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Research Article

**A CROSS-SECTIONAL RESEARCH ON SUSCEPTIBILITY
PATTERN OF MYCOBACTERIUM TUBERCULOSIS (MTB) IN
SPUTUM SAMPLES****Dr. Muhammad Naseer ud Din, Dr. Komal Rubab, Dr. Shabana Amir
THQ Kallur Kot****Abstract:**

Objective: The research was aimed at knowing the drugs susceptibility pattern in (MTB) i.e. Mycobacterium Tuberculosis in the attendants of multi-drug resistant tuberculosis patients.

Methods & Material: The research was performed at Services Hospital, Lahore (August, 16 to March, 17). The research was cross-sectional in that 480 sputum samples from the patients of MDR-TB attendants were under taken for identification of (M. TB). It was performed by Ziehl Neelsen staining, i.e. (BACTEC MGIT-960 culture) line probe assay and also by Lowenstein-Jensen.

Results: There were 480 samples, six at 2.1 percent were observed +ve for M. TB by the method of Ziehl Neelsen staining and mean while ten at 2.8 percent were +ve by the use of (LJ & BACTEC MGIT-960 culture). For the aim of taking drugs susceptibility testing, 10 +ve samples were under taken. To find out ethambutol resistant, streptomycin, rifampicin & isoniazid, process of line probes assay test was employed. It was observed that six M. TB isolates were resistant but four were found to be sensitive to both isoniazid & rifampicin. Out of the six resistant M. TB strains, four showed mutations in the "rpo B" gene at three codons as 526, 531 & 516.

Conclusion: Most of the attendants of MDR-TB patients had drug-resistant tuberculosis & also the ratio of the drug susceptible T.B was found lesser.

Keywords: Tuberculosis, Assay, Sputum, Multi-drug resistant tuberculosis.

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INTRODUCTION:

The tuberculosis is cause of mortality rate in women of developing countries & its number is 7th in fatal ailments. (WHO) has demonstrated that Pakistan is at 6th number in tuberculosis cases [1]. (MDR-TB) because of Mycobacterium "TB" resistant to rifampicin (RIF) and isoniazid (INH) which is a warning to global (T.B) reduction program. Above 450,000 MDR – TB cases are hoped to happen all over the world annually [1, 2]. The diagnosis of (MDR – TB) was made after the employment of (Anti – TB) drugs. It was through (STR) description that is streptomycin resistance [3, 4].

The (MDR – TB) is also recognized through RIF & INH resistance [5]. Moreover, the (M. TB) is a resistant to (STR), (INH), rifampicin & (EMB) ethambutol is because of mutations in several genes in the genome of Mycobacterium Tuberculosis [6]. Because of "rpo B" gene coding mutations, RNA beta subunit polymerase, (M. TB) also makes itself resistant to RIF.

The mutations have been observed greater than ninety percent which were found in 81 bp ranging from (507 – 533) "codons core region" of (RNA) polymerase "rpo B" beta subunit gene [6, 7]. The line probe assay to identify (MDR – TB) in 20088 was recommended by Riordan *et al*. The culture is currently gold standard diagnostic tool & mandatory to identify negative cases of smear microscopy. The range of the identification was (100 bacteria per ml) of sputum samples [9].

In Eastern Mediterranean region comprises six percent world (T.B) & 4.3 % (MDR – TB) patients. Prevalent rate of (MDR – TB) is 3.3 % of all "TB" patients & out of them, only fifty percent cases are there in Pakistan [10]. The identification rate of (MDR-TB) cases is relatively less as compare to actual spread of the disease. The low detection ratio in Eastern Mediterranean area is genuinely due to less identification rate in Afghanistan & Pakistan. In future, the big start of (T.B) may be expected in Pakistan if it remained unexplored [10, 11].

