PREVALENCE AND PATTERN OF RESISTANCE TO ANTI TUBERCULOSIS DRUGS IN OUR COMMUNITY

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ABSTRACT

OBJECTIVE: To find out prevalence of drug resistance in patients with tuberculosis.

DESIGN: Descriptive cross sectional study.

PLACE: Medical wards and out patient department of Civil Hospital Karachi.

SAMPLE SIZE: One hundred patients

DURATION: From November 1999 to April 2001.

PATIENTS AND METHODS: All the patients above 12 years of age of both sexes having sputum smear positive pulmonary tuberculosis were included in the study. A detailed clinical history, clinical examination were carried out and base line investigations including Blood CP, ESR, chest x-ray were done and sputum was inoculated for AFB culture & sensitivity. The data of each patient was collected on a separate Performa.

RESULTS: In this study 100 patients were included among those 52 patients were male and 48 were female and age ranged from 16 to 68 years. Sixty four patients had Hb less than 11g%, ESR was raised in 93 patients. Chest radiographs revealed unilateral lung involvement in 56 patients while 44 patients had bilateral lung involvement. Out of 100 culture positive cases enrolled in our study 48 patients were sensitive to all four drugs tested, 16 patients were resistant to one drug, 16 were resistant to two drugs, 12 were resistant to three drugs and 8 patients were found resistant to all four drugs. **CONCLUSION:** In conclusion factors responsible for drug resistance seemed to be related to the patient as well as health provider. Low socioeconomic status, improper dose schedule, under dosage and lack of health education apparently seemed to be

responsible for most cases of drug resistance and if the spread of drug resistance is to be halted, it looks imperative that proper attention to be paid to each of these factors and a well managed and effective national TB control program with compliance of DOTS strategy is our need today.

KEYWORDS: Tuberculosis, Drug resistance, MDRTB.

INTRODUCTION

Tuberculosis is a chronic granulomatous infection caused by mycobacterium tuberculosis. Although disease can occur in any part of body, it predominantly affects lungs and pulmonary tuberculosis, being infectious is also most dangerous form for the community.

TB is a worldwide major health problem and especially so for the developing countries including Pakistan. It is estimated that more than 95% of all the cases are in developing countries. It is estimated that about 8.8 million new cases of tuberculosis occur each year and TB causes more than 3 million deaths per year.

The treatment of tuberculosis rests on the efficacy of effective chemotherapy. Various chemotherapeutic agents have been used to treat tuberculosis but the most effective regimes are found to be those containing Rifampicin and Isoniazid combined with other anti tuberculous drugs. Improper chemotherapeutic regimes, under dosage and irregular treatment has led to the emergence of drug resistant strains of mycobacterium tuberculosis and the recent worldwide epidemic of MDRTB has led the research fellows to look for alternate drugs which may be cost effective as well as having significant anti Mycobacterial activity. Although the threat of MDRTB is high still various WHO programs have shown that supervised chemotherapy comprising of five first line anti TB drugs, taken regularly is still the most potent weapon to fight TB and is able to treat most cases of the apparent treatment failure because of the poor compliance and non adherence to the prescribed treatment.[1]

The advocation of DOTS strategy by WHO and implementation of the strategy in various TB control programs in countries like Bangladesh, Tanzania, China & even in USA has led an up to 95% cure rate in these countries and a considerable decline in the prevalence of drug resistance amongst TB patients. The priority is to prevent the emergence of drug resistance as this single epidemiological tool can forecast the situation expected in the near future.

The present study is undertaken to find out the prevalence of drug resistance among TB patients so that measures be undertaken to prevent the future emergence of MDRTB.

MATERIALS AND METHODS

This descriptive cross sectional study of 100 patients was carried over a period of six months from November 1999 to April 2001 at all medical wards as well as in outpatient's department of Civil Hospital Karachi. Patients who were suspected to be having tuberculosis on the basis of their symptoms and radiological findings were subjected to sputum smear examination for AFB on random basis. Smear positive sputum of 100 of these patients were inoculated for culture and sensitivity.

