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A Study on Drug Resistance of *Mycobacterium tuberculosis* Isolated from a Pulmonary Iraqi Patient

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ABSTRACT

Background: Tuberculosis (TB) is a contagious and infectious disease caused by *Mycobacterium tuberculosis* (MTB) and its remaining parts, one of the major contagious bacterial diseases around the world. There is an increasing rate of the drug resistance tuberculosis strain toward the drugs for curing of tuberculosis in large sector of risk groups, the problems of emergency multi-drug resistant TB (MDR) is globally in the world. **Aims of present Study:** To carry out Isolation, cultivation and identification of *Mycobacterium Tuberculosis* by culture on (LJ AND Bactec MGIT 960) and GeneXpert MTB-RIF system. To perform Comparative study of drug resistance using LJ media and Bactec MGIT 960 and GeneXpert MTB/RIF specials (rifampicin resistance). Comparison between routine diagnostic methods (culture and GeneXpert) for pulmonary AFB positive sample and Attempts to classify drug resistance to (mono-resistance, poly Resistance and multiple drug resistance) from referred cases of TB and newly diagnosed cases. **Methods:** Seventy five (75) specimens were examined, patients were classified into two main groups: selected MDR patients, which were referred from different hospitals and health, respiratory center in Iraq represented 46 (61.3%) in order to compare different diagnostic methods for identification of resistance isolates, while 29 (38.7%) were randomly isolated and including in this study, 43 (57.3%) were males and 32 (42.7%) were females, with age range between 16- 80 years and the mean age of patients was 49.1 years. All patients were examined by conventional methods by classical solid culture (LJ) media and molecular or advance methods by Gene-Xpert MTB-RIF test and liquid culture (Bactec MGIT 960) to diagnosis of MTB and drug susceptibility testing (DST). **Results:** Different advanced and classical methods were used in this study to compare the pattern of resistance to the first line anti-TB drugs including Rifampicin, INH, Streptomycin and Ethambutol. The resistance patterns showed almost similar rate of resistance to primary drugs INH and Rifampicin by all methods, while the resistance to secondary drugs (Streptomycin and Ethambutol) were significantly higher between solid media and Bactec MGIT 960 methods. **Conclusion:** Culture on solid media (Lowenstein-Jensen media) is golden method to detect of MTB and drug-resistant tuberculosis. Advanced methods (GeneXpert and Bactec MGIT 960) are highly specific and accurate to determine of multi-drug resistance (MDR).The Gene-Xpert MTB/ RIF system is a genetic method that required few hours to identify MTB and also can detect Rifampicin resistance.

INTRODUCTION

Tuberculosis primarily focused in the lung, is called pulmonary Tuberculosis, additionally spread to different parts of the body, which is called extra-pulmonary TB, for example, organs in the nervous, lymphatic, and the circulating blood system among others **[1].** There is an increasing rate of the drug resistance tuberculosis strain toward the drugs for curing of tuberculosis in large sectors of risk groups, the problems of emergency multi-drug resistant TB (MDR) is globally rising in the world **[2].** The drug susceptibility testing (DST) in the tuberculosis laboratory is important for estimation and detected of drug resistance tuberculosis, the classical methods by solid media (Lowenstein-Jensen medium) are remained as a gold stander to estimation of DST, but these methods required long time (weeks to months), the several new laboratory techniques have been developed by world health organization (WHO) for faster detection of drug resistance tuberculosis. These new laboratory methods that are classified into phenotypic methods (liquid culture) such as by Bactec MGIT 960 and genotypic methods (molecular PCR technique) by Gene Xpert system and Line probe assay. Among the new laboratory methods, DST for first-line drug anti-TB is more accurate for Isoniazid (INH) and Rifampicin (RPM) and less reliable and sensitive to Ethambutol (EMB) and Streptomycin (STM) **[3]**.

PATIENTS AND METHODS

Sputum samples

Early morning 5 ml sputum samples were collected from patient by a clean, straightforward and non-delicate or mixture plastic holders with a tight top and a wide spout. All sputum samples were transported to microbiology laboratory examinations that include direct examination (AFB), classical culture (LJ), Advance Liquid culture (Bactec MGIT 960) system and GeneXpert MTB-RIF test.

Safety and disinfection

All microbiological tests were done on the Biosafety class II cabinet as represented in informative supplement E. Gloves and mask (N 95) were utilized amid the research facility work, Autoclave was utilized at 121°C at 1bar for 20-30 minutes for disinfecting all society, the media and Oven were utilized to clean and sterile all glass products at 200°C for 2 hours **[4].** Phenol 5% and ethanol 70% were utilized to clean and purify the seats and hood **[5].**

Classical Laboratory methods

1- Direct sputum examination (AFB)

Principle

Ziehl–Neelsen stain is a microbiological stain used to identify and detect acid-fast organisms, mainly *Mycobacteria*. Acid fast organisms like *Mycobacterium tuberculosis* contain high amounts of lipid substances within their cell walls. These acids resist staining by other methods such as a Gram stain **[6].**

Sampling Procedure of (AFB)

