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EDITORIAL

Original Articles

Case Study

AIMS AND SCOPE:

The SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS is the official journal of the SAARC TB and HIV/AIDS Centre (STAC). The Journal's main aim is to continuing education of personnel and the dissemination of the most up-to-date information in the field of tuberculosis, lung diseases and HIV/AIDS. It is devoted to dissemination of knowledge concerning various aspects of tuberculosis, lung diseases and HIV/AIDS. All articles and health research relevant to the practice of this Journal are published. This Journal is a forum for the publication of articles concerning the social, economic, public health, epidemiology, diagnostics, genetics etc. in the area of tuberculosis, lung diseases and HIV/AIDS. The scientific manuscripts presenting the results of public health importance are encouraged. The novel case reports which adds to the existing knowledge and consistent with the scope of Journal will be considered for publication. The Journal accepts review/mini-review, case report, short communications, and letters to editors within the scope.

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Editorial

In 2010, The World Health Organization (WHO) endorsed an automated molecular test machine named GeneXpert which could detect tuberculosis as well as rifampicin resistance tuberculosis as the preferred diagnostic method for individuals presumed to have multi-drug resistant TB (MDR-TB) or HIV-associated TB. Initially it was recommended to diagnose HIV associated TB or presumptive MDR-TB. Three years later the recommendation was extended conditionally and availability of resources to cover initial diagnostic testing for all adults presumed to have TB.

Globally, in 2017, tuberculosis is one of the top ten causes of death with an estimated 1.3 million deaths among HIV-negative people and there were additional 300 000 deaths from TB among HIV-positive people. The only recommended rapid test to diagnose the TB disease is the GeneXpertMTB/RIF assay. It can provide results within two hours, and was initially recommended for diagnosis of pulmonary TB in adults. Since 2013, recommendation has been updated to use the tool in diagnosing TB and rifampicin resistance in pulmonary, extra pulmonary and pediatric TB. The test has better accuracy than sputum smear microscopy.

Although GeneXpert machines can be placed from a peripheral clinic to a reference laboratory, the selection of the site must take in consideration of the workload, efficiency of referral networks, infrastructure human resources and cost effectiveness. Several studies assessing the cost of TB and drug-resistant TB diagnosis by GeneXpert globally reports that it costs lower than the conventional methods of diagnosing TB.

In this issue “Challenges in the diagnosis of drug-resistant tuberculosis by GeneXpert MTB/RIF assay in Nepal-2017” has emphasized the importance of GeneXpert MTB/RIF assay as a tool in the diagnosis of drug-resistant TB in Nepal. The study revealed the challenges, limitation and its optimal utilization. It emphasized the training support for operations, wider dissemination of the updated diagnostic algorithms among the clinicians and need of identification of a focal point in the central TB reference laboratory for maintenance, cartridge supply and machine calibration. The study identified lack of human resource and inadequate diagnostic algorithm as the main challenges. There is also the difficulty in operation of the machine due to frequent power failure, temperature differences and none/less availability of the cartridges and lack of expertise to store and dispose them. The most important issue identified is the lack of decentralized availability of the GeneXpert machine across the country.

It is assumed that the numbers of missing cases are not negligible and late/non detection of TB increases the risk of transmitting the disease, eventually distress and economic hardship for nation and oneself also. Progress in controlling TB and mitigating its consequences can be expedited through early diagnosis and treatment. Hence, several studies have revealed that the GeneXpert MTB/RIF assay has increased case-finding of TB and MDR TB. However, the performance of the existing diagnostic centres needs to be improved, strengthened and accelerated throughout the countries in the region. Subsequently, there is an urgent need to be addressed and identified challenges to optimize the future scale-up of GeneXpert centres to meet the target of End TB by 2030.

Chief Editor
Director, STAC

PREVALENCE OF MULTI-DRUG RESISTANCE AND ITS RISK FACTORS AMONG TUBERCULOSIS PATIENTS IN KASKI, NEPAL

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ABSTRACT

Introduction: Multidrug-resistant tuberculosis is an intense and feared problem, difficult to control and has shown a trend of increase worldwide. MDR-TB poses a therapeutic and infection control challenge with significantly higher rates of morbidity and mortality. Hence, this study was conducted with objective to assess prevalence of multidrug resistance and its risk factors among Tuberculosis patients in Kaski district.

Methods: The main component of the study comprised institutional based cross sectional study design which was conducted in directly observed treatment short course (DOTS) centers in Kaski district. The study period was from July 2016 to December 2016. The sample size for the study was 175 participants. Data collection was done through interview with used interview schedule, and review of patient treatment cards. Data were entered in Epidata software and analyzed by using SPSS 20 version software.

Results: The prevalence of multidrug resistance in Kaski district was 5.7 per cent. Variables such as TB history in past, TB treatment in past, and cured in past are found statistically significant ($p<0.005$). People with prior history of TB were shown to be 19 times more likely to get MDR TB than those with no prior history (OR=19.056, CI: 4.522-80.294). People with complete TB treatment in past were 0.2 times less likely to get MDR TB than those with incomplete TB treatment in past (OR=0.182, CI: 0.075-0.441).

Conclusion: Present of previous TB infection and prior treatment outcome (to be defaulted or failed in treatment) were also identified as the risk factors for developing MDR TB. Proper surveillance system is to be established in terms of complete treatment to all TB patients that leads the prevention from MDRTB and prevent potent expensive costs from medical care for MDRTB patients.

Key words: Tuberculosis, multidrug resistance, directly observed treatment short course

INTRODUCTION

Multidrug resistant TB (MDR-TB) is defined as resistance to the two most important first-line drug treatments, isoniazid and rifampicin [(1)]. Multidrug-resistant tuberculosis is an intense and feared problem, i.e. difficult to control and has shown a trend of increase worldwide.

Treatment of multidrug resistant tuberculosis is lengthy, toxic, expensive, and has generally poor [(2)]. MDR-TB poses a therapeutic and infection control challenge with significantly higher rates of morbidity and mortality [(3)]. Globally, in 2008, 440,000 cases of MDR tuberculosis are said to have occurred [(4)]. In Nepal, the latest estimate of proportion of MDR case was 2.9% among new cases and 11.7% among retreated cases. The NTP has planned to identify and treat approximately 300 cases of MDR-TB per year [(5)]. This study was

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conducted with objective to assess prevalence of multidrug resistance and its risk factors among Tuberculosis patients in Kaski district.

MATERIALS AND METHODS

Study design

This study was carried out as an institutional based cross sectional study design in DOTS centers of Kaski district of Nepal.

Study period

The study period was from July 2016 to December 2016.

Study setting

The study was conducted in DOTS centers of Kaski district of western development region in Nepal.

Sample size and sampling procedures

The sample size was determined by assuming latest drug resistance surveillance in Nepal by 2011. So, the prevalence among previously treated cases is 11.7%

Prevalence (P) = 0. 117
Maximum Allowable Error (d) = 5 % = 0.05
Sample size ($n_{1)} = z^2p$ (1-p)/d^2
Where,

Z = Z statistic at level of confidence
p = Expected Proportion
d = Maximum Allowable Error
P = 11.7/100000*100
= 0.117
q = 1-p
= 0.883
Now, n = z^2 pq / d^2
=159
Non response rate= 10% of 159
=15.9
~16
Therefore, the total sample size (N) = 159 + 16 =175

Simple random sampling technique was used to select the DOTS centers and sequential sampling method had been used for selection of participants.

For the selection of DOTs centers firstly, listed all DOTS centers and were chosen required DOTS centres (22 out of 62 DOTS centres).

For the data collecting, all the patients taking anti tuberculosis drug more than 2 month of the selected DOTS centers were included. In this study, sequential method was used for data collection.

Study population

Study population was the tuberculosis patients under DOTS therapy.

Inclusion criteria and Exclusion criteria

Tuberculosis patients were receiving medication under DOTS therapy at least two months were included in the study as study participants.

Patients who were unavailable on the day of data collection were excluded. Tuberculosis patient did not confirm diagnosed and patient did not register under DOTS centre.

Data collection tools and techniques

Data were collected through interview using structured questionnaire, and also reviewed of patient treatment card for necessary information. Face to face interview and review of treatment card had been carried out. Enough time had been provided to recall the information and to respond for questions.

Formulation of Questionnaire

Simple and clear colloquial language was used in the formulation of questions. A single variable was measured from each question. Questionnaire consisted of following components:

- Demographic information
- Behavioral information
- Previous TB and health service related factors
- Present of co-morbidities

Statistical analysis

Data were entered in Epidata software and analyzed by using SPSS 20 version software. Data were analyzed by using descriptive and inferential statistics. To describe characteristic

of participants, frequency distribution and cross tabulation between dependent and independent variables was done. Chi square test was used to establish the relationships between the dependent and independent variables.

Ethical considerations

Ethical approval was obtained from the Institutional Review Committee of Pokhara University Research Center, Pokhara University. Written informed consent was taken from participants before initiation of data collection. Participants were made clear about the purpose of study, benefits of the study to them and assuring them about the confidentiality of the information. The interview was conducted considering comfort for the respondent such as; sitting arrangement, not dominating the respondent and/or using a dominating voice and also leading questions were not been asked. Administration approval was taken from the district public health office of Kaski, Nepal

RESULTS

A total 175 TB patients were participated in this study. Table 1 shows mean age of participants was 35.18 16.72 years, and sex wise distribution shows that more than half (56.6%) were males. Highest proportion of participants was in the age group 21-40 years (52.6%). Around two third of the participants were Hindu followed by Buddhist and Islam.

Figure 1 shows that out of 175, 10 participants had suffered from MDR TB (5.7 %).

