RESULTS

This study was done at Chest Out Patient Clinic of Benha University hospital, Benha Chest hospital and Microbiology & Immunology Department of Benha Faculty of Medicine from January to June (2010) on 50 patients (37 males and 13 females). Their age ranged from 18-67 years. The selection of patients was done according to clinical and radiological data suspecting pulmonary tuberculous infections. Patients were classified into two groups:

I- Twenty nine patients with no history of antituberculous treatment

II- Twenty one patients receiving rifampicin: those patients were subdivided into 2 subgroups:

1- Thirteen patients starting antituberculous treatment with no problems.

2- Eight patients with previous history of failed antituberculous treatment.

The collected sputum samples were subjected to ZN stain; out of 50 studied patients 42 (84%) and 8 (16%) were ZN smear positive and negative respectively.

Out of those 42 (84%) ZN smear positive patients, 25 (59.5%) were not receiving any antituberculous treatment, 17 (40.5%) were under antituberculous treatment including rifampicin for more than 2 months. While out of 8 (16%) smear negative patients 4 (50%) had no history of antituberculous treatment and the other 4 (50%) were under antituberculous treatment including rifampicin.

All 50 studied sputum samples from 50 studied patients were positive for FDA/EB stain.

The age of patients under study ranged from 15 to 67 years old 33.4±12.4. There were 5 (10%) cases in age group 10-≤20 years, 18 (36%) cases in age group 20-≤30 years, 15 (30%) cases in age group 30-≤40 years, 7(14%) patients in age group 40-≤50 years, 3 (6%) patients in age group 50-≤60 years and 2(4%) cases in age group 60-≤70 years.

Table (1):Distribution of tuberculosis among different age groups of the studied patients:

Age groups / years	**No**	**%**
10- ≤20	5	10%
20- ≤30	18	36%
30- ≤40	15	30%
40- ≤50	7	14%
50- ≤60	3	6%
60- ≤70	2	4%
Total	50	100%

Table (1)**:** shows that the highest rate of tuberculosis was 18 (36%) out of 50 cases in the age group 20- ≤30.

Figure (1) Distribution of tuberculosis among different age groups of the studied patients:

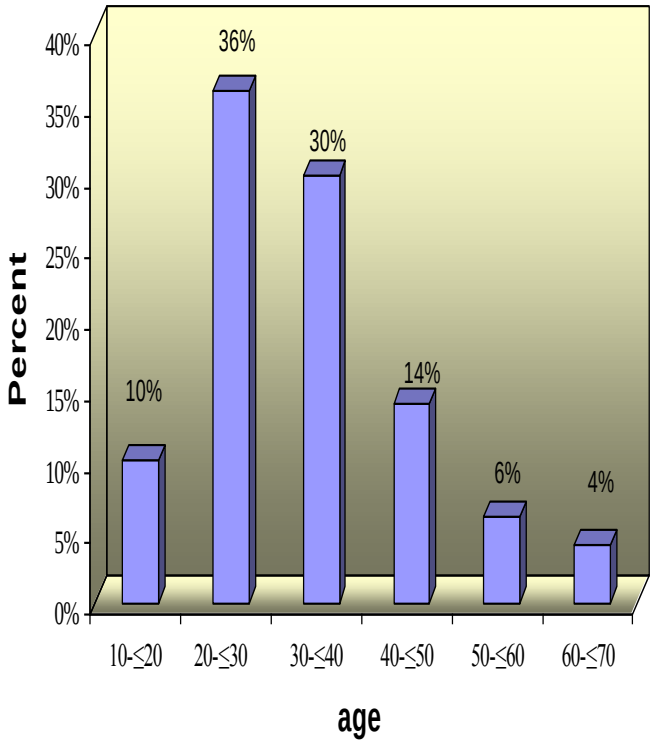


Table (2): Distribution of tuberculosis according to sex of the studied patients:

Sex	**No**	**%**
Male	37	74%
Female	13	26%
Total	50	100%
Z	4.8	
P	<0.05	

Table (2): shows that out of 50 patients; 37 (74%) were males and 13 (26%) were females.

There is a significance statistical difference ($p < 0.05$) as regards the sex of the studied patients.

Figure (2) Distribution of tuberculosis according to sex of the studied patients:

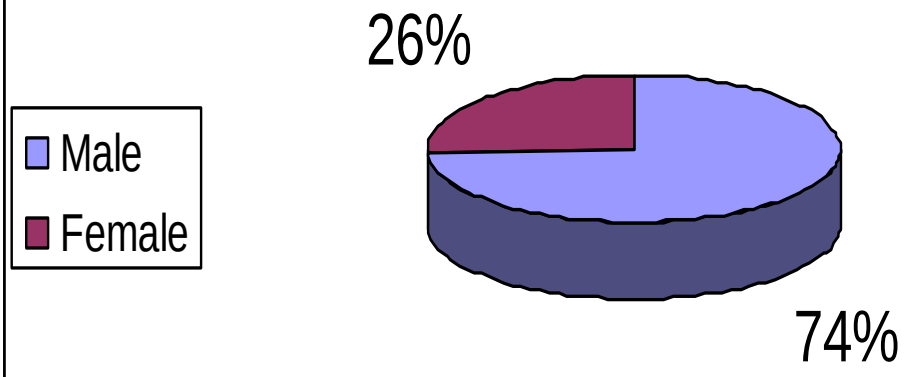


Table (3): Effect of occupation and smoking habit on the tuberculous infection

	NO	%
Occupation		
♦ Workers	31	62%
♦ Housewives	13	26%
♦ Employers	6	12%
Smoking habits		
♦ Cigarette smokers	16	32%
♦ Goza smokers	14	28%
♦ **Cigarette and goza smokers**	6	12%
Non smokers	14	28%
Total	50	100%

Table (3): shows that out of 50 studied patients 31 (62%) were workers, 13 (26%) housewives and 6 (12%) employers.

