ORIGINAL ARTICLE

RISING TREND OF MULTI DRUG RESISTANT TUBERCULOSIS: A THREAT TO COMMUNITY

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ABSTRACT

Background: To determine the frequency and resistance pattern of *Mycobacterium tuberculosis* in our settings. **Methods:** This study was carried out in Department of Microbiology, Armed Forces Institute of Pathology Rawalpindi, from January 2007 through December 2009. A total of 4159 specimens were received during the three years study period. All specimens were cultured on conventional LJ medium, BACTEC 460 system and fully automated MGIT 960 system. Sensitivity testing was performed on BACTEC 460 and MGIT 960 system. **Results:** Most of the specimens were received from hospitalized patients. Out of total 4159 specimens, 693 were culture positive and out of them 262 were multi drug resistant (MDR). Percentage of MDR isolates in 2007 till 2009 were 33, 42.1 and 40.4% respectively in AFIP. Maximum number of MDR isolates was recovered in 2008. **Conclusion:** The rising pattern of MDR tuberculosis is of great concern. There is an urgent need for the early diagnosis, drug susceptibility testing and isolation of patients with MDR tuberculosis.

KEY WORDS: Multi-drug resistant tuberculosis, *Mycobacterium tuberculosis*, Tuberculosis.

INTRODUCTION

Multidrug resistant tuberculosis (MDR-TB) strains are emerging as a real challenge in the combat to control tuberculosis.[1] The situation is even grave in developing countries. The global prevalence of MDR-TB is 1.4% in primary cases and in previously treated persons it is 13%.[2] Pakistan ranks sixth among the high burden TB countries having an incidence of 181/100,000 and prevalence 359/100,000 population.[3] MDR tuberculosis is defined as resistance to at least two first line drugs that is isoniazid and rifampicin which are the most effective drugs in the initial intensive therapy against tuberculosis.[4] The problem multiplies in resource poor but high burden countries where the second line drugs such as quino-lones are expensive and facilities for their sensitivities are only available in reference laboratories.[5]

Causes of MDR tuberculosis include inappropriate therapy, poor compliance, low quality medicines inadequate diagnostic facilities, late diagnosis and side effects of anti-tuberculous drugs resulting in the survival of resistant bacilli. The resistant strains are transmitted in the community from person to person through droplet nuclei.[7] Two dominant MDR strains in South East Asia are the Beijing strain and Central Asian strain 1 type (CAS1) or Delhi type genogroup which are expanding rapidly.[3]

This laboratory based study was aimed to determine the three years resistance pattern of *Mycobacterium tuberculosis* against the first line anti-tuberculosis drugs in targeted population (Northern areas) of Pakistan.

MATERIAL AND METHODS

This retrospective study was carried out in the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi from January 2007 through December 2009. This laboratory receives specimens from most of the Northern areas of Pakistan. All the samples received for MTB culture during the three years were included in the study. A total of 4159 consecutive clinical samples (sputum 1439, bronchoalveolar lavage 258, endobronchial washing1132, pus 251, tissue 417, pleural fluid 401, lymph node 129, urine 7, and stool 6) were dealt during the study period. Repeat samples from the same patient, blood and bone marrow specimens and improper specimens like saliva and pus swabs were excluded from the study. All the specimens (except sterile body fluids) were subjected to standard N-acetyl-L-cysteine NaOH digestion-decontamination method as described by Kent and Kubica.[8] Ziehl-Neelsen (ZN) acid fast staining was done and the remaining suspension was used for inoculation onto Lowenstein Jensen slant (LJ slant, Oxoid) and broth based system BACTEC 460 (Becton Dickinson Diagnostic Sys-