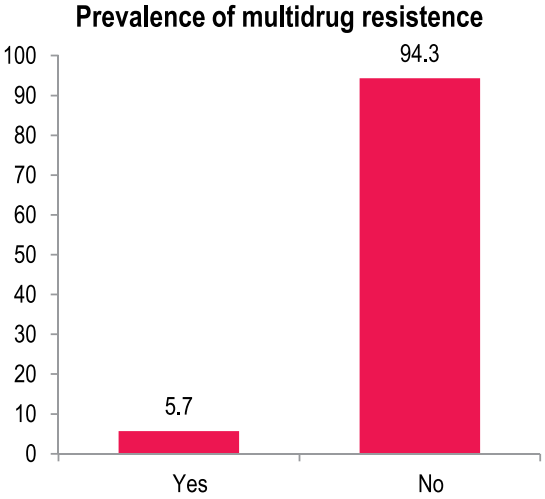

Figure 1: Frequency of multidrug resistance

Table 1 : Socio-demographic information of participants

Characteristics	Number	%
Age in years		
10-20	29	16.6
21-40	92	52.6
41-60	30	17.1
61-82	24	13.7
(MeanSD=35.18 16.72)		
Sex		
Male	99	56.6
Female	76	43.4
Ethnicity		
Dalit	25	14.3
Disadvantaged janajati	21	12
Disadvantaged non-dalit terai	4	2.3
Relatively advantaged janjati	56	32
Religious minorities	45	25.7
Upper caste group	24	13.7
Religion		
Hindu	163	93.1
Buddhist	11	6.3
Islam	1	0.6
Marital status		
Married	99	56.6
Unmarried	59	33.7
Widow	16	9.11
Divorce	1	0.6
Educational status		
Illiterate	23	13.1
Non-formal	30	17.1
Primary level	6	3.4
Secondary level	63	36
Higher secondary and above	53	30.3
Family type		
Nuclear	92	52.6
Joint	83	47.4
Number of members		
<5 members	95	54.3
≥ 5 members	80	45.7

Table 2 revealed that nearly one-third of the participants (29.1%) were ever smoked any tobacco product. Similarly, 31.4 per cent of participants were ever consumed any alcohol product.

Table 2 : Distribution of behavior related factors among participants		
Characteristics	Number	%
Ever smoke any tobacco product Yes No	 51 124	 29.1 70.9
Ever consumed any alcohol Yes No	 55 120	 31.4 68.6

Table 3 shows that 14.3% of the participants were suffered from TB in past. Similarly, 88% of participants had complete TB treatment history in past. Treatment outcome in past showed that 80% of the participants were cured by DOTS therapy in previously.

Table 3 : Distribution of health service related factors among participants		
Characteristics	Number	%
TB in past Yes No	 25 150	 14.3 85.7
Complete TB treatment in past(n=25) Yes No	 22 3	 88.0 12.0
Treatment outcome in past(n=25) Cured Defaulted Failure	 20 4 1	 80.0 16.0 4.0

Three-fifth (60%) of the participants was suffered from pulmonary TB and remaining of them (40%) was suffered from extra pulmonary. Regarding treatment category, fourth-fifth (81.1%) of participants were in category I while 17.7% were in category II and remaining of them (1.1%) were in category III. One-fourth (25.1%) of the participants had other disease along with TB. More than half (54.5%) of the participants had COPD along with TB, followed by other disease such as CVD and kidney related diseases and 11.4% of the participants had diabetes.

Table 4 : Distribution of disease related factors among participants		
Characteristics	Number	%
Types of TB Pulmonary Extra pulmonary	 105 70	 60.0 40.0
Types of treatment category Category I Category II Category III	 142 31 7	 81.1 17.7 1.1
Present of other chronic diseases (n=44) HIV/AIDS Diabetes COPD Others (CVD and kidney related diseases)	 3 5 24 12	 6.8 11.4 54.5 27.3

Table 5 shows age, sex, caste, religion, marital status, educational status, family type and number

Table 5 : Socio demographic variables and MDR TB				
Characteristics	MDR patients (10)	Non-MDR patients (165)	X^2	p-value
Age Below than 30 30 and above	 3(3.4) 7(8.1)	 86(96.6) 79(91.9)	1.89	0.169#
Sex Male Female	 7(7.1) 3(3.9)	 92(92.9) 73(96.1)	0.778	0.369#
Caste Upper caste & Relatively advtg. Janjaati Others	 6(10.7) 4(3.4)	 50(89.3) 115(96.6)	3.519	0.061#
Religion Hindu Non hindu	 9(5.5) 1(8.3)	 154(94.5) 11(91.7)	0.146	0.702#
Marital status Married Others	 7(7.1) 3(3.9)	 92(92.9) 73(96.1)	0.807	0.369#

Characteristics	MDR patients (10)	Non-MDR patients (165)	χ^2	p-value
Educational status Below secondary level Secondary level and above	 5(8.5) 5(4.3)	 54(91.5) 111(95.7)	1.194	0.275
Family type Nuclear Joint	 2(2.2%) 8(9.6%)	 90(97.8) 75(90.4)	4.757	0.29#
Number of members Less than 5 More than 5	 4(4.2) 6(7.5)	 91(95.8) 74(92.5)	0.870	0.351#

(#shows likelihood values)

of members were not significantly associated with MDR patients.

Table 6 shows that the association of the behavioral risk factors and MDR TB. Both behavioral risk factors such as ever smoked and ever consumed alcohol were found not significantly associated with MDR TB.

Table 6 : Association of behavioral risk factors and MDR TB

Characteristics	MDR patients (10)	Non-MDR patients (165)	χ^2	p-value
Ever smoke Yes No	 4(7.8) 6(4.8)	 47(92.2) 118(95.2)	0.65	0.43#
Ever consumed alcohol Yes No	 3(5.5) 7(5.8)	 52(94.5) 113(94.2)	0.01	0.92#

Table 7 shows that the association between TB treatment history and MDR TB. People with prior history of TB were shown to be 19 times more likely to get MDR TB than those with no prior history (OR=19.056, CI: 74.52-80.29). People with incomplete TB treatment in past were 12 times more likely to get MDR TB than those with complete TB treatment in past (OR=12.75, CI: 1.03-157.14). MDR TB showed that those being failed on treatment or defaulted from the treatment were associated with MDR TB.

Table 8 shows that association between presence of co-morbidity and MDR TB and its' associated with MDR TB with a protective effect. It indicated that those with co-morbidities were 0.778 times more likely to get MDR TB (OR=0.77, CI: 0.66-0.9). Problem suffered from HIV/AIDS, Diabetes, COPD, and other diseases (kidney diseases and CVD) were not statically significant with MDR.

Table 7 : Association between TB treatment history and MDR TB

Characteristics	MDR patients (10)	Non-MDR patients (165)	χ^2	p-value	OR	95% CI
TB in past (n=25) Yes No	 7(70) 3(30)	 18(10.9) 147(89.1)	26.88	<0.001#*	19.05	4.52-80.29
Complete TB treatment in past (n=25) Yes No	 4(18.2) 3(75)	 17(81.8) 1(25)	5.21	0.02#*	12.75	1.03-157.14
Treatment outcome in past (n=25) Cured Defaulted	 2(10) 5(83.3)	 17(90) 1(16.7)	11.99	<0.001#*	42.5	3.15-571.81

*(#shows likelihood values and *statically significant)*

Table 8 : Association between presence of co-morbidities and MDR TB						
Characteristics	MDR patients (10)	Non-MDR patients (165)	χ^2	p-value	OR	95% CI
Other disease along with TB (n=44) Yes No	 9(25.7) 1(0.76)	 35(74.3) 130(99.24)	 30.64	 <0.001#	 0.77	 0.66-0.9
Types of Problem suffered (n=44) HIV/AIDS Others	 3(37.5) 7(19.4)	 5(62.5) 29(80.6)	 1.112	 0.355#	 2.48	 0.47-12.97

(#shows likelihood value)

DISCUSSION

This study reveals that, 5.7% participants were suffered from MDR TB. Sixty per cent of the participants were suffering from pulmonary TB and remaining of them were from extra pulmonary. About 81.1% participants are in category I while 17.7% are in category II and remaining of them were 1.1% were in category III. Extra pulmonary TB shows that significantly associated with MDR patients. A cohort study completed in South Africa shows that MDR patients from 84% were smear positive [5].

This study revealed that nearly one-third of the participants (29.1%) were ever smoked any tobacco product. Similarly, 31.4 per cent of participants were ever consumed any alcohol product But there is no association between alcohol and smoking with MDR. The study conducted in Nepal and India also show that there is no association between smoking and alcohol with MDR TB [1, 2].

Study revealed that association between TB treatment history and MDR TB. People with prior history of TB were shown to be 19 times more likely to get MDR TB than those with no prior history (OR=19.056, CI: 74.52-80.29). People with incomplete TB treatment in past were 12 times more likely to get MDR TB than those with complete TB treatment in past (OR=12.75, CI: 1.03-157.14). MDR TB showed that those being failed on treatment or defaulted from the treatment were associated with MDR TB. The study completed in northwest Ethopia, Bangladesh and Pakistan also shows that there were significant association between MDR-TB and history of previous anti-TB treatment [6, 7, 8, 9]. A descriptive case-series study conducted at the DOTS Plus clinic at Bhim Hospital, Bhairahawa revealed that 67% had missed at least a few weeks of drugs during their previous treatment and 13% had been marked as defaulters and 20% had treatment failure [11].

Study shows that association between presence of co-morbidity and MDR TB and Its' associated with MDR TB with a protective effect. It indicated that those with co-morbidities were 0.778 times more likely to get MDR TB (OR=0.77, CI: 0.66-0.9). Problem suffered from HIV/AIDS, Diabetes, COPD, and other diseases (kidney diseases and CVD) were not statically significant with MDR but the research study completed in Georgia and England shows that there is highly association between HIV and DM with MDR TB [12, 13].

CONCLUSION

Behavioral risk factors such as smoking and alcohol were not found to be significantly associated with MDR TB. Presence of extra pulmonary TB had good strength to develop the MDR TB. Disease past history and treatment related factors; presence of TB in past and prior treatment outcome (to be defaulted or failed in treatment) were also identified as the independent risk factors for developing MDR TB.

RECOMMENDATIONS

1. Proper surveillance system is to be established in terms of complete treatment to all TB patients that leads the prevention from MDR

TB and prevent potent expensive costs from medical care for MDR TB patients.

2. Improper treatment of TB further creates the problem of MDR TB. So follow-up should be done properly at community levels.

3. Proper orientation and health education about MDR TB needs to be provided to all the MDR TB patients.

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CONFLICT OF INTEREST

None

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CHALLENGES IN THE DIAGNOSIS OF DRUG- RESISTANT TUBERCULOSIS BY GENE-XPERT MTB/RIF IN NEPAL

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ABSTRACT

Introduction: GeneXpert MTB/Rif assay is an automated, cartridge-based nucleic acid amplification test that can accurately detect both tuberculosis and Rifampicin resistance. Since its introduction, there has been a steady uptake of this technology by the National Tuberculosis Program of Nepal. Nevertheless, a large number of drug-resistant TB cases remains undiagnosed. This study aims to examine the challenges in diagnosis of drug-resistant tuberculosis by the GeneXpert MTB/Rif assay in Nepal and explore the possible solutions.