The frequency of this disease in Pakistan is changing from 2.3 percent in un-treated patients to a dangerous rate of 17.9 percent in those people who were treated in the past for this disease. Many researches have showed a high spread rate of (MDR-TB) in Pakistan [12, 14]. A current research has proved that consistent increase in number of (MDR-TB) cases ranging from 1990 to 2007 were greater than fifteen thousand cases observed in the research [15]. Up till now, no research has ever been performed to know

the happening of (MDR-TB) in all attendants of MDR-TB patients & then this study was conducted to know drugs susceptibility pattern in the attendants of MDR-TB patients.

MATERIAL AND METHODS:

The research was done at Services Hospital, Lahore (August, 16 to March, 17). All the data & sputum samples were gathered from the attendants of (MDR-TB) patients. Both the ethical & scientific committee recommended this study.

WHO sample size calculator was used to measure sample size, absolute precision required was nine percent, confidence level was kept at ninety five percent, expected population proportion was taken as 0.843 & non-probability purposive sampling technique was used. The sample size was 480 specimens based on all above parameters.

Both genders were included in age ranging from eight to sixty-five years. While people (under 8 or above 65) years and also reported co-infection and no history of it were not included in the research. By using (NaOH) & (N-acetyl-L-cysteine), 480 sputum samples were decontaminated & digested.

By using sterile wire loop, the smear was prepared by the sputum sample on slide of microscope. This was found under oil immersion for mycobacteria presence right after staining of Ziehl-Neelsen. Confirmation of positive growth was made through "Nitrate reductase". With a true growth culture, (DST) drug susceptibility testing was done by inoculating media. Drug solution's critical concentration was added to labelled (BACTEC "MGIT – 960") each tube with culture media as (25, 5.0, 1.0, 0.1 and 1.0) µg for pyrazinamide (EMB, STR, INH and RIF) respectively. The (DNA) sample removed from the culture +ve isolates was put under 30 (PCR) cycles for its amplification. The 1st denaturation was performed at 95°C for a time of fifteen minutes and ten cycles of denaturation were done at 95°C for a time of thirty seconds. The initial elongation was performed at a temperature of 58°C for two minutes. Then twenty cycles were performed for a final denaturation at a temperature of 95°C for a time of twenty-five seconds in which annealing was at 53°C for a time of forty seconds and elongation at a temperature of 70°C for a time of forty seconds. After it, the final elongation was performed at 70°C for a time of eight minutes. For hybridization, amplified tubes were detached from PCR.

Strips were used for the hybridization process in which double stranded (DNA) amplicons were

denatured to the single strands in the twin cubator with the help of adding twenty μ l of denaturing buffer & twenty μ l of DNA amplicons. It was followed by one ml of sterile distilled water, 1 ml stringent wash buffer & one ml Rinse solution. Strips developed were made dry & they were sent to Genotype (MTBDR) plus score sheet in order to check M. TB target DNA sequences linked along with RIF & INH resistance. The frequencies were measured for different variables & results were analysed by using graph-pad prism of version 5.0 percentages.

RESULTS:

There were 480 samples, six at 2.1 percent were observed +ve for M. TB by the method of Ziehl Neelsen staining and mean while ten at 2.8 percent were +ve by the use of (LJ & BACTEC MGIT-960 culture). For the aim of taking drugs susceptibility testing, 10 +ve samples were under taken. To find out ethambutol resistant, streptomycin, rifampicin & isoniazid, process of line probes assay test was employed. It was observed that six M. TB isolates were resistant but four were found to be sensitive to both isoniazid & rifampicin. Out of the six resistant M. TB strains, four showed mutations in the "rpo B" gene at three codons as 526, 531 & 516.

Detail outcomes have been tabulated below:

Table – I: Frequency of mycobacterium tuberculosis in MDR-TB patients' attendants reported through ZN staining, LJ and MGIT-960 culture

Tests	Total Participants	Positive	Percentage
ZN staining	480	6	1.25
LJ culture	480	10	2.08
MGIT-960 culture	480	10	2.08

