



Frequency of MTB and Rifampicin Resistance using Xpert-MTB/RIF Assay among Patients Attending National TB Control Program in Sana'a, Yemen

Tawfique. K.A. Alzubiery

Associate professor in clinical Microbiology

Department of medical laboratory Faculty of Medical
and Health Sciences. Taiz University Al-Turbah branch

Talal. Alharazi

professor in Medical Parasitology Department of Medical
Microbiology and Immunology, Faculty of Medicine
and Health Sciences, Taiz University

Aref Mohammed Alhakimi

Associate professor of Medical Microbiology and
Immunology, Faculty of Assistance Medical Sciences
Department of Laboratory Medicine, Dar Al-Salam
International University for Science & Technology

Mohammed A. Alqubati

BSc. medical laboratory- National TB Control Program

Received: 7/11/2025

Accepted: 20/12/2025

Journal Website:

<https://journal.alsaeeduni.edu.ye>

Frequency of MTB and Rifampicin Resistance using Xpert-MTB/RIF Assay among Patients Attending National TB Control Program in Sana'a, Yemen

Tawfique. K.A. Alzubiery

Associate professor in clinical Microbiology,
Department of medical laboratory Faculty of Medical
and Health Sciences. Taiz University Al-Turbah branch.

Talal. Alharazi

professor in Medical Parasitology Department of Medical
Microbiology and Immunology, Faculty of Medicine
and Health Sciences, Taiz University,

Aref Mohmmmed Alhakimi

Associate professor of Medical Microbiology and
Immunology, Faculty of Assistance Medical Sciences
Department of Laboratory Medicine, Dar Al-Salam
International University for Science & Technology

Mohammed A. Alqubati

BSc. medical laboratory- National TB Control Program

Abstract

Background: Mycobacterium tuberculosis is one of the major health problems in world. It is the second-highest cause of death among communicable diseases. According to the World Health Organization, 9 million people fell ill with TB and 1.5 million died from TB. Over 95% of TB deaths occur in low- and middle-income countries. TB incidence rate is falling, but not fast enough. In Yemen, MTB is a major public health problem, it was estimated that, Yemen MTB endemicity comes lower-middle-income. With an incidence ranged (177 and 277) of MTB in Yemen per 100000 population and mortality rate ranged 12.2% to 21%. So we aimed this study to determine the Frequency of MTB and Rifampicin Resistance MTB among Patients Attending National TB Control Program. **Material and method:** this study is a cross sectional study. **Method:** A total of 8896 specimens were

collected from suspected Tuberculosis patients and all of them were initially screened for acid-fast bacilli through Zeihl Neelson (ZN) microscopy and positive samples were further assayed by Real-time Polymerase Chain Reaction (GeneXpert). **Result:** A total 551 (6.1%) were positive by either and or by both GeneXpert and ZN staining. of these, 476 (5.4%) were positive by both ZN and GeneXpert. whereas, the result of 75 remaining specimens was 65 of specimens were positive by GeneXpert and negative by ZN stain. While, ten specimens were negative by GeneXpert and positive for ZN stain. The frequency of MTB among males (6.4%) was higher than female (5.6%), but the risk was found higher among female ($X^2 = 0.8, p < 0.361$ and $OR=1.1$) as compared to males. the rate of infection was found higher 9.5% and 6.0% among age groups 21-30, and 31- 40 years old respectively. High prevalence rate of MBT infection among cases referrals from Taiz governorate (13.9% and $OR= 0.4$) followed by Amran, Al-Hodeida, Sana'a, and Mareb governorates with an account 9.4%, 7.9%, 7.0 and 6.2% respectively. Recent study revealed that, 28/541 (5.2%) of MTB were rifampicin **resistance**. high resistant rate was found among males [(7.4%) $P <0.019$, and $OR = 0.4$] as compared to females [(3.0%) $P <0.019$, and $OR = 2.6$]. Insignificant in the difference of rifampicin resistant among different age groups. The rate of resistant was found higher (7.4%) among subjects aged **41-50** years old followed by **21-30**, (6.5%) and **> 60** (5.2%) years old On other hand, significance with high risk of resistant was found in the difference of MTB susceptibility pattern among infected patient resident the capital city of Sana'a.

Conclusion: We conclude that the high TB rates among age group 20 to 40 years old, suggest that this age group may be an appropriate target group for TB vaccine trials including TB vaccine trials aiming to prevent infection.

Keywords: Yemen, *Mycobacterium tuberculosis*, GeneXpert, Rifampicin resistant

Introduction

Mycobacterium tuberculosis (MTB) is an ancient disease that has affected mankind for more than 4,000 years. [1] *Tuberculosis* (TB) is a chronic disease spreads from person to person through air. A patient with infectious pulmonary TB can infect 10-15 persons per year. [2] The global prevalence of TB in 2013 was estimated at 159 cases per 100 000 population. In 2012, the estimated new cases were 9.0 million and 1.5 million TB death. The morbidity and mortality rate were increased by 400,000 and 200,000 cases, respectively [3] with an estimated 10 million people (range 9 to 11.1 million) developing TB disease in 2018, of which 5.8 million, 3.2 million, and 1 million were men, women, and children, respectively with an account two thirds of cases were from eight countries, India (27%), China (9%), Indonesia (8%), Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%), and South Africa (3%) [4].