Methods: This was a cross-sectional study consisting of two parts – a quantitative part assessing the individual details and a qualitative part assessing the challenges on the diagnosis of drug-resistant TB by GeneXpert MTB/Rif assay. Data were collected from the GeneXpert operators, clinicians and program managers from 16 centers across the country and analyzed by IBM SPSS for Windows v23 and QDA Miner 4 Lite. Descriptive statistics were used to summarize the sociodemographic and other characteristics of the study participants using mean, standard deviation and proportions as appropriate.

Results: A total of 48 technical manpower participated in the study. The mean age was 39.95 years and a majority of them (77.3%) were male. The major challenges identified were inadequate training, frequent power failure, difficulty in maintaining appropriate steady temperature, module failure which is often not replaced in time, issues with calibration and timely availability of cartridges as well as appropriate ways to store the new cartridges and safe disposal of the used cartridges.

Conclusion: A number of challenges limit the optimal utilization of GeneXpert MTB/Rif assay warranting action.

Key words: Drug-resistant tuberculosis, GeneXpert MTB/Rif assay, challenges

INTRODUCTION

TB (TB) remains a leading cause of death and the recent epidemic of drug-resistant TB presents a major public health challenge. In 2017, an estimated 10 million people developed TB disease, of which the TB disease of 558 000 was resistant to Rifampicin (RR TB) or to both Rifampicin as well as Isoniazid (MDR TB).[1] The South East Asia region bears a huge burden of TB – in 2015, nearly half of the global TB cases occurred in this region, of which 200000 cases were MDR/RR TB.[2] In Nepal, TB is the sixth leading cause of death and 2.2% of the new TB cases and 15% of the previously treated TB cases have been estimated to have MDR/RR TB.[3]

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Globally, the priorities of TB control programs are to improve early case-detection and to enhance the capacity to diagnose drug-resistant TB. To help achieve this goal, the World Health Organization (WHO), in 2010, approved and endorsed an automated, cartridge-based nucleic acid

amplification test that is based on the GeneXpert multi-disease platform – GeneXpert MTB/Rif assay (Cepheid, Sunnyvale, CA, USA).[4] It can detect both TB and resistance to Rifampicin in less than 2 hours and has been hoped to be a game changer in the TB diagnosis.[5]A Cochrane systematic review and meta-analysis has reported that the sensitivity of the GeneXpert MTB/Rif assay is 89% when used as an initial test, 67% when tested in smear-negative samples and 79% when tested in the samples from HIV positive patients. The specificity is 99%. In comparison with smear microscopy, GeneXpert MTB/RIF increased TB detection by 23%.[6] The recent WHO policy update on GeneXpert MTB/Rif assay has recommended it for lymph node aspirate, gastric aspirate and Cerebrospinal fluid (CSF) as well with the sensitivities of 84.9%, 83.8%, and 79.5% respectively.[7]

In Nepal, GeneXpert services were introduced in December 2011 through the TB REACH Wave 2 funding of the Stop TB partnership.[8] At present, there are more than 50 GeneXpert machines in different locations across the country. Nevertheless, a large number of drug-resistant TB cases still remain undiagnosed. In 2017, out of 496 estimated cases of drug-resistant TB, only 343 cases were diagnosed and enrolled in the treatment program.[3] This study aims to identify the context-specific challenges in the diagnosis of drug-resistant TB in Nepal by the GeneXpert MTB/Rif assay within the existing National TB Control Program framework and explore the possible solutions to the problems identified to provide recommendations to the program.

MATERIALS & METHODS

This was a cross-sectional study consisting of two parts – a quantitative part assessing the individual demographic details and the relevant information based on his or her experience with the GeneXpert MTB/Rif and a qualitative part comprising of a Focus Group Discussion (FGD) on the challenges on the diagnosis of drug-resistant TB by GeneXpert MTB/Rif assay. Data were collected on July 2018 from the GeneXpert operators, clinicians and program managers from 16 representative GeneXpert centers across the country who gathered in five data collection sites.

(Table 1) Two trained data collectors in each of the study sites collected the data. A sign in sheet collected the information on the basic demographics, details on their work experience and the challenges faced in GeneXpert MTB/Rif assay utilization. It also served as an informed consent. The second part of the data collection consisted of an FGD using a structured format and recording the output of the discussion in the form of note-taking. A set of questions guided the discussions facilitated by probe questions when necessary.

The data obtained from the sign-in sheets were entered in Microsoft Excel (MS Office 2013, Microsoft Corporation, Washington, United States), cleaned, transported to and analyzed by IBM SPSS for Windows v23 (IBM Statistical Package for Social Sciences, 2015 IBM Corporation, New York, United States). For the analysis of FGD data, the transcripts were entered into and analyzed by QDA Miner 4 Lite (QDA Miner 4 Lite, 2012 Provalis Research, Montreal, Canada). Descriptive statistics were used to summarize the sociodemographic and other characteristics of the study participants using mean, standard deviation and proportions as appropriate.

RESULTS

A total of 48 participants were included in the study (Table 2). The information on age and sex

Table 1. Study centers and data collection sites.

Development Regions	Data collection sites	Additional study centers	Total no. of centers
Eastern	NATA Morang, Biratnagar	BPKIHS, Okhaldhunga	3
Central	NTC, Bhaktapur	GENETUP, Dhulikhel, HERD	4
Western	RTC, Pokhara	UMN Palpa, Lumbini Zonal	3
Mid-Western	TB Nepal, Nepalgunj	Kohalpur Medical College, Dailekh	3
Far-Western	Seti Zonal, Dhangadhi	Bayalpata Achham, Doti DHO	3
Total			16

was available for 44 participants. The mean age was 39.95 years (SD ± 11.5) and a majority of the participants (77.3%) were male. The mean work experience in TB was 11.7 years (SD ± 9.9) and that with the GeneXpert MTB/Rif assay was 3.61 years (SD ± 2.19).

Table 2. Details of the study participants.

Position	Service area	Number	Total
Operators of GeneXpert Machine	National Reference Labs	8	22
	Teaching Hospitals	3	
	Community Hospitals	3	
	Government Centers	4	
	Non-Government Organizations	4	
Clinicians			13
Program Managers			13
		Total	48

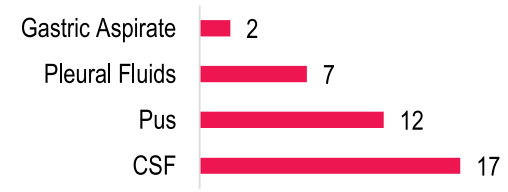
Operators:

Five of the 22 respondents (22.7%) reported that they had not received any formal training on the operations of GeneXpert MTB/Rif assay. Most of GeneXpert MTB/Rif operators reported that they run two (45.5%) or three (50%) cycles of GeneXpert MTB/Rif assays in a typical working day (Table 3).

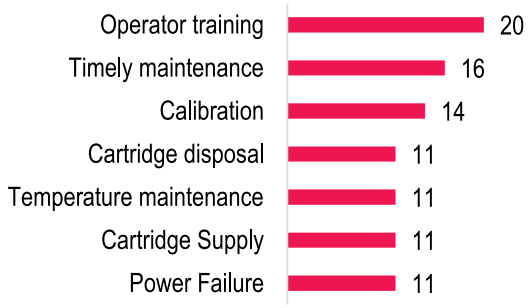
Table 3. Number of cycles of GeneXpert MTB/Rif tests per day.

SN	Number of cycles	Frequency	Percent
1	2	10	45.5
2	3	11	50
3	4	1	4.5

Just over half of the operators reported that they dispatched the GeneXpert MTB/Rif assay reports on the same day while the rest did on the next working day. All the participant operators responded that they had been conducting the GeneXpert MTB/Rif analysis of extra-pulmonary samples as well with CSF being the most commonly analyzed extra-pulmonary sample (Figure 1).



Among the problems reported by the operators, the most common was inadequate operator training (20 out of 22 responses) (Figure 2).



Clinicians:

The average number of presumptive cases of drug-resistant TB encountered was reported to be 1.31 (SD ± 0.48) and the average number of GeneXpert MTB/Rif assay requests made in a typical working day was 4.85 (SD ± 2.82). Only two (15.3%) of the 13 respondents reported that they were confident in selecting appropriate patients for GeneXpert MTB/Rif assay analysis and/or interpreting the test results.

All the respondents reported that they had been sending extra-pulmonary samples as well for GeneXpert MTB/Rif analysis with CSF being the most common extra-pulmonary sample for whom GeneXpert MTB/Rif assay was requested (12 respondents) followed by pleural fluid (9 respondents) (Figure 3).

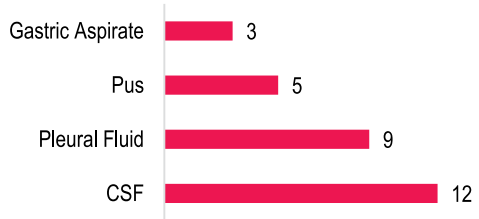


Figure 3. Extra-pulmonary samples requested for GeneXpert by clinicians.

Among the problems reported by the clinicians, the most common was the delayed availability of the GeneXpert MTB/Rif assay results and long waiting time to submit a sample for GeneXpert MTB/Rif assay (9 out of 13 responses) (Figure 4).

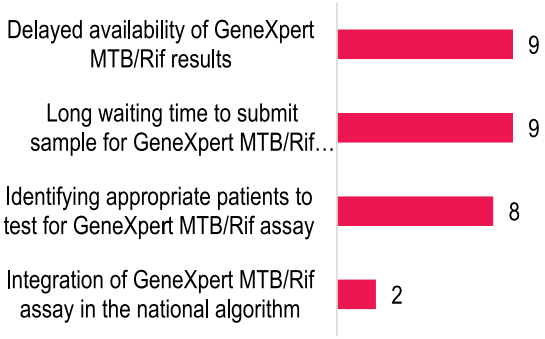


Figure 4. Challenges on GeneXpert identified by clinicians.

Program managers:

The average number of GeneXpert MTB/Rif assay test centers in the catchment program areas were reported to be 8.45 (SD ± 8.39). All except one participant responded that they thought the GeneXpert MTB/Rif assays were placed at the appropriate level of healthcare delivery system and the cost incurred justified the benefits (12 respondents each).

