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Magnitude of *Mycobacterium tuberculosis*, drug resistance and associated factors among presumptive tuberculosis patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

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Abstract

Background

Mycobacterium tuberculosis (*M. tuberculosis*) remains one of the most significant causes of death and a major public health problem in the community. As a result, the aim of this study was to determine magnitude of *Mycobacterium tuberculosis*, its drug resistance, and associated factors among presumptive tuberculosis (TB) patients at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Methods

Cross-sectional study was conducted at St. Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia from January to July 2019. Demographic and clinical data were collected by structured questionnaire through face to face interview. Using microscopic examination and GeneXpert MTB/RIF assay and culturing in the Lowenstein-Jensen (LJ) culture media, we collected and analyzed both pulmonary and extra-pulmonary clinical samples. Data were analyzed by SPSS version 23. Binary logistic regression was done to identify the associated risk factors and p-value less than 0.05 was taken as significant association.

Results

Of the total 436 respondents, 223(51%) were male. The mean ±SD age of the participants was 38±17years. Overall, 27/436(6.2%) of the participants had confirmed *Mycobacterium tuberculosis* using the GeneXpert MTB/RIF assay and LJ culture media, and two isolates were resistant to RIF and one to INH medication, with two (0.5%) being MDR-TB. MTB infection was associated with previous TB contact history, patient weight loss, and CD4+ T-cell counts of 200-350/mm3 of blood.

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Abbreviations: ATCC, American Type Culture Collection; BCG, Bacillus Calmette–Guérin; DOT, Directly Observed Therapy; DST, Drug Susceptibility Test; FMOH, Federal Ministry of Health; HIV, Human Immunodeficiency Virus; INH, Isoniazid; IQC, Internal Quality Controls; MDR-TB, Multidrug-Resistant Tuberculosis; MOTT, Mycobacteria other than TB; MTB, *Mycobacterium Tuberculosis*; OADC, Oleic Acid Albumin Dextrose Complex; PPE, Personal protective equipment; PTB, Pulmonary Tuberculosis; RIF, Rifampicin; RMR, Rifampicin Mono-Resistant; SOP, Standard Operating Procedures; SPHMMC, Saint Paul's Hospital Millennium Medical College; SR, sample reagent; STM, Streptomycin; TB, Tuberculosis; TTD, Time to detection; WHO, World Health Organization.

Conclusion

The magnitude of *M. tuberculosis* and MDR-TB in this study underscores the need for improved early case detection and management of MDR-TB in order to reduce transmission and patient suffering.

Background

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* complex bacteria. The most common mode of transmission is through the respiratory system when an infected person coughs or sneezes near healthy people. However, *Mycobacterium bovis* can infect us while we are consuming and drinking unboiled milk from infected cattle [1]. World Health Organization (WHO) estimated that 9.9 million people developed tuberculosis (TB) and 1.5 million died of TB globally in 2020 and 22% of the world population are infected with latent *Mycobacterium tuberculosis*, of which 95% cases occur in resource limited settings [2]. According to a WHO report published in 2019, estimates that 3.4% of new patients, and 18% of previously treated cases have MDR-TB [3].

According to this report, the incidence of tuberculosis in Ethiopia is estimated to be 247 per 100,000, placing the country seventh in the world and fourth among Sub-Saharan African countries with significant TB burdens [3, 4]. In one study which was conducted in Ethiopia in 2018, the prevalence of MDR-TB in Ethiopia was 7.24%, of which 2.1% was new cases and 21.1% relapse cases [4].

Studies conducted in Ethiopia showed higher mortality rate in different health institutions; 11.3% patients died in Mekelle Hospital and Ayder Comprehensive Hospital [5], 14% children with TB and HIV co-infected from University of Gondar Comprehensive Specialized Hospital [6], and 29.5% of the patients died from MDR-TB in different hospitals of Amhara region, Northwest Ethiopia [7]. In general, the TB mortality rate in Ethiopia decreased from 393.8/100,000 to 100/100,000 between 1990 and 2016 with 75% decline, indicating a slow decline. As a result, study suggested that males had a higher TB mortality rate than females [8]. Microscopic examination in sputum stained smear detection rate for *M. tuberculosis* using light microscope up 80% in the case of fluorescence method applications ranged from 20% to 80% respectively.