The distribution of TB in different geographic regions is characterized by the prevalence of different MTB strains with varied virulence and drug resistance. Both environmental and host factors are responsible for the transmission and prevalence of different MTB strains. [5] World health organization (WHO), reported that, Ethiopia, Kenya, Ghana, Democratic Republic of Congo, Angola, Uganda and South Africa, constituted high TB-HIV burden countries in Africa. [6] The spread of multidrug-resistant tuberculosis (MDR TB) in the world remains a major public health problem and an obstacle to effective global TB control. [7] Tuberculosis remains a major public health problem worldwide. It is the second-highest cause of death among communicable diseases. According to the WHO, in 2013, 9 million people fell ill with TB and 1.5 million died from TB. Over 95% of TB deaths occur in low- and middle-income countries, and TB is among the top five causes of death for women 15 to 44 years of age. [8] TB treatment and control have been severely compromised in recent years due to the increasing prevalence of multidrug-resistant (MDR) and extensively drug resistant (XDR) TB. [9]

The global number of TB deaths fell by 42% between 2000 and 2017 the TB incidence rate (currently 1.5%) is falling, but not fast enough to reach the first milestone of the End TB Strategy; that is, a 20% reduction between 2015 and 202.. [10] Worldwide, the cumulative reduction from 2015 to 2019 was

9% (from 142 to 130 new cases per 100000 population), including a reduction of 2.3% between 2018 and 2019. [11]

An increasing challenge to public health and to TB prevention is that of transmission of drug-resistant strains of *M. tuberculosis*. Initial evidence suggested reduced transmissibility of resistant strains; however, it is now clear that primary transmission of drug-resistant bacteria (as opposed to acquired resistance) is the dominant mechanism sustaining the global transmission of drug-resistant TB (DRTB) cases. [13]

Rifampicin is arguably the most important drug in the chemotherapy of tuberculosis. Rifampicin mono-resistance is rare. It is mostly observed in association with resistance to isoniazid. Thus, about 90% of rifampicin resistant PTB are actually multi drug resistant TB (MDR-TB). [12]

In Yemen, MTB is a major public health problem, it was estimated that, Yemen MTB endemicity comes lower-middle-income. [13] The smear-positive case notification rate, which had shown a steady annual increase, reaching a peak in 2000, declined from 27 to 15 per 100 000 population between 2001 and 2006, with an average annual decline of 11.4%. The smear-positive case detection rate was 46% in 2007. [14] On other hand, a survey tuberculin survey in Yemen during the period 1991–2007, enrolling 31 276 school children aged between 7 and 12 years, In comparison with the 1991 tuberculin survey reported an average annual decline of ARTI was 9.0% by the mirror method and 5.5% by the mixture method. [15]

Shibl et al [16] reported that, yearly high in incidence (177 and 277) and prevalence rate (49 and 71) of MTB in Yemen per100000 population compared to other countries of Arabian Peninsula and Egypt in 1990 and 2010. The mortality rate of TB is high in Yemen with an account ranged 12.2% to 21% [17, 18], due to low success rate for curing TB relative to the WHO's outcome standard of 90 %. [19]. So, the purpose of this study was to determine the frequency of MTB and rate of rifampicin resistance among *M. tuberculosis* infected patients.

Microscopy, culture, and antibiogram susceptibility testing are standard methods used in the diagnosis of MTB all over the world, but they are low sensitive and time consuming methods. [20] So, doctors in the developing world often rely only on chest X-rays. Moreover, diagnosis of

extrapulmonary TB is difficult to establish due to the low number of bacteria in clinical specimens. Rapid and accurate diagnosis of pulmonary as well as extrapulmonary TB is still a great challenge in sub developing countries due to limited resources and a lack of laboratory expertise. [21] The emergence and spread of drug resistance represents a challenge for healthcare systems and jeopardizes TB infection control efforts. [22]

In recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies, owing to their rapidity, sensitivity, and specificity. One of the latest systems, the GeneXpert MTB/RIF (Xpert) assay, was recently evaluated in large studies. The Xpert assay uses heminested real-time PCR to amplify an *M. tuberculosis*-specific sequence of the *rpoB* gene. To determine rifampin (RMP) resistance, the rifampin resistance-determining region of the *rpoB* gene is probed with molecular beacons. [23] The assay can be carried out in a nearly fully automated manner, including bacterial lysis, nucleic acid extraction and amplification, and amplicon detection. The test runs on the GeneXpert platform (Cepheid, Sunnyvale, CA) using a disposable plastic cartridge with all required reagents. [24] Xpert assay detected pulmonary TB in all TB patients, including over 90% of smear-negative patients, with a high sensitivity of over 97%. [25] The Xpert MTB/RIF assay (GX) has been evaluated in detail, and several studies have demonstrated its utility for direct detection in pulmonary specimens. [26, 27]

Material and methods

Study design

This study is cross sectional descriptive study

Location, target population, study period and ethics

This study carried out on MTB obtained from sub-culturing of different clinical specimens sent of patients referred to MTB center for MTB investigating according to physician orders in the hospitals or clinics of Sana'a city, Yemen from Jan to Dec 2020. Relevant information like age, sex, type of specimen and residency of all selected cases gathered from request form and medical records. Consent to do this study was achieved from the management of MTB center. All of these specimens were a part of the routine diagnosis. We consider all related ethics.

