Molecular Detection Of Rifampicin Resistance Among Mycobacterium Tuberculosis (MTB) Patients In Iraq

Ehsan Joudah Hussein[1]*, Ahmed Asmer Mankhi[2]

[1]*MSc microbiology, Maysan health department, Email: Ehsan_bio2002@yahoo.com
[2]MB.CH.B Ph.D molecular pathology, Ministry of Health, Email: ahmedman1966@yahoo.com

***Corresponding Author: -** Ihsan Joudah Hussein
*MSc microbiology, Maysan health department, Email: Ehsan_bio2002@yahoo.com

Abstract:
It was found that the rate of resistance to the standard drug Rifampicin for patients with Mycobacterium tuberculosis (MTB) and non-recovery in most Iraqi governorates was 13%, while the rate of sensitivity to the standard drug Rifampicin was 87% according to the DST test, while the rate of resistance to Rifampicin was 68.4% and the sensitivity was 31.6% according to (L.P.A) test for the same samples under study, and it is clear that the most infected regions of Iraq with Mycobacterium tuberculosis (MTB) were in the city of Baghdad and the least in the province is Anbar, and the most response ages to the standard drug Rifampicin were the ages confined between 10 years to the age of 50 years and the highest rate of response Between 40-50 years, the response rate deteriorated after the age of 70 until the age of 80.
It turned out that the incidence of Mycobacterium tuberculosis (MTB) for males was higher than for females. There was a clear significant difference between the infections of Mycobacterium tuberculosis (MTB) between the people who live in urban areas and between people who they live in rural areas.
It was also found that the Resistance type for (MDR), (RR) and (MONO) were 47.1%, 23.5% and 17.6%, respectively. This indicates that the best antibiotic that may be used in the treatment of Mycobacterium tuberculosis (MTB) is Rifampicin.

Keywords: rifampicin, Iraqi provinces, (MTB), (L.P.A) test.

Indications:
Tuberculosis (tuberculosis - tuberculosis - pulmonary tuberculosis) is one of the top ten causes of death worldwide. It has ancient origins. It is an infectious bacterial disease that can be treated and prevented. Tuberculosis (TB) did not become an epidemic among humans until the early seventeenth century, when it was allowed Western Europe increased urbanization of these airborne bacteria by spreading efficiently from person to person. This epidemic, dubbed the "Great White Plague", spread from England to engulf most of Western Europe .Within 200 years, most of the European population was infected, perhaps a quarter of the population died . (Singh, al et, 2020) Tuberculosis infection is caused by bacteria called mycobacterium tuberculosis. Bacteria belonging to the same family as mycobacteria (such as Mycobacterium bovis and Mycobacterium africanum) can sometimes cause diseases similar to tuberculosis. These bacteria, along with Mycobacterium tuberculosis and some others, are called complex Mycobacterium tuberculosis. The tuberculosis Mycobacterium is the main factor responsible for causing pulmonary tuberculosis, or what is called tuberculosis It is characterized by being complex and mainly interacting with its human host, and by its ability to survive for a long time in phagocytic cells, The large crops grow very slowly and need a long period of 2-12 weeks. During this time they multiply and in fact These reasons contribute to the delay in the therapeutic response and externally32 hours and no longer able to secrete toxins internally Bacillus approx. Cellular immunity until the number of bacilli reaches 103(Cantres- Fonseca, al et ،2019).
These bacilli mainly affect the lung because they have the ability to attack other parts of the body such as the organ The central nervous system, the lymphatic system, the circulatory system, the genitourinary system, the digestive system, and the bones as well. (Koch & Mizrahi, 2018).

The aim of this study is to find out the extent of response to the standard drug Rifampicin for tuberculosis patients in Iraq at different age and geographical levels.

