ORIGINAL RESEARCH ARTICLE

Diagnostic Accuracy of The TrueNat MTB/RIF Assay and Comparison with The Reference Standards to Detect Pulmonary Tuberculosis and Rifampicin Resistance in Sputum Samples from Patients Attending a Tertiary Care Hospital in Bhubaneswar, India

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ABSTRACT

Background: A rapid and accurate diagnostic tool is necessary for correct diagnosis and treatment of TB. This study evaluated and compared the sensitivity, specificity and concordance of TrueNAT MTB/RIF assay with smear microscopy, Xpert MTB/RIF and MGIT culture.

Methods: In all, 4500 patients (1500 each of patients with Diabetes, elderly and HIV-positive patients) attending the Chest and TB department of Capital Hospital, Bhubaneswar were screened. 392 sputum samples were collected from presumptive TB patients. Standard diagnostic procedures (Smear Microscopy, Xpert MTB/RIF, TrueNAT MTB tests and MGIT culture) were performed.

Results: This diagnostic efficiency of rapid molecular TrueNAT MTB/RIF assays have similar properties as Xpert MTB/RIF and may be used for the diagnosis of TB and Rifampicin resistance. Among participants, the TrueNAT MTB shows the sensitivity of 80%, 100% and 78.26% while the specificity was 97.9%, 95.83% and 100%. The concordance rates between all tests were calculated and the TrueNAT MTB showed good agreement with the culture method among study participants (κ = 0.793, 0.554, and 0.862, respectively).

Conclusion: The TrueNAT assays are sensitive for diagnosis of TB patients with faster turnabout time from testing to treatment and economical.

Keywords: Diagnostic accuracy; TrueNAT MTB/RIF Assay; pulmonary tuberculosis; RIF resistance

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INTRODUCTION

Tuberculosis (TB) is the major cause of mortality due to *Mycobacterium tuberculosis* in recent years. In the developing countries, several factors like poor healthcare settings, under nutrition, overcrowding and a higher percentage of people living with HIV (PLHIV) contribute to the expanding load of TB.[1,2,3] There is a huge divergence (2.9 million) in TB case notification due to reduced efficiency of the investigative procedures. Despite quality-assured services, TB control is insufficient due to delayed case diagnosis, which results in dissemination of disease, affliction and loss of life.[4] The standard procedures for TB diagnosis are radiography of chest, smear microscopy and culture testing. The gold standards for the diagnosis of TB include culture method (solid/liquid) and sensitivity tests.[5,6,7] Diagnosis of TB using sputum smear microscopy is a low-cost technique with a low sensitivity (20-78.3%).[8,9] Patient with smear-negative TB is less contagious than smear-positive TB, although smear-negative TB and culture-positive pulmonary TB patients can transmit *M. tuberculosis*.

