International Journal of Advanced Biological and Biomedical Research
Available online at http:www.ijabbr.com
Volume 10, Issue 1 (2022) pp. 1-17 **DOI**:10.22034/ijabbr.2021.534391.1362

Original Article

A study of *Mycobacterium tuberculosis* Zopf, 1883 (Mycobacteriaceae) in Iraq

Haidar Khalid Mostafa[1], Mohsen Hashim Risan[2*], Mohammed Al-Faham[3]

[1]Ministry of Science and Technology, Environment and water Directorate, Iraq, Baghdad
[2]Department of Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad-Iraq
[3]Baghdad University, College of Medicine, Iraq

*Corresponding Author E-mail: M_risan@yahoo.com

Received: 30 July 2021, Revised: 09 September 2021, Accepted: 16 December 2021

ABSTRACT

Background: Tuberculosis (TB) is considered to be one of the deadliest bacterial infections in the world and it can have a large impact on global health, drawing international attention with increasing number of cases worldwide in developed and developing countries. *Mycobacterium tuberculosis* (MTB) is the main causative agent of TB which is an aerobic pathogenic bacillus that establishes infection in the lungs. It is believed that two billion people are carrying non-eradicated intra-granulomatous Tuberculosis bacilli as LTBI (short for latent tuberculosis infection) and around 10% of those people will be infected with active tuberculosis during their lifetime. Therefore, this study aimed to study *Mycobacterium tuberculosis* in Iraq.

Methods: All bacteriological tests were done in biological safety cabinet class II. Gloves and masks (N 95) were used during the laboratory work. Phenol 5% and ethanol alcohol 70% were used to sterilize and disinfect the benches and hood (IUATLD, 1996). Autoclave was used at 121 °C at 1.5 bar for 20-30 minutes for sterilizing all culture media. The study was conducted at National Reference Lab (NRL) of tuberculosis/Baghdad from July 2016 to December 2016. A total of 188 sputum samples were collected from suspected patient. In addition, 80 samples were collected from healthy subjects Specimens were amassed within the outdoor and as away as feasible from different human beings. Each subject was advised to inhale deeply 2-3 instances, cough out deep from the chest and spit the sputum into sterile prevalent container. We needed to ensure that the specimen was of enough extent (3 to 5 mL) and that it incorporated strong or purulent material. Two samples were gathered from the subject. The first was taken when they reached the institute and the second was taken early morning, earlier than breakfast. The early morning collection represents the pulmonary secretions accrued in a single day, and consequently it typically has a better positivity. Gathered specimens were stored at –20 °C until use.

Results: Totally, 118 Tuberculosis suspected samples were used throughout this study;

103 (54.78%) cases were positive using direct assay (AFB smear microscopy) and 85 (45.21%) were negative cases. From the 103 TB patients, 88 (85.43%) represented as new TB cases; the others 15 (14.57%) patients appeared to follow up assessment of the directly observed treatment (DOT) program. The percentage of infected patients was 73 (70.87%) for males to 30 (29.12 %) for females, with over all male to female ratio of 2.43 (73/30) with a highly significant difference (P≤0.01). The age of the study patients ranged from 14 years to 75 years. The mean age was 36 and the median was 30 with highly significant difference between age group. As in many developing countries, direct sputum microscopy is the widely used method for the diagnosis of pulmonary TB in Iraq. Only 103 from 188 patients (suspected) were positive by using ZN smears (Ziehl-Neelsen stain technique). This study for those 188 suspected showed that 119 (63.29%) cases were positive by culture (Lowenstein–Jensen medium) and 69 (36.7%) cases were negative by L.J Culture. This study showed that the percentage of sensitivity, specificity, positive predictive value, and negative predictive value was 74.78%, 79.7%, 86.4% and 64.7%, respectively, using AFB method. The results revealed out of the total 188 specimens, 49 (26.06%) of the specimens were positive by DiaSpot (Tuberculosis Rapid Test on serum) and 139 (73.93%) specimens were negative. This study reported the sensitivity of 33.6% and the specificity of 86.95 % PPV 81.6 and NPV 43.16 for DiaSpot TB Rapid test.

Conclusion: Males aged 15 to 45 years are at high risk for TB infection. *M. tuberculosis* plays a major role for causing tuberculosis in human in Baghdad.

Keywords: Tuberculosis, *Mycobacterium tuberculosis,* patients, infection, Baghdad.

1. Introduction

Tuberculosis (TB) is considered to be one of the deadliest bacterial infections in the world, which can exert a large impact on global health, drawing international attention with increasing number of cases worldwide in developed and developing countries [1].

Mycobacterium tuberculosis Zopf, 1883 (Mycobacteriaceae) (MTB) is the main causative agent of TB, which is an aerobic pathogenic bacillus that establishes infection in the lungs. It is believed that two billion people are carrying non-eradicated intra-granulomatous *Tuberculosis bacilli* as LTBI (short for latent tuberculosis infection) and around 10% of those people will be infected with active tuberculosis during their lifetime [2, 3]. MTB is a pathogenic bacterial species in the genus *Mycobacterium* Lehmann and Neumann 1896 and the causative agent of most cases of tuberculosis. This bacillus was first described by Robert Koch, and for that reason it is referred to as Kochs bacillus, then they thought that MTB had evolved from *M. bovis* due to the animal pathogens adaption to the human host [4].

M. tuberculosis is highly aerobic and requires high levels of oxygen. Phenotypic methods which discriminate infection and mechanism of transmitting drug resistant strains, genotypic methods also known as molecular fingerprinting or genotyping, which offer higher specificity, sensitivity and discriminating *M. tuberculosis* strains[5]. The MTB complex consists of *M. tuberculosis*, *M. canetti*, *M. microti*, *M. pinipedi*, *M. africanum*, *M. bovis*, and *M. caprae*. Most of infections in humans are caused by *M. tuberculosis* with a small number of

infections caused by *M. africanum* and *M. bovis* [6].

It is estimated that 1/3 of the population (around 2 billion people) are infected with TB bacilli; however, only 5-10% of people get infected with the active disease. The remaining 90-95% will initially be experienced no symptoms of the disease but will experience latent disease that may be due to reactivation. The World Health Organization (WHO) estimated that in 2008 there were 9.4 million cases of TB, leading to 1.3 million deaths. The incidence of infections and disease varies greatly geographically as the estimated number of cases in 2008 occurred in Asia (55%) and Africa (30%). Iraq is one of the 7 countries of the region with a high infection rate of TB and has 3% of total number of cases worldwide with a number of 20,000 TB patients and more than 4000 estimated deaths due to TB[1, 7].

