

Multi-drug Resistant Tuberculosis in Northern Pakistan

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Introduction

Tuberculosis is one of the leading causes of death due to any single infectious agent with more than 2 million deaths annually; most of them in the under-developed world.¹ In Pakistan, its incidence is estimated to be 171 per 100,000 of population.² Multi-drug resistant tuberculosis (MDR TB), defined as simultaneous resistance of *Mycobacterium tuberculosis* to both isoniazid and rifampicin³, is an emerging worldwide public health problem especially in the developing world. Both biological as well as socioeconomic factors have been responsible for the emergence of drug-resistant TB, which is a purely man-made phenomenon; a result of sub-optimal chemotherapy.^{3,4}

Early detection and proper treatment of MDR strains of *Mycobacterium tuberculosis* are the most effective measures for the control of MDR TB.⁵⁻⁷ Local knowledge of the drug susceptibility pattern of MDR clinical isolates is necessary to design an appropriate treatment regimen, thus preventing treatment failures and reducing the number of secondary cases of MDR TB.⁶ Our study is part of on-going laboratory-based surveillance of drug-resistance among *Mycobacterium tuberculosis* isolates in Northern Pakistan.

Material and Methods

The study was carried out at the Armed Forces Institute of Pathology, Rawalpindi, between September 2000 and August 2002. A total of 1359 pulmonary and extra pulmonary non-duplicate specimens were collected for mycobacterial culture and susceptibility testing from both known and suspected patients of tuberculosis, referred from various civil and military hospitals in Rawalpindi-Islamabad. Clinical suspicion of tuberculosis was based upon general findings suggestive of tuberculosis such as low-grade fever, malaise, weight loss, night sweats, anaemia, raised erythrocyte sedimentation rate, a reactive tuberculin

skin test (area of induration = 10 mm after intradermal dose of 5 TU) and organ-specific findings. Patients with past as well as present history of anti-tuberculosis treatment were included in the study. Multi-drug resistant tuberculosis (MDR TB) was defined as simultaneous resistance of an isolate to isoniazid and rifampicin.

Pulmonary specimens consisted of 2-5 ml early morning sputa and bronchial washings. Specimens from extra-pulmonary sites chiefly consisted of pus, lymph nodes, endometrium, urine, pleural and cerebrospinal fluids. Isolation of *Mycobacterium tuberculosis* from extra-pulmonary sites by culture was taken as the sole criterion for labeling the case as that of extra-pulmonary tuberculosis.

The specimens were processed by the standard N-acetyl-L-cysteine sodium hydroxide digestion-decontamination technique. Specimens like cerebrospinal, pleural and ascitic fluids were taken as sterile and processed as such for Ziehl-Neelsen (ZN) staining and culture centrifugation at 2000g for 20 minutes.

Culture was done on the radiometric Bactec 460 TB mycobacterial broth culture system (Becton Dickinson Instrument Systems, Towson, Md. USA). The specimens were incubated aerobically at 37°C Centigrade for up to 8 weeks. The Bactec 460 TB vials were monitored by instrument twice weekly for the first 2 weeks and then weekly. Vials showing a growth index (GI) of 10 or more were monitored daily. Those vials in which the GI reached 100 were taken as positive. Mycobacterial growth was confirmed on microscopy by a ZN acid-fast smear. Contamination of the medium was checked by Gram staining and by inoculation on to blood agar.

Mycobacterial isolates were identified as either *Mycobacterium tuberculosis* complex or nontuberculous mycobacteria (NTM) by the radiometric p-nitro-a-acetylamino-b-hydroxy propiophenone (NAP) test (Becton Dickinson

Diagnostic Systems) and the niacin test. Identification was performed once the GI of the culture had reached 300 or more.

Antimicrobial susceptibility testing was done by single concentration break point method using Bactec 460 TB system. Four first line anti-tuberculosis drugs were used: streptomycin (2.0 µg/ml), isoniazid (0.1 µg/ml), rifampicin (2.0 µg/ml) and ethambutol (2.5 µg/ml). Susceptibility testing was performed at a GI of 500 or more.

Results

Out of 1359 clinical specimens processed, 899 were sputa/bronchial washings and 460 were specimens from extra-pulmonary sites. Mycobacteria were isolated from 410 specimens. Among the pulmonary isolates (n=312), 237 (76%) were Mycobacterium tuberculosis and 75 (24%) were NTM. Among the extra-pulmonary isolates (n=98), Mycobacterium tuberculosis were isolated from 88 (90%)

Table 1. Mycobacterial isolates from pulmonary and extra-pulmonary specimens (n=1359)

	Pulmonary (n=899)	Extra-Pulmonary (n=460)
MTB (n=325)	237	88
NTM (n=85)	75	10
Total (n=410)	312	98

MTB = Mycobacterium tuberculosis

NTM = Nontuberculous mycobacteria

Specimens while NTM were isolated from 10 (10%) Specimens (Table 1.)