To address the challenge within the diagnosis of TB, attention has been shifted to culture methods like liquid mycobacterial growth indicator tube (MGIT) which is programmed and more sensitive than sputum smear microscopy. However, culture includes a longer work time, higher contamination rate and very often requires transportation of specimens.[10] Its contribution to case finding of TB needs to be conscientiously assessed, as many patients with suspected TB might have started treatment or died even before the results of culture are accessible. Usually, chest radiographs are non-specific and not used alone for diagnosis of TB but are sensitive in detecting individuals with presumptive TB. The role of chest radiography for the diagnosis of TB must be reanalyzed.[11,12] Pulmonary TB patients having negative smear contribute to 12.6% of transmission, therefore, in nations with low incidence of TB and adequate public health resources, contact investigations of smear-negative TB as well as assisting smear-positive TB patients is necessary. *M. tuberculosis* exposure directly correlates with a higher incidence of TB. Some patients namely the elderly, those with type 2 diabetes, HIV-positive, people who have just received a transplant, people who have chronic kidney failure and people who are receiving treatments with tumour necrosis factor antagonists are more at risk of developing active disease from Latent Tuberculosis (LTBI).[13-17] The underlying immunologic pathways, the degree of immunodeficiency, the duration and timing of prior *Mycobacterium tuberculosis* exposure and other factors together affect the susceptibility of immuno-compromised persons for TB infection. The level of immune-suppression in the patient affects how clinically TB manifests in those who are immune-suppressed.[18] The relationship between these TB and diabetes has an impact as a result of the global rise in the prevalence of diabetes, which has been especially noticeable in TB-endemic nations. Along with under nutrition and HIV/AIDS, type 2 diabetes is currently one of the most prominent co-morbidities and risk factors among TB patients. Among elderly, a number of variables co-exist to make TB a specific problem. Age-related immunodeficiency, the possibility of additional immune-depressive conditions related to other aged co-morbidities, acute or chronic diseases, protective barriers, impaired microbial clearance mechanisms contribute to the anticipated under nutrition and the physiological changes linked with ageing can derange age-related decline in cellular immune responses to microbes like *M.tb.* The WHO (world health organization) has endorsed accessible, high sensitive technologies based on nucleic acid amplification assay (NAA) in order to enhance approach to TB detection in the backdrop of significant burden for faster diagnosis and RIF (rifampicin) resistance in around two-three hours.[19-22] Commercially available NAA tests namely Xpert MTB/RIF and line probe assay (LPA) have been widely used for the identification of *M.tb* complex and RIF resistance in 24-48 hrs.[23] Hence, a quick and precise tool for the diagnosis is necessary for the correct detection of smear-positive and smear-negative TB to confirm rapid diagnosis and active treatment which, in turn, might decrease the affliction and prevent transmission. The TrueNAT™MTB (Molbio Diagnostics, Goa) is a chip-based NAA test performed on the Truelab-UNO™ analyzer for detection of *M. tuberculosis*. Sputum is processed using Trueprep-MAG™ (a procedure based on nanoparticles) and real-time polymerase chain reaction (PCR).[24-27]

The present study was performed to evaluate and correlate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of TrueNAT MTB/RIF assay in patients with presumptive pulmonary TB with smear microscopy (ZN stain), Xpert MTB/RIF and Liquid MGIT culture.

METHODOLOGY

Ethics approval: The protocol of the study was reviewed and approved by the Ethics Committee of ICMR-RMRC, Bhubaneswar vide IRB No. ECR/911/-Inst/OR/2017 on 5.2.2018. All of the procedures were performed accordant with applicable rule and regulations. Informed consent was obtained from all individual participants and/or their legal guardian(s) included in the study.

Settings: Sputum specimens were collected from 3 groups of patients' namely patients with Diabetes, elderly and HIV-positive patients, with presumptive pulmonary TB attending the out-patient's department of Capital Hospital, Bhubaneswar. Standard diagnostic procedures (Smear Microscopy, Xpert MTB/RIF, TrueNAT MTB/RIF tests and MGIT culture) were performed for all patients by trained technical staff at the National Reference Laboratory.

Study population and specimens: A total of 4500 patients (1500 each of patients with Diabetes, elderly and HIV-positive patients) were screened from which 392 sputum samples were collected from patients with presumptive Tuberculosis (A patient exhibits symptoms or signs suggestive of TB disease).

Microscopic examination: Four numbers of sputum samples were collected from the symptomatic individual (two on the spot samples- Day 1 visit) and two more samples were collected on the Day 2 visit (one morning and one on the spot).

Microscopy (ZN Staining): AFB microscopy (acid-fast bacillus) was performed on the collected sputum samples. This involves staining the samples using the Ziehl-Neelsen (ZN) staining technique, which helps to visualize acid-fast bacilli under a microscope. The stained sputum samples were then graded based on the criteria recommended by the WHO.[8] This grading helps to assess the amount of AFB present in the samples, which can provide information about the severity of the infection. After microscopy, the samples were treated to improve the detection using a solution called 2% N-acetyl-L-cysteine (NALC) combined with sodium hydroxide (NaOH). This solution helps to break down mucus and other debris in the sputum, making it easier to concentrate the bacteria for further testing.[28,29, 30]

Xpert MTB/RIF: A new diagnostic approach called Xpert MTB/RIF, with the automated nucleic acid amplification mechanism detects *M.tuberculosis*, and the rpoB gene mutations that accord with RIF resistance, has the potency to address the functional difficulties. This assay substantially reduces the duration to diagnose tuberculosis. With this assay the median time to diagnose is 0 days, in contrary to 1 day for microscopy (traditional method), about 16 days and 20 days for liquid and solid culture.[21, 22] It reduces the time it takes to initiate treatment from a median of 56 days to only 5 days. Smear-negative TB cases can be challenging to diagnose using conventional methods, so this rapid diagnosis and treatment initiation can be life-saving. This assay helps to decrease the rate of untreated smear-negative culture-positive TB cases. Before using the assay, 39.3% of these cases went untreated. With the assay guiding treatment initiation, this rate dropped to 14.7%.[25]