1. Samples were smeared onto slide.
2. The Slide smear was dried for 10 min. at 60°C, and heat-fixed for 10 min. at 90°C, and then flooded with carbol fuchsin.
3. A flame was held beneath the slide until steam appears but didn't allow it to boil.
4. The hot slide was allowed to sit for 3 to 5 min. and was rinsed with tap water.
5. A slide was flooded with 25% sulfuric acid.
6. The slide was allowed to sit 1min. and was rinsed with tap water, then flooded with methylene blue stain.
7. The slide was allowed to sit 1 min. and rinsed with tap water
8. The slide was allowed to dry.
9. Slide was examined under oil immersion lens **[7].**

2-Preparation of Lowenstein –Jensen medium (L.J) solid media:

Principle

A wide range of solid media is accessible for growth of *Mycobacterium tuberculosis*. Most are varieties of egg-potato base or egg white agar base media. There is no broad agreement on which medium is best for isolation of MTB

Procedure of Pulmonary sample cultivation on solid media (LJ)

The sample cultivation was performed according to Petroff's decontamination method **[8].**

Preparation of Petroff's solutions

1. **Phenol red (1%) indicator**

By adding 1g of phenol red to 100 ml of sterile distilled water.

2. NaOH (4%) solution

By adding 4 g of sodium hydroxide (NaOH) to 100 ml of sterile distilled water.

3. Neutralizing solution

Prepared by adding 72 ml of HCl (73%) to 1 ml of phenol red (1%) and the volume made up to 1L with sterile distilled water**.**

Procedure of sputum sampling upon (LJ) solid media

- The sputum sample was transferred to cap centrifuge tube
- NaOH buffer (4%) was added to volume equivalent the amount of sputum sample and left for 15 minutes to less of liquefaction sampling.
- The sample was centrifuged at 3000 RPM speed for 15 minutes, refrigerated centrifugation used to utilize of increase recuperation of Mycobacterium .
- The supernatant was emptied precisely into a reasonable compartment containing a mycobactericidal disinfectant
- Neutralizing buffer was added by Pasteur pipette until the shading is yellow (balance point) or (neutralizing point)
- Two vials of L.J medium used and added about 2-3 drops of the pellet and incubated in slant position and in semi-closed vial for 3 days at 37°C
- After this time period, the vials were tightly closed and incubated vertically at 37°C for 6 weeks
- Then, the outcomes were recorded as positive or negative.
- Positive result means development and growth of *Mycobacterium tuberculosis* in (LJ) media.

Turbidity methods by (McFarland)

- Preparation : Buffer 1 : added 0.1 ml $BaCl_2$ to 9.9 ml H_2SO_4
- Buffer 2 : added 0.05 ml $BaCl_2$ to 9.95 ml H_2SO_4

Drug susceptibility testing (DST) for MTB

The (DST) was tested by two methods

- Upon (LJ) solid media-1
- Upon 7H9 middle brook (Bactec MGIT 960) liquid media

Drug susceptibility testing (DST) for MTB upon solid media (LJ)

There were many methods for estimation of (DST) in the laboratory tuberculosis center. The proportional methods are used in this study to detect of drug susceptibility testing upon solid media (LJ). The Lowenstein- Jensen medium was prepared and added to 4 sterile volumetric flasks (200 ml for each) and mixed with different volume antibiotic as shown in table (1).

Table (1) the volume and concentration of anti-tuberculosis drug in LJ media (SIRE)

Antibiotic	Abbreviation	Conc.in LJ Media	Conc. of stock solution Insolvent	Preparated of antibiotic dilution	Amount of 200 ml LJ preparation
Rifampicin	RMP	40	100 µg/ml Ethyl Glycol	20 µg/ml	2 ml of dilution
Isoniazid	INH	0.2	1000 µg/ml D. W	20 µg/ml	0.4 ml of dilution
streptomycin	SM	4	2000 µg/ml D. W	4000 µg/ml	1.6 ml of dilution
Ethambutol	EMP	2	1000 µg/ml D. W	20 µg/ml	4 ml of dilution

The mixture of media was added to a screw-cap bottle for slant position and dispensed at 85^0C for 45 minutes **[9].**

Mycobacterium suspension was prepared by taking the active growth colony (less than 1 month old) upon (LJ) media by bacteriology loops in 1 ml of sterile distilled water. The suspension was adjusted in turbidity to 0.5 McFarland standard preparation of five tube serial dilution (10-1 – 10-5). Inoculated (LJ) media with and without antibiotic from (10-1 -10-3 – 10-5) diluted only and were discarded the tube dilution (10-2 and 10-4). The incubated (LJ) media kept at 37^0C for 3-4 weeks **[9]**.