Every second a new person is infected and every 15 seconds a new one dies because of TB infection due to the development of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) strains together with the spread of risk factors such as human immunodeficiency virus (HIV) acquired immunodeficiency syndrome (AIDS) and diabetes [8], proving the need to develop treatment for infection strategies and continue to make MTB a health concern in developed countries.

In 2012, new cases of TB were around 8.2 million in Asia and 14 million worldwide and around 1.3 million deaths. This is equivalent to every 100,000 people; around 122 people infected with TB most of the cases were in Asia (58%) and Africa (27%) [7]. MTB mainly infects the lungs and causes coughing depending on the stage of tuberculosis. The clinical manifestations of tuberculosis can vary. The stages of infection disease include latency, primary disease, progressive (active) disease and extra pulmonary disease. The disease spreads via small airborne droplets called droplet nuclei, and is primarily transmitted through air. Sputum smear microscopy is the most frequent method for diagnosing tuberculosis [9-11].

2. Material and methods

2.1. Safety and disinfection

All bacteriological tests were done in biological safety cabinet class II as illustrated in appendix E. Gloves and masks (N 95) were used during the laboratory work [16]. Phenol 5% and ethanol alcohol 70% were used to sterilize and disinfect the benches and hood (IUATLD, 1996). Autoclave was used at 121°C at 1.5 bar for 20-30 minutes for sterilizing all culture media. Oven was used to sterilize all glass wares at 200 °C for 2 hours [12].

2.2. Patients and Sample Collection

The study was conducted at National Reference Lab (NRL) of tuberculosis/Baghdad from July 2016 to December 2016. A total of 188 sputum samples were collected from suspected patient. In addition, 80 samples were collected from healthy subjects.

Specimens were amassed within the outdoor and as a way as possible from different human beings. Each subject was advised to inhale deeply 2-3 instances, cough out deep from the chest and spit the sputum into sterile prevalent container. We needed to ensure that the specimen was of enough extent (3 to 5 mL) and that it incorporated strong or purulent material. Two samples were gathered from the subjects. The first was taken when they reached the institute, and the second one was taken early morning earlier than breakfast; the early morning collection represents the pulmonary secretions accrued in a single

day, and consequently it typically has a better positivity. Gathered specimens were stored at–20°C until use [13]. The sample collected was evaluated in terms of its acceptability, proper labeling such as full name, age, sex, serial number of the patient, date of collection.

2.3. Ziehl–Neelsen Staining (Acid Fast Staining)

The solution was prepared as follows:

2.3.1. Preparation of solutions

2.3.1.1. Carbol Fuchsin solution

solution A (saturated alcoholic fuchsine 3%) was prepared by dissolving 3g of carbol fuchsin in a 100 mL of ethanol 96%.

Solution B (aqueous phenol solution 5%) was prepared with the aid of adding 5 mL of phenol to 95 mL of distilled water. Then, solution A became blended with solution B.

2.3.1.2. Preparation of decolorizing agent (aqueous sulfuric acid solution 25%)

It was prepared by adding 250 mL of concentrated sulfuric acid to 1000 mL of distilled water.

2.3.1.3. Counter stain (methylene blue solution 0.3%)

It was prepared by dissolving 0.3 g of methylene blue in 100 mL of distilled water.

2.3.1.4. Smears preparation for Acid Fast Bacilli (AFB)

The specimen was blended well with a pipette and approximately one drop placed onto a clean microscope slide and left to dry in air. The Slide was warmed at 65-75 ºC for 10 minutes. Smears were flooded with carbol fuchsin stain, and the slides were heated slowly until steaming; the stain was left for five minutes, and then rinsed properly with tap water. Decolorization was done with 25% H2SO4 for two minutes, followed by washing once more with tap water. Methylene blue was added to slides for one minute as a counter stain, then rinsed with tap water and allowed for drying. Smears were examined under light microscopy with an oil immersion 100 x objective. The result was recorded as positive or negative. Positive result means the presence of acid-fast bacilli in smear. Negative result means no acid-fast bacilli in smear. The report of the patients' results was based on National Tuberculosis Management Guideline [14].

2.3.1.5. Preparation of Lowenstein – Jensen medium (L.J)

The medium was prepared according to the guideline [15].

2.3.2. Egg solution preparation

Fresh eggs turned into washed properly and were soaked at liquid cleaning soap for half-hour, then washed with tap water and dried with smooth gauze then placed in alcohol solution (70%) for 15 mins. The eggs were broken separately in sterilized flask, combined and filtered through multilayered sterilized gauze through sterilized funnel.

2.3.3. Malachite green solution 20 mL (2%)

It was prepared by blending 2 g of malachite green in 100 mL of sterilized distilled water, then the mixture turned into dissolved for half-hour

2.3.4. The whole medium preparation

It was prepared through mixing 1L of egg solution with 20 mL of malachite green solution (2%) after salts refrigeration 6 mL of the mixture changed into poured in sterilized screw capped vials.

2.3.5. Inspissation

The vials were put in slant position at 85 °C for 50 minutes; temperature of inspissation should not be greater than 85 °C to prevent air bubbles production.

2.3.6. Procedure of specimen's cultivation on solid media

Based on Petroff decontamination method (1915) the specimens were cultivated as described in [15]:

2.3.7. Preparation of Petroff solutions

1. NaOH (4%) solution: It was prepared through adding 4 g of sodium hydroxide to 100 mL of distilled water.
2. Phenol red indicator (1%): It was prepared by adding 1g of phenol red to 100 mL of distilled water.
3. Neutralizing solution: It was prepared by adding 72 mL of HCl (73%) to 1 mL of phenol red indicator (1%) and volume completed to 1L with distilled water.

2.3.8. Procedure

The sputum was transferred in a 50 mL screw capped centrifuge tube. NaOH solution (4%) was delivered in a quantity equal to the quantity of specimen and left for 15 minutes; the specimen was centrifuged at a speed of 3000 rpm for 15 minutes. The supernatant was decanted cautiously into a suitable box containing a myco bactericidal disinfectant. Drops of neutralizing solution were added by Louis Pasteur pipet until the color turned yellow (neutralization point). Two vials of L.J medium were inoculated with 2-3 drops of the pellet and incubated in slant position and in semi-closed position for 3 days at 37 °C. After this period, the vials were tightly closed and incubated vertically at 37 °C for 6 weeks. The results were recorded as positive or negative. Positive result means growth of *M. tuberculosis*, and negative result means no growth.