Literature Review:
Mycobacterium tuberculosis colonies, This bacterium is the main cause of tuberculosis. Many of the unique clinical features of this pathogen are due to its high fat content. Every 16-20 hours, the bacillus is divided. Other bacteria are slower than it, which takes less than an hour to spread. Mycobacteria's outer membrane is composed of lipid bilayers(Deb,et al.,2009). MTB is resistant to weak disinfectants and can survive for weeks in dry conditions. Bacteria

can only be produced inside the cell of a host organism in nature, although Mycobacterium tuberculosis can be cultured in the laboratory (Mishra and Singh,2012).

Antibiotic resistance in M. tuberculosis generally develops as a result of the accumulation of mutations in the antibiotic's target genes ordifferent a change in drug titration. M. tuberculosis is a multidrug - resistant strain (MDR TB) that has evolved resistance to the most commonly used antibiotics, rifampicin and isoniazid. Furthermore, extensively drug-resistant tuberculosis (XDR TB) is defined by resistance to isoniazid and rifampin, as well as any fluoroquinolone and at least one of three by injectable suspension second - line drugs (ie , amikacin, kanamycin, or capromycin) (Muller, et al., 2013؛ Miller ,et al.,2013).

Tuberculosis may be detected By Molecular detection using GeneXpert and line probe assay (LPA). GeneXpert By using polymerase chain reaction, the Xpert MTB/RIF identifies DNA sequences unique for Mycobacterium tuberculosis and rifampicin resistance. It's based on the Cepheid GeneXpert technology, a nucleic acid amplification test that's quick and easy to use (NAAT). LPA is a polymerase chain reaction (PCR)-based fast approach for detecting "Mycobacterium tuberculosis (MTB) complex as well as drug sensitivity to rifampicin (RPM) and isoniazid (INH). Blood checks to detect antibodies are not accurate or ticklish, so they are not recommended" (Dinnes,et al.,2007). MDR-TB is defined as resistance to both rifampicin and isoniazid, which are effective first-line TB drugs. Tuberculosis that is highly drug resistant is renitent to three or more of the six second-class drugs. TB is completely drug resistant to all the drugs currently in use". Tuberculosis that is fully drug-resistant was first reported in Italy in 2003, but has not been widely reported since 2012. Multidrug-resistant TB (MDR-TB) cases in 2013 were about 3.5% in new cases and" 20.5% in old and previous cases. It was estimated that there were approximately 300,000 cases" of MDR-TB in 2013, of which 136,000 (approximately 45%) were identified and reported (Mekonnen, et al.,2015).

Method and Material:

Samples

Patient suspected TB cases or confirmed TB cases who receive ANTI-TB treatment who visit the national tuberculosis center, Baghdad, Iraq, all age, without excepted samples of study maybe (sputum), Sample: Sputum from suspected or confirmed tuberculosis (TB) subjects (100 positive).

Tests

The positive samples collected from the 100 patients, which were positive in the direct examination of sputum (AFB), were done for bacterial culture with drug sensitivity Test (DST) for the first (1st) and second lines (2st), molecular assays using the Gene Expert device (Realtime PCR) MTB / RIF and PCR examination using the Line Probe Assay.

Questionnaires

All patients also filled out questionnaires by providing answer to important information.

The questionnaires were been used in assessing the new case or retreatment question included in the questionnaires included, age, gender and clinical symptoms. Such as fever, cough, chest pain, night sweeting and others (Ely, et al.,2002).

Direct smear (Acid fast stain bacilli) by zielneelsen stain. (Hot staining)

1- A portion of the sputum sample was taken and spread in the middle of the slide using sterile pipette.
2- Allow the smear to dry complete, Fix the smear by transit the slide 3-4 times through the flame of Bunsen burner.
3- The slide was completely covered with carbol fuchsine dye, Use the Bunsen burner heat the slide until the steaming then we counts 5 Minutes.
4- The slide was washed with water.
5- Wash the slide with alcohol acid solution during 30 sec.
6- Immediately wash with distilled water.
7- Flood the slide with counter stain (Methylene Blue) so one minute.
8- Rinse different the slide thoroughly with water, Dry the slide by the air.
9- Examine under oil immersion (Ayyash S.,2016).

Anti TB drug susceptibility Test (DST the proportion method) [113,114].