This method can be used to detect TB when the clinical sample contains sufficient tuberculosis micro-organism without testing of the resistance pattern, but with less detection rate in immunocompromised people like HIV infected individuals and children, since they could not produce enough sputum [9].

Hence, GeneXpert MTB/RIF assay test should be used as an initial diagnostic test for TB and rifampicin resistance detection in patients suspected of having TB, MDR-TB or HIV-associated TB, as this test has high sensitivity and specificity [10]. Therefore, the aim of this study was to determine magnitude of *Mycobacterium tuberculosis*, magnitude of drug resistance and associated factors among presumptive TB patients referred to St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Materials and methods

Study area

The study was conducted in St Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. The hospital serves patients from all over the country. It has 392 beds, with

catchment population of more than 5 million. On average, the microbiology laboratory receives and process five sputum samples for pulmonary tuberculosis and two extra-pulmonary tuberculosis clinical samples per day.

Study design and period

From January to July 2019, a cross-sectional study was conducted at SPHMMC in Addis Ababa, Ethiopia. All patients who visited the hospital were the source population, and all patients with the diagnosis of presumptive pulmonary tuberculosis who visited the microbiology laboratory and met the inclusion criteria were the study population.

Inclusion and exclusion criteria

All patients with a presumptive diagnosis of *Mycobacterium tuberculosis* who visited a microbiology lab were included in the study. Patients with insufficient specimens or a history of known *Mycobacterium tuberculosis* treatment resistance were excluded from the study.

Variables

Magnitude of *Mycobacterium tuberculosis* and its drug resistance pattern among presumptive patients were dependent variables. Whereas, socio-demographic characteristics, possible risk factors like; TB contact history, previous treatment for TB, presumptive Drug Resistance Tuberculosis (DR-TB), BCG vaccination status, $CD4^{+}$ and HIV viral load counts were independent variables.

Sample size and sampling technique

To get the maximum sample size, the sample size was determined using the assumption of a single population proportion formula, taking into account a proportion of 50%, a margin of error of 5%, and a confidence level of 95%. The calculation result was as follows:

$$n = \frac{(z^{\alpha}/_{2})^{2}\ p\,(1-p)}{d^{2}} \Rightarrow \frac{(1.96)^{2}\ 0.5(1-0.5)}{(0.05)^{2}} = 384\ \text{study subjects.}$$

Where: n = minimum sample size,
P = estimated proportion of patients with *Mycobacterium tuberculosis* for the study population, and taking 10% non-response rate, the final sample size become 422 participants.
d = the margin of sample error, $z^{\alpha}/_{2}$ = the standard normal variable at 1-$^{\alpha}/_{2}$ confidence level and we used consecutive sampling technique was used to select the study population.

Data collection procedure

Data collectors were given training and instructions on how to collect the information. The study participants' socio-demographic status and related risk variables were collected using a structured questionnaire. For each patient with a presumptive diagnosis of *Mycobacterium tuberculosis*, a 2–4 ml of clinical sputum, lymph node aspirate or peritoneal and pleural fluid, and gastric aspirate samples were collected. For children who were unable to cough up sputum, we used gastric aspirate or induced sputum with physician assistance.

Laboratory procedures

For all samples, GeneXpert MTB/RIF, microscopy, and culture tests were done in parallel.

In the case of sputum samples, pellets were used for GeneXpert MTB/RIF assay. Sample reagent (1.5 ml) was added to 0.5 ml of the re-suspended sputum pellet and manually agitated twice at room temperature during a 15-minutes period.

In the case of other clinical body fluids, we transferred the entire specimen to a conical centrifuge tube, and concentrate the specimen at 3000 g for 15 minutes, carefully poured off the supernatant through a funnel into a discard can containing 5% sodium hypochlorite, re-suspend the deposit to a final volume of 2 ml by Phosphate Buffer Saline (PBS), using transfer pipette, we added a double volume of the GeneXpert MTB/RIF sample Reagent (1.4 ml) to 0.7 ml (2:1) of suspension, then 2 ml transferred to the concentrate and load to the GeneXpert MTB/RIF cartridge. The GeneXpert MTB/RIF purifies *M*. *tuberculosis* from these clinical samples. The genetic content of *M*. *tuberculosis* captured and subsequently amplifies the genomic DNA by polymerase chain reaction (PCR). In addition, it identifies RIF's resistance mutations in the RNA polymerase beta (rpoB) gene of *M*. *tuberculosis* in all clinically important samples within 2 hours [11].