TrueNAT MTB/RIF test: The TrueNAT MTB chip amplifies a specific portion of the ribonucleoside-diphosphatereductase gene, nrdB. This gene is specific to *Mycobacterium tuberculosis* and serves as a target for detection. The assay's limit of detection (LOD) is around 100 CFU (colony forming unit) per milliliter of sputum sample. Liquefaction buffer (two drop) was added to 0.5 ml of collected sputum sample for liquefaction and incubated it at room temperature for five minutes. Liquefaction buffer (two drop) and 0.5ml of processed sputum which was liquefied previously was added to lysis buffer and kept it at room temperature and then transferred to the cartridge's sample chamber and MTB DNA was extracted using Truepep Auto Chip interface and the Trueprep-MAG Sputum kit. 5 µL of the isolated DNA was processed through TrueNAT MTB microchip that contains freeze-dried mastermix (lypholized), which includes primers and a probe specific for *Mycobacterium tuberculosis*. The polymerase chain reaction (PCR) was performed through a pre-programmed setup on the device. The remaining of the same DNA extract was analyzed by TrueNat MTB-RIF Dx chip for MTB positive results.[23-26]

Data Analysis: All the data related to the demographical, social and clinical profile of the entrant of this study were validated by comparison and analyzed using SPSS v.20 software (IBM, USA). Descriptive statistics were used to characterize the participants. Sensitivity, specificity, accuracy and κ (kappa) values of smear microscopy, Xpert MTB and TrueNAT MTB assay were compared with culture method with estimated 95% confidence interval.[31]

RESULTS

A total of 4500 patients [1500 each of the patients with Diabetes, elderly and HIV-positive patients] attending routinely to the hospital were enrolled in this study and screened. Table 1 shows the social, demographical and clinical profile of study participants. Data are presented as median (inter-quartile range) or number (%)). Of these, 59% (2666) were males, 40% (1811) were females and 0.5% (23) were transgender. 0.5% (8) & 10.4% (156) of patients with Diabetes & HIV-positive patients had a history of TB, 1.8% (27) & 5.6% (84) had abnormal X-ray findings and 0.14% (2) & 0.4% (6) had hemoptysis. RIF resistance was positive in 0.06% (1) of the patients with Diabetes & 0.13% (2) with HIV-positive patients. 0.3% (5) of the elderly persons had a history of TB, 2.5% (38) had abnormal X-ray findings and 0.2% (3) had hemoptysis.

Table 2 shows the rate of sensitivity and specificity of the ZN Microscopy, TrueNAT MTB/RIF, Xpert MTB-RIF assays in contrast to reference standard of MGIT culture. Screen positive denotes symptoms of active TB disease, including: cough for more than three weeks, coughing up blood (hemoptysis), chest pain, sputum production, sweating at night, loss of appetite, uncontrolled weight loss, and feebleness or fatigue/exhaustion.

As per the reports of ZN staining, the sensitivity is 66.67% among the smear positive and 50% among the smear negative samples with diabetes. The specificity is 75% among smear positive and 100% among the smear negative samples.

The sensitivity and specificity are 100% among the elderly patients. Among the HIV-positive patients, sensitivity is 88% among the smear positive and 66% among the smear negative samples. The overall sensitivity of ZN staining is 83% and specificity is 100%.

Table 1: The clinical and demographic profile of study participants (n = 1500)

Parameters	Elderly	Patients with Diabetes	HIV positive patients
Age	75 ± 10	38.5 ± 10	38.5 ± 10
Gender			
Male	906 (60.4)	776 (51.73)	984 (65.6)
Female	594 (39.6)	724 (48.26)	493 (32.86)
Transgender	0	0	23 (1.53)
Last History of TB			
Yes	5 (0.33)	8 (0.53)	156 (10.4)
No	1495 (99.67)	1492 (99.47)	1344 (89.6)
Abnormal X-ray			
Yes	38 (2.54)	27 (1.8)	84 (5.6)
No	1462 (97.46)	1473 (98.2)	1416 (94.4)
Hemoptysis			
Yes	3 (0.2)	2 (0.14)	6 (0.4)
No	1497 (99.8)	1498 (99.86)	1494 (99.6)
RIF Resistance			
Xpert MTB/RIF	0	1 (0.06)	2 (0.13)
True NAT MTB/RIF	0	1 (0.06)	2 (0.13)
MGIT Culture	0	1 (0.06)	2 (0.13)