1. Transfer the colonies to a sterile glass tube containing 5-6 ml sterile saline solution.
2. Homogenization of the suspended solution using (glass beads) and then put it in vortex for 2-3 minutes ensure that the tubes are properly closed.
3. Leave the tube for 10 minutes draw 2-4 ml the supernatant and transfer it to a tube sterile.
4. Adjust the turbidity of the suspension solution to match the standard McFarland.
5. The tubes were marked with the sample number and drug name in addition to the 1,2 control sample tubes (cultivars ordinary does not contain anti –tuberculosis)
6. Using an automatic pipette with a sterile tip 0.1 of the suspension was taken at 10^{2} the control sample no.1 and all tubes containing tuberculosis medicine.
7. 0.1 of the suspension was added at dilution of 10^{4} to control sample tubes no.2.
8. Incubate in 37 at angle.

Read the results (DST)

Microbes are considered resistant if the growth in the culture medium containing the antibiotic is diluted 10^{2} it is greater than the growth on the culture media that does not contain the antibiotic 10^{4} , The microbe is considered sensitive if the growth in the culture medium containing the antibiotic is diluted 10^{2} less growth on culture medium that does not contain , antibiotic in diluted 10^{4}

Table (1) Line probe assay (LPA)

Genotype MTBDRsl VER 2.0	Strips	Strips membrane (96)
	DEN	Denaturation solation (1-92 ml)
	HYB	Hybridization butter 96 ml
	STR	Stringent wash solution 96 ml
	RIN	Rise solation 288 ml
	CON - C	Conjugate concentrate 960 ml
	CON - D	Conjugate butter 96ml
	SUB - C	Substrate concentrate 960 ml
	SUB - D	Substrate concentrate 96ml
	AM-A	Amplification mix A 960 ml
	AM-B	Amplification mix B 3.36 ml

Table (2) Gene xpert kit (Cepheid)

Name	Contains
Sample reagent	Contains": sodium hydroxide (5-8%) and isopropyl alcohol (10-15%) 50% 8.0 ml"
Gene expert kit	Detection of my tuberculosis complex DNA and probe gene core mutation associated with rifampicin resistance (50 kit)

Statistical Analysis

(Sensitivity = a/a + b, Specificity = d/c + d) where a represents true positive, b represents false positive, c represents false negative, and d represents true negative (Parshall, 2013).

Discussion:

Globally, Tuberculosis has two types of infection: Latent tuberculosis: that the person is a carrier of the bacteria; Where the bacteria remain dormant inside the body without showing any symptoms on the person; due to the body's resistance to it. Also, the person is not contagious, and cannot spread the infection to others; But latent infection may turn into active disease.

Active tuberculosis: that the person has active bacteria; Where the person shows symptoms of infection, and the infection can be transmitted to others when accompanying the infected person for a long period of time. Symptoms also appear several weeks after infection, and may not appear until months or years later. DST accessibility is" limited in low- and middle-income countries by its high cost and complexity, as well as by technological restrictions (maintenance and environmental conditions). "Due to these problems, improper treatments may result in the moving in and development of highly Dr - Mtb strains (Van, et al., 2013؛ Schnettger , et al.,2017).

As shown in Tables 3, 4, and 5, and Figures 1 and 2, this study proves findings indicated that a total of 100 samples were collected, including 57 males and 43 females. 68 percent of patients came from metropolitan areas, while 32% came from rural areas. Patients ranged in age. That is typically between the ages of 30 and 39. (24 percent). Additionally, patients came from other provinces around Iraq. However, the majority of them were from Baghdad, the capital city. Patients had a disease stature of 68 percent that was freshly diagnosed. The participants in this research presented with a variety of signs and symptoms. For example, 82% of instances had a productive cough, while 75% of patients had weight loss and appetite reduction. The majority of symptoms were seen in both sensitive and resistant individuals; however, hemoptysis occurred in 65% of resistant patients but only 25% of sensitive patients. The patients in this research revealed a variety of radiological characteristics, some of which were unique to each group. Cavity lesions in the right apex, for example, were seen in three and thirteen instances of resistance and sensitivity, respectively. Similarly, infiltration occurred in the right mid-upper zone in 1 and 12 resistant and sensitive instances, respectively. Other radiological characteristics followed a similar trend (Ibrahim, et al.,2018).