Regarding microscopic examination, all sputum smears are prepared from decontaminated and concentrated specimens. The smears stained with Ziehl-Neelsen (ZN) staining techniques, could be used to count both viable and non-viable bacilli as acid-fast bacilli (AFB).

This method uses a carbolfuchsin as primary stain, acid alcohol as decolorizer, and methylene blue as counterstain. Acid-fast organisms stain red, while the background of debris stains blue. The ZN stain confirms the acid-fast property of mycobacteria using microscopy examination. Bacillary density will be graded as scanty, 1+, 2+, and 3+, and all such smears will be defined as "smear-positive".

Lowenstein-Jensen (LJ) culture medium was used which incorporates congo red and malachite green to inhibit unwanted bacteria for culturing. Once good growth was obtained, the positive slants were stored in a cool, dark place to archive the positive *M*. *tuberculosis* isolates.

Data quality assurance

The questionnaire was pre-tested and proper training was given for data collectors. The quality of data was maintained following the pre-analytical, analytical and post-analytical steps through each day supervision using standard laboratory procedures (SOPs).

Data analysis and interpretation

The collected data were entered to EPI info 2002 version 3.32 after data cleaning it was exported to SPSS version 23 windows software computer program for analysis. The logistic regression was employed to assess the association between TB and its risk factors. A p-value of less than 0.05 was considered as statistical significance.

Ethical considerations

This study was approved by Department of Medical Laboratory Science, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia. IRB also obtained from St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. Written informed consent was secured from each participant greater than 18 years old and assents were obtained for those less than 18 years old. Infected patients and/or those who had resistance *M*. *tuberculosis* were informed to their health care provider for better care and management.

Operational definition

MDR-TB: is non-susceptibility of *M*. *tuberculosis* at least two first line TB drugs (isoniazid and rifampicin).

Presumptive TB: a patient who presents with symptoms or signs suggestive of TB including cough >2week, fever >2week, significant weight loss, haemoptysis, any abnormality chest radiograph.

Presumptive MDR-TB: smear positive previously treated patients who define as relapse, return after default, and failure; new smear positive pulmonary TB patients who sputum remains smear positive at month 2 or 3 of treatment.

Results

Socio-demographic characteristics

A total of 436 participants were enrolled during the study period, of this 223 (51%) were male. The mean ± SD age the participants were 38±17years. The highest age range was 35–49 years old, while the youngest was under 15 years old. Majority of the respondents were 240 (55%) urban residents, and 214 (49%) had monthly income of 100–1000 Ethiopian Birr (Table 1).

Table 1. Socio-demographic characteristics among presumptive TB patients at SPHMMC, Addis Ababa, Ethiopia, 2019.

Variables/ characteristics		No. of Participants	Percentages (%)
Sex	Male	224	51
	Female	212	49
Age groups	<15 years	39	9
	15–24 years	56	13
	25–34 years	98	22
	35–49 years	127	29
	>50 years	116	27
Residence	Urban	240	55
	Rural	196	45
Family size/house	1–3	152	35
	4–6	220	50
	>6	64	15
Marital status	Single	146	33
	married	238	55
	Divorced	20	5
	Widowed	32	7
Occupational status	Laborer	97	22
	Government workers	97	22
	Private workers	109	25
	House wife	70	16
	Student	63	15
Educational status	No formal Education	119	27
	1-8th grades	147	34
	9-12th grades	106	24
	>12th grade	64	15
Monthly Income	<100 Birr	60	14
	100–1000 Birr	83	19
	1001–2000 Birr	155	35.
	2001–3000 Birr	59	14
	3001–4000 Birr	32	7
	4001–5000 Birr	25	6
	>5001 Birr	22	5.0

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Clinical data

There were a total of 374 (85.8%) pulmonary tuberculosis presumptive patients and 62 (14.2%) extra-pulmonary tuberculosis presumptive patients, with 130 (30%) of them being HIV positive. Presumptive TB was discovered in 422 (96.8%) of the individuals, while presumptive DRTB was found in 14 (3.2%) of the total participants.