A detailed clinical history as regarding the age, sex, occupation, and history of contact with tuberculosis patient, duration of illness and presenting symptoms with duration was noted. History regarding the previous use of ATT was given special emphasis with all means applied to find out exact dose of drugs, the combinations used and the duration and regularity of treatment with dose schedule noted. A detailed physical examination of the patient was done which included all physical stigmata of respiratory diseases like clubbing, cyanosis, pedal edema etc. Systemic examination of all major systems was done. The data of each patient was recorded on standard Performa

Inclusion criteria

Smear positive patients of Tuberculosis

Exclusion criteria

- Children below 12 years of age
- Seriously ill patients already on ATT in whom drugs may not be stopped prior to inoculation

- Patients who are not expectorating

LABORATORY INVESTIGATIONS

All enrolled patients had their CBC & ESR, blood sugar, LFTs, urea, urine DR, x-ray chest were carried out in all patients in standard PA view. Lateral view was done in two patients who had apparently wide mediastnal shadow and decubitus view done in one patient to detect subpulmonic pleural effusion. The radiological findings were recorded as unilateral or bilateral and mild moderate or extensive depending upon the extent of pulmonary parenchymal involvement on radiograph.

Sputa were sent for direct smear examination and culture of 100 positive smear applied for Mycobacteria.

CULTURE MEDIA

The culture media used in our study was OGAWA media which is a modification of LJ media and indirect method of inoculation was employed for susceptibility testing. Mycobacterial strains of H37RV were used as controls.The concentration of drug used for testing was in accordance with the WHO recommendations for sensitivity testing and were as follows. [2]

INH	0.2 µgm/ml
Rifampicin	40 µgm/ml
Ethambutol	2 µgm/ml

Streptomycin 4 µgm/ml

These drugs were chosen because they are and have been widely used through out the world as first line agents, their susceptibilities can be reliably measured by standardized technique, they have been studied for many years and background knowledge already exists to which new information can be added. Given the difficulties in standardizing susceptibility testing for PZA due to instability of the drug at high PH, this drug was not included for susceptibility testing as is also the WHO recommendation. [2]

Each specimen, after routine processing with a 3% NaOH solution and NALC, was centrifuged at 30000 x g for 20 minutes. The supernant fluid was than discarded and sediment was divided into two parts 100 times apart (direct and 1: 100 dilution). Both control and drug containing media were inoculated with both the strength of the specimen in accordance with modified proportional susceptibility testing method [3] Inoculums were incubated at 37ºC and growth observed weekly up to 4 weeks.

A principal of Mycobacterial drug susceptibility testing is based on the in vivo correlation between the clinical response to an anti Mycobacterial and the result of in vitro susceptibility testing. It has been found that if more than 1% of patients tubercle bacilli are resistant to a drug in vitro, therapy with that drug is not clinically useful.[4] results were thus declared resistant if the drug containing medium showed a growth of 1% or more of INH, Rifampicin and Ethambutol and 10% or more for Streptomycin in comparison with the control slant [4]

Statistical Analysis

Statistical analysis was done using SPSS version 6.0 and test of significance applied at a p value of 0.01

RESULTS

One hundred patients were enrolled in this study which was aimed at finding out the prevalence of drug resistance against the first line anti TB drugs in our community. Out of these 100 patients, 52 were male and 48 were female. The age wise distribution of these patients is shown in Table 1

Out of these 100 patients 31 belonged to rural Sindh, 10 from Balouchistan and 9 belonged to Punjab but had been residing in Sindh for over two years and four from NWFP, 7 immigrants were included in this study of which 4 belonged to Bangladesh and the other three were Afghan refugees. The remaining 39 patients belonged to urban area of Sindh.