As regards the effect of smoking habits in tuberculous infection 16 (32%) out of 50 cases under this study were cigarette smokers, 14 (28%) were goza smokers and 6 (12%) were both cigarette and goza smokers.

Table (4): Results of microscopic examination of ZN stained smear

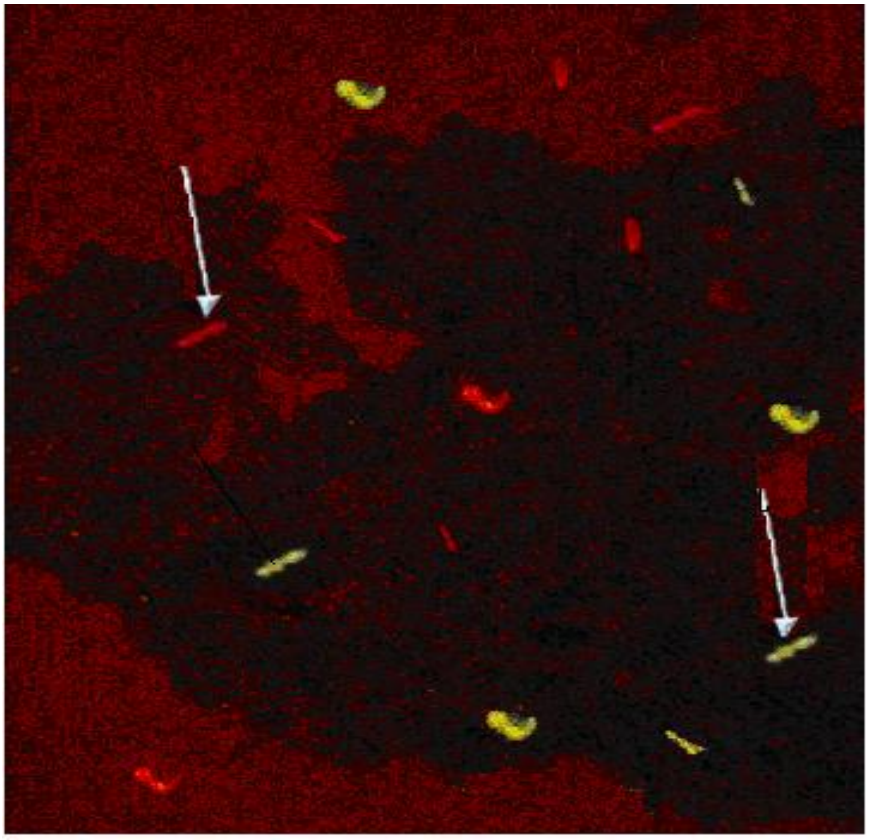
ZN stained smears	NO	%
Positive	42	84%
Negative	8	16%
total	50	100%

Table (4): shows that out of 50 ZN stained smear from 50 studied patients 42 (84%) were ZN positive and 8 (16%) were ZN negative.

Table (5): Results of microscopic examination of Fluorescein diacetate ethidium bromide (FDA/EB) stained smear

FDA/EB stained smears	NO	%
Positive	50	100%
Negative	0	0
total	50	100%

Table (5): shows that all 50 studied sputum samples from 50 studied patients were positive for FDA/EB stain



Figure(3): FDA/EB stained smear shows that FDA stained viable *M.TB*. bacilli (greenish yellow) while EB stained dead *M.TB.* bacilli (red) as examined by fluorescent microscope (400x)

Table (6): Results of microscopic examination of FDA/EB stained smear

FDA/EB stained smears	**Viable**		**Dead**		**Viable and dead**		**Total**	
	NO	%	NO	%	NO	%	NO	%
	23	46	4	8	23	46	50	100