Case definitions

The cases of TB were divided into four categories

- **Positive case:** Patient with TB suggestive of symptoms, history of diseases and X ray abnormalities and laboratory investigation revealed positive by PCR examination and/or AFB examination.
- **Positive new case:** patient with sputum (TB) or other specimen who has never had treatment for TB or has taken anti TB drug
- **Old case:** Pretreated for TB or has taken anti TB drug including Follow up, Relapse case. Failure case and return after default.
- **Negative case-** patient with TB suggestive of symptoms and at least 2 sputum negative by PCR examination and/or AFB examination.

Methods

Specimen Collection and Processing

All collected samples were liquefied and decontaminated using a standard procedure using sodium hydroxide and isopropanol for the digestion and decontamination of sputum specimens and processed through Zeihl Neelsen (ZN) staining microscopy and real-time PCR (Gene Xpert).

The eligible patients were asked to provide at least 1 expectorated sputum specimen to the maximum of 3 specimens. Smear examination for presence of acid fast bacilli (AFB) was carried out routinely on fresh specimen at the Laboratory for all specimens. Smears were prepared, fixed, and stained with ZN stain and then visualized under light microscope. The remaining deposit of the specimen was used for PCR investigation using the Xpert MTB/RIF assay [28, 29] (Cepheid, Maurens-Scopont, France). One milliliter of the remaining deposit of clinical sample was transferred to a screw-capped tube containing 2 mL of sample reagent at a ratio of 1:2; this reagent inactivates the sample with NaOH and isopropanol. The mixture was then incubated for 15 minutes at room temperature and mixed every five minutes until liquefied with no visible clumps. The mixture was transferred into the Xpert MTB/RIF cartridge using the sterile pipette provided until the meniscus was above the minimum mark. The Xpert MTB/RIF cartridge includes an internal control for sample processing (DNA extraction and for PCR presence inhibitors). The inoculated cartridge was placed into the GeneXpert instrument (GX). Results were available in less than two hours and interpreted by the GX system automatically as follows: positive or negative results were related to the presence or absence of MTB DNA, while false results were due to the presence of PCR inhibitors. [28, 30]

Statistical analysis

Descriptive statistics of MTB results and other characteristics of the target population were computed and expressed as a percentage. A *p* value less than 0.05 was calculated to be statistically significant. The statistical difference was also evaluated by applying the Chi-square test. All the statistical analysis was done using the Statistical Package for Social Sciences (SPSS) software package version 20. (SPSS Inc. Chicago, Illinois, USAT).

Results

Socio-demographics characteristics of MTB infection

A total of 8986 suspected MTB infection cases were enrolled in this study with age ranged from 1 to 110 years old (mean of age 40.1 SD 18.8). Of these 4654 (51.8%) were females and 4332 (48.2%) were males. All cases were subjected for clinical, and laboratory investigations, where all collected specimens were examined microscopically for AFB following staining by ZN stain. According to our case definition, it was found that, out of total of 8896 referrals patients, 7928/ 8896 (89.1%) were negative (patient with absent MTB suggestive of symptoms, history of diseases and X ray abnormalities absent and at least 2 sputum negative by AFB examination). While, 968/8896 (10.9%) were patients or suggestive cases (with TB suggestive of symptoms, history of diseases and/ or X ray abnormalities) were subjected for confirmation of MTB infection by GeneXpert.

The results of this study revealed that, 551/8896 (6.1%) of specimens were positive for MTB by both GeneXpert and/ or ZN stain which are represents the overall prevalence rate of MTB infection among all MBT suspected cases. while, the prevalence of MBT by GeneXpert was 541/8896 (6.1%) and 486/ 8896 (5.5%) by ZN stain. On other hand, it was found that, out of total 551 specimens, 65 were positive only by one of each GeneXpert and ZN stain. Where, 10 cases were positive only by ZN and negative by GeneXpert technique compared to 55 were positive only by GeneXpert and negative by ZN technique.

The results of this study revealed that, 551/8896 (6.1%) of specimens were positive for MTB by both GeneXpert and/ or ZN stain which are represents the overall prevalence rate of MTB infection among all MBT suspected cases.

Out of 551 positive cases, 65 were positive only by one of each GeneXpert or ZN stain. Out of total 65 positive cases, only 55 were positive by

GeneXpert and negative by ZN technique, compared to 10 were positive only by ZN and negative by GeneXpert technique. Meanwhile, the prevalence of MBT by GeneXpert was 541/8896 (6.1%) and 486/8896 (5.5%) by ZN stain. On other hand, it was found that, out of total 551 specimens, 65 were positive only by one of each GeneXpert and ZN stain. Where, 10 cases were positive only by ZN and negative by GeneXpert technique compared to 55 were positive only by GeneXpert and negative by ZN technique.

Frequency and risk of MTB infection

The result of recent study showed that, the frequency of MTB among males (6.4%) was higher than female (5.6%), but the risk was found higher among female ($X^2 = 0.8$, $p < 0.361$ and $OR = 1.1$) as compared to males with no statistical significance was found in the difference of prevalence rate of infection among males and females. No statistical significance but the rate of risk was higher ($OR = 2.0$) in the rate of positive subjects (3.2%) aged 1-10 years old the rate of infection was found higher 9.5% and 6.0% among age groups 21-30, and 31- 40 years old respectively, but the risk was found low with no statistical significance as compared to others age group.