Drug susceptibility test (DST) by Bactec MGIT 960 system

Principle

The (MGIT) tube contains 7 ml of Middle brook 7H9 stock. The complete medium, with dextrose, Oleic acid, catalase and albumin (ODAC) improvement and PANTA (Nalidixic Acid, Amphotericin B, Polymyxin B, Azlocillin and Trimethoprim) antibiotic mix, this component is usually utilized liquid media for the growth of *Mycobacterium tuberculosis* (MTB). The fluorescent compound dissolved, highly sensitive to oxygen in the broth. At first, the high measure of the dissolved amount of oxygen extinguishers discharge of the compound and little fluorescence can be identified. MTB microorganisms receive the oxygen and the fluorescence to be identified. Bactec MGIT 960 is automated device that adventures the fluorescence of an oxygen sensor to distinguished development of *Mycobacterium tuberculosis* in culture and incubated at 37°C. The Bactec MGIT 960 system scans, the MGIT tube every 60 minutes for expanded fluorescence. Examination of the fluorescence is utilized to if MGIT tube is a positive result **[10].**

3-3-3-1 Bactec MGIT 960 system consist of following

1 Mycobacteria Growth Indicator Tube ((MGIT)

Contains 7.0 ml Middle brook 7H9 broth and 0.1 ml fluorescent indicator. The fluorescent indicator composed of 7-diphenyl-1, Tris 4 and 1 phenanthroline ruthenium chloride pentahydrate, in silicon rubber base.

2 PANTA vial antimicrobial agent (lyophilized vial)

Approximate formula/vial lyophilized

Polymyxin B	6000 unit
Trimethoprim	600 ug
Amphotericin	600 ug
Nalidixic acid	2400 ug
Azlocillin	600 ug

3- ODAC (Bactec growth supplement) contain 15 ml of middle brook (Oleic acid, Albumin, Dextrose, Catalase) enrichment

Approximate formula/liter water

Oleic acid	0.1 ml
Dextrose	20 gm
Bovine albumin	50 gm
Catalase	0.03 gm
Polyoxyethylene stearate	1.1 gm

The PANTA antibiotic vial was reconstituted with ODAC MGIT supplement and then 0.8 ml of mix was used.

Drug susceptibility testing (DST) upon (SIRE) kit in Bactec 960 system

1- Name and number of five 7 ml MGIT tubes to including GC (Growth Control), STR (Streptomycin), INH (Isoniazid), RIF Rifampicin), EMB Ethambutol.

2- Tubes were placed in the accompanying arrangement in the 5 tube from left to right: GC, STR, INH, RIF, and EMB.

3- 0.8 ml of the BACTEC MGIT SIRE Supplement was added to each SIRE tube and growth control tube.

4- 100 µl of the each drug solution was pipetted into the MGIT tube; e.g., include 100 µl of the 83 µg/ml MGIT (RMP) with except the growth control tube.

5-Loop having full active Mycobacterium growth colony was taken to mix with sterile normal saline in glass tube and mix well.

6- Suspension turbidity was measured using a 1 McFarland standard (9.9 ml H_2SO_4 + 0.1 $BaCl_2$) and waiting for 15 minutes. After this time suspension was transferred to another glass tube to measure the turbidity from a 2 McFarland standard (9.95 ml H_2SO_4 + 0.05 $BaCl_2$) and left also 15 minutes.

7- 1 ml suspension was added to the tube containing 4 ml normal saline to make 1:5 dilution (for used to drug SIRE tube) and 0.1 ml from same suspension was added to 9.9 ml normal saline in another glass tube to make 1:10 dilution (for used in growth control tube)

8- 500 µl (dilution 1:5) transfer was added to each MGIT tube, which contains drug (SIRE), and 500 µl (dilution 1:10) was added to growth control tube .

9- All tube were incubated in automated Bactec MGIT 960 system and the results appear through report after 7-21 days **(NTP, 2011).**

Table 2. Concentration of MGIT 960 SIRE drug Kit and volumes added to MGIT tube

Drug	Concentration of drug	Lyophilized drug vial	Volume added to MGIT tube for test	Final concentration in MGIT tube
MGIT/R	8.3 µg/ml	332 µg	100 µl	1.0 µg/ml
MGIT/S	8.3 µg/ml	332 µg	100 µl	1.0 µg/ml
MGIT/E	415 µg/ml	1660 µg	100 µl	5.0 µg/ml
MGIT/I	8.3 µg/ml	33.2 µg	100 µl	0.1 µg/ml

The molecular detection by using Gene-Xpert system MTB/RIF (Cepheid, USA).

Principle

The Gene Xpert System integrates, automates specimen processing, nucleic acid amplification, and detection of the target sequences in specimens using real-time PCR and reverse transcriptase PCR. The system consists of an instrument, barcode scanner, computer, and preloaded software for running tests and viewing the results.

The systemic use and requires single disposable GeneXpert cartridges that hold the reagents and host of the PCR process.

The primers in the gene Xpert system have amplified a portion of the *rpoB* gene containing the 81 base pair “core” region. The probes are can able to differentiate between the conserved wild-type nucleic acid sequence and mutations in the core region that are associated with RIF's detection **[11-12].**

Reagents of GeneXpert MTB-RIF

Table 3. Reagents of GeneXpert (Cephid) system

Reagents	Quantities
Sample reagent (sodium hydroxide and isopropanol)	10 x 8 ml bottles
Tris Buffer, EDTA	3 ml per cartridge

Procedure of GeneXpert MTB/RIF taken from solid media

1-Equal volumes of sputum sample and 4% sodium hydroxide were mixed, shaken and incubated at room temperature for 15 minutes, the mixture was shaken regularly by a mechanical shaker every 5 minutes.