Table 2: Sensitivity and specificity of the ZN Microscopy, TrueNAT MTB/RIF, Xpert TB/RIF assay compared to the reference standard of MGIT culture

Assay and groups	Screen Positive	True Positive	False Positive	False Negative	True Negative	Sensitivity	Specificity
ZN Microscopy							
Patients with Diabetes (n=1500)	141	3	0	1	137	75 (19.43-99.3)	100 (97.32 - 100)
Elderly (n=1500)	74	2	0	0	72	100 (15.81-100)	100 (97.32-100)
HIV-positive (n=1500)	177	20	0	1	156	95.24(76.8-99.8)	100 (97.6 -100)
Overall (n=4500)	392	25	0	5	362	83.33 (65.2 – 94.3)	100 (98.97 - 100)
TrueNAT MTB							
Patients with Diabetes (n=1500)	141	4	1	1	135	80 (28.36-99.49)	97.79 (95.97-9.89)
Elderly (n=1500)	74	2	3	0	69	100 (15.81 – 100)	95.83 (88.30-9.13)
HIV-positive (n=1500)	177	18	0	5	154	78.26(56.30-92.54)	100 (97.63 -100)
Overall (n=4500)	392	22	4	8	358	73.33 (54.11-87.72)	98.90 (97.20-9.70)
Xpert MTB							
Patients with Diabetes (n=1500)	141	4	0	1	136	80.00 (28.36 - 99.49)	100 (97.32-100)
Elderly (n=1500)	74	2	0	0	72	100 (28.36 -100)	100 (95.01 -100)
HIV-positive (n=1500)	177	22	0	1	154	96.65 (78.05 – 99.89)	100 (97.63 -100)
Overall (n=4500)	392	28	0	2	362	93.33 (77.19-99.13)	100 (98.99-100)
MGIT Culture							
Patients with Diabetes (n=1500)	141	5	-	-	136	100 (47.82 – 100)	100 (97.32 -100)
Elderly (n=1500)	74	2	-	-	72	100 (15.82 – 100)	100 (95.01 -100)
HIV-positive (n=1500)	177	23	-	-	154	100 (85.18 – 100)	100 (97.63 -100)
Overall (n=4500)	392	30	-	-	362	100 (88.43 – 100)	100 (98.99-100)

Table 3: Accuracy and concordance of the Smear Microscopy, TrueNAT MTB/RIF, Xpert MTB/RIF assay compared to the reference standard of MGIT culture

	Culture +Ve	Culture -Ve	Se	Sp	PPV (95% CI)	NPV (95% CI)	Accuracy	Kappa
Patients with Diabetes								
Smear Microscopy (+Ve/-Ve)	3/1	0/137	75	100	100 [29.24 - 100]	99.27 [96.17 -99.87]	99.29	0.885
Xpert (+Ve/-Ve)	4/1	0/136	80	100	80 [39.76 -100]	99.26 [95.93 - 99.87]	99.29	0.885
TrueNAT (+Ve/-Ve)	4/1	1/135	80	99.26	80 [35.09 - 96.73]	98.54 [95.90 - 99.87]	98.58	0.793
Elderly patients								
Smear Microscopy (+Ve/-Ve)	2/0	0/72	100	100	100 [15.81 - 100]	100 [95.01 – 100]	95.14	0.654
Xpert (+Ve/-Ve)	2/0	0/72	100	100	100 [15.81 - 100]	100 [95.01 – 100]	95.14	1
TrueNAT (+Ve/-Ve)	2/0	3/69	100	95.83	40 [18.05 -66.87]	100 [94.79 - 100]	95.95	0.554
HIV-positive								
Smear Microscopy (+Ve/-Ve)	20/3	0/154	86.96	100	100 [15.81 - 100]	98.09 [83.16 - 100]	96.05	0.903
Xpert (+Ve/-Ve)	22/1	0/154	95.65	100	95.65 [84.56 - 100]	100 [95.77 - 99.90]	99.44	0.974
TrueNAT (+Ve/-Ve)	18/5	0/154	78.26	100	100 [81.47 - 100]	96.73 [93.41 - 98.53]	97.18	0.862