The prevalence of MBT infection was found statistically significant and increasing in risk of infection among subjects aged 41 to 50, 51-60 and more than 60 years old [(4.1%) $P < 0.001$, and $OR = 1$], [(4.5%) $P < 0.007$, and $OR = 1.5$] and [(3.2%) $P < 0.000$, and $OR = 2.1$] respectively. Moreover, as compared to others age groups, the rate of MBT infection was found higher (9.5%) among age group 21 to 30 years old followed by (6.3%) age group 11 to 20 years old and old 31 to 40 years old (9.5%). Less prevalence rate (3.2%) and higher risk ($OR \geq 2$) of MBT infection was found among age groups up to ten years old and more than 60 years old but the risk was found.

The frequency of MBT infection was found statistically insignificant with increasing in rate and risk of infection among preschool (4.1%) $P < 0.356$, and $OR = 1.5$ and primary school aged children, (4.6%) $P < 0.156$, and $OR = 1.4$. Despite the rate of MBT infection was higher among secondary school aged children than preschool and primary school aged children but the risk was found less ($OR = 0.8$) among secondary school aged children compared to preschool and primary school aged children.

Our study showed, high prevalence rate of MBT infection among cases referrals from Taiz governorate (13.9% and $OR = 0.4$) followed by Amran,

Al-Hodeida, Sana'a, and Mareb governorates with an account 9.4%, 7.9%, 7.0 and 6.2% respectively. The prevalence rate (5.6 % $P < 0.007$, and $OR = 1.5$) of MBT infection in Capital City of Sana'a was found statistically significance. Less (4.6% and $OR = 1.4$) frequency of MBT infection was found among cases referrals from Ibb governorates followed by Al-Baeda (5.6% and $OR = 1.1$) governorate.

Rifampicin resistant

Rifampicin resistant among 541 the result of recent study revealed that, 28/541 (5.2%) of MTB were rifampicin resistance. high resistant rate was found among males [(7.4%) $P < 0.019$, and $OR = 0.4$] as compared to females [(3.0%) $P < 0.019$, and $OR = 2.6$]. The difference in the resistant among gender was found statistically significance with high risk of resistant among females. table2

The difference of rifampicin resistant among different age groups was found statistically insignificance. Where, the rate of rifampicin resistant was found higher (7.4%) among subjects aged 41-50 years old followed by 21-30, (6.5%) and > 60 (5.2%) years old. No rifampicin resistant was found among children aged up to 10 years.

On other hand, significance with high risk of resistant was found in the difference of MTB susceptibility pattern among infected patient resident the capital city of Sana'a. In addition, the risk of resistant was found higher non-significant in resistant among infected patients residence Sana'a [(2.1%) $P < 0.311$, and $OR = 2.7$] and Amran [(3.4%) $P < 0.530$, and $OR = 1.6$] governorates which are nearby capital city of Sana'a. Moreover, the resistant rate was found higher with low risk among patients resident in Mareb (25.0%), Ibb (19.2%) and Al-Hodeida (11.1%) governorates followed by Taiz and Al-Baeda governorate with an account 10.0% and 7.7% respectively.

Recent study revealed significant high resistant rate [(13.3%) $P < 0.002$, and $OR = 0.3$] among old MTB infected cases as compared to new MTB infected cases [(4.2%) $P < 0.002$, and $OR = 3.6$], but the risk of resistant was found higher among new MTB infected cases.

Table 1.

Distribution of *Mycobacterium TB* according to demographic characteristics

Gender	Positive N(551)		negative N(8435)		Total N(8986)		χ^2	OR	CI	P
	N	%	N	%	N	%				
Male	276	6.4	4056	93.6	4332	48.2	0.8	0.9	0.8-1.1	0.361
Female	275	5.9	4379	94.1	4654	51.8	0.8	1.1	0.9-1.3	0.361
Age groups										
1-10	5	3.2	149	96.8	154	1.7	2.3	2.0	0.8-4.8	0.132
11-20	54	6.3	798	93.7	852	9.5	0.1	0.96	0.7-1.3	0.792
21-30	252	9.5	2399	90.5	2651	29.5	74.4	0.5	0.4-0.6	0.000
31-40	87	6.0	1359	94.0	1446	16.1	0.04	1.0	0.8-1.3	0.842
41-50	53	4.1	1233	95.9	1286	14.3	10.5	1.6	1.2-2.2	0.001*
51-60	59	4.5	1259	95.5	1318	14.7	7.4	1.5	1.1-1.9	0.007*
>60	41	3.2	1238	96.8	1279	14.2	22.2	2.1	1.5-3.0	0.000*
Preschool aged children 7	5	4.1	116	95.9	121	1.3	0.9	1.5	0.6-3.7	0.356
Primary school aged children 7-14	23	4.6	472	95.4	495	5.5	2.0	1.4	0.9-2.1	0.156
Aldoses 15-18	31	7.9	359	92.1	390	4.3	2.3	0.8	0.5-1.1	0.126
19-25	168	10.7	1408	89.3	1576	17.5	68.1	0.5	0.4-0.6	0.000
More than 25	324	5.1	6080	94.9	6404	71.3	44.5	1.8	1.5-2.2	0.000*
governorates										
Capital city Sana'a	313	5.6	5313	94.4	5626	62.6	8.4	1.3	1.1-1.5	0.004*
Sana'a governorate	51	7.0	675	93.0	726	8.1	1.1	0.9	0.6-1.2	0.296
Taiz	21	13.9	130	86.1	151	1.7	16.0	0.4	0.2-0.6	0.000
Amran	58	9.4	558	90.6	616	6.9	12.4	0.6	0.5-0.8	0.000
Thamar	40	5.8	653	94.2	693	7.7	0.2	1.1	0.5-2.0	0.681
Al-Hodeida	18	7.9	210	92.1	228	2.5	1.3	0.8	0.8-1.2	0.261
Ibb	26	4.6	543	95.4	569	6.3	2.5	1.4	0.9-2.1	0.108
Al-Baeda	13	5.6	218	94.4	231	2.6	0.1	1.1	0.6-1.9	0.746
Mareb	8	6.2	120	93.8	128	1.4	0.003	0.98	0.5-2.0	0.955
Others	3	16.7	15	83.3	18	0.2	3.5	0.3	0.1-1.1	0.062
New	489	5.9	7851	94.1	9340	92.8	14.5	1.7	1.3-2.4	0.000*
Old	62	6.9	584	93.1	646	7.2	14.5	0.6	0.4-0.8	0.000