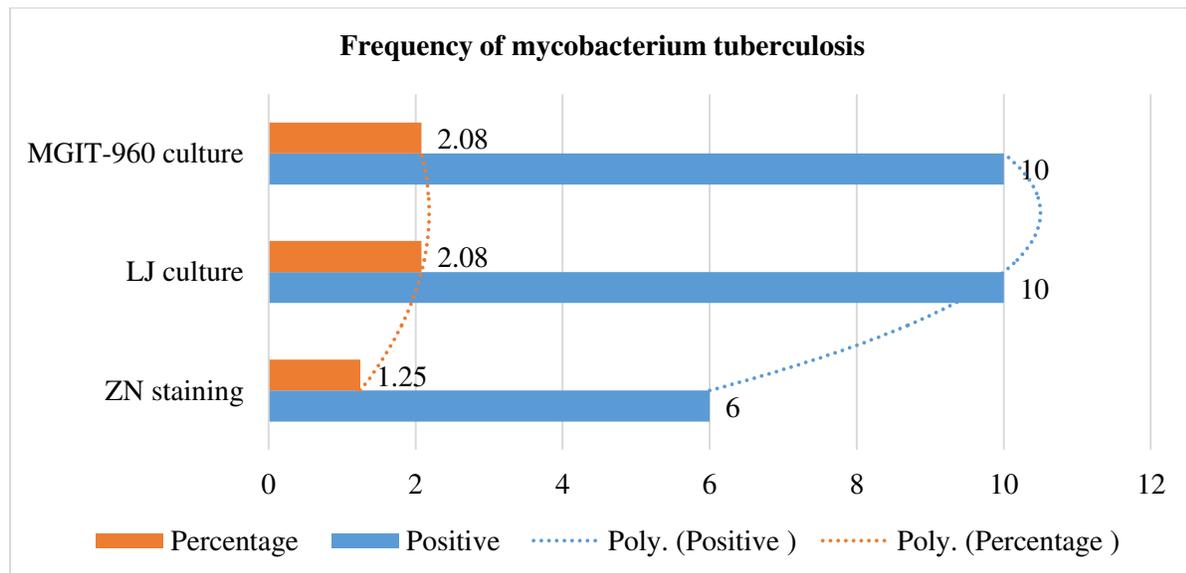
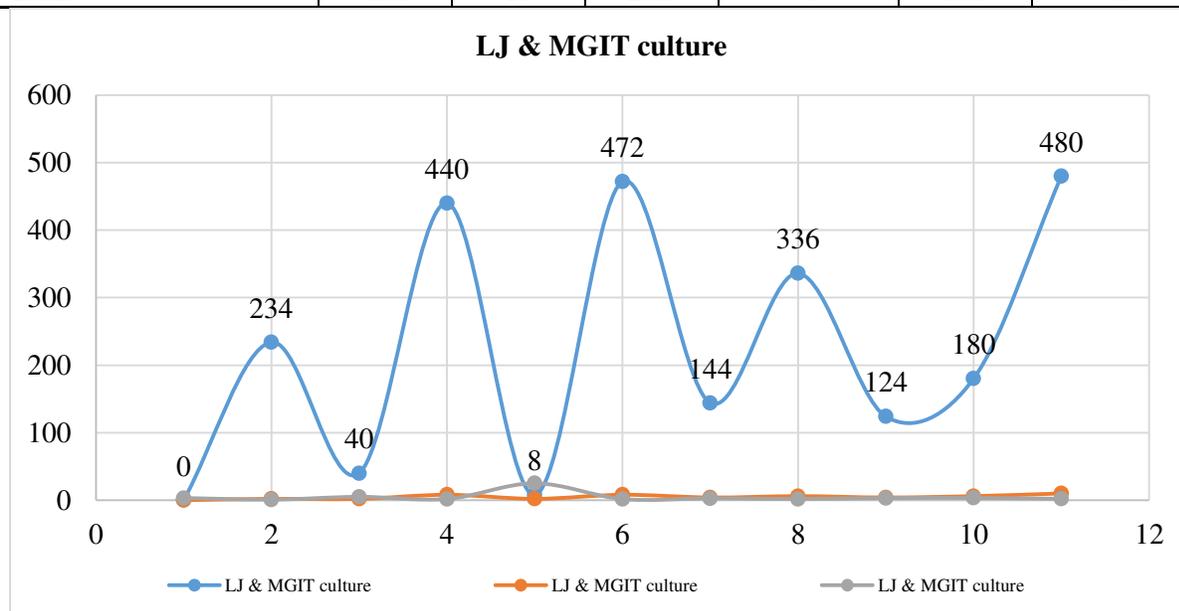