2-Then specimen was centrifuged in a sterile tube by cooling centrifuge at high speed (> 13 000 rpm), for 15 minutes.

3-The supernatant was discarded and the sediment immediately neutralized by adding, drop by drop of 37% HCl containing 20 ml of phenol red solution per liter (L) until the mixture loses pink color.

4-GeneXpert MTB/RIF cartridge was labeled with the sample number.

5-Half ml of resuspension was transferred to tube for the Xpert MTB/RIF by using a sterile pipette.

6- 1.5 ml of Xpert MTB/RIF Sample reagent was added to 0.5 ml of resuspended sediment sample and was shaken vigorously 10–20 times.

7- The Specimen was incubated for 15 min. at room temperature.

8-Two ml of sputum was transferred into the GeneXpert MTB/RIF cartridge, and then cover was closed.

9-The instrument door was opened with the blinking green light and the cartridge was loaded.

10-GeneXpert DX instrument was turned on.

11-The door was closed, the test was started and the green light was stopped blinking.

12-GeneXpert DX instrument has released the door after 2 hours and the results were read.

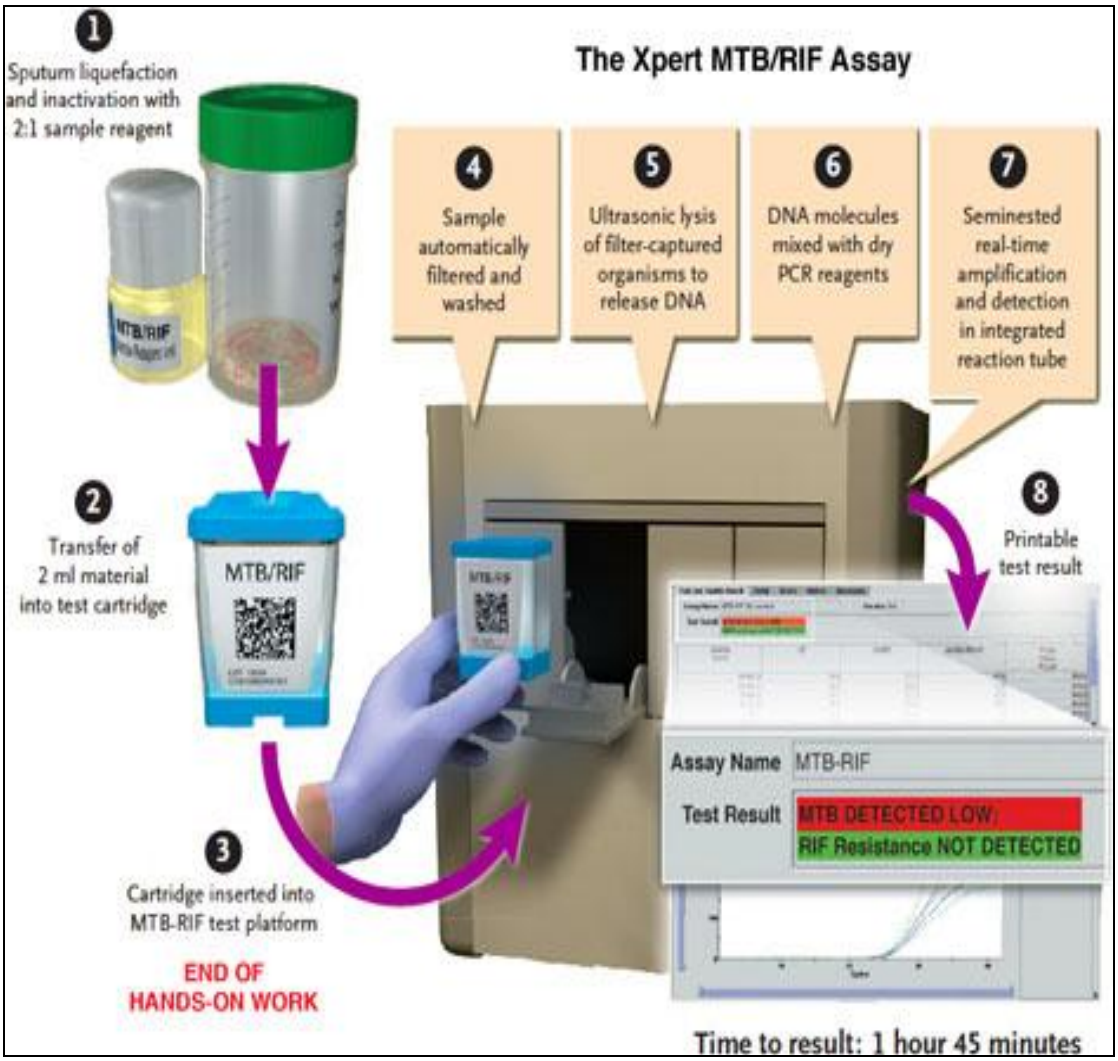

Figure 1. Gene-Xpert MTB/RIF Assay [13]

Interpretation of results of GeneXpert MTB-RIF assay

1- MTB detected: detected of DNA MTB.