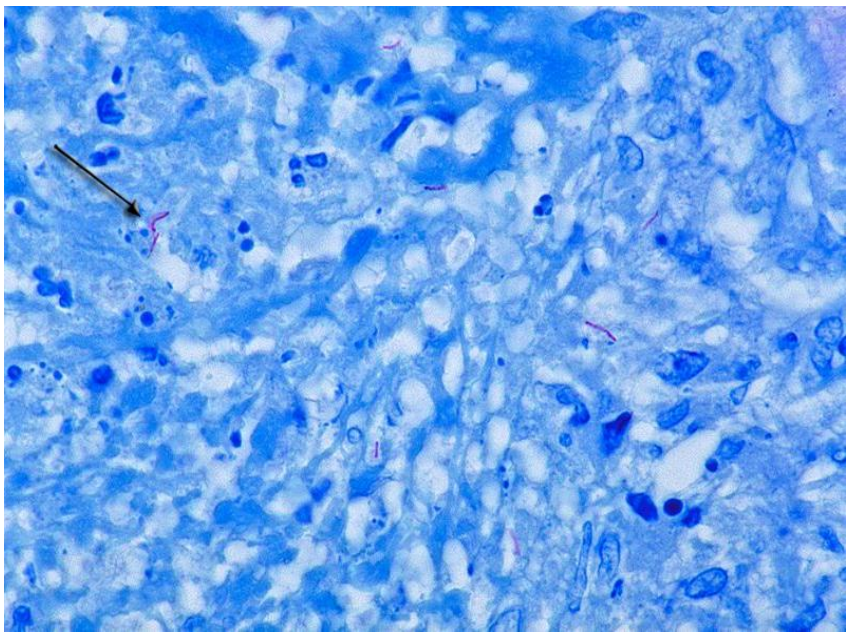
Table (6): Shows that out of 50 FDA/EB stained smear from 50 studied patients 23 (46%) showed viable bacilli, 4 showed dead bacilli and 23 (46%) showed both viable and dead bacilli.

Table (7): Grades of microscopic examination of 50 ZN stained sputum smears from 50 studied patients

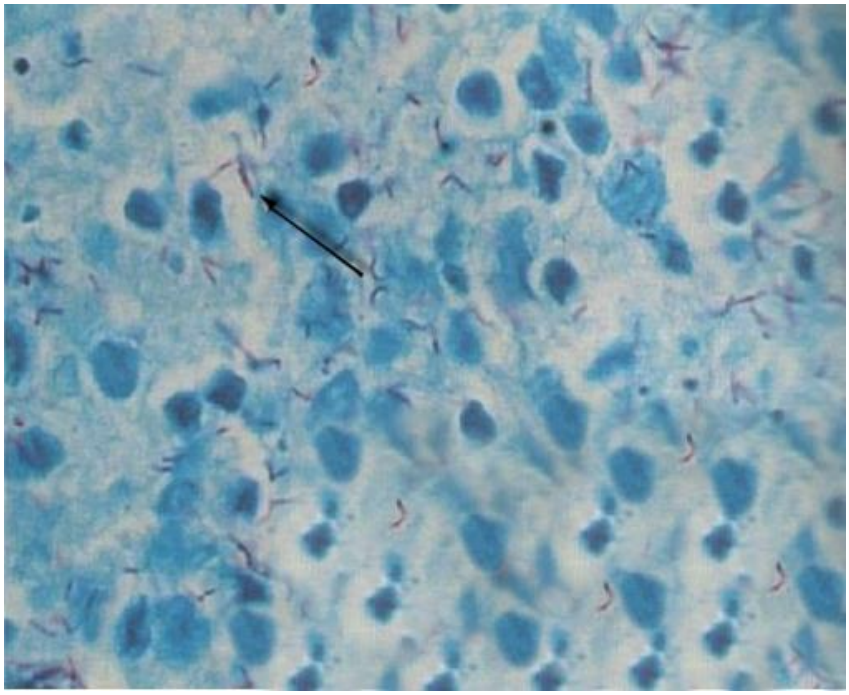
ZN stained smears	NO	%
Negative	8	16%
Positive		
1. Grade +	10	20%
2. Grade ++	24	48%
3. Grade +++	8	16%
Total	50	100%

Grades of microscopic examination of ZN stained sputum smear according to **(*American Thoracic Society, 1981*)**

- Negative: No AFB per 100 fields by oil immersion lens1000x
- Scanty: 1-9 AFB per 100 fields by oil immersion lens 1000x
- 1+: 10-99 AFB per 100 fields by oil immersion lens 1000x
- 2+: 1-10 AFB per one field by oil immersion lens 1000x
- 3+: more than 10 AFB per one field by oil immersion lens 1000x



Figure(4): Positive ZN stained smear
Grade (+) according to (*American Thoracic Society, 1981*)



Figure(5): Positive ZN stained smear
Grade (+++) according to (American *Thoracic Society, 1981*)

Table (8): Grades of microscopic examination of 50 FDA/EB stained sputum smears from 50 studied patients

FDA/EB stained smears		Total	
Viable bacilli	**Dead bacilli**	**NO**	**%**
-	+	4	8%
scanty	++	8	16%
+	+	4	8%
+	-	2	4%
++	+	12	24%
++	-	10	20%
+++	-	10	20%
		50	100%

Grades of microscopic examination of FDA/EB stained sputum smears according to ***(Nakamura and Kinomoto, 1990):***

- Negative: No AFB per 100 oil immersion field (400x)
- Scanty: 1-9 AFB per 100 fields (400x)
- 1+: 10-99 AFB per 100 fields(400x)
- 2+: 1-10 AFB per individual field(400x)
- 3+: 10 to 100 AFB per individual field (400x)

Table (9): Results of FDA versus ZN stained sputum smears from 50 studied patients.