Additionally, the mean age of all persons who exhibited resistance was 44.5. 82.4 percent of these patients were male, and 76.5 percent of resistant patients lived in cities. 47.1 percent of patients had MDR resistance, whereas only 5.9 percent had XDR resistance. Between the resistant and sensitive groups, there was no significant difference in age, clinical characteristics, or years after declaring cure (p value >0.05). However, several parameters vary between the two groups. For instance, each groups have distinct sex and case classifications (Day and Gray ,2017).

All culture - positive Mycobacterium tuberculosis isolates acquired with newly diagnosed PTB patients were subjected to a molecular drug susceptibility screening. Similarly, the results of our study were more than prior findings from various regions of the nation. Similarly, medication naive tuberculosis patients in East Africa have been shown to have a lower degree of Isoniazaid (INH) and RIF resistance. According to a published review and (WHO) programmatic management

of DR - TB guidelines, variation in the overall prevalence of DR - TB across study settings could be explained by sample size variation, ineffective TB case management difference diagnosis setup, and irregular anti-TB drug supply. One may argue that the time period of the research is crucial. Drug resistance is a result of clones that circulate around the world", some more effectively than others and fluctuating in frequency over time (Nasiri, et al.,2014).

Conclusion

The current study clearly indicated that TB infection is one of the most important health burdens. In all cases of this study the resistance were high prevalence.

Persons previously infected with Tuberculosis tend to have more drug resistance than newly diagnosed persons as 17 drug resistance cases were recorded in the study 13 of which were for person previously infected with Tuberculosis, Additionally, the mean age of all persons who exhibited resistance was 44.5. 82.4 percent of these patients were male, and 76.5 percent of resistant patients lived in cities, The majority of symptoms hemoptysis occurred in 65% of resistant patients

Table (3) Drug sensitivity tests findings among TB cases included in the study.

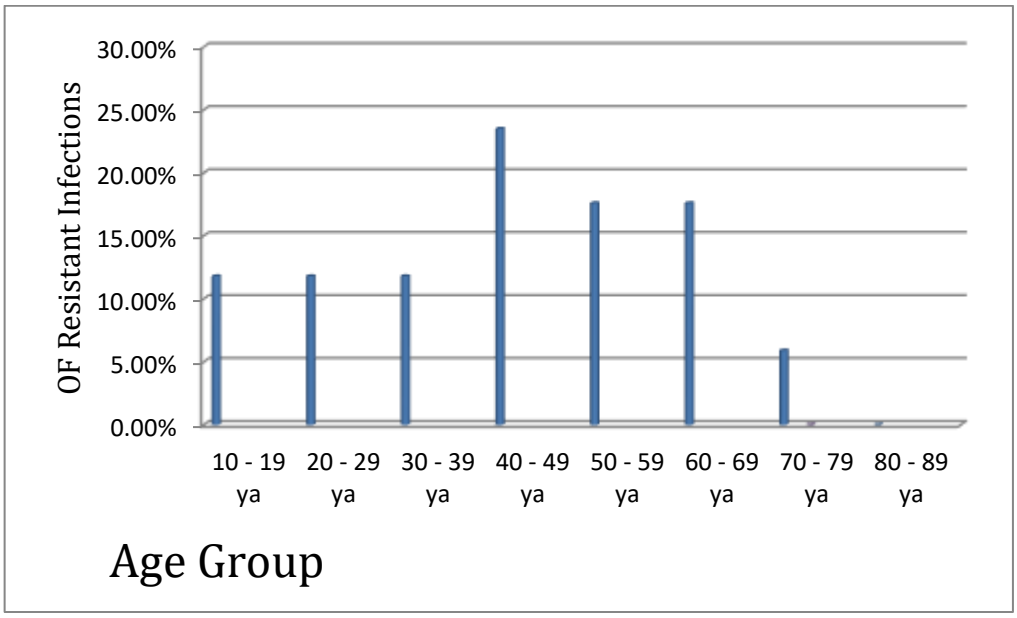
Test		**Resistant**		**Sensitive**	
		No.	%	No.	%
Gene expert for Rifampicin resistance (total 100)		13	13%	87	87%
DST (total 100)	Rifampicin	13	13%	87	87%
L.P.A (total 19)	Rifampicin	13	68.4%	6	31.6%
Final classification (total 100)		17	17%	83	83%