2.4. Drug susceptibility test

2.4.1. Testing the susceptibility by using proportion method

Susceptibility test was conducted by using proportion method as described in [15], as follows:

Rifampicin preparation (R)

1. Stock solution (2000 µg/mL) was prepared by dissolving 40 mg of rifampicin in 5 mL of ethylene glycol and the mixture heated in water bath at 70 °C;
2. after dissolving, 15 mL of sterilized distilled water was added;
3. five mL of the stock solution was added to 245 mL of L.J medium; and
4. the final concentration (critical concentration) of the drug in L.J medium was 40 µg/mL.

Isoniazid preparation (I)

1. Stock solution (1000 µg/mL) was prepared by 10 mg of isoniazid in 10 mL of sterilized distilled water;
2. solution A (100 µg/mL) was prepared by diluting 5 mL of stock solution in 45 mL of sterilized distilled water;
3. solution B (10 µg/mL) was prepared by diluting 5 mL of solution A in 45 mL of sterilized distilled water;
4. five mL of solution B was added to 245 mL of L.J medium; and
5. the final concentration (critical concentration) of the drug in L.J medium was 0.2 µg/mL.

2.4.2. Preparation of McFarland solution

This solution was used to give approximate number of bacterial cells in culture suspension with concentration of (1mg/mL); McFarland 1 gives a number of 3×10^8cell/mL. while McFarland 0.5 gives a number of $1.5x10^8$ cells/ mL and was prepared as follows:

Part I: Prepared by dissolving 1.175 g of hydrated barium chloride (BaCl2.H2O) in 100 mL of distilled water;

Part II: Prepared by adding 1 mL of concentrated sulpheric acid (H2SO4) to 100 mL of distilled water;

McFarland 1: Prepared by adding 0.1mL of part I to 9.9 mL of part II; and

McFarland 0.5: Prepared by adding 0.05 mL of part I to 9.95 mL of part II [16].

Preparation of culture suspension

1. Growth on solid medium was used within 15 days of appearance of positive growth, many colonies scraped as possible with the help of a sterile loop;
2. the growth was transferred into another tube and crushed with Pasteur pipette with adding drops of saline;

a. the turbidity of the suspension was compared with McFarland 1 by adding drops of normal saline until the suspension and McFarland 1 had the same turbidity;

b. series of dilutions tubes from 10^{-1} to 10^{-5} was prepared by adding 4.5 mL of saline to each tube and then, 0.5 mL of culture suspension was added to first tube to obtain 10^{-1};

c. to obtain 10-2 dilution, 0.5 mL of 10-1 dilution was added to the second tube and so on to five dilutions and the last 0.5 mL of 10^{-5} dilution was discarded;

d. each drug medium and control (free of drug) was inoculated with 0.1 mL of 10^{-1}, 10^{-3} and 10^{-5} dilutions, respectively, and incubated diagonally and semi closed for 3 days at 37 °C, after this period, the vials were closed tightly and incubated vertically for 1 month at 37 °C; and

e. then, the result was recorded as sensitive or resistant by comparing the drug medium with control medium.

2.4.3. Tuberculosis Rapid Test (Cassette) on Serum or plasma

1. Allowing the test cassette, specimen, buffer to equilibrate to room temperature (15 – 30 °C) prior to testing;
2. removing the test cassette from the sealed foil pouch and use it as soon as possible;
3. peeling off the tape from the test card, and stick the test cassette in the middle of the test card with arrows pointing downwards;
4. holding the dropper vertically, transfer 2-3 drops of serum or plasma (approximately 60-90 µl) to the sample pad of test cassette and then start the timer; The colored line(s) to appear. The result should be read at 15 minutes.
5. then, recording the results as positive, negative and invalid. The following statues were represented as positive;
6. in addition to the presence of C band, if only T1 band is developed, it indicates the presence of IgM anti-TB in the specimen; and
7. in addition to the presence of C band, both T1 and T2 band is developed, indicating the presence of IgM anti-TB and IgG anti-TB in the specimen.

3. Results

3.1. Database Analysis

Out of 188 suspected specimens, 103 (54.78%) cases were positive using direct assay (AFB smear microscopy) and 85 (45.21%) were considered to be negative cases by direct assay AFB smear.

3.2. Gender Studies

The data set in Table (1) exhibited the gender distribution percentage of infected patients were 73 males constituting 70.872% to 33 females 29.12%, with male to female ratio of 2.21 with a highly significant difference ($P \leq 0.01$).

Table 1. Distribution of TB patients according to gender

Gender	subjects			
	No. of healthy	No. of suspected	No. of suspected	No. of suspected
	AFB-ve (%)	AFB (%)	AFB+ve (%)	AFB –ve (%)
Male	42 (52.5%)	110 (58.51%)	73 (70.87%)	37 (43.52)
Female	38 (47.5%)	78 (41.49)	33 (29.12%)	48 (56.47)
Total	80 (100%)	188 (100%)	103 (100%)	85(100%)

AFB = Acid Fast Bacilli

3.3. Subjects Age

The age of patients ranged between 14 years to 75 years. The mean age was 36 years and the median was 30 years with highly significant difference between age group (Fig. 1, Table 2).

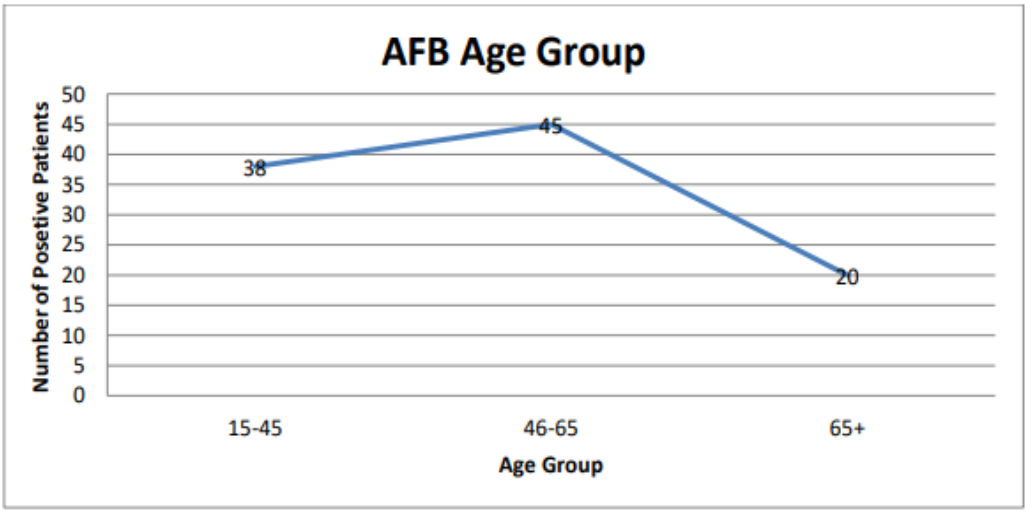

Figure 1. Distribution of TB patients according to their age group

Table 2. Distribution of TB patients according to their age group.