FDA stain=46 samples	ZN		Total
	Positive	**Negative**	
Positive	42	4	46
Negative	0	4	4
Total	42	8	50

Table (9): shows that out of 50 sputum samples 42 were positive by FDA and ZN stains, 4 samples were positive and negative by FDA and ZN stains respectively while 4 were negative by both .

For FDA stain.

Sensitivity = 100%

Specificity = 50%

PPV =91.3%

NPV=100%

Table (10): LJ culture results of 50 studied sputum samples

LJ culture	NO	%
Positive	46	92%
Negative	4	8%
Total	50	100%

Table (10): shows that out of 50 sputum samples 46 (92%) samples showed growth on LJ medium while 4 (8%) had no growth.

Figure(6): Mycobacterial growth on LJ medium

Colonies are rough, yellow and cauliflower

Table (11): Results of Z.N stain versus L.J culture for detecting acid fast bacilli in sputum samples.

Z.N stain	L.J culture		Total
	+ ve	- ve	
+ ve	42	-	42
- ve	4	4	4
Total	46	4	50

Table (11): shows that out of 50 sputum samples 42 were positive for tuberculosis by both Z.N stain and culture on LJ media, 4 were negative by both methods and 4 were negative by Z.N

For Z.N stain:
Sensitivity = 91.3%
Specificity = 100%
Positive Predictive value = 100%
Negative predictive value = 100%

Table (12): Results of FDA stain (viability stain) versus LJ culture method for sputum samples of 50 studied patients.

FDA stain	LJ culture		Total
	Positive	**Negative**	
Positive	46	_	46
Negative	_	4	4
Total	46	4	50

Table (12): shows that out of 50 sputum samples 46 were positive by FDA stain and LJ culture, 4 were negative by both methods.

For FDA stain.

Sensitivity = 100%

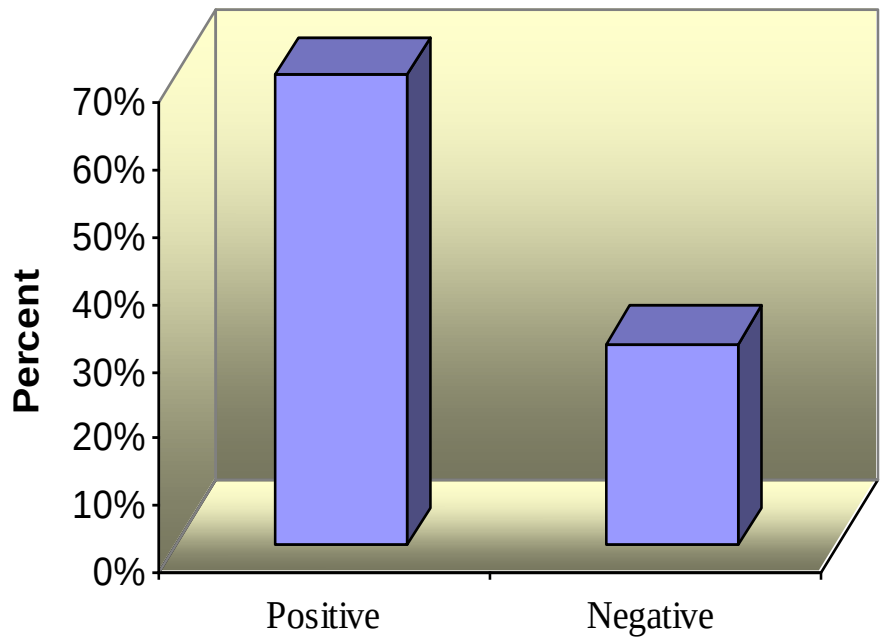
Specificity = 100%

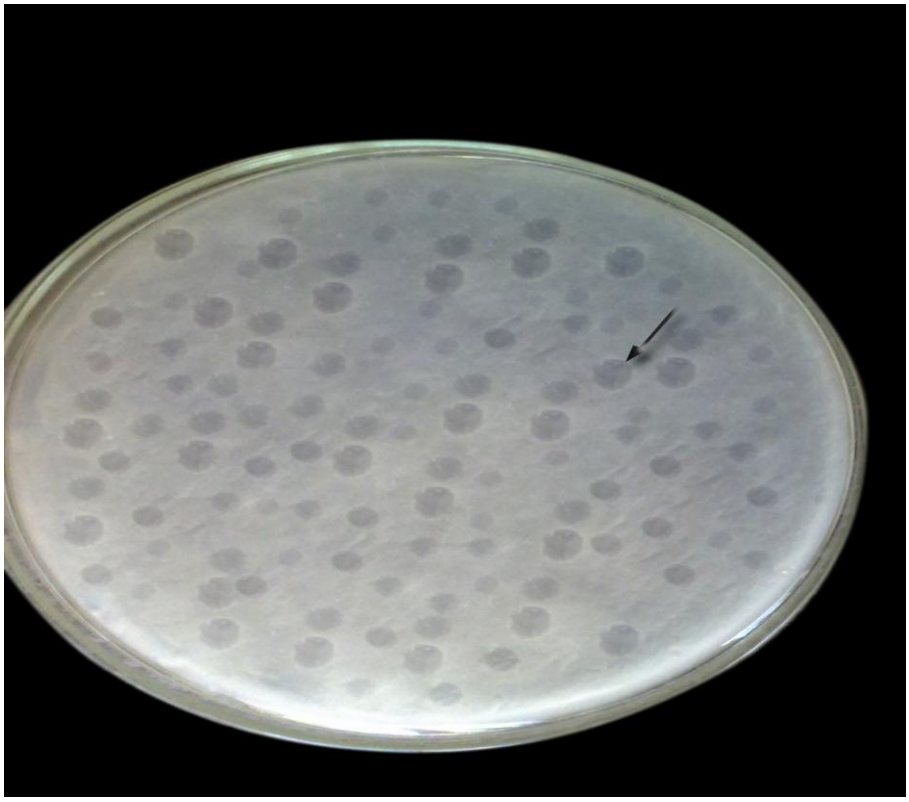
Table (13): Results of fast plaque response(TM) assay of 50 studied sputum samples