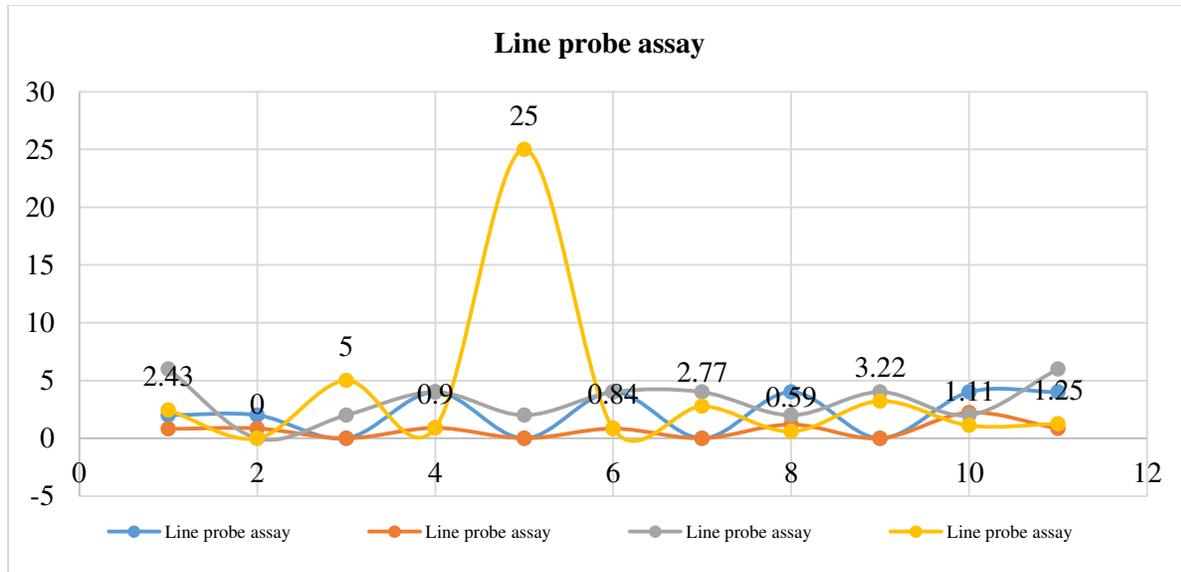
Table – II: Representative patterns of drugs resistant and drugs sensitive *M. tuberculosis* reported through drugs susceptibility testing

Positive Samples	Drugs Susceptibility Testing				
	INH	RIF	STR	ETH	PZA
1	R	R	S	S	R
2	R	R	R	S	R
3	R	R	R	R	R
4	S	S	S	S	S
5	S	S	S	S	S
6	R	R	S	S	R
7	R	R	R	R	R
8	R	R	R	R	R
9	S	S	S	S	S
10	S	S	S	S	S
S: Sensitive			R: Resistive		

Table – III: Percentage distribution of *M. tuberculosis* and MDR-TB on the basis of different factors

Actors	LJ & MGIT culture			Line probe assay			
			%	Sensitive to INH & RIF	%	MDR-TB	%
Rural			3.25	2	0.81	6	2.43
Urban	234	2	0.85	2	0.85	0	0
Smoker	40	2	5	0	0	2	5
Non-Smoker	440	8	1.81	4	0.9	4	0.9
Medicated participants	8	2	25	0	0	2	25
Non - Medicated participants	472	8	1.69	4	0.84	4	0.84
Income/month (≥ 5000)	144	4	2.77	0	0	4	2.77
Income/month (≤ 6000)	336	6	1.78	4	1.19	2	0.59
Group 1 (5-20)	124	4	3.22	0	0	4	3.22
Group 2 (21-36)	180	6	3.33	4	2.22	2	1.11
Total	480	10	2.08	4	0.83	6	1.25





DISCUSSION:

National Directly Observed Treatment Short course in Pakistan aims to give early diagnosis & cure for (MDR-TB) patients and it may minimize the mortality and morbidity in society [16]. The attendants of (MDR-TB) patients are at bigger danger of involving in the (MDR-TB) or (TB).