Table (4) Main characteristics of the TB cases included in the study revealed a resistance to anti TB drugs

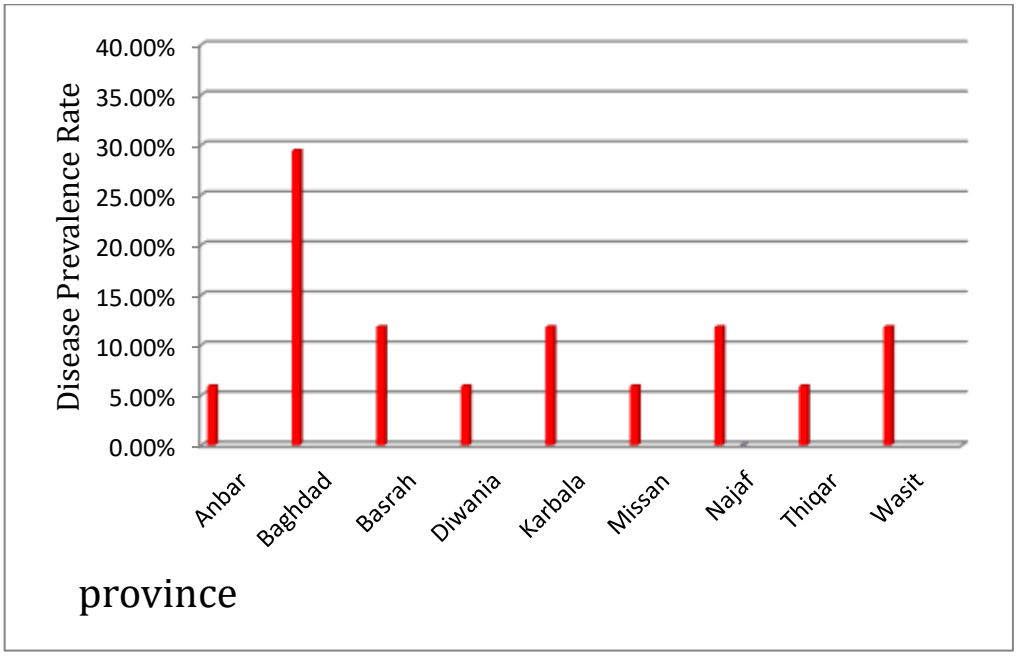
Characteristic	Total of 17	
	Mean (± SD)	
Age (year)	44.5(± 16.3)	
Sex	No.	%
Male	14	82.4%
Female	3	17.6%
Residency		
Urban	15	76.5%
Rural	4	23.5%
Case classification		
Newly diagnosed	3	17.6%
Retreatment	14	82.4%
Resistance type		
MDR	8	47.1%
RR	4	23.5%
Mono	3	17.6%

Table (5) The difference between means of specific characteristics and the outcome among the study group.

Characteristic		Outcome		P value
		Resistant	Sensitive	
Age (year)	Mean (± SD)	44.5 (±16.3)	42.5 (±16)	0.64
No. of clinical features	Mean (± SD)	4.71 (±1.4)	4.7 (±1.7)	0.98
No. of years since declaring cure (retreatment group)	Mean (± SD)	3.8 (±1.4)	4 (±1.45)	0.68



Figure(1) Most ages respond to standard drug rifampicin



Figure(2) Prevalence of TB disease by geographical location.