Among the problems reported by the program managers, the most common was lack of decentralization in the distribution of the GeneXpert MTB/Rif assay centers within their program areas (9 responses) (Figure 5).

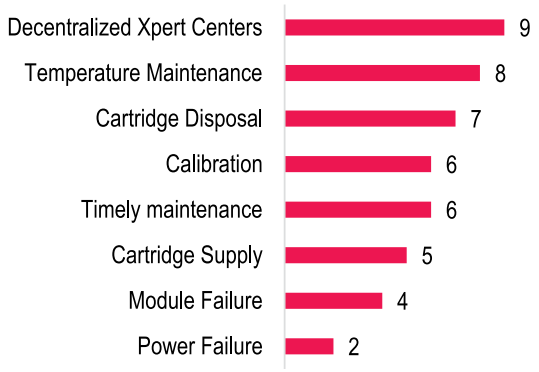


Figure 5. Challengeson GeneXpert identified by the program managers.

Focus Group Discussion

There were five sessions of the Focus Group Discussions conducted – one session each in the five development region capitals. Each Focus Group comprised of three operators, three clinicians, and two program managers. The Program managers were Regional and District level managers in the four development region capitals and National level managers in the Focus Group of the session at the National TB Center. The discussions lasted about one to two hours and were moderated by the two trained facilitators from the National TB Center, who were themselves operators or clinicians or program managers. A set of questions guided the discussions and note-taking accompanied the discussions. The moderators facilitated the discussions where necessary with aid of probe questions.

Key findings

Challenges in human resource and diagnostic algorithms

Most of the FGD participants shared that the human resource utilizing the GeneXpert MTB/Rif assay have not been well oriented to the updated diagnostic algorithms. The reported need for training support included that of basic operations, software handling, testing strategies and interpretation of the test results, especially when there is discordance.

Challenges in the maintenance and operations of GeneXpert MTB/Rif assay

The major challenges shared in the Focus Group Discussions were a frequent power failure, difficulty in maintaining appropriate steady temperature, module failure that is often not replaced in time, issues with calibration and timely availability of cartridges as well as appropriate ways to store the new cartridges and safe disposal of the used cartridges. These issues have been shown to have an impact on the erroneous reports.

Managerial challenges

One of the important managerial issues identified was the lack of decentralized availability of the GeneXpert machines across the country. The sputum courier system intended to fill the gap has often been inadequate. As a result, many patients with presumptive drug-resistant TB fail to be tested by GeneXpert. Often, the central TB lab has poorly responded to the requests for machine

maintenance or cartridge supply or calibration issues. Appropriate facilities to store the cartridges also seem to be lacking in many locations.

Recommendations and solutions

The focus Group Discussions have come up with a number of possible solutions to the challenges identified. First, ensure wide dissemination of the updated diagnostic algorithms among the clinicians as well as operators to minimize the confusions. This can be done by refresher training for the operators and workshops for the clinicians. Identify a focal point in the central TB reference lab in National TB Center, which can be a person or a group of persons who will respond to the requests for maintenance, cartridge supply, machine calibration etc. from the GeneXpert centers and proceed accordingly. The GeneXpert centers should be encouraged to manage the problems which can be managed locally. It was recommended to maintain a buffer stock of the modules (at least 25% of the total number of modules being operated) at the center so that they can be replaced soon to avoid or minimize lapses in services. These GeneXpert machines and the modules should be established according to the caseloads to avoid prolonged waiting times. National TB Center has been urged to come up with nationally agreed algorithms and recommendations in the use of extra-pulmonary samples for the diagnosis of TB, particularly drug-resistant TB.

DISCUSSION

Though GeneXpert machines can be placed anywhere, from a peripheral clinic to a reference laboratory, the selection of a site depends on the workload, efficiency of referral networks, the infrastructure requirements, the human resources capacity and running costs. These considerations often result in GeneXpert machines being placed above the peripheral level, which requires the establishment of the reliable specimen or patient-referral networks.[4] A study from India showed that the installation and operations of the GeneXpert MTB/Rif assay in the lower levels of health systems were feasible and required minimal infrastructural modifications.The Program Managers participated in the study felt that the GeneXpert MTB/Rif assays have been placed at the appropriate level of healthcare delivery system in Nepal.

Available studies have demonstrated that the diagnosis of TB is cost-effective in different settings from low-income countries like Tanzania to high-income low TB burden countries like the United States.[10,11] A study assessing the cost of TB and drug-resistant TB diagnosis by GeneXpert globally as well as in 36 high-burden countries reported that diagnosis of drug-resistant TB by GeneXpert would cost US$70–90 million per year globally and be lower cost than conventional diagnostics.[12] No studies could be found that have examined the cost-effectiveness of the GeneXpert within the National TB Control Program of Nepal. However, the TB Program managers studied have been involved in the planning and budgeting activities of the programs and all except one respondent were of the opinion that the cost incurred by the GeneXpert MTB/Rif assays was justified for the benefits obtained.

Among the operators who participated in the study, 95.5% reported that they run 2 to 3 cycles of GeneXpert testing in a typical working day. While the number of test outputs in a day also depends on the number of modules available, given the less than 2 hour turn-around time, the World Health Organization estimates 16 tests in an 8-hour working day using 4 module GeneXpert machine that amounts to 4 cycles of tests per day.[4] Increasing the number of test cycles per day, therefore, could potentially maximize the use of GeneXpert MTB/Rif assay. Considering the average number of GeneXpert MTB/Rif assay requests made by clinicians studied in a typical working day as 4.85, the number of test cycles per day needs to be balanced against the number of requests of GeneXpert received for optimal outcome

Among the extra-pulmonary samples reportedly being tested for GeneXpert MTB/Rif assay, CSF was the most common sample, followed by pus and gastric aspirate. The policy update on GeneXpert MTB/Rif assay by the WHO now recommends considering CSF, pus, gastric aspirate only for GeneXpert analysis.[7] Seven operators and nine clinicians participating in our study reported that they have been obtaining GeneXpert MTB/Rif assay samples for pleural fluids as well. While this may be justified for a limited number of reference labs or academic centers, routine testing of pleural

fluids and other body fluids should be limited. Perhaps, updating the operators and clinicians with the latest national algorithms and evidence base can help achieve this goal.

Although the GeneXpert MTB/Rif assay procedure takes less than 2 hours, many clinicians report that they receive their results days later. Frequently cited problems include laboratory operations issues, including limited staff, practices of batching specimens, and other logistical barriers such as inefficient specimen referral and transport networks. In order to expedite the test results delivery to the clinicians, an SMS based tool called XpertSMS has been rolled out in TB REACH projects in a number of countries, including Pakistan and Bangladesh.[13] These services can potentially minimize the delays in treatment decision by the clinicians.

The National TB Center conducts periodic training on identifying the individuals to test for GeneXpert MTB/Rif assays, the test results interpretations and clinical decision-making. However, these activities seem to be inadequate in building up the confidence in operations and/or test results interpretations. While an escalation of training activities seem to be an obvious initial response, a further assessment of the specific needs and tailored approaches would ensure optimal utilization of the available resources. A short-term hands-on training at National Reference Labs by the trained colleagues/superiors can be an option for the selected operators. For clinicians, wider dissemination of the most up-to-date diagnostic algorithms is recommended. Workshops for clinicians can be especially helpful in minimizing the use of GeneXpert MTB/Rif assay for treatment response monitoring, guiding the interpretations of discordant results and clinical decision-making. An easy access to the technical advisory group can supplement in the clinical decision-making process.

The GeneXpert machine requires a stable electric power supply. Interruptions in power may cause damage to the machine, results to be lost, cartridges to be wasted, and the need to obtain another specimen.[4] Therefore, a power stabilizer and an uninterrupted power supply unit (UPS) are recommended for the GeneXpert instrument. In the testing locations with frequent and longer power outages, an additional infrastructure to prevent the test cycle is needed. This will, in turn, prevent cartridges from being wasted and protect the equipment. Depending upon the frequency and duration of power outages, this may include an inverter with an external battery that can provide power for both the instrument and the computer for the average duration of the test – that is, 2 hours. An operational research in Uganda has demonstrated the feasibility of solar power at the district/sub-district level with abundant sunlight.[14] A guideline from the Foundation for Innovative New Diagnostics is available that provides a clear guidance on GeneXpert power backup solutions to meet different power supply scenarios.[15]

The power supply irregularities also have implications in the GeneXpert machine operations as well as cartridge storage conditions. The manufacturer recommends that the ambient operating temperature be maintained between 15 °C and 30 °C for the GeneXpert machine operations and the cartridges and the specimen reagent be stored at 2–28°C.[4] The operating temperature recommended is not different from the operating temperatures recommended for a wide range of other laboratory equipment, household appliances, and computers. Therefore, an airconditioningis required in many locations, especially with a humid climate, in Nepal, the Southern Terai region of the country. Ensuring that appropriate temperature is of utmost importance because operating outside the recommended temperature range may increase the error rates since extreme temperatures interfere with thermo-cycling during the test. Though the manufacturer has stated that the cartridges are stable if kept at 2-45 °C for less than 6 weeks at 75% relative humidity, an uninterrupted power supply to the refrigerator storing the cartridges is important.

Equipment maintenance is an important problem in many resource-limited settings, including in Nepal. One of the important maintenance issues is the module failure. One possible solution is the provision of buffer modules and spare parts within the country, which can potentially minimize or prevent service interruptions. Resources from the manufacturer can be a useful resource to try tackling the maintenance problem locally.[16] The GeneXpert modules require annual calibration.

A remote calibration option is available that uses a kit containing special cartridges that are run on each module without specimens. The process lasts approximately 20 minutes and each kit can calibrate 4 modules. However, in some cases, remote calibration will not be sufficient. Therefore, provision of maintenance services and support for shipping and distribution of supplies, including calibration have been identified as key demands.

CONCLUSION

While GeneXpert MTB/Rif assay is an important tool in the diagnosis of drug-resistant TB, various challenges limit its optimal utilization. Several recommendations can be formulated based on the current study. These include training support for operators, wider dissemination of the updated diagnostic algorithms among the clinicians as well as operators, identification of a focal point in the central TB reference lab in National TB Center who will respond to the requests for maintenance, cartridge supply, machine calibration etc. from the GeneXpert centers and proceed accordingly. Maintain a buffer stock of the modules (at least 25% of the total number of modules being operated) at the center is also recommended so that they can be replaced soon to avoid or minimize lapses in services.