In this study 33(7.5%) of the participants had history of contact with a TB patient, 68 (15.6%) had history of alcohol drinking, and 22 (5%) were cigarette smokers. Among the study participants 319 (73.1%) had fever, 311 (71.3%) had night sweating, and 365(83.7%) had cough. Out of 130 HIV positive participants, 104 (81%) were on antiretroviral (ART) treatment and were monitored based on their $CD4^+$ T-cells count. In addition, 119 (91.5%) participants were tested for HIV viral load. Higher magnitude *M*. *tuberculosis* seems to be appeared for those who have $CD4^+$count 200-350/mm^3 (5/34) and viral load $\geq$1000/mm^3 (6/90) (Table 2).

Magnitude of *M*. *tuberculosis* and its resistance pattern

Out of the total participants of clinical specimen, *M*. *tuberculosis* was detected in 36(8.3%) of the samples with GeneXpert MTB/RIF, and of which only 2 (0.5%) of them were RIF resistant. Regarding culture result, 27(6.2%) of the participants were confirmed for M. tuberculosis infection and one *M*. *tuberculosis* strain was resistant for Isozianide drug (mono-resistant) and 2 were resistant for Isozianide and RIF (Multidrug resistant TB).

Factors associated to *M*. *tuberculosis*

The bivariate logistic regression analysis of socio-demographic characteristics revealed that participants under the age of 15 years old were 1.8 times (95% CI: 0.4, 8.1) more likely to develop M. tuberculosis than those over 50 years old. Widowed participants were 2.6 times (95% CI: 0.4, 17) more likely to have *M*. *tuberculosis* than unmarried ones, and government workers were 1.8 times (95% CI: 0.6, 5.9) higher than housewives (Table 3). On bivariate logistic analysis, contact history with tuberculosis-infected patients, pneumonia confirmed by chest X-ray examination, and $CD4^+$results were associated factors for *M*. *Tuberculosis*; however, none of these factors were associated in the multivariate analysis (Table 4).

Discussion

People aged 35 to 49 years old, as well as those living in families with 4–6 members, had the highest frequency of tuberculosis. In terms of occupation, laborers earning between 1000 and 2000 Ethiopian Birr per month were the most susceptible to tuberculosis. This could be because these age groups are more likely to be subjected to high workloads and have a greater range of motion.

The current study found that as the number of people living together increases (5–6 family size), *M*. *tuberculosis* positivity increases as well. Other studies have found that having a larger family and malnutrition contribute to the development of tuberculosis [12], however, the present study found no association between family size/household and *M*. *tuberculosis*.

Higher *M*. *tuberculosis* was detected among participants in presumptive diagnosis of tuberculosis 25/436 (5.7%), non-vaccinated for BCG 18/436 (4.1%) than vaccinated, non-alcoholic drinkers 21/436 (4.8%) than alcoholic drinkers, and non-cigarette smokers 25/436 (5.7%) than smokers.

Again, among symptomatic tuberculosis patients, higher *M*. *tuberculosis* results were observed in those with night sweating 23/436 (5.2%), fever 22/436 (5.0%), weight loss 20/436

Table 2. Clinical characteristics participants among presumptive TB patients at SPHMMC, Addis Ababa, Ethiopia, 2019.

Variables/ Characteristics		Number of participants	Percentages (%)
Reason for diagnosis	Presumptive TB	422	97
	Presumptive DR-TB	14	3
BCG vaccination	Vaccinated	156	36
	Non-Vaccinated	280	64
TB contact history	Yes	33	8
	No	403	92
Alcohol drinking	Yes	68	16
	No	368	84
Cigarette smoking	Smokers	22	5
	Non-smokers	414	95
Night sweating	Yes	310	71
	No	126	29
Presence of fever	Yes	318	73
	No	118	27
Weight loss	Yes	200	46
	No	236	54
Presence of Cough	Yes	364	83
	No	72	17
Loss of appetite	Yes	285	65
	No	151	35
Presence of chest pain	Yes	206	47
	No	230	53
Presence of diarrhea	Yes	57	13
	No	379	87
Presence of dyspnea	Yes	140	32
	No	296	68
External-adenopathy	Yes	63	14
	No	373	86
Anti-TB Treatment	Previously treated	110	25
	Previously untreated	326	75
Presumptive DR-TB	New	384	88
	Relapse	46	11
	Failure	6	1
HIV status	Positive	130	30
	Negative	306	70
Tuberculosis type	PTB	373	86
	EPTB	63	14
$CD4^+$ count	<200 cells/mm^3	16	16
	200-350/mm^3	34	33
	>350/mm^3	53	51
HIV viral load	<1000/ mm^3	29	24
	≥ 1000/ mm^3	90	76