Of these 100 patients, 76 belonged to low socioeconomic group with average income of under Rs.4000/ month where as 24 patients belonged to the middle class family with an average income of more than Rs.4000/month.the average duration of symptoms before seeking medical advice was more than 6 months in 63 patients where as it was less than 6 months in remaining 37 patients. Forty eight patients were new cases who after intensive questioning did not disclose a history of taking anittuberculous medicine before coming to hospital and thus belonged to category I of the WHO treatment protocol where as 58 patients had history of ATT. Twenty out of these 52 patients had completed full course of ATT supervised or unsupervised where as other 32 patients had taken the medicine irregularly or have interrupted the course after 2-3 months. Sixteen out of these 52 patients had taken supervised chemotherapy and thus belonged to category IV of the WHO treatment protocols where as the other 36 patients belonged to the category II of the WHO protocol. (Fig 1)

Questioning about the ATT dosage and schedule disclosed under dosage in 13 patients where as split dose regime was adopted by 17 patients, 6 of whom employed the split

dose regime upon the prescribers advice where as the other 11 patients did so upon relatives advice or social taboos. Of the 32 patients who took irregular treatment, 18 patients were female and 14 male. The reason of interrupting therapy was cost of the medicine in 20 out of 32 patients where as other 12 patients left medicine for various other reasons.

All 100 patients had fever at the onset of the symptoms. Cough with sputum was present in all 100 patients, 40 patients had hemoptysis at some time during their symptoms, chest pain was experienced by 56 patients, and shortness of breath was present in 60 patients. There was a history of significant weight loss in 56 patients. Clubbing was noted in 24 patients and pedal edema was present in 20 patients. Four patients had hepatosplenomegaly, four patients had evidence of DVT and all four of them were addicts. Cervical lymphnodes were enlarged in 6 patients where as another 4 patients had superficial inguinal lymphadenopathy. FNAC was performed in these patients and the results revealed non specific inflammatory changes suggestive of reactive lymphnode hyperplasia in seven patients where as 3 patients had evidence of chronic granulomatous inflammation consistent with tuberculosis. The stained smear was negative for AFB by ZN staining procedure.

The ESR was raised in 93 patients where as it was normal in 7 patients. Out of 100 patients 24 had an ESR less than 50 mmHg in 1^{st} hour by westergren method, 44 patients had an ESR between 50 & 100 mmHg in 1^{st} hour and 32 patients had an ESR of more than 100.the TLC was within normal range in 65 patients where as it was raised in 35 patients. The blood urea was raised in 5 patients and 3 patients were detected to have Diabetes Mellitus which was undiagnosed before. The Urine DR showed protienuria in 7 patients and another 5 patients had WBC in the urine.

The radiographic results were grouped and recorded as being unilateral or bilateral involvement and minimal, moderate and extensive lesions depending upon pulmonary

parenchymal involvement as evident on x-ray film. Fifty six patients had evidence of unilateral lung involvement, out of these 56 patients, 24 had minimal disease (less than 1/3 lung involved), 24 had moderate disease and 4 had extensive disease. Forty four patients had bilateral lung involvement. Out of these 44 patients 24 had moderate pulmonary parenchymal involvement 20 patients had bilateral extensive disease. Two patients out of these 44 had pneumothorax, there was evidence of fibrosis in 38 patients and pleural effusion was noted in 9 patients.

The sputum direct smear examination by ZN stain was positive in all 100 patients as only sputum positive cases were enrolled for study purpose.

Out of the 100 culture positive cases enrolled in our study 48 were sensitive to all 4 (Fig 2) drugs tested 28 out of theses 48 patients were male and 20 females. Sixteen patients were found resistant to one drug, 16 resistant to 2 drugs, 12 resistant to 3 and 8 patients were found resistant to all 4 drugs tested. (Fig 3) The percentage of MDRTB as defined by the combined resistance to Rifampicin and INH was found to be 20% (20 out of 100 cases).

