbiology Laboratory. Specializes in Microbiological and molecular diagnosis of Infectious diseases. Consultant Microbiologist, Care Hospital, Nagpur. MBBS from Govt. Medical College, Nagpur, MD in Microbiology from Govt. Medical College, Nagpur. M.A. in Public Administration from Nagpur University. Former Professor at Peoples Medical College, Bhopal and Indira Gandhi Govt. Medical College, Nagpur. 30 Research Articles published in National & International Journals. Authored a book on Microbiology for MBBS students. Co-authored three manuals on Clinical Microbiology and Immunology. Authored a manual on Hospital Infection Control Methods. Past President of Maharashtra Chapter of Indian Association of Medical Microbiologists. Past President of Vidarbha Association of Medical Microbiologists. Associated with Masonic Lodge, Lions and PEACE Foundation.

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Ref. No. smm/388/21-22

Date : 08/02/2022

To,
Dr. Yagnesh Thakar
Director,
Vishakha Clinical Microbiology Laboratory,
Nagpur

Subject: **Invitation to deliver a Guest Lecture at our College.**

Respected Sir,

I may be excused for disturbing your extremely busy studded schedule to apprise you with this letter that you are invited to deliver a ***Guest Lecture*** for the students of **B.Voc. Course in "Medical Laboratory and Molecular Diagnostic Technology"** of Sevadal Mahila Mahavidyalaya, Nagpur.

Organization of your Guest Lecture will be helpful to our students as a part of their curriculum.

Topic of the Lecture: **"PCR, an Effective Molecular Diagnostic Tool"**

Date and Time : **9th February, 2022 at 11.30 am.**

I hope your eminent lecture will definitely boost up the knowledge of our students.

We shall always remain grateful to you.

Thanking you.

Yours,

(Prof. Pravin Charde)
Principal,
Sevadal Mahila Mahavidyalaya
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Ref. No. Smm/427/2021-22.

Date : 24.02.2022.

To,
Dr. Yagnesh Thakar
Director,
Vishakha Clinical Microbiology Laboratory,
Nagpur

Letter of Thanks for accepting invitation as a Guest Speaker

Sir,

We thank you for the Guest Lecture on the topic, **"PCR, an Effective Molecular Diagnostic Tool"** organized by B.Voc. Medical Laboratory and Molecular Diagnostic Technology of Sevadal Mahila Mahavidyalaya, Nagpur on **9th February 2022.**

We once again thank you for guiding our students on this topic and also informing them about various types of PCR and its applications.

Thanking you.

Yours,

(Prof. Pravin Charde)
Principal,
Sevadal Mahila Mahavidyalaya
Nagpur

25/2/22
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