BCG = Bacillus Calmette–Guérin, DRTB = Drug Resistance Tuberculosis, EPTB = Extra Pulmonary Tuberculosis HIV = Human Immunodeficiency Virus, MDR-TB = Multidrug-Resistant Tuberculosis, MTB = Mycobacterium Tuberculosis, PTB = Pulmonary Tuberculosis

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Table 3. Socio-demographic factor analysis of *M. Tuberculosis* among presumptive TB patients at SPHMMC, Addis Ababa, Ethiopia, 2019.

Variables/ characteristics		#M.TB Not detected (%)	#M.TB Detected (%)	#Total (%)	COR (95% CI)	P-value
Sex	Male	208 (93)	16 (7)	224 (51)	1.4(0.6–3.1)	0.4
	Female	201 (95)	11 (5)	212 (49)	1	
Age groups	<15 years	36 (92)	3 (8)	39 (9)	1.8(0.4, 8.1)	0.41
	15–24 years	52 (93)	4 (7)	56 (13)	1.7(0.4, 6.6)	0.44
	25–34 years	92 (94)	6 (6)	98 (22)	1.5(0.4, 4.9)	0.55
	35–49 years	118 (93)	9 (7)	127 (29)	1.7(0.6, 5.2)	0.36
	>50 years	111(96)	5 (4)	116 (27)	1	
Residence	Urban	227 (95)	13 (5)	240 (55)	1	
	Rural	182 (93)	14 (7)	196 (45)	1.4(0.6, 2.9)	0.4
Family size/house	1–3	144 (95)	8 (5)	152 (35)	1	
	4–6	204 (93)	16 (7)	220 (50)	1.4(0.6, 3.4)	0.4
	>6	61 (95)	3 (5)	64 (15)	0.9(0.3, 3.5)	0.8
Marital status	Single	136 (93)	10 (7)	146 (33)	1	
	Married	226 (95)	12 (5)	238 (55)	1(0.2, 5.3)	0.9
	Divorced	17 (85)	3 (5)	20 (5)	0.8(0.2, 3.7)	0.7
	Widowed	30 (94)	2(9)	32 (7)	2.6(0.4, 17)	0.3
Occupational status	Laborer	89 (92)	8 (8)	97 (22)	1.4(0.4, 4.6)	0.6
	Government workers	91(94)	6 (6)	97 (22)	1.8(0.6, 5.9)	0.3
	Private workers	58 (92)	5 (8)	63 (15)	1.7(0.4, 6.4)	0.4
	Student	67 (96)	3 (4)	70 (16)	0.9(0.2, 4.0)	0.9
	House wife	104 (95)	5 (5)	109 (25)	1	
Educational status	Illiterate	112 (94)	7 (6)	119(72)	0.7(0.3, 2.4)	0.6
	1-8th grades	140 (95)	7 (5)	147(34)	0.6(0.2, 1.9)	0.4
	9-12th grades	98 (92)	8 (8)	106(24)	0.9(0.3, 3.0)	0.9
	>12th grade	59 (92)	5 (8)	64 (15)	1	
Monthly Income	<100 Birr	56 (93)	4 (7)	60 (14)	1.4(0.2, 13)	0.8
	100–1000 Birr	79 (95)	4 (5)	83 (19)	1(0.1, 9)	0.9
	1001–2000 Birr	147 (95)	8 (5)	155(36)	1(0.3, 9)	0.9
	2001–3000 Birr	54 (92)	5 (8)	59 (14)	1.8(0.2, 16)	0.6
	3001–4000 Birr	32 (100)	0 ()	32 (7)	-	0.9
	4001–5000 Birr	20 (80)	5 (20)	25 (6)	5(0.5, 46)	0.2
	>5001 Birr	20 (91)	2 (9)	22 (5)	1	

N.B: BCG = Bacillus Calmette–Guérin, COR = Crude Odds Ratio, DRTB = Drug Resistance Tuberculosis, HIV = Human Immunodeficiency Virus, MDR-TB = Multidrug-Resistant Tuberculosis, MTB = Mycobacterium Tuberculosis, TB = Tuberculosis

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(4.5%), cough 24/436 (5.5%), loss of appetite 20/436 (4.5%), and chest pain 16/436 (3.7%). The lowest results were reported in those patients with dyspnea 9/436 (2.0%), diarrhea 3/436 (0.7%), and palpable lymphadenopathy 3/436 (0.7%).