In our research, just 1.2 percent +ve sputum samples were observed by following Z.N staining method. These results are almost same with another study who identified 1.1 percent pulmonary T.B prevalence in newly diagnosed patients [17]. In the city of Faisalabad-Pakistan, Siddiqui et al [18] observed fifteen percent +ve cases by L.J & (BACTEC-MGIT-960) media culture. A report on several (TB) diagnostic methods has contrasted +ve culture growth of (M. TB) on solid (L.J) culture & (BACTEC-MGIT-960) culture. Out of 527 sputum samples, 411 at 78 percent by L.J cultures media and 428 at 81 percent +ve cases were observed by (BACTEC- MGIT-960) culture [19].

Then 9.4 percent resistant cases to one anti-T. B drug & fifteen percent (MDR-TB) cases were reported by Andrea et al [20]. At Rawalpindi, Holy Family Hospital showed to find out (DST) for (M. TB) & they observed greater resistant rate for PZA, STR & EMB [21]. More study was done on T.B to know frequency of T.B in MDR-TB contacts [22]. In total of 2112 MDR-TB contacts just 108 at five percent developed T.B. The results of our study are supported by Siddiqui et al [18] who found five percent +ve cases by using ZN staining in the suspected T.B patients in Faisalabad.

There was a prevalence of M. TB in eight patients treated in the past & 472 were non-treated participants for the T.B disease in our study. Out of treated only two at 25 percent were found +ve for TB & they were all (MDR-TB) cases. In 472 non-treated patients only eight were +ve for T.B in which four at 1.69 percent were (MDR-TB) & four at 0.84 percent were found drugs sensitive cases. A study done in Rawalpindi, Pakistan, in thirty cases, 83.3 percent had taken anti-T. B drugs previously [21].

In present study, +ve growth cultures found were put under (DST) & it was observed that only six cultures were resistant to RIF & INH. Out of these, six (MDR-TB) isolates and four at 3.22 percent were found in 1st group of age between 5 to 20 years. Just two at 1.11 percent were reported in 2nd group of age 21 to 36 years. The spread of (MDR-TB) in our participants with an income (\geq 5000) per month was 4 at 2.77 percent.

286 cases were studied in a survey in Pakistan, in which thirty-six were resistant to minimum 1 drug & thirty-seven isolates were resistant to (INH), (30 to PZA), four to (RIF), (15 to STR), & (13 to EMB). The participants at 88.7 percent were less than age of 40 years & 9.4 percent had an income less than 100 US dollars/month [23]. In 6 (M. TB) cases, resistant strains found patterns of resistant were located at the point of rpo (BMUT3) & (KatGMUT1) mutation with (RIF) & (INH) resistant, (rpo BMUT1) and (KatGMUT1) mutation, (rpo BMUT1), (KatGMUT1) mutation. Four showed mutation in rpo B gene at 526, 531 and 516 codons. Another two showed (MDR-TB) strain mutation at the KatG gene.

A research also examined total of 108 smear +ve pulmonary tuberculosis samples by using (LPA) & found M. TB resistance rate. The M. TB resistance rates were observed as 92.5 percent for RIF & 76.3 percent for INH [24]. The mutations at 533 & 531 codons of rpo B gene were identified. The mutation of (kat G) gene was identified in 55.9 percent & (inhA) promoter mutations were observed in 11.9 percent of (INH) resistant isolates. These were done at the laboratory of (Aga Khan University), Karachi in Pakistan [24].

CONCLUSION:

Many attendants of (MDR-TB) patients have developed drug resistant tuberculosis & also the ratio of the drug susceptible T.B was found quite lesser.

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