Se – Sensitivity; Sp - Specificity

With regard to TrueNAT MTB assay, the sensitivity is 80% and specificity is 97% among the patients with diabetes. Among the elderly patients, the sensitivity is 100% and specificity is 95.8%. Among the HIV-positive patients, the sensitivity is 78.2% and specificity is 100%. The overall sensitivity of TrueNAT MTB assay is 73.3% and specificity is 98.9%. The sensitivity of Xpert MTB is 80% and specificity is 100% among the patients with diabetes. Among the elderly patients, the sensitivity and specificity is 100%. Among the HIV-positive patients, the sensitivity is 96.6% and specificity is 100%. The overall sensitivity of Xpert MTB assay is 93% and specificity is 100%. With reference to MGIT assay, both the sensitivity and specificity are 100% among the patients with diabetes, elderly patients and the HIV-positive patients. The sensitivity and specificity of MGIT assay are 100% in general.

Table 3 shows the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), concordance and its accuracy in compared to the culture method are all displayed in the table below. Confidence intervals (CIs) of 95% were calculated in accordance with the binomial distribution. The κ coefficients were used to determine how closely test results agreed. The κ (kappa) values of all tests were assessed and the TrueNAT MTB/RIF showed significant agreement similar to the culture method among elderly patients, patients with Diabetes and HIV-positive patients (κ = 0.793, 0.554, and 0.862, respectively). The accuracy of TrueNAT MTB was 98.58%, 95.95% and 97.18% among elderly, patients with Diabetes and HIV-positive patients. As per the assessment microscopy and Xpert MTB/RIF shows the similar diagnostic precision as TrueNAT MTB/RIF.

Discussion

In this study, the TrueNAT MTB/RIF assay exhibit a remarkable compliance with smear microscopy, Xpert MTB and culture method. As a result, the assay could be a possible, rapid and accurate tool for diagnosis of TB positive cases among population with high risk in high TB burden countries like India.

In the clinical management of various mycobacterial diseases quick diagnosis of mycobacterial infections is essential. Traditional diagnostic assays for diagnosis of infections caused by *mycobacterium* are ZN staining (smear microscopy) and culture (liquid/solid). Smear microscopy is effective because it is quick, inexpensive, and simple to use, although it occasionally suffers from a lack of specificity and sensitivity. For the diagnosis of tuberculosis, culture method remains as the gold standard with its high sensitivity and specificity rate but the disadvantages are lengthened time and lack of laboratory infrastructure, that is only confined to the reference laboratory national wide.[23,24,25] PCR-based nucleic acid amplification test with its high sensitivity and specificity has been used to detect the mycobacterial DNA. In the present study, we correlated the TrueNAT MTB/RIF with ZN staining (smear microscopy), Xpert MTB/RIF and culture method.

Results showed that among elderly, patients with diabetes and HIV-positive patients, the TrueNAT MTB/RIF shows the sensitivity of 80%, 100% and 78.26% while the specificity was 97.9%, 95.83% and 100%. The κ (kappa) values of all the tests were assessed and the TrueNAT MTB/RIF shown significant agreement with the standard culture method among patients with Diabetes, elderly and HIV-positive patients (κ = 0.793, 0.554, and 0.862, respectively). The accuracy of TrueNAT MTB/RIF was 98.58%, 95.95% and 97.18% among elderly, patients with diabetes and HIV-positive patients. It was shown that microscopy and Xpert MTB/RIF have similar accuracy as TrueNAT MTB/RIF. Different survey displayed the related outcome as our present study. Though a few studies show the lower sensitivity rate of 50-70%.