Although tuberculosis has been frequently observed in people who have had a history of contact with TB infected person who has already developed a cough, as well as cigarette smokers and alcohol users, our data suggest that these people are not significantly more likely to develop tuberculosis. These findings were different from the studies done in Addis Ababa, Ethiopia in 2011 and north Gondar in 2015 [13, 14].

The possible reason could be the lower number of participants diagnosed with presumptive DRTB and the fact that most participants live in an urban area. Higher results were observed

Table 4. Clinical factors associated with magnitude of *M. tuberculosis* among presumptive TB patients at SPHMMC, Addis Ababa, Ethiopia, 2019.

Variables/ characteristics		#M. TB not detected (%)	#M.TB detected (%)	#Total (%)	COR (95% CI)	P-value
Reason for Diagnosis	Presumptive TB	397 (94)	25 (6)	422 (97)	1	
	Presumptive DRTB	12 (86)	2 (14)	14 (3)	2.6(0.6, 12)	0.2
BCG Vaccination	Vaccinated	147 (94)	9 (6)	156 (36)	1	
	Non-Vaccinated	262 (94)	18 (6)	280 (64)	1.1(0.5, 2.6)	0.7
TB contact History	Yes	28 (85)	5 (15)	33 (8)	3.1(1.1, 8.7)	0.03
	No	381 (95)	22 (5)	403 (92)	1	
Alcohol Drinking	Yes	62 (91)	6 (9)	68 (16)	1.6(0.6, 4.1)	0.3
	No	347(94)	21(6)	368 (84)	1	
Cigarette smoking	Smokers	20 (91)	2 (9)	22 (5)	1.6(0.3, 7.0)	0.5
	Non-smokers	389 (94)	25 (6)	414 (95)	1	
Chest X-ray	Pneumonia	25 (89)	3 (11)	28 (7)	3(33, 319)	0.02
	Interstitial	28 (90)	3 (10)	31(7)	3(0.3, 30)	1.0
	Bronchiectasis	11(92)	1 (8)	12 (3)	2.6(0.3, 27)	0.34
	Bilateral	6 (43)	8 (57)	14 (3)	9(0.9, 8)	0.4
	Unilateral	14 (74)	5 (24)	19 (4)	0.5(0.6, 4.3)	0.5
	Normal	324 (98)	7 (2)	331(76)	1	
Anti-TB treatment	Untreated	103 (94)	7(6)	110 (25)	1	
	Previously treated	306 (94)	20 (6)	326 (75)	1.1(0.4, 2.5)	0.9
Presumptive DRTB	New	362 (94)	24 (6)	386 (89)	1	
	Relapse	44 (91)	2 (9)	46 (11)	0.7(0.2, 3)	0.6
	Failure	3 (75)	1(25)	4 (1)	5.0(0.5, 5.0)	0.2
HIV status	Positive	120 (92)	10 (8)	130 (30)	1.4(0.6, 3.1)	0.4
	Negative	289 (94)	17 (6)	306 (70)	1	
CD4 count/ mm^3 blood	<200	16 (100)	0 (0)	16 (15)	1.2(0.9, 2.4)	0.9
	200–350	29 (85)	5 (15)	34 (33)	8.9(0.5, 0.9)	0.049
	≥350	52 (96)	2 (4)	54 (52)	1	
Viral Load /mm^3 blood	<1000	27 (93)	2 (7)	29 (24)	1	
	≥1000	84 (93)	6 (7)	90 (76)	0.9(0.2, 5.0)	0.9

BCG = Bacillus Calmette–Guérin, CD = Cluster of Differentiation, Crude Odds Ratio, DRTB = Drug Resistance Tuberculosis, HIV = Human Immunodeficiency Virus, MDR-TB = Multidrug-Resistant Tuberculosis, MTB = Mycobacterium Tuberculosis, TB = Tuberculosis

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in patients who had previously been treated with anti-TB medications (20/436, or 4.5%), as well as new patients with a presumptive diagnosis of drug-resistant tuberculosis 24/436 (5.5%).