Evaluating the resistance on individual basis, it was found that only 8 out of 48 patients belonging to category I showed resistance to one or more drugs giving figure of 16.66% for primary or initial drug resistance. For the retreatment cases 44 out of the 52 patients enrolled showed resistance against one or more drugs giving a figure of 84.61%for acquired resistance.(Fig 4) The overall resistance found in our study was thus 52 patients out of 100 or 52% drug resistance (combined primary and acquired)

The resistance pattern for individual drug was 28% for INH (4% primary and 28% acquired drug resistance), 24% for Rifampicin, all 24 patient being retreatment cases and thus 24 % acquired drug resistance and no evidence of primary drug resistance was detected in our study. Thirty two percent resistance was detected for Streptomycin out of

which 8% was primary drug resistance and 24% acquired drug resistance. Ethambutol showed 28% resistance, all cases being for of the acquired type. (Fig 5)

Sex wise prevalence figures showed 28 out of the 48 females to be resistant to one or more drugs (58.33% resistance) where as the resistance in males was 24 out of 52 patients (46.15%). Of the different regional groups included in our study, it was observed that out of 31 patients belonging to rural Sindh, 23 had resistance against one or more drugs, 7 patients belonging to Balouchistan province, 3 patients belonging to Punjab, 15 patients belonging to urban Sindh showed resistance against one or more drugs. Of the immigrants 2 of the three Afghan refugees and 2 of the 4 patients belonging Bangladesh showed resistance to antituberculous drugs where as none of the patients from NWFP showed resistance. It was observed that resistance was least in the in16 to 26 year age group as only 4 out of 28 patients belonging to this age group showed resistance in comparison to the middle age and the elderly groups which harbored the most number of resistant cases.

DISCUSSION

The problem of tuberculosis is not new to mention and the breakthrough in the field of treatment made earlier on by the discovery of the anti tuberculous activity of the INH and Streptomycin and later on by the addition, to the already existing treatment protocols, of Rifampicin made short course chemotherapy, best available weapon against this not an infrequently deadly disease but albeit for short period of time. Wrong treatment protocols, poorly organized control programs and war and femine in different parts of the world has led to resurgence of the disease which in this world of fast air travel is no more limited to the underdeveloped nations. The reports of MDRTB arising from the United States in recent past, specially in association with AIDS syndrome has caused great concern owing to the very high case fatality rates.[5]

Currently, therapy for tuberculosis involves multidrug chemotherapy for a period of several months. The most effective treatment regime includes INH and Rifampicin the two most effective drugs available in the tuberculosis armamentarium, at least for part if not all of the treatment regime.[6] Other potent drugs in common use include Pyrazinzmide, Streptomycin and Ethambutol. These 5 drugs are the most widely used antituberculous drugs available at this time. Our study gives a very important impression of situation of drug resistant TB in the province of Sindh. The study group although small and not being truly representative sample of the community as a whole, does gives us an insight regarding the emerging and up to some extent an already established problem of MDRTB.

Comparing the results of our study with another recently conducted study at the Mayo Hospital Lahore by Dr. Shamshad Rasool and others, the Lahore study [7] gave an overall resistance rate of 36%. In Lahore study 14% strains were resistant to one drug, 13% to two drugs, 5% were resistant to three drugs and about 4% were resistant to four drugs. The figures in our study are in the order of 52%. The resistance pattern for single drug was 16%, another 16% were resistant to two drugs, 12% were resistant to three drugs and 8% were found to be resistant to all four drugs. The prevalence of MDRTB in the Lahore study was 11% where as the figures of our study were 20%. The resistance pattern for individual drugs in the Lahore study was 25% for INH, 19% for Streptomycin, 15% for Rifampicin and 12% for Ethambutol where as the figures in our study are 28% for INH, 32% for Streptomycin.24% for Rifampicin and 28% for Ethambutol. The comparatively higher percentage of resistance noted in our study may be a reflection of higher prevalence of drug resistant TB in province of Sindh as compared to Punjab province. In our province the neglected health facilities in the rural areas and relatively greater degree of poverty, the large number of immigrants from Bangladesh and Afghanistan that we are hosting, may be contributing factors responsible for a relatively high degree of resistance. Moreover the Lahore study included 61% newly diagnosed cases where as only 39% cases were of the retreatment category thus denoting acquired drug resistance where as

the percentage in our study was 48% for new cases and 58%for retreatment cases. As we know that the drug resistant tuberculosis is more prevalent amongst the retreatment cases as compared to new cases (initial as compared to acquired drug resistance), the disparity between the number of patients included under each category in Lahore study may be responsible for the slightly lower figures as compared to our study.