Age Group	Male	Female
15-45 year	32	11
46-65 year	27	7
Above 65 year	14	12

3.4. Positive tuberculosis cases using AFB stain

As in many developing countries, direct sputum microscopy is the widely used method for the diagnosis of pulmonary TB in Iraq. Only 103 from 263 subjects (suspected and healthy) were positive by using ZN smears (Ziehl-Neelsen stain technique); they were therefore regarded as presence of *Mycobacterium* in the specimen and further investigation was performed for those 103 samples stained by ZN, showing acid-fast bacilli, which appeared red, straight or slightly curved rods,

occurring singly or in clumps and with size ranges from 0.3-0.6 µm x 0.6-1 µm (Fig 2 and Fig 3).

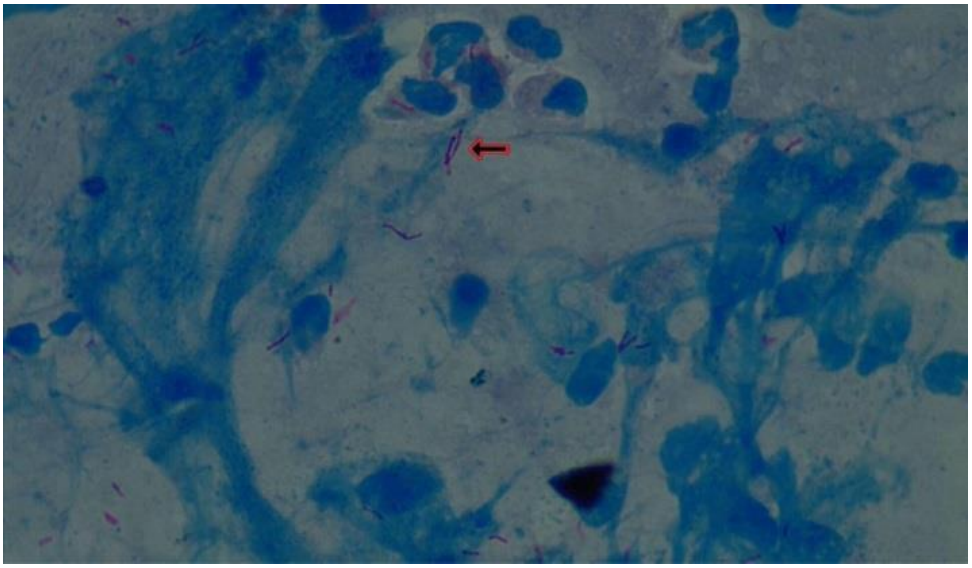

Figure 2. Acid Fast Bacilli positive case stained by ZN

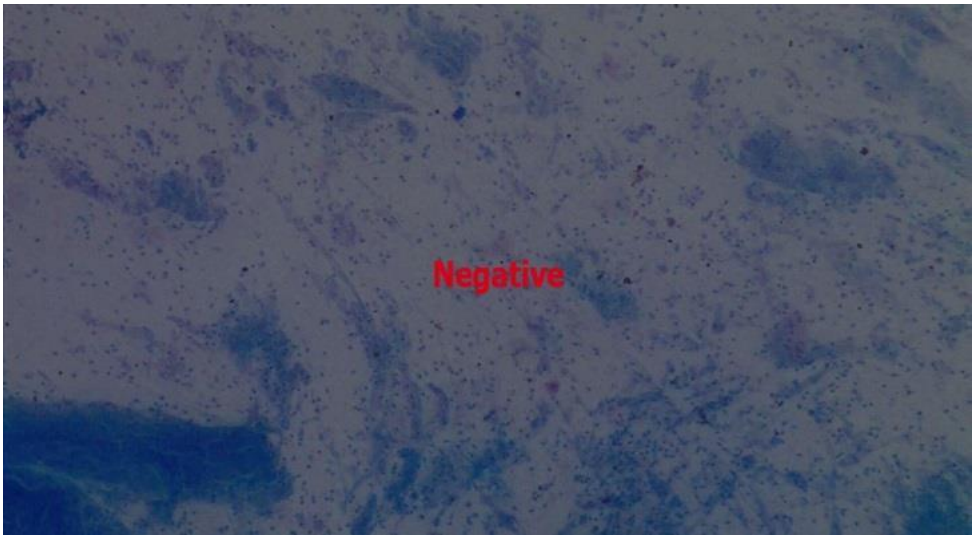

Figure 3. Acid Fast Bacilli negative case stained by ZN

3.5. Detection Positive tuberculosis cases using Lowenstein–Jensen culture

Out of 188 suspected cases, 119 (63.29%) were positive by culture (Lowenstein–Jensen medium) and 69 (36.7%) were negative specimens (Table 3).

Table 3. Distribution of TB patients according to their gender using Lowenstein–Jensen culture

Gender	No. of Healthy Subjects (%) L.J culture	No. of Suspected subjects (%) L.J culture	No. of Suspected subjects (%) L.J culture +ve	No. of Suspected subjects (%) L.J culture -ve
Male	42 (52.5%)	110 (58.51%)	83 (69.74%)	27 (39.13%)
Female	38 (47.5%)	78 (41.49)	36 (30.25%)	42 (60.86%)
Total	80 (100%)	188 (100%)	119 (100)%	69 (100%)

L.J = Lowenstein –Jensen culture.