*Statistically significant, χ^2 : Chi-square, p: probability. ($\chi^2 \geq 3.84$, $p < 0.05$: significant). N: number, %; percentage

Table 2.

Distribution of Rifampicin susceptibility pattern of MTB strains

Gender	RR N=28 (5.2%)		RS N=513 (94.8%)		Total N(541)		χ^2	OR	CI	P
	N	%	N	%	N	%				
Male	20	7.4	250	92.6	270	50.1	5.5	0.4	0.2-0.9	0.019
Female	8	3.0	263	97.0	271	49.9	5.5	2.6	1.1-6.1	0.019*
Age groups/years										
0-10	0	0.0	5	100.0	5	0.9	0.3	-	-	0.600
11-20	2	3.7	52	96.3	54	10.0	0.3	1.5	0.3-6.4	0.607
21-30	16	6.5	232	93.5	248	45.8	1.5	0.6	0.3-1.3	0.218
31-40	3	3.5	83	96.5	86	15.9	0.6	1.6	0.5-5.5	0.441
41-50	4	7.4	47	92.6	51	9.4	0.8	0.6	0.2-1.8	0.366
51-60	1	1.7	57	98.3	58	10.8	1.6	3.4	0.5-25.3	0.209
>60	2	5.2	37	96.8	39	7.2	0.00	1.0	0.2-4.4	0.989
Preschool aged children < 7	0	0.0	5	100.0	5	0.9	0.3	-	-	0.600
Primary school aged children 7-14	0	0.0	3	100.0	3	0.6	0.2	-	-	0.685
Aldoses 15-18	2	3.9	49	96.1	51	9.4	0.2	1.4	0.3-6.0	0.671
19-25	12	7.1	154	92.8	166	30.7	2.1	0.6	0.3-1.2	0.151
> 25	14	4.4	302	95.6	316	58.4	0.9	1.4	0.7-3.1	0.354
governorates										
Capital city Sana'a	9	2.9	299	97.1	308	56.9	7.4	3.0	1.3-6.6	0.007*
Sana'a governorate	1	2.1	47	97.9	48	8.9	1.0	2.7	0.4-20.5	0.311
Taiz	2	10.0	18	90.0	20	3.7	1.0	0.5	0.1-2.1	0.321
Amran	2	3.4	56	96.6	58	10.7	0.4	1.6	0.4-6.9	0.530
Thamar	2	5.1	37	94.9	39	7.2	0.00	1.0	0.3-4.4	0.989
Al-Hodeida	2	11.1	16	88.9	18	3.3	1.3	0.5	0.1-1.9	0.248
Ibb	5	19.2	21	80.8	26	4.8	11.0	0.2	0.1-0.6	0.001
Al-Baeda	1	7.7	12	92.3	13	2.4	0.2	0.7	0.1-5.2	0.678
Mareb	2	25.0	6	75.0	8	1.5	6.5	0.2	0.03-0.8	0.011
Others	2	66.7	1	33.3	3	0.6	23.2	0.03	0.002-0.3	0.000
New	20	4.2	461	95.8	481	88.9	9.2	3.6	1.5-8.5	0.002*
Old	8	13.3	52	86.7	60	11.1	9.2	0.3	0.1-0.7	0.000

*Statistically significant, χ^2 : Chi-square, p: probability. ($\chi^2 \geq 3.84$, $p < 0.05$: significant). N: number, %; percentage, RR: rifampicin resistant, RS: rifampicin sensitive

Discussion

The prevalence of MTB in present study (6.1%) was slightly more than that reported in Iran (5.55%) [31] and less than (12.9%) reported by Valenc *et al*, [32] and Wasihun *et al*, [33] (7.9%) in Brazil and Ethiopia respectively.