Fast plaque response(TM) assay	NO	%
Positive	35	70%
Negative	15	30%
Total	50	100%

Table (13): shows that out of 50 sputum samples 35 (70%) were positive while 15 (30%) were negative by fast plaque response(TM) assay.

Figure (8): Results of fast plaque response(TM) assay of 50 studied sputum samples





Figure(8): Positive fast plaque response(TM) assay

Figure(9): Negative fast plaque response(TM) assay

Table (14): Results of fast plaque response(TM) assay of 50 studied sputum samples versus LJ culture.

fast plaque response (TM) assay	LJ culture		Total
	Positive	**Negative**	
Positive	35	_	35
Negative	11	4	15
Total	46	4	50

Table (14): shows that out of 50 sputum samples 35 were positive by Fast plaque assay and LJ culture, 4 were negative by both methods and 11 were negative by Fast plaque assay .

For Fast plaque assay .

Sensitivity = 76.1%

Specificity = 100%

Positive Predictive value = 100%

Negative predictive value = 26.7%

Table (15): Time of isolation of mycobacterial isolates of 50 studied sputum samples by LJ culture and Fast plaque response(TM) assay

Time / days	**LJ culture**	**Fast plaque response(TM) assay**
Range	22-41 days	2 days
Mean	27.5	2
S.D. ±	9.59	-
S.E. ±	1.35	-

S.D: Standered deviation

S.E: Standered error

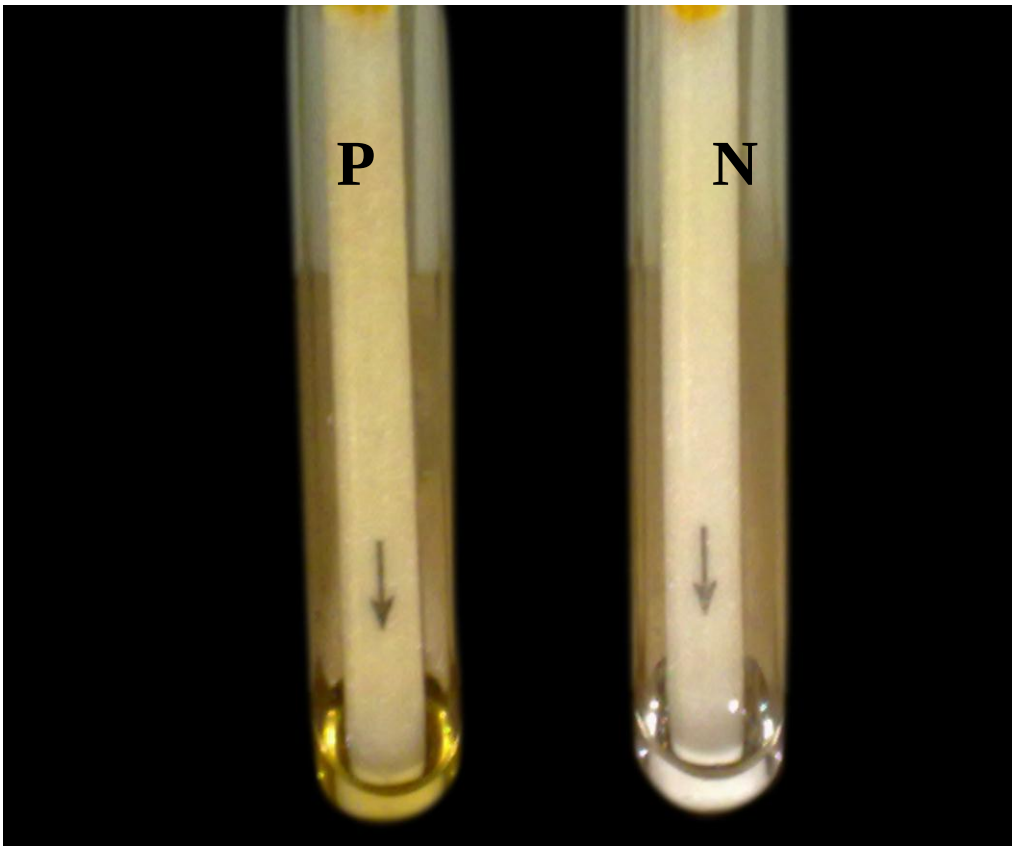
Table (16): Number of *M.TB.* and MOTT strains isolated on LJ culture medium:

LJ culture		*M.TB.*		MOTT	
NO	**%**	**NO**	**%**	**NO**	**%**
46	100%	41	89.1%	5	10.9%

M.TB.: mycobacterium tuberculosis

MOTT: mycobacterium other than tuberculosis

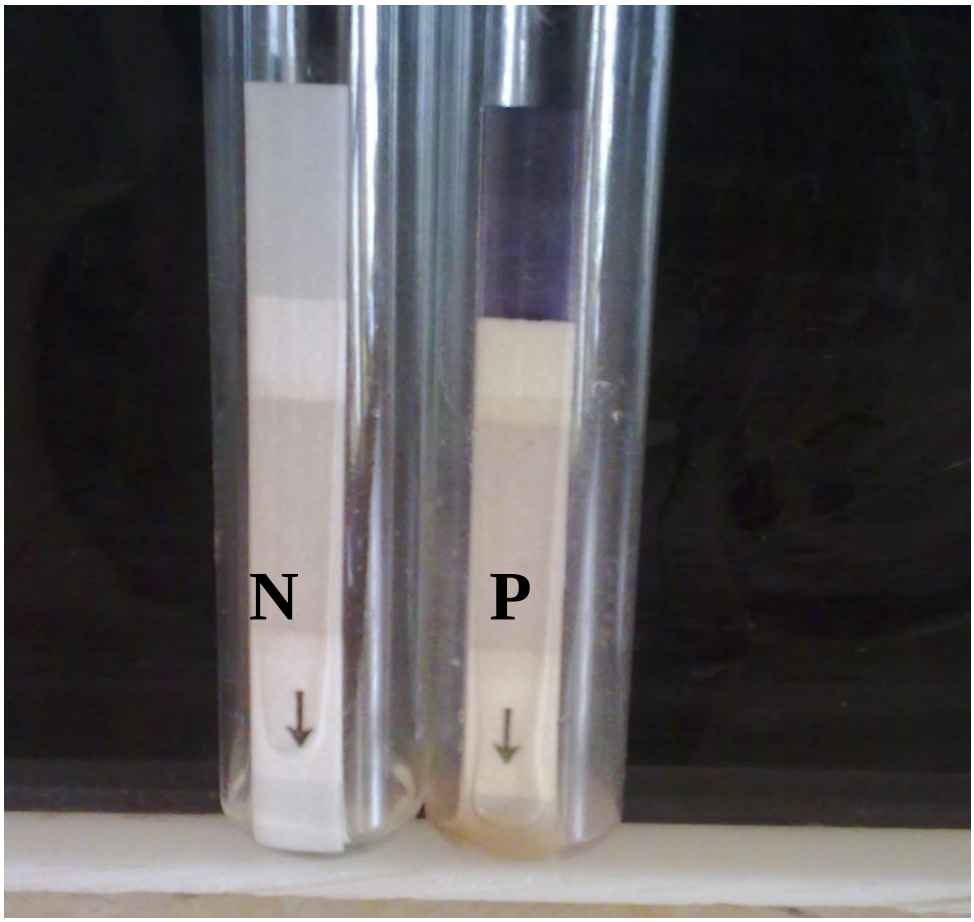
By using niacin production and nitrate reduction tests; out of 46 mycobacterial isolates,41 (89.1%) were biochemically typed as being *M. tuberculosis* whereas the other 5 (10.9%) were typed as MOTT.



Figure(10): NIACIN TEST

"P" positive : Yellow color

"N" negative: No color



Figure(11): NITRATE REDUCTION TEST

- "P" positive : a blue color in the top portion of the strip.
- "N" negative: No color

Table (17):Relation between Z.N stain results and antituberculous drug intake including rifampicin:

Antituberculous drug intake	Z.N stain				Total		Z	p
	positive		negative					
	NO	%	NO	%	NO	%		
No history of antituberculous treatment	25	86	4	14	29	100	0.5	>0.05 NS
Antituberculous treatment	17	80.9	4	19.1	21	100		
♦ **Antituberculous treatment response**	9	69.2	4	30.8	13	100	1.7	>0.05 NS
♦ **Antituberculous treatment failure**	8	100	-	-	8	100		
Total	42	84	8	16	50	100		

Table (17): shows there is insignificant statistical difference (P>0.05) between the results of ZN stained smear in both patients with no history of antituberculous treatment and patients under treatment.

Table (18): Relation between the diagnosis of tuberculous infection by FDA/EB stain method and antituberculous drug intake including rifampicin:

Antituberculous drug intake	FDA/EB stain method						Total	
	Viable		Dead		Both			
	NO	%	NO	%	NO	%	NO	%
No history of antituberculous treatment	20	68.97	-	-	9	31.03	29	100
Antituberculous treatment	3	14.3	4	19.1	14	66.6	21	100
♦ **Antituberculous treatment response**	-	-	4	30.8	9	69.2	13	100
♦ **Antituberculous treatment failure**	3	37.5	-	-	5	62.5	8	100
Total	23	46	4	8	23	46	50	100

Table (18): shows that by FDA/EB stain method 20 patients (68.97%) with no history of antituberculous treatment had viable bacilli while 3 (37.5%) with history of antituberculous treatment failure had viable bacilli. There is 4 (19.1%) patients had dead bacilli among those had history of antituberculous treatment. While in patients with no history of antituberculous treatment no one had dead bacilli.