CONFLICT OF INTEREST

None

ACKNOWLEDGEMENT

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TUBERCULOSIS INFECTION CONTROL MEASURES AT HEALTH FACILITIES PROVIDING TUBERCULOSIS SERVICES IN NEPAL

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ABSTRACT

Introduction: Globally there were an estimated 10.6 million new tuberculosis patients and 1.7 million deaths from TB in 2016. There is an evidence of tuberculosis transmission at health care settings where health care workers and patients come in contact with people having tuberculosis. This study aims to explore infection control measures at health facilities in terms of administrative, environmental and personal protective measures needed for infection control.

Methods: This is a cross-sectional study carried out at 79 health facilities across the country. The study continued for three months starting from January 2018 to March 2018. Trained enumerators from health sciences background collected the information using semi-structured questionnaire. Written consent was obtained prior interview.

Results: All the selected health facilities participated in the study. Around 44% of health facilities have infection prevention plan, but very few of them have budgeted for tuberculosis infection control activities. Less than one third of health facilities (24 out of 79 HFs) have provision to separate presumptive tuberculosis patients, however, only 50% (12 HFs) have turned such provision into action. Only 15 HFs (38%) out of 40 HFs having N95 or FPP2 mask for health workers. Around half of the HFs (44%, 35 out of 79) was found to have cross ventilation.

Conclusion: Tuberculosis infection plan needs to be developed and implemented by all the health facilities to strengthen administrative, managerial, and environmental and person protective measures of inaction control to minimize the risk of TB transmission at health facilities.

Key words: Infection control, infection prevention, tuberculosis, TB, Nepal

INTRODUCTION

Tuberculosis (TB) is one of the leading cause of death worldwide.[1]Globally there were an estimated 10.6 million new TB patients and 1.7 million deaths from TB in 2016.[2]Moreover it is a leading killer disease among HIV positive people accounting 40% of total death among HIV positive.[3]Besides, the emergence of drug resistant forms of TB has threaten the TB prevention and treatment efforts.[4]

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In Nepal, tuberculosis ranks among the top ten diseases causing morbidity and mortality.[5]TB incidence is152 per 100000 population.[6] In 2016, National Tuberculosis program registered 32,056 TB cases, half (53%) of them were new and relapsed pulmonary smear positive TB cases. National Tuberculosis Program provides TB diagnostics and treatment services free of cost to all TB patients across the country.[7]

There is an evidence of TB transmission in health care settings where health care workers and patients come in contact with people who have TB disease.[8,9]Insufficient tuberculosis infection control (TB-IC) measures at the facility pose serious risk to health workers and other patients attending health facilities.[10]Even, TB-IC is one of the WHO recommended 12 collaborative TB/HIV

activities.[11]The absence of TB-IC policy, guidelines and appropriate interventionsat health facility needs immediate attention to reduce the risk of TB transmission. Thus, this study aims to explore infection control measures at health facilities in terms of administrative, environmental and personal protective measures needed for infection control.

METHODS

This is a cross-sectional study carried out at health facilities providing TB diagnosis and treatment services. All the health facilities offering TB services (DOTS center, Microscopy center, Culture lab, DR center/sub center) were included in the sampling frame. This study continued for three months starting from January 2018 to March 2018.

Sample size for the study was determined based on the sampling manual for health facility surveys.[12]A total of 205 HFs was initially planned to visit for assessment. However, due to budget and time constraints, only 79 HFs (including 23 microscopy centers) from 8 districts (Morang, Khotang, Saptari, Sindhupalchowk, Tanahun, Rupandehi, Surkhet, Kailali) were selected for the study purpose. The cluster design adopted by Nepal Demographic and Health Surveys (NDHS) stratifies Nepal into three topographic zones (mountain, hill and Terai), five development regions. The same 15 sub-regional domain was planned to be used in this assessment.[13]Due to study limitations, we could not follow aforementioned technique. Thus, we randomly selected districts from each province for this assessment. A proportionate allocation of service delivery sites was done to select microscopy and DOTS center from selected districts.

This assessment majorly focused on three dimensions of infection assessment i.e. Administrative, Environmental and Personal protective equipment. The required information was collected using semi-structured questionnaire. Questionnaire was developed based on WHO health facility assessment checklist, and CDC TB-IC checklist. Similarly, a further consultation with the program and laboratory focal persons at National Tuberculosis Center (NTC) was done to contextualize the questionnaire in country's setting.

Trained enumerator collected information using face to face interview technique. Written consent was obtained from all the health workers prior the interview. A database was prepared in CSPRO 7 for data entry. Different checks (range checks, skip) were applied to maintain data quality. Data was further exported to STATA 14.0 for further analysis. Descriptive and exploratory data analysis (summary statistics, frequency distributions) was performed to assess the situation of tuberculosis infection control measures at the study sites.

RESULTS

All health facility participated (100%) in this assessment. This section elaborats the situation of managerial, administrative, and environmental measures adopted by health facilities for the tuberculosis infection control (Table 1).

Facility level managerial activities

Out of 79 health facilities (HFs), less than half (44%, 35 HFs) had a general infection prevention plan. Of those health facilities having infection prevention plan, only 24 health facilities had TB infection control (IC) plan included in their overall IC plan. Less than one third (28%, 22 HFs) had a focal person for infection control. Only 9 service delivery sites were found to have IC committee.

Administrative information of service delivery sites

Majority of HFs (89%, 70 out of 79 HFs) was found to screen patients for TB. However, less than one third (30%, 24 HFs)had provision for separation of presumptive TB patients. Among them, majority (80%, 19 out of 24 HFs) were found to separate presumptive TB patients. Around one third of HFs (34%, 24 HFs) had provision of mask for suspected or TB patients, while 19% (15 HFs) had provision of tissues for TB patients. Similarly, more than two third (71%, 56 HFs) had dustbin to dispose used tissue as a part of respiratory hygiene practice. Nearly half of the HFs (48%, 38 out of 79 HFs) had IEC materials on coughing etiquette. Among them, majority (90%, 34 out of 38 HFs) had placed IEC material at visible place to all patients. Nearly all HFs replied to provide health education to all TB patients. Health worker focused on use of tissue/ handkerchief while coughing (38%), followed by use of mask (24%), use of hand while coughing

(20%) while providing health education. However, only 14% of health workers were screened for TB by the respective HFs. More than half (56%, 13 out of 23) of the HFs had separate room for sputum sample collection followed by sputum collection inside the lab and near to the lab (26% and 18% respectively). Half of HFs used to disinfect the remaining sputum collection followed by burying it with other waste and bury it (40 % and 9% respectively).

Personal protective equipment

Only half of HFs (51%, 40 out of 79) had mask available at HF. Among them, more than one third (38%, 15 out of 40) had N95 or FPP2 mask. However, no HFs practice fit test for respirator before doing their regular work using masks. Half of health workers were found to have (49%) used gloves during lab work, while one fifth (27%) used gloves during sputum sample collection from suspected TB patients. Six out of every ten (61%) HFs had apron available for health worker. Among them, health workers from three fifth of HFs (77%, 37 out of 48) were found to have used apron. Only 17% HFs has provision to keep personal and lab apron separately. Only 3 HFs (13%) were found to practice wearing special shoes in lab. Majority of microscopy centers (20 out of 23) were found to disinfectant or bury remaining sputum after sample collection.

Environmental controls

Among them, around half of the HFs (44%, 35 out of 79) had cross ventilation. Specifically, among the microscopy centers, (91%, 21 out of 23) had proper sunlight at lab. More than one third of microscopy centers (26%, 6 out of 23) had exhaust fan in their lab. However, only 2 of them had exhaust fan properly placed to control direction of air. Majority of laboratory had wall (96%, 22 out of 23) and floor (96%, 22 out of 23) smooth to reduce the risk of TB transmission. Very few (4 out of 79) HFs had pick flow present at HFS to measure Air change per hour (ACH). Only 3 of them were found to have used pick flow to measure ACH and had maintained the record. Similarly, only 3 HFs had UVGI light, which was found installed by technical person. Only 10 HFs had biosafety cabinet available, of which only 6 were working. Three fifth (75%, 59 out of 79) of them had disinfectant available. Majority of HFs had Phenol and Hypochlorite at their disposal for the purpose of disinfection. All HFswere found to have basin. Two fifth of the lab (74%, 17 of 23) were found to prepare sputum slide on table, while rest of them prepared on slab.

Table 1: Details of TB infection control measures at health facilities

TB Infection Control (TBIC) measures at health facilities	Number (%) (n=79)
Managerial measures	
HFs having Infection prevention plan	35 (44%)
TB-IC included in Infection prevention plan (n=35)	24 (69%)
Budget allocated for TBIC (n=24)	12 (50%)
HFs has focal person for IC	22 (28%)
HFs has IC committee	9 (11%)
Previously IC assessment done in HFs	20 (25%)
Administrative measures	
Screening of TB patients in HFs	70 (89%)
Provision for separation of presumptive TB patients	24 (30%)
Practice of separation of presumptive TB patients (n=24)	19 (80%)
Provision of mask for patients	27 (34%)
Provision of tissue for patients	15 (19%)
Provision of dustbin to dispose used mask and tissue	56 (71%)
Personal protective measures	
Apron available for HW	48 (61%)
Apron used by HW	37 (77%)
Provision to keep personal clothes and lab apron separately	13 (17%)
Provision to keep used and clean apron separately	18 (23%)
HW uses special shoes in lab (n=23)	3 (13%)
Environmental Control measures	
Pick flow present at HFs	4 (5%)
ACH measured in HFs (n= 4)	3 (75%)
ACH flow recorded and maintained	3 (75%)
24-hour electricity available at HFs	72 (91%)
Adequate water facility at HFs	72 (91%)
Equipment for infection control	
Autoclave available at HFs	71 (91%)
Autoclave in working condition (n=71)	71 (100%)
Records of time, pressure maintained (n=71)	46 (65%)
Disinfectant in HFs	59 (75%)
Availability of buckets for waste collection	48 (63%)

DISCUSSION

Less than half of HFs had infection prevention plan and only few of them had budgeted for TB IC. Dedicated focal person to implement and monitor IC activities are crucial; however only 28% HFs had dedicated focal person to oversee infection control activities. A systematic review along with similar studies conducted in India, China and Nigeria have also underlined the need of administrative and managerial support for TB infection control measures.[14,15,16,17]A proper infection plan, designated focal person and adequate budget allocation are inevitable for proper planning and implementation of infection control activities at facility level. Similarly, prompt identification and separation of people with TB symptoms (i.e. triage) is crucial. However, this study found very few health facilities have provision for the separation of presumptive TB patients and very few of them has practiced it at their HF. Evidences have shown that cough etiquette alone is a successful measure for TB infection control and have highlighted the need of Information, Education and Communication materials and mechanism at HFs.[18]In this study, only 33% HFs has provision of mask for TB patients. Less than half of health facilities do not have IEC materials available on HFs.