2-RMP resistance detected: a mutation in the *rpo* B gene has been detected.

3-RMP resistance not detected: no mutation in the *rpo* B gene has been detected.

Statistical Analysis:

The Database was first collected with excel file then transported into SPSS file (statistical package for social sciences v20) for data analysis.

Continuous variables presented as means with standard deviation and discrete variables presented as numbers and percentages as appropriate.

Chi-square test for independence used (in SPSS format) to test the significance of the association between discrete variables. Findings with P value less than 0.05 were considered significant.

Epicalc 2000 statistical package was used for further analysis of test validities yielding validity indicators with their 95% confidence intervals (95% CI).

RESULTS

Seventy five patients were included in this study, patients were grouped according to age and gender, were grouped to: (15-45), (46-65) years and above 65 years old, (41.3%) of patients were in the age group (46-65) years, (37.3%) of age group (15-45) years and (21.3 %) were above 65 years old. The mean age of patients was 49.1 years and the standard deviation was 16.2 years old. According to gender, 43 (57.3%) were males and 32 (42.7%) females. Patients were classified into two main groups: selected MDR patients, which were referred from different hospitals and health, respiratory center in Iraq represented 46 (61.3%) in order to compare different diagnostic methods for identification of resistance isolates, while 29 (38.7%) were randomly isolated.

Table 4. Grouping of patients according to age, gender and referred sample:

Variables		N(75)	100%
Age Group	• 15-45 y	28	37.3%
	• 46-65 y	31	41.3%
	• > 65 y	16	21.3%
Sex	• Male	43	57.3%
	• Female	32	42.7%
Referred Study Samples	• MDR	46	61.3 %
	• Random	29	38.7 %

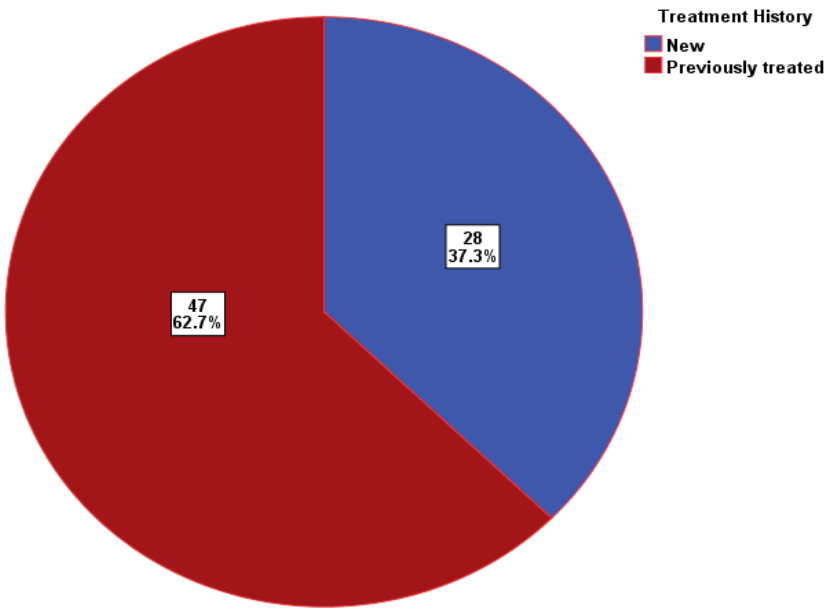

Figure 2. Demonstration of the Distribution of patient groups according to treatment history

Moreover, as the resistance patterns showed almost similar rate of resistance to primary drugs INH and Rifampicin by both methods, while the resistance to secondary drugs (Streptomycin and Ethambutol) were significantly higher by solid media methods as shown in table 5.

Table 5. Pattern of drug susceptibility testing (DST) using different Laboratory methods

Method of testing drug susceptibility	Drug	Resistant		Sensitive	
		N	%	N	%
Solid Media	Streptomycin	42	56.0%	33	44.0%
	INH	41	54.7%	34	45.3%
	Rifampicin	47	62.7%	28	37.3%
	Ethambutol	38	50.7%	37	49.3%
GeneXpert	Rifampicin	48	64.0%	27	36.0%
Bactec	Streptomycin	9	12.0%	66	88.0%
	INH	44	58.7%	31	41.3%
	Rifampicin	45	60.0%	30	40.0%
	Ethambutol	6	8.0%	69	92.0%

Table 6. Shows the drug resistance to anti-TB drugs as obtained in this study, the highest MDR for 4 drugs (SIRE) was found to be 18 (24.0%) cases, while resistance to 3 drugs was ranging between (4.0%-10.7 %). Resistance to 2 drugs was found to be (1.3% - 9.3%), while resistance to single anti-TB drug on solid media technique was ranging between (2.7% - 10.7%).