tems) or MGIT 960 (Becton Dickinson Diagnostic Systems) according to recommended protocol for either method.[9] Inoculation into BACTEC 460 and MGIT 960 12B vials was carried out according to the manufacturer's recommendations. Yielded growth was identified as *Mycobacterium tuberculosis* complex by BACTEC NAP (p-nitro-a acetylamino-b-hydroxy propiophenone) test on BACTEC 460, MGIT paranitrobenzoic acid (PNB) test and LJ slant with PNB.[10] Sensitivity testing on all the positive isolates was performed either on BACTEC 460 system or MGIT 960 system. In doubtful MDR cases, sensitivity pattern was confirmed by the detection of *inh* A, *rpo* B and *kat* G genes by polymerase chain reaction.[11] In case of BACTEC 460 system, anti-microbial susceptibility of mycobacterial isolates for primary drugs was carried out using stock solutions of Isoniazid (INH) 4 µg/ml, Rifampicin (RIF) 80 µg/ml, Ethambutol (ETH) 300 µg/ml and Streptomycin (STREPT) 240 µg/ml in liquid 7H12 Middle Brook Medium (12B vials BACTEC 460). The prepared final concentrations were as follows: INH 0.1 µg/ml, RIF 2 µg/ml, ETH 7.5 µg/ml and STREPT 6 µg/ml of the medium. The results were read as per the recommended protocols. In case of MGIT 960, lyophilized drugs (BACTEC MGIT 960 SIRE kit, Becton Dickinson, Baltimore, MD) were dissolved in diluent according to the manufacturer's instructions. From the dissolved drug solutions, 100 µl was pipetted into a 7-ml MGIT 960 tube. The final drug concentrations used were 1.0 and 4.0 µg/ml for STR; 0.1 and 0.4 µg/ml for INH; 5.0 and 7.5 µg/ml for EMB; 1.0 µg/ml for RIF. Readings were interpreted and reported.

ATCC 25177 of *Mycobacterium tuberculosis* was used as positive control. An uninoculated MIGIT tube was used as negative control. Institutional control strain of *Mycobacterium chelonei* and *Mycobacterium fortuitum* was used to check MIGIT PNB and BACTEC NAP.

RESULTS

Out of total 4159 samples received, 87 were contaminated which were not included in the study. Among the remaining 4072 samples 693 yielded mycobacterial growth (*Mycobacterium tuberculosis* complex, n=686; non-tuberculous mycobacteria, n=7) .There was only one sputum sample in which both *Mycobacterium tuberculosis* and *Mycobacterium fortuitum* was recovered. The three years data along with the previous two studies conducted at AFIP for culture positivity, number of MDR cases and overall resistance is shown in Table 1. Maximum percentage (42.1%) of MDR cases was in the year 2008 followed by 2009. Pulmonary specimens were the dominant yielding growth of MTB and MDR as shown in Fig. 1. The three years data for monoresistant, polyresistant (resistance to more than one drug excluding MDR), MDR and panresistant isolates is shown in Table 2. Out of total 686 positive *Mycobacterium tuberculosis* isolates in three years, 242 (35.3%) were sensitive to all the four first line drugs while only 11 (1.6%) were panresistant. Among the four first line drugs, resistance to isoniazid was dominant (53%) as shown in Fig 2.

Table 1: AFIP data of MDR isolates from 2004 to 2009.

Year	Total specimen	Culture positive	MDR (%)	Overall resistance (%)
2004[12]	1359	325	91(28)	49
2005-2006[5]	1247	290	89(31)	48
2007	1354	254	84(33)	62
2008	1396	192	81(42.1)	65.1
2009	1322	240	97(40.4)	67

Table 2: Resistance pattern of culture positive isolates (n=686)

Year	Monoresistance (n)	Polyresistance (excluding MDR)	MDR (n)	Panresistance (n)	Pansensitive (n)
2007	48	21	84	05	96
2008	31	11	81	02	67
2009	46	14	97	04	79