Only half of HFs had respirators available for health workers. Furthermore, only 15 HFs has N95 or FPP2 mask. In line with other study, this study also highlights the needs of particulate respirators in HFs in order to have additional protection from risk of TB transmission.[19] Likewise, only half of the HFs had cross ventilation. Moreover, only 3 HFs had UVGI.Adequate ventilation and sufficient UVGI in health-care facilities is essential for preventing transmission of airborne infections and is strongly recommended for controlling spread of TB and respiratory infections.[20]

This study has couple of important limitations. First, this study couldn't cover all the facilities as determined by the sampling methodology due to budgetary and time constraints. It affected the generalize ability of this study. Similarly, private sector providing tuberculosis diagnosis and treatment services were not included in the sampling frame of the study. Expanding sampling frame beyond HFs under NTP could have brought additional evidences.

CONCLUSION

There is the risk of TB transmission at health facilities. Tuberculosis infection control measures at health facilities needs to be assessed and strengthened specifically the administrative, managerial, environmental and personal protective measures to minimize the risk of tuberculosis transmission. Different divisions/centers under Ministry of Health and Population (like National Health Training Center, National Health Education Information and Communication Center, Logistics Management Division, National Center for AIDS and STD Control, Management Division), National Tuberculosis Program, province and local level administrative bodies and health facilities should collaborate to strengthen the efforts and place TB infection control intervention among priority interventions.

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CONFLICT OF INTEREST

None

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PROTEOMIC PROFILE OF PULMONARY AND EXTRA PULMONARY TB SAMPLES

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ABSTRACT

Introduction: Tuberculosis (TB) is a major health problem in India, so early diagnosis and treatment of Mycobacterium tuberculosis (*M.tb*.) infection can prevent deaths from this pathogen. The secretion of proteins by *M.tb.* is important in diagnostic purposes for generation of therapeutic drugs and vaccines candidates for TB. The objective of this study was to identify the protein expression (biomarkers) in TB and Tuberculosis meningitis (TBM) using proteomic approach.

Methods: In this study, using Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDSPAGE), we analyzed the secretory proteins of M.tb. in serum, cerebrospinal fluid (CSF) samples. The identified proteins were determined by Total Lab- 100 Quantity One densitometry software.

Results: Our study showed that protein bands expressed in CSF samples reveals the presence of 72kD, 70kD, 44kD, 40kD & 16kD predominantly in TBM patients compared to healthy individuals. The electrophoretogram identified 97kD, 72kD, 44kD, 38kD, 29kD & 16kD predominant proteins in serum samples of TB patients.

Conclusion: The detection of secretory proteins in serum and CSF samples of *M.tb.* in TB and TBM patients gives reliable and early diagnosis of TB and TBM. The secretory proteins can be useful as immunodiagnostic and vaccine targets which can serve as important biomarkers

Key words: Tuberculosis, Mycobacterium tuberculosis, Tuberculosis meningitis, Electrophoretogram, Biomarkers.

INTRODUCTION

Tuberculosis (TB) is one of the most challenging infectious diseases which is caused byz single infectious disease agent *i.e. Mycobacterium tuberculosis (M.tb).* Globally, 9.2 million new cases and 1.7 million deaths occur due to this disease which is an impediment to human development in developing countries. [1] Furthermore, due to reactivation of dormant bacteria and emergence of multi-drug resistant (MDR) strains leads to serious threat in controlling the global TB efforts. [2] The World Health Organization (WHO) estimated the new MDR-TB cases and 60% of these cases were located in India and China. [3] So, it is prerequisite to study the genetics and physiology of *M.tb.* to understand the *M.tb*-host interaction, to learn how

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these bacteria circumvent host defense and causes disease in order to develop new anti-tubercular agents which made a significant progress in this field. [4]

TB is a communicable disease and patients with pulmonary TB are the most important source of infection. According to the site of infections, TB can be classified as pulmonary and extrapulmonary disease. The primary route of infection involves the lungs called as pulmonary TB, whereas the infection causing the meninges of brain called as extra pulmonary TB. The infection of TB is initiated by inhalation of droplet nuclei (1-5μm), expectorated by patients with active pulmonary TB (open TB), typically when the patient coughs, sneeze or talking. The risk of infection is dependent on several factors such as infectiousness of the source, the closeness of contact, the bacillary load inhaled, and the immune status of potential host. [5] Some times, the bacteria escapes from treatment of anti-TB drugs and cross blood brain barrier causes infections to meninges of host brain called tuberculosis meningitis (TBM). [6]

Early diagnosis of TB is helpful in treatment of patients and also aids in curbing the further transmission of disease. The various techniques available for the diagnosis of tuberculosis infection are microscopy (or) culture methods, fluorescent staming, antigen detection, ELISA based assays against various antigen preparation [7],but they possess certain limitations i.e. interpretation is difficult because sensitization with non-tuberculosis mycobacteria leads to false-positive tests and laborious processes etc. [8] The set of proteins encoded by genome referred as proteomics has become a useful tool in the study of microbial physiology. This technique has been used to assess differences in levels of protein between different mycobacterial species, clinical isolates and in response to certain stimuli. [9] Secretory proteins are the proteins released by *M.tb.* to the surrounding medium and are extremely important for diagnostic purposes and generation of therapeutic drugs and vaccines which are the useful biomarkers for immunodiagnostics of infection. [10, 11, 12] It was reported that enzyme based immunoassay technique have been developed for diagnosis of pulmonary and extra pulmonary TB and promising results have been observed in these studies using crude mycobacterium extracts. [13, 14] But, identification of mycobacterial proteins and its isolates by one and two-dimensional (2D) electrophoresis has been largely applied to broth grown cultures, because abundant protein amount are available here for both analysis and comparison. [15,16]

To the best of our knowledge the proteomic profiles of TB, TBM and its samples have been fragmentary. Thus, the present study was undertaken to observe variable pattern of proteomic profile in serum and cerebrospinal fluid (CSF) samples of *M.tb.* for the identification [3] and characterization of secretory proteins as potential biomarkers.

MATERIALS & METHODS

The study was conducted at the Research Laboratory in Central India Institute of Medical Sciences (CIIMS), Nagpur, Maharashtra, India and approved by the Institutional ethical committee and written informed consent was obtained from the patients. We prospectively selected serum samples from 20 active TB patients (13 male, 7 female; 23-61 years) and as well as 20 healthy individuals with no signs of clinical impairment and normal chest radiographs, were included as controls.

To diagnose active TB, sputum microscopy was done on two serial sputum samples by staining with Ziehl Neelson Stain as per the guidelines of India's Revised National Tuberculosis Control Programme. TB was confirmed if acid fact bacilli (AFB) and/or culture of sputum specimens were positive for *M.tb*. When both tests were negative, the patients were diagnosed by clinical symptoms. Clinical suspicion of tuberculosis was based on symptoms a) Chronic cough with or without expectoration or past history of TB b) Fever more than 2–3 weeks c) Progressive weight loss d) loss of appetite e) night sweats. The TBM was diagnosed based on the clinical features, which included subacute or chronic fever and signs of meningeal irritation with or without other features of central nervous system (CNS) abnormality.

Specimens

Venous blood was collected from all the patients and control subjects and allowed to clot, and after centrifugation (1000×*g*, 10 min) the serum was separated and stored at -20°C untill analysis.

The CSF samples were confirmed on the basis of BacT/Alert-3D culturing and examination includes total and differential cell count, biochemistry, and microscopic evaluation after Gram, India ink, and AFB staining.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

One and two dimensional electrophoresis:

The serum and CSF samples obtained from confirmed TB and TBM patients were subjected to SDS-PAGE. SDS-PAGE was performed with a vertical slab gel electrophoresis system (Broviga, India) using the standard Laemmali method. [17] Electrophoresis was performed with vertical slab gel electrophoresis system by using 10% running gel and 5% stacking gel. The *M.tb*. samples are prepared by mixing with 10µL of distilled water and SDS sample buffer and incubate on heating chamber at 65°C for 15min and electrophoresis was carried out at 250 volts/50 mAmps. After completion of electrophoresis, gels were developed using both with Coomassie brilliant blue GR-250 and silver staining method. The protein migration was allowed to proceed until the dye had migrated to the bottom of gel. The protein patterns were visualized using Total Lab-100 Quantity One densitometry software. Band size (i.e., molecular weight) was estimated using molecular weight markers (Genei, Bangalore, India) in a parallel lane.

The samples were subjected to 2DPAGE and were applied to isoelectric focusing strips with linear pH 4 to 7 gradients and focusing conducted using a ZOOM IPGRunner (Invitrogen, Carlsbad, CA). Strips were resolved by SDS-PAGE using 5 to 12% Bis-Tris gels (Invitrogen) followed by either staining with Coomassie or transfer to nitrocellulose. The developed gels were analyzed by using PD Quest Advanced software (Bio-Rad, Hercules, CA, USA).[5]

Data analysis

The sensitivities and specificities of serum and CSF samples for TB and TBM group were calculated. All the values obtained were expressed as mean and standard deviation (SD) and *P*-value <0.05 was considered as statistically significant. Statistical analysis was carried out using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA) (Data not shown).

RESULTS

The present study focused on the proteins expressed by *M. tb.* in serum and CSF samples. The expressed protein profiles were studied by 1D and 2D PAGE. According to the experimental plan, we selected five serum and CSF samples of TB and TBM patients, five samples of healthy individuals and two samples as controls. The serum samples were separated out and analyzed through SDS-PAG Electrophoresis. In the gel, 13 lanes were prepared, five were serum samples of TB patients, five samples of healthy individuals and two as controls in order to compare between TB patients, healthy individuals and control samples. Gels were developed by staining with Coomassie brilliant Blue and silver staining. The gel pattern of serum fraction was then compared with the pattern of direct serum of TB patients.