Similar high prevalence rate among age group aged 20 to 40 years old was reported in Egypt [34] and in Nigeria, [35] whereas, the recent study showed high rate of MTB infection (9.5%) among age group 21-30 years old followed by subjects aged 11-20 and 31-40 years old (6.3% and 6.0%) respectively which was in agreement with that reported by Jaber *et al*, [36] who showed that, the majority of TB cases were found in patient age 16–25 (41.9%). High prevalence rate of MTB among this age group could be related to smoking habits [37] and non-restricted movement and contact with others.

Moreover, the prevalence of MTB among all age groups (ranged 3.2% to 9.5%) in present study which was less than that reported (ranged 10.3% to 24.6%) in Brazil and Ethiopia. [33]

Otherwise, the result of recent study reveal low prevalence rate of MTB among preschool aged children which gradually increased among school aged, adolescents and reach up to 10.7% among young adult aged 25 years old, then the rate and the number of MTB cases decreased among age group 45 years old, which is in consent with that reported by WHO [38] and documented all over the world. [39] Less frequent MTB infection among preschool aged children (0-4) as compared to age group 5-15 years old also reported by Ramose *et al*. [40]

Low rate of MTB among children up to 10 years old as compared to others age groups could be related to several factors such as less contact of this age group with others population and their restricted movement and activity. On other hand, recent study demonstrated that MBT infection was found statistically insignificant with increasing in rate and risk of infection among preschool and primary school aged children which call there is an urgent need for poster immunization of school aged children.

High prevalence rate of MBT infection among cases referrals from Taiz governorate as compared to others governorates of Yemen, could be due to that the most referrals from Taiz governorate were sent to National TB Control Program in Sana'a city not just as suspected MTB cases but many of

them were previously diagnosed as either presumptive or confirmed MTB cases as well as for follow up of infected cases in Sana'a city due to stop of working in the branch of National TB Control Program at Taiz city as result of civil war there in addition to increasing in number of population in Taiz governorate as compared to others governorates of Yemen.

Similar rifampicin resistant was (5.1%) reported in Ghana by Ameke. [41] Rifampicin resistant was more than that reported in Bahrain, (3.0%) [42]. and less than that reported in Saudi Arabia (5.9% and 7.4) [43,44] and in Nigeria (7.3%). [45] In Yemen it was found that, 20.0% and 18.7% of isolates were resistant to rifampicin at two time interval in 2002 and 2009 respectively [46], in addition it was reported that the incidence of multi-drug resistance of MTB to rifampicin and Isoniazid at two time intervals resistant in Yemen was 9% in 2003 and 13.5% in 2009. [46] compared to 24.2% rifampicin and Isoniazid resistant rate reported in Yemen in 2019. [18]

High rate of old MTB cases (52 cases) with high rifampicin resistant (13.8%) among them could be related lack of follow-up during the initial months of therapy. Where, high resistant and failure of therapy was reported 45/147 (30.6%) in Saudi Arabia. [47]

Conclusion

We conclude that the high TB rates among age group 20 to 40 years old, suggest that this age group may be an appropriate target group for TB vaccine trials including TB vaccine trials aiming to prevent infection.

Recommendations

Further studies regarding TB infection among school aged children, aldos and young adult to get more clear knowledge on prevalence of TB infection

Acknowledgments

We would like to thank all coworkers in general and in particular, all staff members in National TB Control Program in Sana'a Yemen. The authors are grateful to the members of National TB Control Program teams for technical help.

References

- [1] Albert R. Zink, Christophe Sola, Hans Wolf, Udo Reischl, Waltraud Grabner, Nalin Rastogi and Andreas G. Nerlich. 2003. Characterization of *Mycobacterium tuberculosis* Complex DNAs from Egyptian Mummies by Spoligotyping. *journal of clinical microbiology*. Vol. 41: (1): 359–367
- [2] Vasantha M, Gopi PG, Subramani R. 2008. Survival of tuberculosis patients treated under dots in a rural tuberculosis unit (TU), South India Indian J Tuberc; 55(2): 64-69.
- [3] World Health Organization (WHO), Global Tuberculosis Report 2014, Geneva, Switzerland.
- [4] Global tuberculosis report 2018, World Health Organization, Licence: CC BY-NC-SA 3.0 IGO, Geneva, 2018, Jan 2019. (https://www.who.int/tb/publications/global_report/en/).
- [5] P. Supply, R. M. Warren, A. L. Baulus et al. 2003. “Linkage disequilibrium between minisatellite loci supports clonal evolution of *Mycobacterium tuberculosis* in a high tuberculosis incidence area,” *Molecular Microbiology*. vol. 47 (2): 529–538,
- [6] World Health Organization Global Tuberculosis Report. Geneva. 2019
- [7] L.P. Ormerod, Multidrug-resistant tuberculosis (MDR-TB): epidemiology, prevention and treatment, Br. Med. Bull. 73-74 (2005) 17-24.
- [8] World Health Organization (2014) Global Tuberculosis Control Report 2014. Available:
http://www.who.int/tb/publications/global_report/en/.Accessed 8 August 2015.
- [9] Abubakar I, Zignol M, Falzon D, Raviglione M, Ditiu L, Masham S, Adetifa I, Ford N, Cox H, Lawn SD, Marais BJ, McHugh TD, Mwaba P, Bates M, Lipman M, Zijenah L, Logan S, McNerney R, Zumla A, Sarda K, Nahid P, Hoelscher M, Pletschette M, Memish ZA, Kim P, Hafner R, Cole S, Migliori GB, Maeurer M, Schito M, Zumla A. (2013). Drug-resistant tuberculosis: time for visionary political leadership. *Lancet Infect Dis* 13: 529-539.
- [10] WHO Guidelines on Tuberculosis Infection Prevention and Control 2019 UPDATE
- [11] Global tuberculosis report 2020. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.