The chance of locating acid-fast bacilli in sputum samples through smear microscopy is at once associated with the concentration of bacilli inside the sputum when the concentrations are less than 1000 microorganisms per mL. The probability of getting bacilli in a smear will reduce by 10% if we compare it with culture procedure that is able to detect 100 microorganisms per mL. Another advantage of culture method is its ability in recognizing mycobacterial species depending on biochemical test. On the other hand, AFB could not recognize pathogenic and non-pathogenic mycobacteria; all of them have the same morphology. For the above reasons, culture method represents the gold standard procedure in the diagnosis of tuberculosis, so culture technique is characterized with high percentage of the sensitivity and the specificity comparing with smear procedure.

The calculation of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for smear procedure will depend on the result of the standard procedure, i.e. culture method. Therefore, we consider real positive case if it is positive in culture method (Fig. 4) and the same situation for the negative case.

Figure 4. Positive growth on Lowenstein–Jensen media

This study showed that the percentage of sensitivity, specificity, positive predictive value, and negative predictive value is 74.78%, 79.7%, 86.4% and 64.7%, respectively, for AFB method (Table 4).

Table 4. The percentage of sensitivity, specificity, PPV and NPV of AFB smear compared with sputum culture

AFB Smear	L.J +ve	L.J - ve	Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Smear +ve	89	14	103	74.78	79.7	86.4	64.7
Smear -ve	30	55	85				
Total	119	69	188				

L.J = Lowenstein–Jensen culture, AFB= Acid Fast Bacilli, PPV= Positive predictive value, NPV=Negative predictive value.

3.6. Positive tuberculosis cases using Tuberculosis Rapid Test (Cassette) on Serum or plasma

The results revealed that among total 188 specimens (Suspected Group), 49 (26.06%) of the specimens were positive by DiaSpot (Tuberculosis Rapid Test on serum) and 139 (73.93%) negative specimens by Diaspot (Table 5). Positive results were shown as two lines, one for control and other for sample. While negative result had only one line to appear as the control line and the invalid results when the control line did not appear (Fig 5 and Fig 6).

Table 5. Detection of TB in patients according to their gender using Tuberculosis Rapid Test

Gender	No. of Healthy subjects (%) DiaSpot	No. of Suspected subjects (%) DiaSpot	No. of Suspected subjects (%) DiaSpot +ve	No. of Suspected subjects (%) DiaSpot -ve
Male	42 (52.5%)	110 (58.51%)	35 (71.42%)	75 (53.95%)
Female	38 (47.5%)	78 (41.49)	14 (28.57%)	64 (46.04%)
Total	80 (100%)	188 (100%)	49 (100%)	139 (100%)

Figure 5. Tuberculosis rapid test represented as positive result

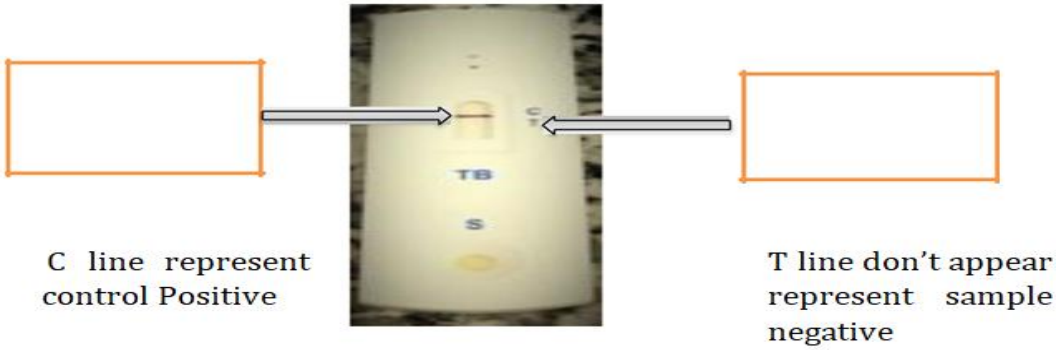

Figure 5. Tuberculosis rapid test represented as negative result

We found a sensitivity of 33.6% and a specificity of 86.95% PPV 81.6 and NPV 43.16 for DiaSpot TB Rapid test in this study (Table 6) as against the manufacturer's reported sensitivity of 33.6% and 98.9%. Other investigators have reported low sensitivities for TB rapid tests in various studies.

The performance of DiaSpot TB Rapid test in this setting was not as good as sputum smear microscopy. However, given the simplicity, the speed and relatively low cost of this test, it may be used in combination with smear microscopy as a screening test to reduce the number of sputum samples to be examined. In such a use, it may lessen the inconveniences of patients and reduce the laboratory workload.

Table 6. The percentage of Sensitivity, Specificity, PPV and NPV of Diaspot in comparison with sputum culture

DiaSpot	L.J +ve	L.J -ve	Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
DiaSpot+	40	9	49				
DiaSpot -	79	60	139	33.6	86.95	81.6	43.16
Total	119	69	188				

L.J = Lowenstein –Jensen culture

4. Discussion

Out of 103 TB patients, 88 (85.43%) were represented as new TB cases; the

rest of 15 (14.57%) were previously diagnostic and the follow up (DOT) program. This study agrees with two recent studies conducted in Baghdad [16], reporting that 84% were new TB cases, and the rest (16%) as follow up (DOT) program. The other recent study in Hilla, Babil governarate, Iraq [17] found that the 80.6% of TB infected patients were classified as new TB patients and 19.4% were from the follow up program.

The smear microscopy plays a critical position inside the early prognosis of mycobacterial infections that is rapid, less expensive and a really useful method to diagnose exceptionally contagious patients. However, this method lacks sensitivity; its usefulness depends in large part on the exceptionality of the sputum specimen and its overall performance of the laboratory [18, 19].

TB patients were interviewed using a dependent questionnaire. The findings indicated that the extensive majority of sufferers were associated with poor housing best, an overcrowding populated vicinity, and low degree of education, and were related to poverty, which is in agreement with the past studies [20]. The infection of TB is transmitted when an infected person with active TB type coughs or sneezes *Mycobacterium* bacilli that turn into droplets approximately of less than 5 μm diameter that can remain suspended in the air for many hours. Therefore, TB transmission occurs with greater prevalence in poorly ventilated and crowded spaces [21]. It is expected that Al-Sadr and Al-Hurriya Cities in Baghdad have the highest ratio of TB infections, being in agreement with a previous study [22], which found a high percentage of tuberculosis in Sadr City because it is the most populated city in Iraq with majority of people living under the poverty line; crowdedness and poverty are two essential requirements for successful tuberculosis transmission.