References:

1. Chakraborty S, Rhee KY.(2015), Tuberculosis Drug Development: History and Evolution of the Mechanism-Based Paradigm. Cold Spring Harb Perspect Med. 2015 Apr 15;5(8):a021147.
2. Deb C, Lee C-M, Dubey VS, Daniel J, Abomoelak B, Sirakova TD, et al. A novel in vitro multiple-stress dormancy model for Mycobacterium tuberculosis generates a lipid-loaded, drug-tolerant, dormant pathogen. PloS one. 2009;4(6):e6077.
3. Mishra NC, Singh K. Comparative computational analysis of Mycobacterium species by using different techniques in study. tuberculosis. 2012;5.
4. Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance inMycobacterium tuberculosis: 1998 update. Tubercle and Lung disease. 1998;79(1):3-29.
5. Müller B, Borrell S, Rose G, Gagneux S. The heterogeneous evolution of multidrug-resistant Mycobacterium tuberculosis. Trends in Genetics. 2013;29(3):160-9.
6. Singh KK, Muralidhar M, Kumar A, Chattopadhyaya T, Kapila K, Singh MK, et al. Comparison of in house polymerase chain reaction with conventional techniques for the detection of Mycobacterium tuberculosis DNA in granulomatous lymphadenopathy. Journal of clinical pathology. 2000;53(5):355- 61.