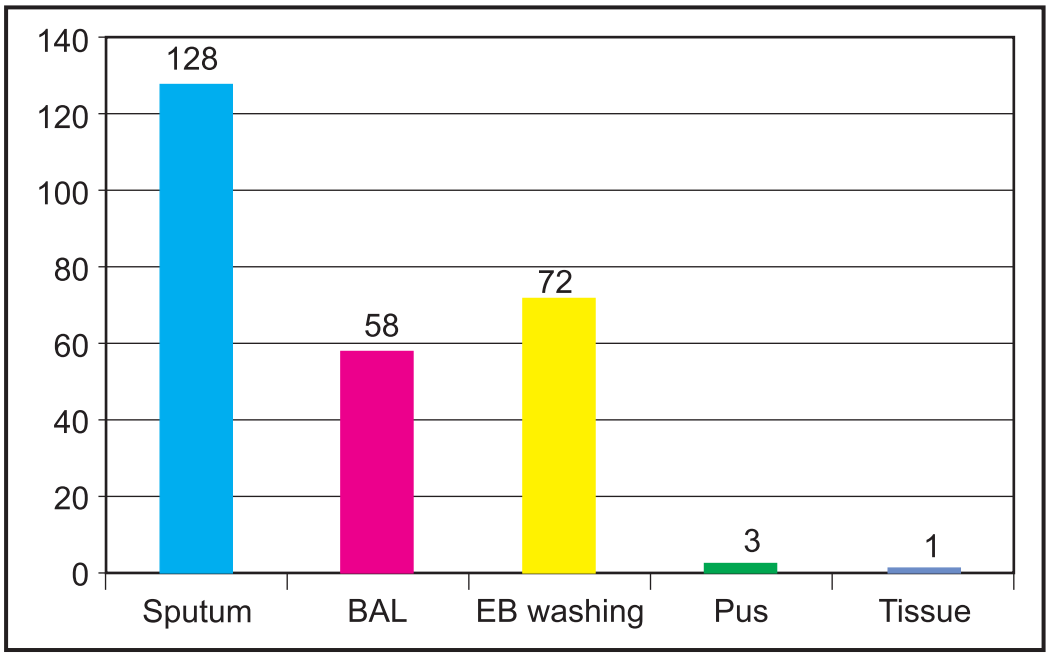

Fig 1: Relationship of MDR isolates with specimen type (n=262).

DISCUSSION

Early diagnosis, prompt treatment and timely isolation of patients are the keys to control the spread of tuberculosis in the community. Over the past few years, the MDR prevalence has increased worldwide.[13] According to the World Health Organization (WHO) International Union against Tuberculosis and Lung Disease working group report on global surveillance for antituberculosis drug resistance, the prevalence of primary MDR and acquired resistance in previously treated patients is 1.4% and 13% respectively.[14] Drug resistant tuberculosis is a real challenge in Pakistan. Except for one reported study, there is no complete data available due to lack of diagnostic and DST facilities in most of the areas.[6] In addition to sparse diagnostic modalities across the country, poor infection control practices are major factors contributing to the spread of these resistant bacilli.

The present study is a continuation of the previously reported studies done at our institute to determine the frequency of MDR-TB in our settings. Since 2004, there is a gradual surge in the MDR-TB cases in our settings as shown in Table 1. This rise is highest in the year 2008 with a frequency of 42.1% and overall resistance of 65.1% which is quite alarming. This high frequency in 2008 may be due to the increased number of received specimens in that year or it may be due to the fact that most of the specimens were from already treated/ complicated cases. However in one study conducted in Karachi, the reported prevalence was 47%.[12] As expected, in all laboratory based studies, the frequency is higher as compared to general population as most of the specimens come from hospitalized patients or from complicated cases. The reported prevalence of MDR-TB in various areas of Pakistan is between 8% to 25%.[15-18]

In our study, monoresistant isolates were 30.3%. Majority of them (53%) were resistant to isoniazid followed by rifampicin. This resistance pattern is comparable to other studies done in our institute previously as well as in various areas of Pakistan.[13, 15] However resistance to either INH or RIF is a major threat to the development of MDR-TB as 70.8% of the patients having resistance to either INH or RIF develop MDR-TB after treatment failure.[19] These two drugs are easily available, cheaper and most effective first line antituberculosis drugs and their resistance lead to resorting to costly and less effective therapeutic agents. Another interesting observation in our study was that the number of panresistant isolates was too low as compared to previous studies done at this institute. Similarly in another study, 35.2% of the total isolates were pan resistant.[13] These isolates are a real problem for the patients as well as for clinicians as they limit the choices for therapy.

As in most studies, pulmonary specimens maximally yielded growth of MTB including MDR. This highlights the importance of early isolation of patients with pulmonary tuberculosis as the dis-

ease is spread by droplet nuclei in most of the cases. However many patients especially in remote areas do not seek medical attention for cough and once they seek advice, the disease is already in the advanced stage. The major drawback of our study is that we could not discriminate between primary and acquired MDR due to lack of clinical details.

Despite improvements in the field of mycobacteriology, the National TB Control Program (NTCP) still faces many challenges. There is a need to strengthen the technical and administrative facilities at district levels as there is a shift of TB control planning from the national to the district level. New MDR-TB cases rose from 2.0 percent in 2003 to 3.2 percent in 2007 according to USAID report May 2009. Pakistan accounts for 57 percent of the MDR-TR burden within WHO's Eastern Mediterranean Region. The national or local data for extensively drug-resistant TB has not yet been reported in the country. However, Pakistan has significantly improved the quality assurance of laboratories and is establishing a National Reference Laboratory, steps that are critical to successful implementation of a MDR-TB treatment program and breaking the TB transmission chain.

CONCLUSION

A continuous rise in the cases of MDR-TB is a matter of great concern in our country. There is an urgent need to raise this grave issue. Use of broth based media will help in early detection and sensitivity of *Mycobacterium tuberculosis*. Multicentric studies at the national level will help in knowing the resistance pattern more accurately. This will help physicians to formulate a more effective empirical therapy, thereby reducing the drug resistance.

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