Comparing our study with another study conducted by Ansari Shoaib in 1994-95 at the Institute of Chest diseases Kotri, the Shoaib study showed an overall resistance of 64% as compared to 52% overall resistance in our study. The resistance figures for individual in the Shoaib study were 56% for INH, 34% for Streptomycin, 20% for Ethambutol, 22% for Rifampicin. The figures in our study were 28%, 32%, 28% and 24% for INH, Streptomycin, Ethambutol and Rifampicin respectively. The slightly higher figure of resistance in the Shoaib study may represent the high prevalence of drug resistance in rural Sindh as compared to urban areas, as in Shoaib study 42% of the patients enrolled belonged to rural Sindh where as the figures in our study were 31% and out of these 31 patients, 23 (77.7%) showed resistance against one or more drugs.

When the results of this study are compared with the other studies conducted in different parts of the world, it was noted that the Lombardy region of Italy, the prevalence of resistance to at least one drug was 28.1% [8] in comparison to 16% in our study and the prevalence of the MDRTB in the same study was 12.9% as compared to 20% for our study. Similarly the data from Estonia and Latvia showed 28% to 38% primary and 46 to 78% acquired drug resistance in their patients where as the figure for primary drug resistance in our 16.66% and 84.61% for acquired drug resistance. The prevalence for MDRTB was from 9-14% for untreated patients and 19-54% in the retreatment group in the region of Estonia and Latvia where as in our study, the prevalence of MDRTB was 20%, all being in the retreatment group with no evidence of MDR in the newly registered patients. These high figures of drug resistance and specially of MDRTB in untreated patients in Latvia and Estonia represents the poor TB surveillance and poorly functioning

TB control program in these areas. In another study conducted at the Russian Research institute of Phthisis & Pulmonology, out of the 50 patients studied, drug resistance was observed in 33 (66%) case. With 6% resistance to one drug, 14% to two drugs, 32% to three drugs, 40% to four drugs and 8% to all five drugs. The resistance to individual drugs was 50% for Rifamycin, 28% for INH, 14% for Streptomycin in comparison to 24%, 28% & 32% in our study. The internationally reported figures for drug resistant TB in Pakistan are 14.7% for INH,5.1% for Rifampicin, 17.7% for Streptomycin and 8.7% for Ethambutol [9] for the year 1998 in Lahore and 30.3% for INH, 15.2% for Rifampicin, 12.1% for Streptomycin and 9.1% for Ethambutol in the year 1993 in Karachi.[10] These rising figures if seen in the light of the results of our study in which there is increase in the resistance against Rifampicin from 5.1% in 1998 to 12.1 in 1993 and now 24% in our study seems to be quite alarming trend which not only denotes the poor functioning of the control program but also warns us of the potential spread of MDRTB. The rising trend in the resistance against various ant TB drugs may be because of inappropriate regimes by the health provider or non adherence by the patient.

Resistant mutants to any single antibiotic occur readily at random in bacilli undergoing replication and may be selected for by not using the adequate combination of drugs. In the tubercle bacilli, the sites of resistance to anti TB drugs are chromosomally located and are not plasmid born. Thus the likelihood of occurrence of a mutant resistance simultaneously to two drugs is the product of individual probabilities.[11] drug resistant mutants exists at different frequencies for given drugs and have a selective advantage in the setting of monotherapy as they are able to survive therapy while the drug susceptible bacilli are killed. When two drugs are included in the treatment, mutants resistant to one drug are killed by the other drug.