7. Ekka, B., Das, G., Aarif, M., & Alalmai, A. (2023). Unveiling the Significance of Sustainability in Tourism: Environmental Conservation, Socioeconomic Development, and Destination Resilience. *Rivista Italiana di Filosofia Analitica Junior*, *14*(1), 918-933.
8. Wang F, Sambandan D, Halder R, Wang J, Batt SM, Weinrick B, et al. Identification of a small molecule with activity against drug-resistant and persistent tuberculosis. Proceedings of the National Academy of Sciences. 2013;110(27):E2510-E7.
9. Mekonnen F, Tessema B, Moges F, Gelaw A, Eshetie S, Kumera G. Multidrug resistant tuberculosis: prevalence and risk factors in districts of metema and west armachiho, Northwest Ethiopia. BMC infectious diseases. 2015;15(1):1-6.
10. Al-Safi, J. K. S., Bansal, A., Aarif, M., Almahairah, M. S. Z., Manoharan, G., & Alotoum, F. J. (2023, January). Assessment Based On IoT For Efficient Information Surveillance Regarding Harmful Strikes Upon Financial Collection. In *2023 International Conference on Computer Communication and Informatics (ICCCI)* (pp. 1-5). IEEE.
11. Khairallah A.S. Mohammed,corresponding author* 1 Ghorob S. Khudhair, 1 and Dheyaa Bekheet Al-Rabeai,(2022), Prevalence and Drug Resistance Pattern of Mycobacterium Tuberculosis Isolated from Tuberculosis Patients in Basra, Iraq, Pol J Microbiol. 2022 Jun; 71(2): 205–215 May 31. doi: 10.33073/pjm-2022-018.
12. WHO (2017a). Frequently Asked Questions about the WHO Technical Expert Consultation Findings on Xpert®MTB / RIF Ultra. Available at: http://www.who.int/tb/areas-of-ork/laboratory/diagnostics/XpertUltraFAQs.pdf
13. Van Deun A, Aung KJ, Bola V, Lebeke R, Hossain MA, de Rijk WB, et al.(2013), Rifampin drug resistance tests for tuberculosis: challenging the gold standard. Journal of clinical microbiology. 2013;51(8):2633-40.
14. M. A. Tripathi, R. Tripathi, F. Effendy, G. Manoharan, M. John Paul and M. Aarif, "An In-Depth Analysis of the Role That ML and Big Data Play in Driving Digital Marketing's Paradigm Shift," 2023 International Conference on Computer Communication and Informatics (ICCCI), Coimbatore, India, 2023, pp. 1-6, doi: 10.1109/ICCCI56745.2023.10128357.
15. Schnettger L, Rodgers A, Repnik U, Lai RP, Pei G, Verdoes M, et al. A Rab20-dependent membrane trafficking pathway controls M. tuberculosis replication by regulating phagosome spaciousness and integrity. Cell host & 66 microbe. 2017;21(5):619-28. e5.
16. Deb C, Lee C-M, Dubey VS, Daniel J, Abomoelak B, Sirakova TD, et al.(2009), A novel in vitro multiple-stress dormancy model for Mycobacterium tuberculosis generates a lipid-loaded, drug-tolerant, dormant pathogen. PloS one;4(6):e6077.
17. Alnamlan, A. A. A. (2023). A Crucial Impact on the Crash of Corporate Governance on Corporate Sustainable Growth. *resmilitaris*, *13*(3), 1355-1364.
18. Mishra NC and Singh K.(2012), Comparative computational analysis of Mycobacterium species by using different techniques in study. tuberculosis. 2012;5.
    Muller B, Borrell S, Rose G, Gagneux S.(2013). The heterogeneous evolution of multidrug-resistant Mycobacterium tuberculosis. Trends in Genetics. 2013;29(3):160-9 .
19. Miller CH. Seleno-amino acids(2013) A novel class of anti-tuberculosis compounds identified through modified culture screening conditions. 2013.
20. N. Madhumithaa, G. Manikandan, S. Kalaivany, R. Selvameena, P. K. Patjoshi and M. Aarif, "Stochastic Sequential Development of Supply Chain Management System and Performance Index in Dynamic Environment," 2022 5th International Conference on Contemporary Computing and Informatics (IC3I), Uttar Pradesh, India, 2022, pp. 2039-2043, doi: 10.1109/IC3I56241.2022.10072721.
21. Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, et al.(2007), A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technology Assessment (Winchester, England).;11(3):1- 196.
22. Mekonnen F, Tessema B, Moges F, Gelaw A, Eshetie S, Kumera G. Multidrug resistant tuberculosis: (2015), prevalence and risk factors in districts of metema and west armachiho, Northwest Ethiopia. BMC infectious diseases. 2015;15(1):1- 6.
23. Ely JW, Osheroff JA, Ebell MH, Chambliss ML, Vinson DC, Stevermer JJ, et al.(2002), Obstacles to answering doctors' questions about patient care with evidence: qualitative study. Bmj. 2002;324(7339):710.
24. Ayyash S.(2016), Fast and Inexpensive Detection of Bacterial Viability and Drug Susceptibility Through Metabolic Monitoring 2016.
25. Parshall MB.(2013) Unpacking the 2× 2 table. Heart & Lung. 2013;42(3):221-6.
26. Ibrahim IR, Palaian S, Ibrahim MI.(2018), Assessment of diarrhea treatment and counseling in community pharmacies in Baghdad, Iraq: A simulated patient study. Pharmacy Practice (Granada). 2018;16(4).
27. Day C. and Gray A.(2017), Health and related indicators. South African health review.;2017(1):217-339.
28. Nasiri MJ, Rezaei F, Zamani S, Darban-Sarokhalil D, Fooladi AAI, Shojaei H, et al.(2014), Drug resistance pattern of Mycobacterium tuberculosis isolates from patients of five provinces of Iran. Asian Pacific journal of tropical medicine. 2014;7(3):193-6.
29. Cantres-Fonseca, O. J., Rodriguez-Cintrón, W., Olmo-arroyo, F. D& ,.
30. Baez-Corujo, S. (2019). Extra Pulmonary Tuberculosis: An Overview. Role of Microbes in Human Health and Diseases, 1-16.
31. Koch, A., & Mizrahi, V. (2018). Mycobacterium tuberculosis. Trends in microbiology, 26(6), 555-556.
32. Singh A., Prasad R., Balasubramanian V., Gupta N. Drug-resistant tuberculosis and hiv infection: Current

perspectives. HIV/AIDS—Res. Palliat. Care. 2020; 12:9–31. doi: 10.2147/HIV.S193059.