There was a statistically significant link between culture-positive pulmonary tuberculosis and TB contact history, pneumonia, and CD 4^+ counts, as well as several tuberculosis patient symptoms such as weight loss. The earlier study also found a link between pulmonary tuberculosis and the number of $CD4^+$ cells in HIV patients and the amount of virus in their blood [13, 15].

The current result seems similar with reports of study conducted in Addis Ababa, Ethiopia in 2017 [16], prisons settings of East Gojjam Zone, Northwest Ethiopia using GeneXpert MTB/RIF, 9(3.4%) [17] and 9.9% of the study conducted in extra pulmonary tuberculosis at University of Gondar, Northwest Ethiopia [18]. This overall culture confirmed *M.tuberculosis*, 27/436(6.2%) magnitude is lower than the study conducted in the Health Centers of Addis Ababa, Ethiopia reported as 46.0% (233/506) [13], from Metehara sugar factory hospital, eastern Ethiopia (14.2%) and 124 (32.2%) of studied in two public hospitals in East Gojjam zone, northwest Ethiopia [19].

We detected a reduced prevalence of tuberculosis (24.6%) when compared to a retrospective study report from the University of Gondar Hospital from January 2013 to August 2015 [20]. Our results were also lower than those of a study conducted in Debre Markos Referral Hospital in Ethiopia, which found a prevalence of 23.2% utilizing the GeneXpert MTB/RIF assay.

The difference could be due to the different diagnostic methods we used; for example, in our cases, we used the sputum sedimentation concentration technique for microscopic smear examination, GeneXpert MTB/RIF assay, and finally, LJ culture for confirmation, whereas in previous studies, a single diagnostic tool was used, such as stained by Ziehl-Neelsen staining and examined by Microscopy in Metehara [18], GeneXpert MTB/RIF in prisons settings of East Gojjam Zone [17]. This low prevalence could also indicate that TB infection control in our study area, Addis Ababa, Ethiopia, is relatively good.

From the overall confirmed *M*. *tuberculosis* 6.2% (27/436), a total of three *M*. *tuberculosis* strain showed resistance pattern to anti-tuberculosis drug, of which two of them were multi drug (INH and RIF) resistance strains. This result was lower than the study conducted in the University of Gondar Hospital, northwest Ethiopia which was reported as 71(15%) of tuberculosis-presumptive cases were resistant to rifampicin [20], and 15.58% of two public hospitals in East Gojjam zone, northwest Ethiopia [19], and 12 (10.3%) patients referred to Debre markos Referral Hospital, Ethiopia [21].

Only 10(7.7%) of the 130 HIV-infected patients tested positive for MTB, one mono (INH) resistant and one MDR-TB (INH+RIF) resistant strain were found in this seropositive figure.

In terms of viral load and tuberculosis, only one mono resistant strain was discovered in the participant serum, which has a high viral load count (1000/mmm3). This could be linked to HIV infection, which causes anti-TB drug mal-absorption and immunological suppression, leading to resistance, and our findings are backed up by previous research [22, 23].

According to the bivariate logistic analysis, patients with a presumptive diagnosis of drug resistance were two times more likely 2.6 times (95% CI 0.6, 12, p = 0.2) to acquire tuberculosis than those with a presumptive diagnosis of tuberculosis. Patients who also had night sweating were two times more likely to get tuberculosis 2.4 times (95% CI 0.8, 7.2, p = 0.1) than those who did not have. When compared to patients who did not have chest pain, having chest pain was also associated with had 1.6 times, (95%t CI 0.8, 3.7, p = 0.2) greater risk of getting *Mycobacterium tuberculosis*.

Conclusion

In general, this study found low-magnitude *M*. *tuberculosis* in patients with presumptive diagnosis of TB at SPHMMC in Addis Ababa, Ethiopia. And three DRTB strains, including two MDR strains, were discovered in individuals with a history of failure, relapse, and previous anti-TB treatment.

Contact with tuberculosis-infected patients, weight loss, pneumonia on radiological examination, and low CD4+ levels were all found to be linked with *M*. *tuberculosis*. To maintain this low illness outcome, health education on tuberculosis, TB control programs, and large community-based studies should be continued. To lessen the incidence of MDR-TB, it is also advised that TB infection control activities be strengthened and DOT be properly implemented.

Supporting information

S1 File. This is the S1 of English and Amharic language version of the questionnaire.
(DOCX)

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