[12] K.N. Jaleta, M. Gizachew, B. Gelaw, H. Tesfa, A. Getaneh, B. Biadgo. 2017. Rifampicinresistant *Mycobacterium tuberculosis* among tuberculosis presumptive cases at University of Gondar Hospital, northwest Ethiopia, *Infest. Drug Resist.* 10, 185–192.

[13] Global tuberculosis report 2015. Geneva: World Health Organization.

[14] World Health Organization. WHO. 2009. Global tuberculosis control: surveillance, planning financing. Geneva, Switzerland.

[15] A. Al-Absi, A. Bassili, H. Abdul Bary, A. Barker, M. Daniels, A. Munim, A. Seita, B. Williams and C. Dye. 2009. The decline of tuberculosis in Yemen: evaluation based on two nationwide tuberculin surveys. *INT J TUBERC LUNG DIS* 13(9):1100–1105

[16] A. Shibli, A. Senok and Z. Memish. 2012. Infectious diseases in the Arabian Peninsula and Egypt. *Clinical Microbiology and Infection* 2012 European Society of Clinical Microbiology and Infectious Diseases, CMI, 18, 1068–1080

[17] Ammar Ali Saleh Jaber and Baharudin Ibrahim. 2019 Evaluation of risk factors associated with drug-resistant tuberculosis in Yemen: data from centres with high drug resistance. *MC Infectious Diseases.* 19:464. <https://doi.org/10.1186/s12879-019-4069-1>

[18] Ammar Ali Saleh Jaber and Baharudin Ibrahim. 2019. Outcome of interim multidrug-resistant tuberculosis treatment in Yemen. *Tropical Journal of Pharmaceutical Research;* 18 (8): 1755-1762

[19] World Health Organization. Global tuberculosis report 2016.

[20] Centers for Disease Control and Prevention. 2009. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. *Morb Mortal Wkly Rep.* 58:7-10.

[21] Zumla A, Raviglione M, Hafner R, Reyn C (2013) Tuberculosis-current perspectives. *N Engl J Med* 368: 745755

[22] Nathanson E, Nunn P, Uplekar M, Floyd K, Jaramillo E, Lönnroth K, Weil D, Raviglione M. 2010. MDR tuberculosis — critical steps for prevention and control. *N Engl J Med* 363:1050-1058

[23] El-Hajj, H. H., S. A. Marras, S. Tyagi, F. R. Kramer, and D. Alland. 2001. Detection of rifampin resistance in *Mycobacterium tuberculosis* in a single tube with molecular beacons. *J. Clin. Microbiol.* 39:4131–4137.

[24] Raja, S., et al. 2005. Technology for automated, rapid, and quantitative PCR or reverse transcription-PCR clinical testing. *Clin. Chem.* 51:882–890.

[25] Boehme, C. C., *et al.* 2010. Rapid molecular detection of tuberculosis and rifampin resistance. *N. Engl. J. Med.* 363:1005–1015.

[26] Boehme CC, *et al.* 2011. Feasibility, diagnostic accuracy, and effectiveness of decentralized use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicenter implementation study. *Lancet* 377:1495–1505.

[27] Moure R, *et al.* 2011. Rapid detection of *Mycobacterium tuberculosis* complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. *J. Clin. Microbiol.* 49: 1137–1139.

[28] Cepheid, Xpert MTB/RIF Assay (Package Insert), Cepheid, Sunnyvale, Calif, USA, 2013.

[29] World Health Organization. 2010. Roadmap for rolling out Xpert MTB/RIF for rapid diagnosis of TB and MDR-TB. Available: http://www.who.int/tb/laboratory/roadmap_xpert_mtbo-rif.pdf. Accessed 8 August 2015.

[30] HUMAIRA ZAFAR.2015. DIAGNOSIS OF MYCOBACTERIUM TB INFECTION WITHGENE XPERT TEST ALONG WITH RIFAMPICIN RESISTANCE (rpoB). *ISRA MEDICAL JOURNAL*; 7 (1); 1-2

[31] Jamal Falahi, Kiarash Gazvini, Fares Bahrami, Marzieh Mirzaei, Araz Majnoni, Hadi Lotfi& Saeid Amel Jamehdar. 2018. Prevalence of *Mycobacterium tuberculosis* Infection in Suspected Patients in a Teaching Hospital in Northeastern Iran: A Cross-Sectional Study. *Journal of Molecular Biology Research*; 8 (1); 95-100.