The gender distribution percentage of infected patients were male 73 constituted(70.872%) to female 33 (29.12 %), with over male to female ratio of 2.21 with a highly significant difference (P≤0.01). These results are in concordance with those of previous research[23].

The reason for higher men incidence is poorly understood, and requires additional research to identify correlating hazard agents. The difference between male and female susceptibility to TB may result partly from biological differences (i.e. sex differences), cultural and the economic state of the society due to gender differences having access to health care. Various studies have introduced evidence for a possible role of the X chromosome and sex hormones (i.e. testosterone) in susceptibility to TB [24]. Hormones related with gender may be a compelling factor for this difference; testosterone for example decreases the macrophage activation process as well as pro-inflammatory cytokines production. On other hand estrogens are pro inflammatory mediator's inducer. Recent study reported that this difference may be due to more presence of men in the community and more unexpected meetings with carriers and the disease's risk factors such as cigarettes and narcotic materials [25].

The current results of infection age groups demonstrated that all age groups were susceptible to infection with tuberculosis but with different proportion. Only one infection (1.78%) of tuberculosis case was 15 years of age, which may refer to BCG vaccine given at young age that protects against *M. tuberculosis* infection Table (4), [26]. The highest percentage of pulmonary tuberculosis positive patients recorded at the age over 15 years are due to many factors such as immune depression factors such diseases of lungs because of smoking [27]. These results are in

resonance with recent studies in Baghdad [28] which found the highest percentage of TB at the age over 15 years. In Iran, the mean age of patients was 41.89 years (range 15-65 years) [29]. The mean age in Syria was 35.75 years [30]. In Pakistan, it was found that the greater number of tuberculosis patients were in age groups of 20-30 years and over 40 years in both genders [31], while a study from India indicated that the mean age among the new cases was at the age of 27.8 [32].

It was evaluated that about 75% of all TB cases are in people less than 50 in developing countries while in developed countries tuberculosis occurs mainly in older adults [33].

Acid-fast staining of sputum is a rapid, inexpensive method for diagnosing pulmonary tuberculosis. Despite low sensitivity of ZN stain as diagnostic tool for TB, it is still an essential process for diagnosis of high bacillary load patients where sputum smears would be positive [34]. It requires 104 CFU/mL of sputum to be visualized by a light microscope [35]. In conclusion, the examination through Ziehl- Neelsen stain is commonly missed in sputum specimen, which may be due to the very few number of acid fast bacilli. Culture from sputum, which is digested and decontaminated, is a very sensitive procedure compared with sputum in microscopically one, but the results of culture are not readily and take time to be read with in several weeks. The consequences lead to real delay in diagnosis as well as treatment, with the probability for transmission of disease.

Out of these, 83 were males accounting for 69.74 and 36 were females that accounted for 30.25 %; with male to female ratio of 2.3 (83/36) with a highly significant difference($P \leq 0.01$) (Fig. 4). These results were in accordance with the previously reported results [23].

This study showed that the percentage of sensitivity, specificity, positive predictive value, and negative predictive value is 74.78%, 79.7%, 86.4% and 64.7%, respectively, for AFB method, which are in agreement with those of past research [27].

Negative cases regarding smear procedure are causing the transmission of TB, with less degree if we compare with sputum smear negative cases; they could be later considered as smear positive when it is left without treatment. It is very difficult to create rapid and efficient procedure to diagnostic paucibacillary disease case. Culture from sputum which is digested and decontaminated is a very sensitive procedure than sputum in microscopically one, but the results of culture are not simple and take time to be read within several weeks. The consequences lead to real delay in diagnosis as well as treatment, with the probability for transmission of disease. Additional problems of diagnosis are that cases do not have a real Tuberculosis but diagnostic as TB in wrong way for false mean positive and then taking treatment as a consequence. In other instances, patients may already be cured and unfortunately the course of treatment will continue. Till now these are no realistic way to enable us to be sure these cases are cured. The laboratory thus has an important role to play in the management of tuberculosis patient [36].

Rapid diagnostic tests (RDTs) detect the presence of specific antibodies directed against immune dominant antigens of TB. These antibody detection (serological) tests in simple rapid immune chromatographic format have greatly simplified the diagnosis of many infectious diseases [37].

A poor performance for SDHO MTB test, with a sensitivity of 20.6% and a specificity of 90.3% was reported in Bangui, Central African Republic [38]. In a laboratory- based evaluation of 19 commercial TB serological tests by TDR

researchers, sensitivities ranged from 0.97% to 59.7% and specificities ranged from 53% to 98.7% [7].

A wide variability in the performance of serological tests is a common feature of several published evaluation results [48]. Differences in the types, the number and the chemical nature of the antigens used in TB serological tests may be partly responsible for this variability in performance [39]. Furthermore, the local epidemiological factors such as the prevalence of HIV, exposure to environmental mycobacteria, vaccination with BCG and the proportion of disease caused by nontuberculosis mycobacteria (NTM) may affect the sensitivity and specificity of a serological antibody test in a particular epidemiological setting [40].

The heterotypic nature of immune responses to antigens of *M. tuberculosis* in different individuals and the different profile of antigenic proteins of *M. tuberculosis* recognized by antibodies at different stages of infection and disease progression are other factors that complicate serological antibody detection in the diagnosis of tuberculosis [41].

5. Conclusion

Males between 15-45 were found to have high risk of TB infection. (The percentage of infected patients were 73 males (70.87%) to 30 females (29.12 %), with over all male to female ratio of 2.43 (73/30). The species *M. tuberculosis* plays a major role for causing tuberculosis in human in Baghdad, Iraq.

Authors' contributions:

Mohsen Hashim Risan, designed this study, Haidar Khalid Mostafa and Mohammed Al-Faham obtained and analyzed the data. Haidar Khalid Mostafa supervised the collection of samples and the identification of plant species studied. Mohsen Hashim Risan and Mohammed Al-Faham proceeded to the data quality control and the manuscript drafting. Mohssen Hashim Risan revised the final version.

Consent for publications:

All authors agree to have read the manuscript and authorize the publication of the final version of the manuscript

Conflict declaration:

The authors declare that there is no conflict.

Conflict of interest:

None of the authors have any conflict of interest to declare.

Availability of data and material:

Data are available on request from the authors.

Funding/Support:

Not applicable

Ethics approval and consent to participate

Sample collection was obtained from sputum samples collected from suspected patients, in addition to 80 samples collected from healthy subjects. The study was conducted at National Reference Lab (NRL) of tuberculosis/Baghdad.