In the population of on million bacilli, one might expect to find 10-15 mutants capable of surviving in the presence of 0.05 mg/l of INH.[12] If the population is only 100, on the other hand, then the probability of finding one resistant organism to the same

concentration of INH is zero. The probability of resistance to multiple drugs is multiplicative. If the mutation rate is 10^6 per reproductive event per bacillus, then the probability of developing resistance simultaneously to 3 drugs, assuring that they act inadequately is the product of the 3 independent probabilities or 10-18 per reproductive event per bacillus. A tuberculous cavity normally harbors 107-109 bacilli. Thus it is theoretically improbable that double or triple spontaneously resistant mutants can be found in most patients.[12] The resistant mutants do not have a selective advantage unless they are exposed to a drug to which they are already resistant while the mass of bacilli are sensitive. In such an instance, the sensitive bacilli are killed while the resistant organism continues to grow and eventually become the dominant forms of the infecting organisms found in that particular patient. Future therapy with the drug in question will be futile as the bacilli will remain resistant. If the patient is then exposed to a second course of drug therapy with yet another drug, the patient may end up with bacilli resistant to two and eventually more drugs as future courses of chemotherapy are used.

This scenario is referred to as serial selection of drug resistance and is predominant mechanism accounting for multi drug resistance to 2 or more drugs. The patient harboring multi drug resistant bacilli constitute a pool of chronic drug resistant infectious cases, propagating primary multi drug resistant disease. There are several possible explanations for the emergence of drug resistance which include lack of knowledge or improper prescription of chemotherapy by health care providers, no standardization of recommended regimes, poor adherence to prescribed drug regimes by the patients, absent or erratic drug supplies, the availability of fixed combinations of unproven bioavailability, availability in the open market of separate INH and Rifampicin prescription which may lead to inappropriate monotherapy and deficient or deteriorating tuberculosis control program resulting in inadequate administration of effective chemotherapy. [13,14] Regardless of the specific reasons which results in the emergence of drug resistance, the rates of acquired drug resistance are usually high as compared to primary drug resistance. It is important to note that primary drug resistance reflects the

consequences over a number of years of transmission from a pool of patients with acquired resistance, and therefore indicates poor program conditions.

An exception to this is the recent transmission in unique situations, such as MDRTB outbreaks in HIV infected patients.[15] In contrast, acquire drug resistance may indicate the current program conditions and is a very sensitive indicator of combined physician and patient adherence to internationally recommended treatment regimes. The high degree of acquired drug resistance in our study is truly alarming as this denotes not only the patient's negligence of his disease but also the treating physicians not following the standard treatment protocols. In a questionnaire survey conducted by the department of Chest Medicine KE Medical College, only 10% doctors in the city of Lahore were giving antituberculous drug according to the body weight, only 10% relied on the sputum smear examination and only 5% doctors were aware of drug resistance in TB. In a similar study conducted at the Agha Khan University Hospital Karachi in which case records of all patients hospitalized for TB were evaluated, it was discovered that only 53% patients had their sputum examined for AFB at the start of treatment, only 41% patients received the WHO recommended treatment for the continuation phase, no sputum smear test were done during treatment and over 70% patients lost to follow up, more than half of these during intensive phase.[16] Such situations in reality provide the necessary soil in which the plant of drug resistant disease grow rapidly. Looking on to the patients side, Shamshad Rasool et al observed compliance in only 49.53% of TB patients Globe et al found 70% of drug resistant cases to be uncomplaint. When we questioned our patients it was reviled that of the 52 cases of the retreatment cases, 32 (61.1%) patients were defaulters who took irregular treatment for some reason. Although one of the major factors responsible for the default is poor socioeconomic status, most of the patients didn't have proper knowledge regarding the proper dose and timing of the drug nor did they have any insight of their disease. Lack of the health education thus appears to be an important factor responsible for the few cure rates and the increase emergence of drug resistant bacilli at least in our society and given due importance, health education, continuous supply of the

medicine and strict adherence to the internationally accepted diagnostic and treatment protocols might help control this dangerous yet treatable disease and prevent the emergence of drug resistant strains.