Table 6. Anti-TB drug resistance using the solid media method

Drug	N	%
SIRE	18	24.0%
IRE	8	10.7%
S	8	10.7%
R	7	9.3%
SE	7	9.3%
IR	6	8.0%
SIR	4	5.3%
I	3	4.0%
SRE	3	4.0%
SI	2	2.7%
E	2	2.7%
RE	1	1.3%
Susceptible to all	6	8.0%

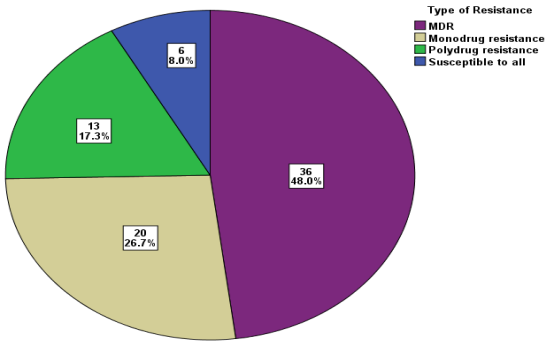

Figure 3. Distribution of isolates according to observed type of drug resistance on solid media

Table 7. Distribution of sampled patients according to streptomycin susceptibility, personal characteristics and another drug susceptibility on solid media

	Streptomycin Susceptibility				
	Resistant		Sensitive		
Variables	**N(42)**	**100%**	**N(33)**	**100%**	**P value**
Age Group					0.113
15-45 y	19	45.2%	9	27.3%	
46-65 y	13	31.0%	18	54.5%	
> 65 y	10	23.8%	6	18.2%	
Sex					0.970
Male	24	57.1%	19	57.6%	
Female	18	42.9%	14	42.4%	
INH Susceptibility					0.627
Resistant	24	57.1%	17	51.5%	
Susceptible	18	42.9%	16	48.5%	
RifampicinSusceptibility					0.526
Resistant	25	59.5%	22	66.7%	
Susceptible	17	40.5%	11	33.3%	
Ethambutol Susceptibility					**0.008**
Resistant	27	64.3%	11	33.3%	
Susceptible	15	35.7%	22	66.7%	

***The Streptomycin drug resistance was associated only with Ethambutol drug resistance (P < 0.05). While the other drug were not significantly associated**

Table 8. Depending upon solid media, distribution of sampled patients, according to INH susceptibility, personal characteristics and another drug susceptibility:

	INH Susceptibility				
	Resistant		Sensitive		
Variables	**N(41)**	**100%**	**N(34)**	**100%**	**P value**
Age Group					0.169
15-45 y	13	31.7%	15	44.1%	
46-65 y	16	39.0%	15	44.1%	
> 65 y	12	29.3%	4	11.8%	
Sex					0.812
Male	23	56.1%	20	58.8%	
Female	18	43.9%	14	41.2%	
Streptomycin Susceptibility					0.627
Resistant	24	58.5%	18	52.9%	
Susceptible	17	41.5%	16	47.1%	
Rifampicin Susceptibility					**<0.001**
Resistant	36	87.8%	11	32.4%	
Susceptible	5	12.2%	23	67.6%	
Ethambutol Susceptibility					**0.015**
Resistant	26	63.4%	12	35.3%	
Susceptible	15	36.6%	22	64.7%	

***The INH drug resistance is significantly associated with Rifampicin resistance and Ethambutol resistance (P < 0.05) and the other drug did not show a significant association**

Table 9. Depending upon solid media, distribution of sampled patients, according to Rifampicin susceptibility, personal characteristics and another drug susceptibility

	Rifampicin Susceptibility				
	Resistant		Sensitive		
Variables	**N(41)**	**100%**	**N(34)**	**100%**	**P value**
Age Group					0.453
15-45 y	15	31.9%	13	46.4%	
46-65 y	21	44.7%	10	35.7%	
> 65 y	11	23.4%	5	17.9%	
Sex					0.648
Male	26	55.3%	17	60.7%	
Female	21	44.7%	11	39.3%	
Streptomycin Susceptibility					0.526
Resistant	25	53.2%	17	60.7%	
Susceptible	22	46.8%	11	39.3%	
INH Susceptibility					**<0.001**
Resistant	36	76.6%	5	17.9%	
Susceptible	11	23.4%	23	82.1%	
Ethambutol Susceptibility					**0.013**
Resistant	29	61.7%	9	32.1%	
Susceptible	18	38.3%	19	67.9%	

***The Rifampicin drug resistance significantly associated with INH resistance and Ethambutol resistance (P<0.05) but not with age, sex or streptomycin resistance.**

Table 10. Depending upon solid media, distribution of sampled patients, according to Ethambutol susceptibility, personal characteristics and another drug susceptibility

	Ethambutol Susceptibility				
	Resistant		Sensitive		
Variables	**N(41)**	**100%**	**N(34)**	**100%**	**P value**
Age Group					0.657
15-45 y	16	42.1%	12	32.4%	
46-65 y	15	39.5%	16	43.2%	
> 65 y	7	18.4%	9	24.3%	
Sex					0.133
Male	25	65.8%	18	48.6%	
Female	13	34.2%	19	51.4%	
Streptomycin Susceptibility					**0.008**
Resistant	27	71.1%	15	40.5%	
Susceptible	11	28.9%	22	59.5%	
INH Susceptibility					**0.015**
Resistant	26	68.4%	15	40.5%	
Susceptible	12	31.6%	22	59.5%	
Rifampicin Susceptibility					**0.013**
Resistant	29	76.3%	18	48.6%	
Susceptible	9	23.7%	19	51.4%	