The expression of low molecular weight (m.w) protein band were indicated by blue arrow whereas, red arrows shows expression of high m.w. protein. In other words, the presence of low m.w. protein in the range of approx. 29kD was obtained only in the serum sample of healthy individuals and absent in the serum of TB patients and control group. **(Figure 1A)**. However, when we compare the proteomic profile of serum samples by using Quantity one densitometric software we found predominant band at 29kD region in TB patients whereas other samples shows the presence of predominant protein band in the region of 97kD, 72kD, 44kD, 38kD as compared to healthy group**(Figure 1B)**.

Similarly, the CSF samples of TBM patients, healthy individuals and controls were evaluated and found the presence of predominant protein bands at the region of 30kD, 68kD and approx. 97.4kD. In **Figure-2A** arrow (Green, Red, Blue, purple) indicates the expression of predominant proteins only in TBM patients as compared to healthy individuals and controls.

Figure (1) One dimensional SDS-PAGE of *M.tb.* Serum Positive Sample & Negative sample

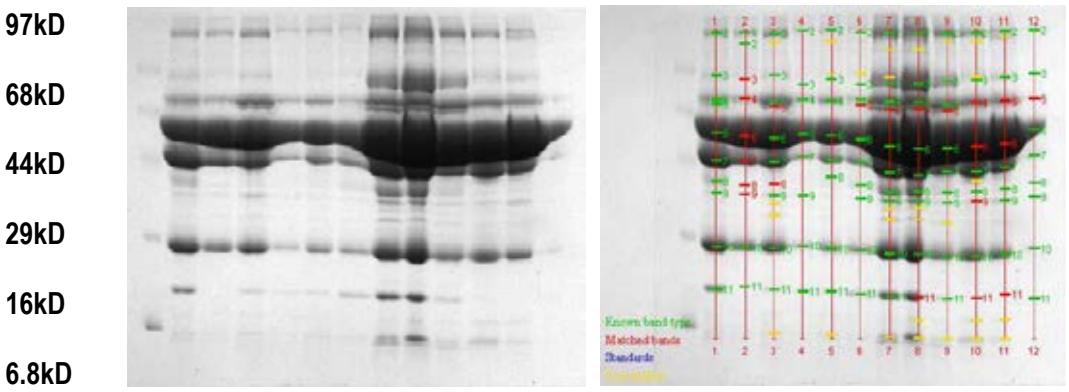

Figure 1(A) **Figure 1(B)**

Figure 1 A): Shows 1D SDS PAGE in *M.tb.* given serum positive, serum negative and the control for compare the sample.

Figure 1 B): Shows densitometry analysis in 1D SDS PAGE in *M.tb.* given serum positive, serum negative sample and the control for compare the sample.

Lane1- Molecular wt marker
Lane2-TB 1
Lane3-TB 2
Lane4-TB 3

Lane5-TB 4
Lane6-TB 5
Lane7-NTB 1
Lane8-NTB 2
Lane9-NTB 3
Lane10-NTB 4
Lane11-NTB 5
Lane12-Control Sample
Lane13--Control Sample

Figure (2): One dimensional SDS-PAGE of CSF Positive & CSF Negative sample 1 2 3 4 5 6 7 1 2 3 4 5 6 7

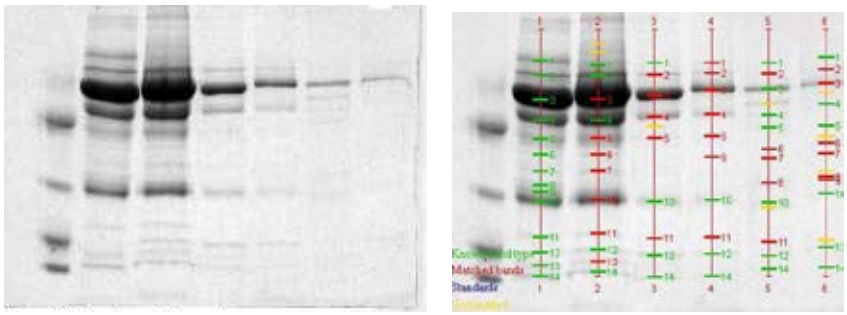

Figure 2(A) **Figure 2(B)**

2 A): Shows 1D SDS PAGE electrophoretogram in given CSF positive & CSF negative and their respective healthy control sample.

2 B): Shows densitometry analysis in 1D SDS PAGE electrophoretogram in given CSF positive & CSF negative and their respective healthy control sample.
Lane 1 - Molecular weight Marker
Lane 2 & Lane 3 - TBM
Lane 4 & Lane 5 – Non TBM
Lane 6 & Lane 7 - CSF Control Sample

DISCUSSION

Tuberculosis is a major health problem and reemerging as a major threat, which is rated as second most common infectious disease with high mortality rate than other infectious diseases. The diagnosis is based on relevant feature and compatible biochemical and cytological data from serum and CSF samples which varies from patient to patient. Similarly, there are various gold standard techniques available for the diagnosis of tuberculosis infections but they possess limitations like cost effectiveness, time consuming, laborious, less sensitivity and specificity.

So, as an alternative Anderson et al [18] have suggested the use of secretory antigens as potential biomarker for serodiagnosis of tuberculosis infection. Secretory antigens are secreted by *M.tb.* to the surrounding medium and most potent in generation of immune response and subsequent protection against tuberculosis infection. [15] Many researchers have attempted the use of various *M.tb.* antigen markers such as Lipoarabinomannan (LAM), 38kD antigen, purified protein derivatives, Antigen 85 complex, heat shock protein of 65kD and 14kD etc., which have now been recognized as the potential markers for the diagnosis of TB and TBM. [19] In our previous studies [14, 20], the 30kD protein band identified in the CSF of TBM pateints including components of the Ag85 complex suggests that this protein can be used as important molecular marker for the early and confirmatory diagnosis of TBM.

In the present study, we detected various protein markers in the serum and CSF of patients with active TB and TBM. We evaluated and compare the CSF samples of TBM, healthy individuals and controls and found there is presence of protein bands at the region of 30kD and 97kD and also a predominant protein band below and above the albumin region (68kD) (Figure 2A &B). In case of serum TB samples, the protein expression bands were decreased however increased in healthy individuals**.** As *M.tb.* secretes variety of protein in serum and CSF samples during their growth, these secreted proteins can be separate out through SDSPAGE electrophoresis which shows different protein pattern in different ranges. When we analyzed the serum and CSF samples with molecular weight (M.W) marker, we can easily detect the ranges of different secretory proteins with molecular weights.

In other words, the presence of low m.w. protein i.e. 29kD was found healthy individuals and absent in the serum of TB patients and control group. However, when we compare the proteomic profile of serum samples by using Quantity one densitometric software we found predominant band at 29kD region in TB patients whereas other samples shows the presence of predominant protein band in the region of 97kD, 72kD, 44kD, 38kD as compared to control group (Figure 1A &B). Our findings are in agreement with Johnson et al [21] and Upadhye et al. [22]

We have performed electrophoretogram analysis of all serum, CSF samples of TB and TBM healthy individuals and controls which found expression of some extra protein bands, in the serum and CSF of TB or TBM patients. In short we can say that in all cases of TB, the secretory proteins of *M. tb.* serve as an important diagnostic marker. These secretory proteins released during the growth of *M.tb.* in diseased person could be of important while differentiating from healthy individual.

Based on our study, it is clear that microorganisms secrete protein in diseased person as well as in healthy individual. The study of these secretory proteins is an important diagnostic criterion for the detection of Tuberculosis. All over the world as well in India many diagnostic methods and various techniques such as Polymerase Chain Reaction (PCR), ELISA, Western blotting etc. has been developed specifically for the diagnosis of TB and TBM. However, in our study we have performed SDS-PAGE, which was significantly helpful to compare serum and CSF samples of diseased and healthy individuals. On the basis of electrophoretogram we can conclude that in serum samples of TB patients 97kD, 72kD & 29kD proteins were predominantly secreted as compared to healthy individuals. Similarly, electrophoretogram of CSF samples reveals the presence of 72kD, 70kD, 44kD, 40kD & 16kD predominantly in case of TBM patients than healthy individuals.

In conclusion, our study highlights the expression of protein bands present in serum and CSF sample of TB or TBM patients gives reliable diagnosis and does not give false results with other non-

tuberculosis diseases. The identified protein bands are more important and useful for the diagnosis of tuberculosis which may prove as potential biomarkers in future.

LIMITATION OF THE STUDY

The characterization of identified protein bands has not been done in the present study and also the densitometric analysis of 2-D gel is not done.

CONFLICT OF INTEREST

None

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Case Study

COMMUNITY ACQUIRED STENOTROPHOMONAS MALTOPHILIA CAUSING EMPYEMA IN AN ADULT WITH HIV

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ABSTRACT

Introduction: *Stenotrophomonas maltophilia (S. maltophilia)* is multidrug resistant (MDR) organism usually associated with hospital acquired infections. Here we report a rare case of community acquired *S. maltophilia* empyema in a human immunodeficiency virus (HIV) positive patient.

Case Report: A 54 year old male presented with cough, breathlessness and chest pain for one month. On investigation, radiological picture was suggestive of massive right empyema. Pleural fluid culture grew *S. maltophilia* repeatedly which was treated with cotrimoxazole and levofloxacin based on antibiogram. Following improvement patient was discharged on anti-retro viral and anti-tubercular treatment.

Conclusion: Community acquired invasive *S. maltophilia* infections should be kept as differential diagnosis in immunocompromised patients. Being MDR, appropriate microbiological identification and susceptibility play an important role in treatment and outcome of these patients.

Key Words: *Stenotrophomonas,* immunocompromised, empyema, HIV

INTRODUCTION

Stenotrophomonas maltophilia (S. maltophilia) is an environmental emerging pathogen which is usually multi-drug-resistant (MDR). *S. maltophilia* is generally associated with hospital acquired infections in patients having co-morbidities or immune suppression. It may cause severe infections including pneumonia, endocarditis, meningitis, urinary tract infection, soft tissue and bone infections, peritonitis, bacteremia, multiple organ dysfunction. It is associated with substantial morbidity and mortality particularly in elderly patients with serious respiratory involvement requiring ventilatory support. However, community-acquired infections have been rarely reported. [(1)] These infections are difficult to treat as it is intrinsically resistant to many drugs including β-lactams.[(2)] Here we report a case of community acquired pneumonia with empyema caused by *S. maltophilia* in an HIV positive patient.