Table 2. Resistance among mycobacterial isolates against first-line anti-tuberculosis drugs* (n=410)

	Single-drug resistance	Multi-drug resistance+ not MDR	Resistant to two drugs but	No resistance
MTB (n=325)	48 (15%)	91 (28%)	21 (6%)	165 (51%)
NTM (n=85)	4 (5%)	65 (76%)	12 (14%)	4 (5%)

MTB = Mycobacterium tuberculosis

NTM = Nontuberculous mycobacteria

* Rifampicin, Isoniazid, streptomycin & ethambutol

+ Simultaneous resistance against Isoniazid & rifampicin (including isolates resistant to 3 or 4 drugs)

Antimicrobial susceptibility of each isolate was tested against streptomycin, isoniazid, rifampicin and ethambutol. Among Mycobacterium tuberculosis (n=325), 48 isolates (15%) were single-drug resistant, 91 (28%) were multi-drug resistant (including isolates resistant to 3 or 4 drugs) and 24 (7%) were resistant to all the four drugs. Twenty one (6%) isolates were resistant to at least two anti-tuberculosis drugs but were not MDR. One hundred and sixty five (51%) isolates were susceptible to all the four drugs. Among the NTM (n=85), 65 (76%) isolates were MDR while 52 (61%) were resistant to all the four drugs (Table 2). Resistance against rifampicin and isoniazid was 32% and 37% respectively among Mycobacterium tuberculosis (Table 3). Cases of primary and acquired resistance were not separated.

Table 3. Individual drug resistance with or without resistance to other drugs among isolates of Mycobacterium tuberculosis (n=325).

Antimicrobial	No. of isolates resistant	Percentage
Streptomycin	61	19
Isoniazid	120	37
Rifampicin	105	32
Ethambutol	54	17

Discussion

Resistance in dividing mycobacteria occurs spontaneously due to random mutations even in

drug-free environment. However, antimicrobials provide the selective rug pressure that allows the pre-existing resistant mutants to become predominant. Development of multi-drug resistance is attributed to inadequate treatment which means inappropriate treatment regimen, sub-standard antimicrobials or poor patient compliance.^{3,4,7}

We observed very high levels of resistance in our isolates. The frequency of single-drug resistance (15%) and especially multi-drug resistance (28%) among Mycobacterium tuberculosis was much higher than the figures reported by two worldwide studies: the World Health Organization (WHO) has reported a global median single-drug resistance prevalence of 11.1% (range 2.9-40.8%) and MDR TB prevalence of 1.8% (range 0-18.1%)³ while in a survey of 35 countries, Pablos-Mendez et al reported 12.6% (range 2.3-42.4%) of Mycobacterium tuberculosis isolates as resistant to at least one anti-tuberculosis drug and 2.2% (range 0 to 22.1%) as multi-drug resistant.⁹

Very high resistance against rifampicin and isoniazid was noted in our population. These two antimicrobials are the most effective anti-tuberculosis drugs available with us⁷ and increasing resistance against them is a matter of great concern as we would be left with only the less effective drugs for treatment of TB.

Karamat et al, in 1999 had reported single-drug resistance among Mycobacterium tuberculosis from both pulmonary and extra-pulmonary isolates at 21%, multi-drug resistance at 14% and resistance to all four first-line drugs at 5% in Rawalpindi-Islamabad.¹³ The increase in multi-drug resistance in the short time between the two studies is quite dramatic. It is likely that secondary resistance was responsible for most of the MDR cases in our study. Failure to discriminate between primary and secondary resistance in our study is a definite short-coming. However it is very difficult to get complete clinical data in our set up, especially when conducting a large scale surveillance study. Even after allowing for the bias towards the higher side, the rates of MDR TB are still very high. A recent study from Karachi has reported MDR TB rates of 24.4%.¹¹ This suggests that the increase in resistance against anti-tuberculosis drugs is not restricted to our setting and is probably a nationwide phenomenon.

In Pakistan tuberculosis is mostly diagnosed on clinical suspicion and on therapeutic response to anti-tuberculosis drugs, rather than on the basis of culture isolation. This results in inappropriate use of anti-tuberculosis drugs. Furthermore, compliance with treatment remains poor. Despite TB having

been declared a national emergency in 2001, implementation of the national TB control programme has been hampered by under-developed health facilities, lack of resources and poor management.

The DOTS strategy adopted in 1995 has shown some progress with DOTS coverage at 24% in 2001 but with high drop-out rates of 17%.² All these factors appear to be responsible for the high levels of MDR TB in our population.

The increasing level of drug resistance among mycobacterial isolates in our population is most alarming. Adequate control strategies have already been devised; what has been lacking is a serious commitment to implement these measures. Unless we find the will and resources to tackle this unfolding crisis on sound scientific grounds, the problem would get totally out of control.

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Abstract

Objective: To determine the resistance pattern of Mycobacterium tuberculosis isolates in Rawalpindi-Islamabad.

Methods: The study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi between September 2000 and August 2002. We examined 1359 pulmonary and extra-pulmonary specimens from suspected cases of tuberculosis. The radiometric Bactec 460 TB system was used for culture and antimicrobial susceptibility testing.

Results: Mycobacterium tuberculosis was isolated from 325 clinical specimens. Antimicrobial susceptibility of the isolates was tested against the four first-line anti-tuberculous drugs (rifampicin, isoniazid, streptomycin and ethambutol). Fifteen percent of the isolates were resistant to a single drug, 28% were multi-drug resistant including 7% which were resistant to all the four drugs. The overall resistance against individual drugs was rifampicin 32%, isoniazid 37%, streptomycin 19% and ethambutol 17%.

Conclusion: The increasing level of drug resistance among mycobacterial isolates in our population is most alarming. Strict implementation of control measures is required to combat this unfolding crisis (JPMA 54:469;2004).