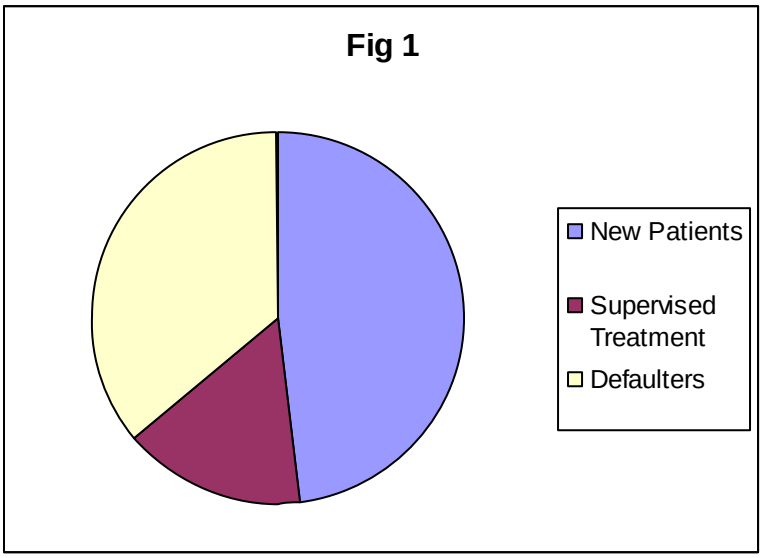
CONCLUSION

In conclusion factors responsible for drug resistance seemed to be related to the patient as well as health provider. Low socioeconomic status, improper dose schedule, under dosage and lack of health education apparently seemed to be responsible for most cases of drug resistance and if the spread of drug resistance is to be halted, it looks imperative that proper attention to be paid to each of these factors and a well managed and effective national TB control program with compliance of DOTS strategy is our need today.