The high Time to positivity (TTP) is a significant hurdle in quick diagnosis although culture is economical. In this study, TrueNAT MTB/RIF test had a TTP of about an hour, facilitating quick diagnosis of MTB DNA. The specimens were tested instantly without waiting for more specimens for processing. Another advantage is the lyophilized master-mix on chip which excluded the requirement of time for reagents to liquefy and reagent contamination leading to false positive results. The possibility of residuum between specimens is also prevented due to disposable, independent chip, designed to be used once. The on-screen results are displayed and transmitted via GSM/Wi-Fi/Bluetooth® for printing. These lightweight, portable devices are adaptable in outlying healthcare facilities.[32,33] The TrueNAT MTB/RIF test conforms to the needs of the resource-constrained health care and has good reactivity and precision for the detection of TB.This study indicated that the diagnostic efficiency of rapid molecular TrueNAT MTB/RIF assays have similar application properties as Xpert MTB/RIF and may be used as preliminary test for the diagnosis of TB as well as Rifampicin resistance in primary healthcare centres. One of the significant patient-centered outcomes is faster turnabout from testing to treatment. The TrueNAT MTB/RIF assays are economical when compared with microscopy and Xpert MTB/RIF. Several other studies have reported that the results of TB if tested by TrueNAT MTB/RIF, Xpert MTB/RIF or culture is treated as microbiologically confirmation of TB and the person with such diagnosis needs to be initiated on TB treatment.[32-36] While culture is regarded the valuable reference standard, its diagnostic performance is based on several points such as the procedure and media used for culture (solid/liquid), the quantity of sample cultured, condition of the samples and the mode of processing of the sample, decontamination process and concentration of the TB bacilli for culture. With its cost effective and rapid detection technologies TrueNAT MTB/RIF

will be used to prevent the spread of TB in low and high occurrence areas, but the limitation is that it unable to diagnose the multidrug resistant TB (MDR-TB), which is one of the major public concerns in developing countries like India.

In conclusion, the TrueNAT MTB/RIF assay has a quicker processing time and diagnoses the TB and Rifampicin resistance within 3 hours of time.

Strengths and Limitations

This study has specific robustness. All the laboratory tests for TB diagnostic testing were carried out at the authorized reference laboratory with robust quality check and commitment process. For specimen flow, 4 ml of sputum sample was collected and homogenized prior to processing. This facilitated the outcome of the TrueNAT MTB/RIF, the comparative (Xpert MTB/RIF) and TB culture on identical specimen. This is the preferable specimen flow to assess the outcome of assays as per latest guidelines for diagnosis of TB using sputum samples. Two specimens from each patient were cultured using standardized procedure in liquid and solid media. There were some constraints in this study. A comparison was made among the TrueNAT MTB/RIF, Smear microscopy, Xpert MTB/RIF and culture for their diagnostic precision (Sensitivity and Specificity) to diagnose TB.

The study is a significant evaluation of diagnostic accuracy of molecular test for TB. The number of HIV-positive patients and Rifampicin-resistant TB cases were very less, leading to improper evaluation of sensitivity in these groups. Therefore, more studies are required for evaluating spectrum of RIF resistance in hospital settings across different landscapes and patient communities. Although there is a visible advantage of rapid detection of TB among elderly, patients with Diabetes and HIV-positive patients, more studies are needed to evaluate the precision of the TrueNAT MTB/RIF assays and lower than expected outcome of the TrueNAT MTB/RIF assays among TB cases which are smear-negative and culture-positive in these at-risk populations.

Conclusion

The study reported that TrueNAT MTB/RIF assay is a sensitive assay for diagnosis of TB, including HIV-positive patients, as endorsed by the WHO. But for better management of patients, more data are required on the analysis and usage of TrueNAT-positive and culture-negative results.

Availability of Data & Materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contributions

Braja Sundar Barik was involved in Conceptualization; Data curation; Formal analysis; original draft. Shritam Das was involved in Data curation; Formal analysis. Khusbu Singh was involved in Investigation; Methodology; documentation of socio-demographic profile and sample collection. Sudatta Chandan was involved in Investigation; Methodology; documentation of socio-demographic profile and sample collection. Subrat Kumar Swain was involved in Methodology; documentation of socio-demographic profile and sample collection. Prashanti Nayak was involved in Investigation; Methodology; documentation of socio-demographic profile and sample collection. Omkar Abadhesh Mishra was involved in Investigation; Methodology; documentation of socio-demographic profile and sample collection. Shally Pandit was involved in data collection and formal analysis. Dr. Tahziba Hussain was involved in Conceptualization; Project administration; Resources; Software; Supervision; Visualization; review & editing. Dr. Dasarathi Das was involved in Validation; facilitated the microbiological work in NRL. Dr. Sanghamitra Pati, Director was involved in Supervision; facilitated the study by providing all necessary support.

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