[32] M. S. Valenc, a, J. L. R. Scaini, F. S. Abileira, C. V. Gonc, alves, A. von Groll, P. E. A. Silva. 2015. Prevalence of tuberculosis in prisons: risk factors and molecular epidemiology. *INT J TUBERC LUNG DIS*; 19(10):1182–1187

[33] Araya Gebreyesus Wasihun, Tsehaye Asmelash Dejene and, Genet Gebrehiwet Hailu. 2020. Frequency of MTB and rifampicin resistance MTB using Xpert-MTB/RIF assay among adult presumptive tuberculosis patients in Tigray, Northern Ethiopia: A cross sectional study. *PLoS ONE* 15(11): e0240361. <https://doi.org/10.1371/journal.pone.0240361>

[34] Eman Sobh, Sayed Abd Elsabour Kinawy, Yasser Mohamad Ahmad Abdelkarim and Mahmoud Ahmed Arafa. 2016. The pattern of tuberculosis in Aswan Chest Hospital, Egypt. *International Journal of Mycobacteriology*; 5: 333– 340

[35] Henry Ukwamedua, Victor Omote, Johnson Etaghene, Matthew Ejike Oseji Imaria Celia Agwai, Harrison Agbroko.2018. Rifampicin resistance among notified pulmonary tuberculosis (PTB) cases in South-Southern Nigeria. *Helion*; 5 (2019) e02096. <https://doi.org/10.1016/j.heliyon.2019.e02096>

[36] Ammar Ali Saleh Jaber, Amer Hayat Khan, Syed Azhar Syed Sulaiman and Nafees Ahmad. 2016. Role of socio-demographical factors on tuberculosis outcome in Yemen. *International Journal of Mycobacteriology* 5 (2016) S 20. Available at www.sciencedirect.com

[37] S den Boon, S W P van Lill, M W Borgdorff, S Verver, E D Bateman, C J Lombard, D A Enarson, and N Beyers. 2005. Association between smoking and tuberculosis infection: a population survey in a high tuberculosis incidence area. *Thorax*; 60:555–557. doi: 10.1136/thx.2004.030924

[38] World Health Organization. Best practices in child and adolescent tuberculosis care.2021

[39] James A Seddon and Delane Shingadia. 2014. Epidemiology and disease burden of tuberculosis in children: a global perspective. *Infection and Drug Resistance* downloaded from <https://www.dovepress.com/ by 178.130.120.187 on 31-Jul-2021. For personal use only>.

[40] José M. Ramos, Mario Pérez-Butrague, Abraham Tesfamariam, Francisco Reyes, Jacob Endirays, Seble Balcha, Tamasghen Elala, Dejene Biru, Belén Comech, Gebre Tiziano and Miguel Grgolas. 2019. Comparing tuberculosis in children aged under 5 versus 5 to 14 years old in a rural hospital in southern Ethiopia: an 18-year retrospective cross-sectional study. *BMC Public Health*; (2019) 19:856. <https://doi.org/10.1186/s12889-019-7206-2>

[41] Selassie Ameke, Prince Asar, Samuel Yaw Aboagye, Isaac Darko Otchere, Stephen Osei-Wusu, Dorothy Yeboah-Manu, Adwoa Asante-Poku. 2021. Molecular epidemiology of *Mycobacterium tuberculosis* complex in the Volta Region of Ghana. *PLoS ONE* 16(3): e0238898. <https://doi.org/10.1371/journal.pone.0238898>

[42] Safaa AlKhawaja.2020. Epidemiology of drug resistant tuberculosis in Bahrain, five years review. *Arch Community Med Public Health* 6(1): 022-025. DOI: <https://dx.doi.org/10.17352/2455-5479.000065>.

[43] Maha Al Ammari, Abdulrahman Al Turaiki, Mohammed Al Essa, Abdulhameed M. Kashkary, Sara A. Eltigani and Anwar E. Ahmed.2018. Drug resistant tuberculosis in Saudi Arabia: an analysis of surveillance data 2014–2015. *Antimicrobial Resistance and Infection Control*; 7:12. DOI 10.1186/s13756-018-0306-4

[44] Mohammad Fouad MohammadKhatib Sambas, Unaib Rabbani, Manal Mansour Mezal Al-Gethamy, Saud Hasan Surbaya, Irmat Alharbi, Faisal Fuwaran Riyadh Ghazi Abdulrahman Ahmad, Hamzah Khalid Hamzah Qul, Safa Mohammed Saeed Nassar, Abdulaziz Khalid Mohammed Ali Maddah, Basel Ali Kabah Darweesh. 2020. Prevalence and Determinants of Multidrug- Resistant Tuberculosis in Makkah, Saudi Arabia. *Infection and Drug Resistance*:13 4031–4038

[45] Henry Ukwamedua, Victor Omote, Johnson Etaghene, Matthew Ejike Oseji Imaria Celia Agwai, Harrison Agbroko.2018. Rifampicin resistance among notified pulmonary tuberculosis (PTB) cases inSouth-Southern Nigeria. *Heliyon* 5 (2019) e02096. <https://doi.org/10.1016/j.heliyon.2019.e02096>

[46] Anwar K Al Madhagi, Khaled Al Moyed and Ahmed Al Hadad. 2013. Rrsistance of Anti-tuberculosis drugs among pulmonary tuberculosis patients in Yemen. *J Fac Med Bagdad* v 55, no. 3, p 250-253.

[47] Sahal Al-Hajoj. Tuberculosis in Saudi Arabia.2014. See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/221924839>.