***Ethambutol drug resistance significantly associated with resistance to each of streptomycin, INH and Rifampicin (P < 0.05) but not with any of age and sex.**

Figure (4) show the comparison of result obtained between the three laboratory methods to estimation of DST especially in Rifampicin detected

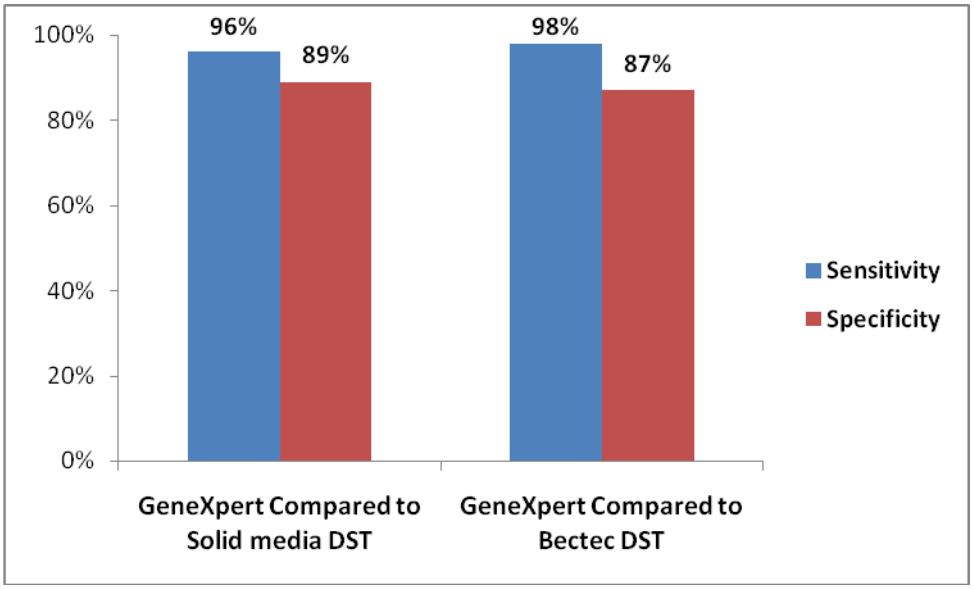

Figure 4. DST Validity of GeneXpert for testing RIF susceptibility compared to solid media & Bactec MGIT 960

***The result of GeneXpert as compared with Bactec MGIT 960 was with high sensitivity (98%) and specificity (87%). While the GeneXpert, when compared with solid media, showed high sensitivity (96%) and specificity (89%).**

DISCUSSION

The World Health Organization (WHO) applied the programs to control and elimination of multiple drug resistance tuberculosis, its works by the National TB control programs (NTP) in many countries of the worldwide **[14].** The quality assurance and quality control are very important and a comprehensive for estimation of false or error in the laboratory of tuberculosis center. The error estimation in the identification of MTB and detected by drug susceptibility testing (DST) through the classical method (solid media) and rapid molecular technique (GeneXpert and Bactec MGIT 960 system). The program of quality control of tuberculosis that is recommended by WHO for the detection of accuracy and error of laboratory worker and also materials used in laboratories of tuberculosis **[15-16].** In table (4), shows the age distribution of pulmonary TB patients, it is observed that the highest common age group infected with MTB between (45-65) years old with a total percentage (41.3%) and the lowest percentage of patients were presented in age group (>65) years old with a total

percentage (21.3 %). For all samples collected and selected. These results that agreed with those results obtained by **[17]** from Romania, [**18]** from Libya, and also agreed with the local study of **[19-20]** and **[21]** from Baghdad, who reported that young people at higher risk for received tuberculosis.