Table (19):Relation between LJ culture results and antituberculous drug intake including rifampicin:

Antituberculous drug intake	LJ culture				Total		Z	p
	positive		negative					
	NO	%	NO	%	NO	%		
No history of antituberculous treatment	29	100	-	-	29	100	2.5	<0.05 S
Antituberculous treatment	17	80.9	4	19.1	21	100		
♦ **Antituberculous treatment response**	9	69.2	4	30.8	13	100	1.7	>0.05 NS
♦ **Antituberculous treatment failure**	8	100	-	-	8	100		
Total	46	92	4	8	50	100		

S= significant

NS=non significant

Table (19): shows that there is significant statistical difference (P<0.05) as regards the diagnosis of tuberculous infection by LJ culture method among patients with no history of antituberculous treatment . While, there is insignificant statistical difference (P>0.05) as regards the diagnosis of tuberculous infection by LJ culture method among patients under antituberculous treatment .

Table (20):Relation between the diagnosis of tuberculous infection by Fast plaque response(TM) assay and antituberculous drug intake including rifampicin:

Antituberculous drug intake	Fast plaque response (TM) assay				Total		Z	p
	positive		negative					
	NO	%	NO	%	NO	%		
No history of antituberculous treatment	26	89.7	3	10.3	29	100	2.01	<.05 S
Antituberculous treatment	9	42.9	12	57.1	21	100		
♦ **Antituberculous treatment response**	1	7.7	12	92.3	13	100	4.2	<.05 S
♦ **Antituberculous treatment failure**	8	100	-	-	8	100		
Total	35	70	15	30	50	100		

Table (20): shows that the diagnosis of tuberculous infection by Fast plaque assay was higher among patients who had no history of antituberculous treatment than those under antituberculous treatment with significant statistical difference**(**P<0.05).

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Table (21): Relation between the demographic data of tuberculous patients and Rifampicin resistance :

Charachter	Rifampicin resistance				Total No=50		Z	P
	Sensitive		Resistant					
	No	%	No	%	No	%		
I- Age								
< 40	23	60.5%	15	39.5%	38	76%	**1.7**	**>0.05 NS**
≥ 40	10	83.3%	2	16.7%	12	24%		
II- Sex								
Male	23	62.2%	14	37.8%	37	74%	**1.5**	**>0.05 NS**
Female	10	75%	3	25%	13	26%		
III-Smoking habits								
♦ Cigarettes	9	56.3%	7	43.7%	16	32%		
♦ Goza	9	46.3%	5	35.7%	14	24%	**1.9**	**>0.05 NS**
♦ Cigarettes and goza	4	66.7%	2	33.3%	6	12%		
Non smokers	10	71.4%	4	28.6%	14	28%		

There are insignificant statistical differences in the relation between sex, age and smoking with rifampicin resistance results.

Table (22): Relation between the clinical data of tuberculous patients and rifampicin resistance.

Charachter	Rifampicin resistance				Total No=50		Z	P
	Sensitive		Resistant					
	No	%	No	%	No	%		
I-History of tuberculosis								
+ve	10	55.6	8	44.4	18	36	1.9	>0.05
-ve	23	71.9	9	28.1	32	64		NS
II-Cavitation								
+ve	3	30%	7	70%	10	76%	1.7	>0.05
-ve	30	75%	10	25%	40	14%		NS
III-Pulmonary T.B only								
+ve								
-ve	23	63.2%	12	36.8%	37	74%	1.5	>0.05
	10	75%	3	25%	13	26%		NS
IV- *D.M								
+ve	4	40	6	60	10	20	3.2	<0.05
-ve	29	72.5	11	27.5	40	80		S
V-History of contact with T.B case								
+ve	7	41.2	10	58.8	17	34	0.5	>0.05
-ve	26	78.8	7	21.2	33	66		NS

* D.M: Diabetes mellitus

There is a significant statistical difference as regards the relation of D.M and rifampicin resistance among tuberculous patients. There are insignificant statistical differences in the relation between history of TB, pulmonary TB cavitation, history of pulmonary TB only and history of contact with T.B case with rifampicin resistance results.