Acknowledgments

We thank Anmar L. Al-hassani**,** Ministry of Health, Respiratory and Chest Centre, Medical City, Iraq, Baghdad**.**

References

1. Organization W H. (2014). Global tuberculosis report 2014: *World Health Organization*. 171 Pages,

2. Rocchetti T T, Silbert S, Gostnell A, Kubasek C, Widen R. (2016). Validation of a multiplex real-time PCR assay for detection of *Mycobacterium* spp., *Mycobacterium tuberculosis* complex, and *Mycobacterium avium* complex directly from clinical samples by use of the BD Max open system. *Journal of Clinical Microbiology*, 54(6): 1644-1647. https://doi.org/10.1128/JCM.00241-16
3. Moure R, Muñoz L, Torres M, Santin M, Martín R, Alcaide F. (2011). Rapid detection of *Mycobacterium tuberculosis* complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. *Journal of Clinical Microbiology*, 49(3): 1137-1139. https://doi.org/10.1128/JCM.01831-10
4. Zakham F, Akrim M, Benjouad A, Ennaji M. (2012). Rapid screening and diagnosis of tuberculosis: a real challenge for the mycobacteriologist. *Cellular and Molecular Biology*, 58(2): 1632-1640.
5. Yusuf E. (2015). A Book Review on: Sherris Medical Microbiology–International Edition. *Frontiers in cellular and infection microbiology*, 5: 34. https://doi.org/10.3389/fcimb.2015.00034
6. LoBue P, Enarson D, Thoen C. (2010). Tuberculosis in humans and animals: an overview [Serialised article. *Tuberculosis*: a re-emerging disease in animals and humans. Number 1 in the series]. *The International Journal of Tuberculosis and Lung Disease*, 14(9): 1075-1078.
7. Chakaya J, Khan M, Ntoumi F, Aklillu E, Fatima R, Mwaba P, Kapata N, Mfinanga S, Hasnain S E, Katoto P D. (2021). Global Tuberculosis Report 2020–Reflections on the Global TB burden, treatment and prevention efforts. *International Journal of Infectious Diseases*, 113: S7-S12. https://doi.org/10.1016/j.ijid.2021.02.107
8. Corbett E L, Watt C J, Walker N, Maher D, Williams B G, Raviglione M C, Dye C. (2003). The growing burden of *tuberculosis*: global trends and interactions with the HIV epidemic. *Archives of Internal Medicine*, 163(9): 1009-1021. https://doi.org/10.1001/archinte.163.9.1009
9. Hopewell P C, Fair E L, Uplekar M. (2014). Updating the International Standards for Tuberculosis Care. Entering the era of molecular diagnostics. *Annals of the American Thoracic Society*, 11(3): 277-285. https://doi.org/10.1513/AnnalsATS.201401-004AR
10. Karabulut F, Parray J A, Mir M Y. (2021). Emerging trends for Harnessing plant metabolome and microbiome for sustainable food Production. *Micro Environer*, 1(1): 33-53 https://doi.org/10.54458/mev.v1i01.6672
11. Abbas-Al-Khafaji Z-K, Aubais-aljelehawy Q h. (2021). Evaluation of antibiotic resistance and prevalence of multi-antibiotic resistant genes among Acinetobacter baumannii strains isolated from patients admitted to al-yarmouk hospital. *Cellular, Molecular and Biomedical Reports*, 1(2): 60-68.
12. Hutchins R J, Phan K L, Saboor A, Miller J D, Muehlenbachs A. (2019). Practical guidance to implementing quality management systems in public health laboratories performing next-generation sequencing: personnel, equipment, and process management (phase 1). *Journal of Clinical Microbiology*, 57(8): e00261-00219. https://doi.org/10.1128/JCM.00261-19
13. Robinson S. (2021). Priorities for Health Promotion and Public Health: *Londen: Routledge*. 111 Pages,
14. Subgroup S T P C T. (2006). Guidance for National Tuberculosis Programmes on the management of tuberculosis in children. Chapter 1: introduction and diagnosis of tuberculosis in children. *The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease*, 10(10): 1091-1097. PMID: 17044200
15. Mankhi A. (2009). Supervised by Hashem, ZS Guidelines of Laboratory manual work of tuberculosis in Iraq. Iraq. Baghdad: *Prepared by Ministry of Health*. 289 Pages,
16. Mostafa H K, Risan M H, Al-Faham M, Tbena M. (2018). Detection of *Mycobacterium tuberculosis* from Clinical Samples using Different Real-Time PCR System. *Int. J. Curr. Microbiol. App. Sci*, 7(12): 2357-2368.