Due to the involvement of young people in increase the habit of smoking, alcohol, air pollution, dangerous jobs and lack of awareness. The smoke exposure is a common risk factor to increase the chance of acquiring tuberculosis **[22]** through smoking the phagocytosis activity in the alveolar macrophage is reduced, that lead to decreasing of immune response (lymphopenia) due to nicotine of cigarettes **[23]**. In alcohol cases, is considered a strong risk factor for tuberculosis **[24]**. The reasons for increasing acquiring TB including to alteration in immune response especially in production of cytokine when exposure to MTB **[25].** In cases of air pollution, the large particulate such as carbon monoxide, formaldehyde and nitrogen oxide that can deposit in the alveolar of the lung that leading to receiving TB **[26]**. The findings of the present study showed that the males gender were more to have pulmonary tuberculosis than females that taken from all sample collected, these results that agreed with many studies reported that the males gender are higher than females to have pulmonary tuberculosis **[17]** from Romania, but the results were not in agreement with **[27]** from India that reported the females is the higher risk group to received TB than males due to the same explanations above. In figure (2) the samples collected is presented as higher 47 (62.7%) MDR-TB referring cases than 28 (37.3%) random cases. The most previously treated (MDR) cases that referred from hospital and respiratory heath center in Baghdad and other regions of Iraq to the National Reference Laboratory (NRL) tuberculosis – Baghdad was added in this study. In table (5) shows, the drug susceptibility testing (DST) for first-line anti-TB as obtained by using solid media, Bactec MGIT 960 and GeneXpert system was compared. The comparative DST investigation between solid media and Bactec MGIT 960 for all drugs of the first line anti-TB, they were presented. The golden stander in which Solid media (LJ) were used, the resistance of cases to anti-TB drugs were 47 (62.7%) to Rifampicin, 41 (54.7%) to Isoniazid, 42 (56%) to Streptomycin and 38 (50.7%) to Ethambutol. Respectively, while by using Bactec MGIT 960 in which advanced liquid media culture used in the laboratory, the resistance patterns showed almost similar rate of drug resistance to primary drugs, anti-TB (Rifampicin and Isoniazid) by both methods. While the resistance to secondary drugs (Streptomycin and Ethambutol) were higher significantly by solid media. In which the resistance to Ethambutol was 38(50.7%) on solid media, but in Bactec MGIT 960

was 6 (8.0%), and the resistance to Streptomycin was 42(56.0%) of cases, while on Bactec MGIT 960 was 9 (12.0%) only. This result is highly sensitivity and accuracy for detecting primary drug resistance (Rifampicin and INH) was in agreement with were the results obtained **[28]**. While not in with same author for (Streptomycin and Ethambutol). The Drug susceptibility testing observed for (Isoniazid and Rifampicin) as a comparison between solid media and Bactec MGIT 960 methods showed no clear pattern of discordant results as both false susceptible and false resistant**.** While different and unacceptable DST result between (Streptomycin and Ethambutol) due to the that Bactec MGIT 960 is highly liable to contamination than solid media, small sample size, multiple drugs used in community for medication and heterogenetic mutation in embB 306 codon for (Ethambutol resistance TB) this is in agreement with the study of **[29]**. Also heterogenetic mutation rpsL and rrs gene of (Streptomycin resistance TB) **[30]**. Especially in solid media to become drug resistance tuberculosis because of long time incubation of *Mycobacterium tuberculosis* than Bactec MGIT 960 methods to become resistance mutants. The GeneXpert methods is a rapid molecular technique that used to identified of MTB and Rifampicin detected, they were used in this study and showed that resistance to Rifampicin, was 48 (64.0%) for all cases included in this study. The classical or conventional drug susceptibility testing DST by solid media (Lowenstein-Jensen medium) remained as a gold stander technique and is still utilized in many countries in the world **[31].** In table (6) the drug resistance to first-line anti-TB drug, the higher MDR-TB for all drugs (SIRE) was found to be 18 (24.0%) isolates, while resistance for three drugs was ranging between (4.0% - 10.7%). And the resistance to 2 drugs was ranging found to be (1.3%-9.3%), while resistance to single drug was ranging between (2.7%-10.7%). The cases were classified according to drug resistance tuberculosis that obtained from the golden stander (solid media), as shown in figure (3). Due to highly selected MDR cases and comparison between different laboratory methods in this study. The drug susceptibility testing (DST) of TB isolates to Streptomycin drug resistance on solid media that shows was associated only with Ethambutol drug resistance ($P<0.05$), while the (Rifampicin and Isoniazid) drugs resistance were not significantly associated, and according to age group that presented was higher isolates obtained from age group (15-45y) as (45.2%) were resistance of all cases, and were Streptomycin resistance on solid media including 24 (57.1%) males and 18 (42.9%) females, that shows in table (7). These results are in agreement with another study by [**32]**. In table (8) the distribution of (DST) on solid to Isoniazid drug estimation presented as the INH drug resistance is significantly associated with Rifampicin resistance and Ethambutol resistance ($P>0.05$). And not significant with

streptomycin drug resistance. The demonstrated Rifampicin drug resistance on solid media was (41) cases shows higher in isolates obtained from age group (46-65) years as 21 (44.7%) of Rifampicin resistance cases, that including 26 (55.3%) males and 21 (44.7%) females, the Rifampicin drug resistance significantly associated with Isoniazid resistance and Ethambutol resistance (P>0.05) but not significantly associated with Streptomycin, gender and age as shown in table (9). Depending upon solid media the distribution of (DST) to demonstrate Ethambutol drug resistance was higher in cases obtained from age group (15-45) years as 16 (42.1%) of 42 cases Ethambutol drug resistance, that including 25 (65.8%) males and 13 (34.2%) females, and the Ethambutol drug resistance on solid media are significant with each group of Streptomycin, Rifampicin and Isoniazid (P>0.05) but not significant with age and gender as showed in table (10). the distribution results of Rifampicin drug resistance between the three methods, the result of GeneXpert as a comparison with Bactec MGIT 960 was high specificity (87%) and sensitivity (98%), while the GeneXpert when comparison with solid media shows high specificity (89%) and sensitivity (96%), as shown in figure (4). This result is in agreement with the study obtained from **[33].**

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