Table (23): Relation between the rifampicin resistance pattern of *M.TB* isolates and severity of disease

Rifampicin Resistance	Severity of disease						Total
	Minimal		Moderate		Advanced		
	No	%	No	%	No	%	
	2	11.8	4	23.6	11	64.7	17

Table (23): shows that out of 17 rifampicin resistant tuberculous patients 2(11.8%) had minimal, 4(23.6%) moderate and 11(64.7%) advanced clinical condition

Table (24) Number of plaques in plates with and without rifampicin

Number of plaques without rifampicin	Number of plaques with rifampicin	Interpretation	Total	
			NO	%
>300	>50	Resistance to rifampicin	5	10
>100	>50	Resistance to rifampicin	12	24
>100	<50	Sensitive to rifampicin	18	36
<100	<50	Absence of TB bacilli	15	30
Total			50	100

According to *Albert* et al., *2002*:

The number of plaques in plates without rifampicin (RIF-)

- >100 plaques is considered positive
- >300 plaques is considered strong positive

The number of plaques in plates with rifampicin (RIF+)

- >50 plaques is considered resistant to rifampicin
- <50 plaques is considered sensitive to rifampicin

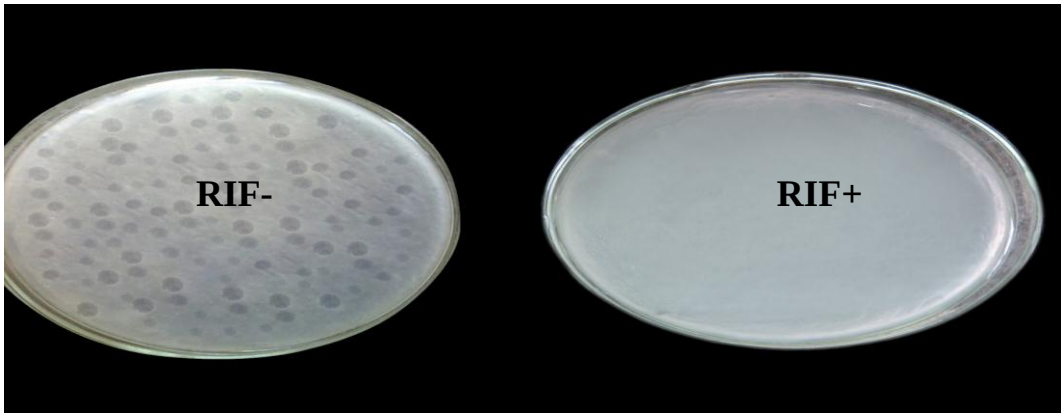

Figure (12): Rifampicin sensitive *M.TB*

The plate on the left, free from rifampicin: contain>100 plaques

The plate on the right with rifampicin: contain no plaques

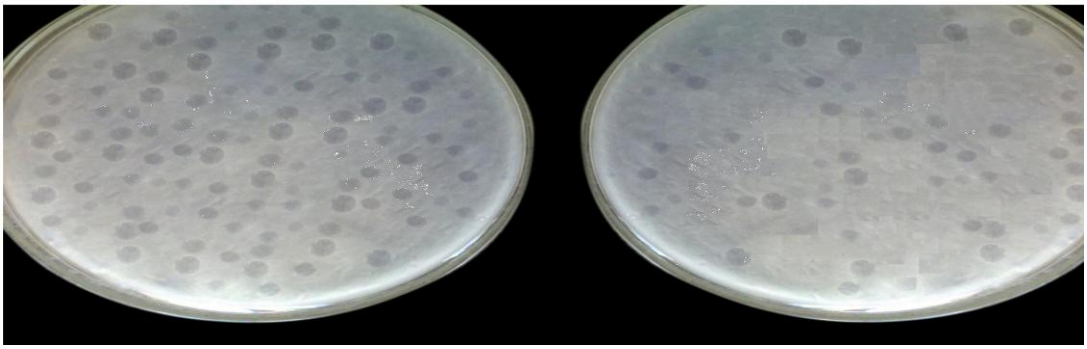

Figure (13): Rifampicin resistant *M.TB*

The plate on the left, free from rifampicin: contain >100 plaqes

The plate on the right with rifampicin: contain >50 plaques

Table (25): Rifampicin resistance using fast plaque response (TM) assay.

Rifampicin resistant		Rifampicin susceptible		Total	
NO	**%**	**NO**	**%**	**NO**	**%**
17	34	33	66	50	100

Table (25): shows that out of 50 studied sputum samples; 17 (34%) mycobacterium isolates were rifampicin resistant and 33(66%) were rifampicin susceptible.

Figure(15)Rifampicin resistance using fast plaque response(TM) assay

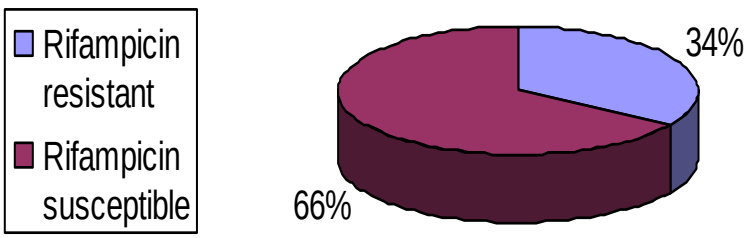

Table (26): Primary and secondary rifampicin resistance among mycobacterium isolates

Primary rifampicin resistance		Secondary rifampicin resistance		Total	
NO	**%**	**NO**	**%**	**NO**	**%**
9	52.9%	8	47.1%	17	100

Table (26): shows that out of 17 rifampicin resistant mycobacterium isolates, 9 (52.9%) had primary rifampicin resistance and 8 (47.1%) had secondary rifampicin resistance.