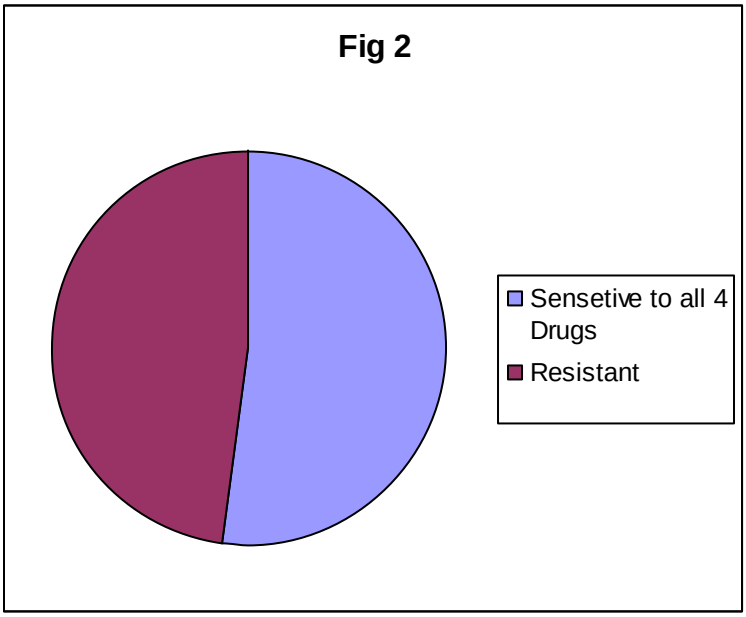
Table 1

Age distribution of Patients

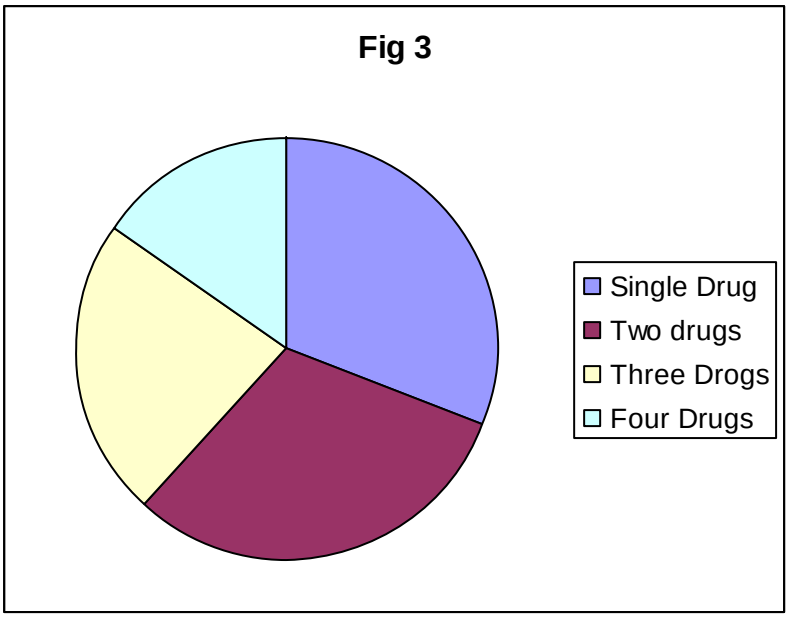
Age Group	**Male**	**Female**	**Total**	**%**
16-26 Years	8	12	20	20%
27-36 Years	12	8	20	20%
37-46 Years	8	16	24	24%
47-56 Years	12	8	20	20%
57 and above	12	4	16	16%
Total	52	48	100	100%



Categorization of Patients



Culture Results



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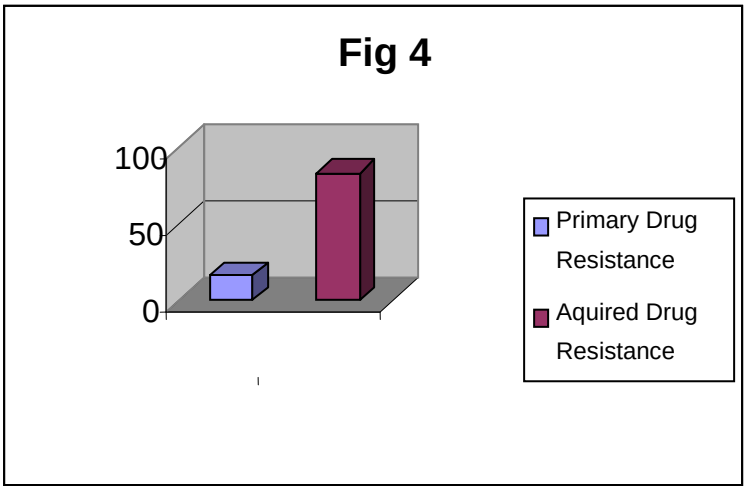
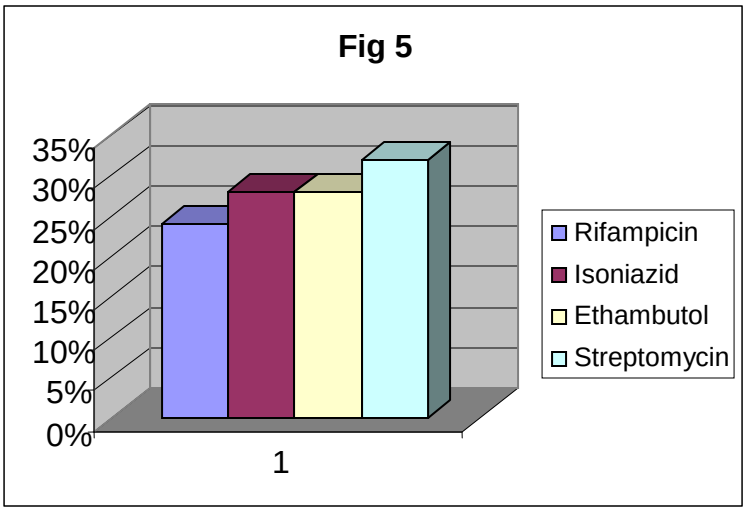


Comparison between Primary and Acquired drug Resistance



Resistance Pattern for Individual Drugs

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