https://doi.org/10.20546/ijcmas.2018.712.267

17. Al-Saadi M, Naher H, Shalan A. (2014). Evaluation of Commercial Real Time PCR and Immunochromatography Techniques in Laboratory Diagnosis of *Tuberculosis*. *Medical Journal of Babylon*, 11(1): 58-69.
18. Rosser E, John Holley M, Choi H, Harvey S A. (2009). Willingness to use and pay for a new diagnostic test for active *tuberculosis* in HIV-positive and HIV-negative individuals: Results from Benin, Peru, and Tanzania. Bethesda: *Center for Human Services*. 71 Pages,
19. Parray J A, Ali U, Mir M Y, Shameem N. (2021). A high throughputs and consistent method for the sampling and isolation of Endophytic bacteria allied to high altitude the medicinal plant *Arnebia benthamii* (Wall ex. G. Don). *Micro Environer*, 1(1): 1-6. https://doi.org/10.54458/mev.v1i01.6668
20. Duyan V, Kurt B, Aktas Z, Duyan G, Kulkul D. (2005). Relationship between quality of life and characteristics of patients hospitalised with *tuberculosis*. *The International Journal of Tuberculosis and Lung Disease*, 9(12): 1361-1366.
21. Djibuti M, Mirvelashvili E, Makharashvili N, Magee M J. (2014). Household income and poor treatment outcome among patients with tuberculosis in Georgia: a cohort study. *BMC Public Health*, 14(1): 1-7. https://doi.org/10.1186/1471-2458-14-88
22. Mohammed S H, Ahmed M M, Ahmed A R H. (2013). First experience with using simple polymerase chain reaction-based methods as an alternative to phenotypic drug susceptibility testing for *Myobacterium tuberculosis* in Iraq. *International Journal of Applied and Basic Medical Research*, 3(2): 98. https://dx.doi.org/10.4103%2F2229-516X.117069
23. Shaker H, Saleh D. (2013). A study on diagnosis and multidrug resistance of Mycobacterium tuberculosis using different methods. Unpublished thesis for the Degree of Master of Science in Microbiology. College of Science/University of Baghdad.
24. Boum Y, Atwine D, Orikiriza P, Assimwe J, Page A-L, Mwanga-Amumpaire J, Bonnet M. (2014). Male Gender is independently associated with pulmonary *tuberculosis* among sputum and non-sputum producers people with presumptive tuberculosis in Southwestern Uganda. *BMC infectious diseases*, 14(1): 1-8. https://doi.org/10.1186/s12879-014-0638-5
25. Babamahmoodi F, Alikhani A, Yazdani Charati J, Ghovvati A, Ahangarkani F, Delavarian L, Babamahmoodi A. (2015). Clinical epidemiology and paraclinical findings in tuberculosis patients in north of Iran. *BioMed research international*, 2015(Article ID 381572): 1-5. https://doi.org/10.1155/2015/381572
26. Roy A, Eisenhut M, Harris R, Rodrigues L, Sridhar S, Habermann S, Snell L, Mangtani P, Adetifa I, Lalvani A. (2014). Effect of BCG vaccination against Mycobacterium tuberculosis infection in children: systematic review and meta-analysis. *Bmj*, 349: g4643 https://doi.org/10.1136/bmj.g4643
27. Singh P N, Yel D, Hurd G, Job J S. (2013). Cigarette smoking and *tuberculosis* in Cambodia: findings from a national sample. *Tobacco Induced Diseases*, 11(1): 1-9. https://doi.org/10.1186/1617-9625-11-8
28. Al-Jebouri M M, Wahid N M. (2014). The interactions between polymerase chain reaction (PCR) and other diagnostic tests among *tuberculosis* patients of various blood groups. *World J. pharm. Pharmaceut. sci*, 3(7): 217-230.
29. Yarahmadi A, Zahmatkesh M M, Ghaffari M, Mohammadi S, Labbafinejad Y, Seyedmehdi S M, Nojomi M, Attarchi M. (2013). Correlation between silica exposure and risk of tuberculosis in Lorestan Province of Iran. *Tanaffos*, 12(2): 34. PMCID: PMC4153244; PMID: 25191460
30. Rahmo A, Hamze M. (2010). Characterization of *Mycobacterium tuberculosis* in Syrian patients by double-repetitive-element polymerase chain reaction. *EMHJ-Eastern Mediterranean Health Journal, 16 (8), 820-830, 2010*, 16(8): 820-830.
31. Khosravi A D, Dezfulian A, Alavi S M. (2006). Detection of Isoniazid and Rifampin resistant *Mycobacterium tuberculosis* isolated from tuberculosis patients using

conventional method and PCR. *PAKISTAN JOURNAL OF MEDICAL SCIENCES*, 22(1): 47-52.
32. Sharma S K, Kaushik G, Jha B, George N, Arora S, Gupta D, Singh U, Hanif M, Vashisht R. (2011). Prevalence of multidrug-resistant *tuberculosis* among newly diagnosed cases of sputum-positive pulmonary tuberculosis. *The Indian Journal of Medical Research*, 133(3): 308. PMCID: PMC3103156; PMID: 21441685
33. Schaaf H S, Collins A, Bekker A, Davies P D. (2010). Tuberculosis at extremes of age. *Respirology*, 15(5): 747-763. https://doi.org/10.1111/j.1440-1843.2010.01784.x
34. Datta S, Sherman J M, Bravard M A, Valencia T, Gilman R H, Evans C A. (2015). Clinical evaluation of tuberculosis viability microscopy for assessing treatment response. *Clinical infectious diseases*, 60(8): 1186-1195. https://doi.org/10.1093/cid/ciu1153
35. Chan C E, Zhao B Z, Cazenave-Gassiot A, Pang S-W, Bendt A K, Wenk M R, MacAry P A, Hanson B J. (2013). Novel phage display-derived mycolic acid-specific antibodies with potential for tuberculosis diagnosis [S]. *Journal of lipid research*, 54(10): 2924-2932. https://doi.org/10.1194/jlr.D036137
36. Udwadia Z F. (2003). Controlling tuberculosis in India. *The New England journal of medicine*, 348(8): 758-759; author reply 758. https://doi.org/10.1056/nejm200302203480817
37. Organization W H. (2006). Diagnostic and treatment delay in tuberculosis. https://apps.who.int/iris/handle/10665/116501.
38. Kassa-Kelembho E, Kassa E, Zandanga G, Service Y-B, Ignaleamoko A, Talarmin A. (2006). Poor performance of a novel serological test for diagnosis of pulmonary tuberculosis in Bangui, Central African Republic. *Clinical and vaccine immunology*, 13(6): 702-703. https://doi.org/10.1128/CVI.00194-05
39. Steingart K R, Henry M, Laal S, Hopewell P C, Ramsay A, Menzies D, Cunningham J, Weldingh K, Pai M. (2007). Commercial serological antibody detection tests for the diagnosis of pulmonary tuberculosis: a systematic review. *PLoS medicine*, 4(6): e202. https://doi.org/10.1371/journal.pmed.0040202
40. Sada E, Aguilar D, Torres M, Herrera T. (1992). Detection of lipoarabinomannan as a diagnostic test for *tuberculosis*. *Journal of Clinical Microbiology*, 30(9): 2415-2418. https://doi.org/10.1128/jcm.30.9.2415-2418.1992
41. Davidow A, Kanaujia G V, Shi L, Kaviar J, Guo X, Sung N, Kaplan G, Menzies D, Gennaro M L. (2005). Antibody profiles characteristic of *Mycobacterium tuberculosis* infection state. *Infection and immunity*, 73(10): 6846-6851. https://doi.org/10.1128/IAI.73.10.6846-6851.2005

How to cite this article: Haidar Khalid Mostafa, Mohsen Hashim Risan*, Mohammed Al-Faham. A study of *Mycobacterium tuberculosis* Zopf, 1883 (*Mycobacteriaceae*) in Iraq. *International Journal of Advanced Biological and Biomedical Research*, 2022, 10(1), 1-17. Link: http://www.ijabbr.com/article_248083.html