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CASE REPORT

A 54-year-old man presented with history of fever, breathlessness, chest pain and productive cough for one month. There was no past history of any chronic illness. General physical examination was unremarkable. On chest examination there were decreased chest movements on right side with stony dull note on percussion and absent breath sounds in right infra-mammary, infra-axillary, inter and infra scapular areas. Rest of the systemic examination was normal. On admission, white blood cell count and absolute neutrophil count were both normal (6.3x 10^3/μL, 4800/μL). An initial chest roentgenogram (Figure 1) showed massive right sided pleural effusion. Ultrasonography also revealed moderate right sided pleural effusion with multiple dense internal echoes suggestive of right loculated empyema. The patient tested positive for human immunodeficiency virus (HIV) infection as per National Aids Control Organization (NACO) guidelines.[(3)] CD4 count was 165/ mL. Antiretroviral therapy (ART) was withheld for two weeks till opportunistic infections were treated.[(3)]

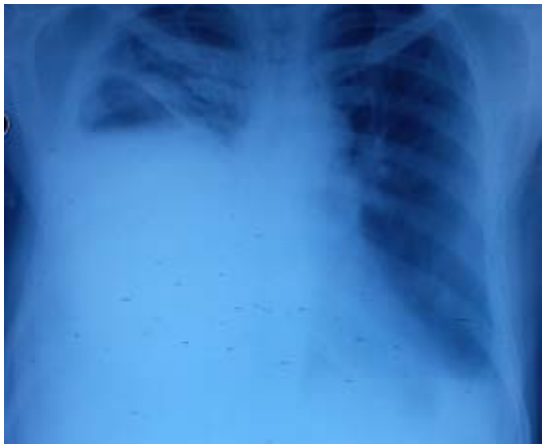

Figure 1. Massive right sided pleural effusion.

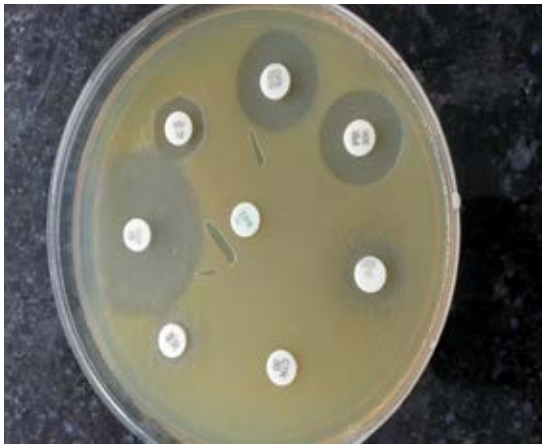

Figure 2. Antibiotic susceptibility by Kirby Bauer method

Empiric treatment with intravenous piperacillin-tazobactam, clindamycin and oral anti-tubercular drugs (ATT) was started along with co-trimoxazole prophylaxis in view of immunocompromised status. Inter-costal chest tube drainage was inserted in right 5th inter-costal space midaxillary line, which drained around one liter of thick pus. This specimen was sent for microbiological examination. Gram stain revealed many pus cells and gram negative bacilli, suggestive of pyogenic etiology. Smear for acid fast bacilli, Gene Xpert for *M. tuberculosis* complex, were negative. Pleural fluid culture grew yellow pigmented, non fermenting smooth colonies. The bacteria were gram negative and motile. Biochemical tests including catalase, citrate utilization, esculin hydrolysis were positive; oxidase, Indole, Methyl red, Voges-Proskauer reaction, Hydrogen sulfide, urea hydrolysis were negative. Based on the above findings, isolate was identified as *Stenotrophomonas maltophilia* and further confirmed by Microscan Autoscan-4 (Beckman Coulter semi automated bacterial identification and susceptibility system). Antibiotic susceptibility by Kirby Bauer method showed sensitivity to cotrimoxazole, levofloxacin, ciprofloxacin, cefoperazone-sulbactam and polymyxin-B but resistance to imipenem, gentamicin, amikacin, ceftriaxone and piperacillin (Figure 2). Following the AST report, cotrimoxazole was increased to therapeutic dose of 1500 mg once a day. The patient started improving clinically. The follow-up chest X-ray showed resolution and right lung expansion; computed tomography scan (CT scan) at day 15 showed only mild pleural collection with thick enhancing right parietal and visceral pleura associated with multiple air foci. Subpleural fibrotic streaks in right lower and right middle lung lobes was seen along with patchy areas of ground glass attenuation with ill defined nodular lesions in right upper lung lobes (Figure 3). The chest drain output decreased and repeat culture of fluid at this time also revealed growth of *S.maltophilia.* Hence intravenous levofloxacin 500mg was also added. There was further clinical and radiological improvement; drain was removed on day 20 and patient was discharged from the hospital. At discharge, patient was advised to continue cotrimoxazole for another 1 week, ATT and ART including fixed dose combination

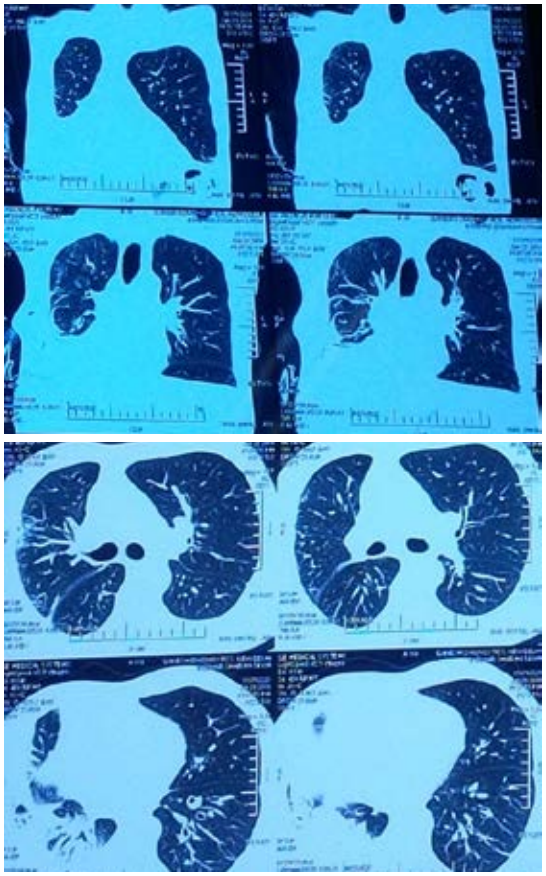

Figure 3. CT scan on day 15 of admission in hospital.

of tenofovir, lamivudine and efavirenz and was advised for further follow up at the ART and directly observed treatment short course (DOTS) centre.

DISCUSSION

S. maltophilia is an aerobic, motile, gram-negative multiple-drug-resistant organism.[4] It is an emerging pathogen which is associated with hospital-acquired infections, rarely community-acquired.[5] Our isolate was community acquired as it was isolated from pleural fluid culture from a patient with no previous history of hospitalization. These infections in community settings usually have an associated co-morbid conditions like prior hospitalization, chronic obstructive pulmonary disease (COPD), malignancy, HIV infection, or other immune suppressive conditions, trauma, prior antibiotic use.[1] It mostly causes pulmonary infections though it has also been known to cause eye, heart, brain, bone & joints and urinary tract infections. [1]

Possible community sources of infection may be water supply systems as these bacteria have been isolated from drains, water pipes, faucets, sponges, etc where they can form biofilms.[1] Biofilm formation is enhanced by *S. maltophilia* fimbriae 1 (SMF-1). Other virulence factors are lipopolysaccharides, diffusible signal factor system, flagella, extracellular hydrolytic enzymes like DNase, RNase, proteases, lipases, esterase, and fibrolysin which are encoded by *S. maltophilia* K279a genome. It can also transfer resistance genes to and fro from other MDR bacteria like *Pseudomonas, Sphingomonas, Serratia, Citrobacter, Proteus, Klebsiella* etc. Global warming is implicated with higher infection rate as the bacterial growth increases in environment which in turn increases the cell concentration leading to more chances of gene exchanges.[1]

In hospitals it has been isolated from tap water, endoscopes, suction tubings.[1] Risk factors for acquisition of this infection include HIV, malignancy, other immune suppressive conditions, COPD, central venous catheterization etc.[6] Our isolate was resistant to many drugs including carbapenems. Patients receiving long term carabapenem pose an increased threat to *S. maltophilia* infection to which it is inherently resistant[5]. Mechanism of drug resistance in *S. maltophilia* include chromosomal or plasmid encoded β lactamases, mobile elements; Class 1 integrons & insertion element common region (ISCR) elements responsible for resistance to cotrimoxazole; phosphoglucomutase (SpgM)-resistance to ceftazidime, gentamicin, nalidixic acid, polymyxin B and E, piperacillin-tazobactam, ticarcillin-clavulanic acid and vancomycin. Other mechanisms include efflux pumps, reduction in outer membrane permeability; modification of antibiotics; mutations of topoisomerase and gyrase genes. Genes for intrinsic resistance has been acquired in natural environment thus indicating the non-clinical settings for resistance transfer.

Cotrimoxazole is considered drug of choice when found to be sensitive, though the sensitivity ranges from >90% to <35%. [7,8] Alternatives being fluoroquinolones,colistin or tigecycline. [1] Our patient improved on therapeutic dose combination of cotrimoxazole with levofloxacin. In vitro pharmacodynamics studies on *S. maltophilia* have proven that combination of TMP-SMX with ciprofloxacin, ceftazidime, or tobramycin demonstrates higher bactericidal efficacy ($P < 0.0001$) than co-trimoxazole alone. [9] *S. maltophilia* being resistant to many drugs like β-lactam antibiotics including cephalosporins and carbapenems, aminoglycosides, macrolides, fluoroquinolones, chloramphenicol, tetracyclines, even TMP-SMX and polymyxins, pose difficulty in treatment leading to treatment failure or even death.[2]

Patient presenting with massive pleural effusion and being HIV positive leads to presumptive diagnosis of tuberculosis in countries like India with high tuberculosis prevalence. In turn, pulmonary tuberculosis is an independent risk factor for MDR organism co-infection like *Stenotrophomonas, Pseudomonas, Enterobacter, Proteus* etc.[10] This patient was also treated for *Mycobacterium tuberculosis* based on clinical diagnosis of Koch's disease along with *S. maltophilia* co-infection.

CONCLUSION

Community acquired invasive *S. maltophilia* infections should be kept as differential diagnosis in immune compromised patients. Being MDR, appropriate microbiological identification and susceptibility testing play an important role in treatment and outcome of these patients.

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