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Drug resistance pattern in *Mycobacterium tuberculosis* to the first line drugs of pulmonary tuberculosis patients at Hazara Region, Pakistan

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SUMMARY

Drug resistance pattern in *Mycobacterium tuberculosis* to the first line drugs of pulmonary tuberculosis patients at Hazara Region, Pakistan

Introduction: Multidrug resistant TB (MDR-TB), defined as resistance to at least rifampicin and isoniazid together, has been rapidly spreading in recent years. In new pulmonary tuberculosis patients, rapid spread of MDR-TB and XDR-TB challenging the effectiveness of national TB control programs especially in many low-income countries. This study was aimed to determine the resistance pattern of *Mycobacterium tuberculosis* among new cases, cured, failure, relapse, defaulted, treatment completed, treatment not evaluated and suspect to be resistant to first line antitubercular drugs of pulmonary tuberculosis (PTB).

Materials and Methods: The study was conducted during 2013-2016 in which 148 patients were enrolled infected with pulmonary TB. Three sputum samples were consecutively collected and transported for drug analysis to the Provincial Reference Laboratory (PRL) at Hayatabad Medical complex Peshawar (HMCP) TB laboratory, within three days of collection at +4°C in a cold box. Using the standard proportion method, drug susceptibility test was performed on 132 (89.2%) sputum samples for rifampicin (R), isoniazid (H), pyrazinamide (Z), ethambutol (E), and streptomycin (S).

Results: Prevalence of resistance to one drug was 5 (3.4%). The highest proportion of mono-drug resistance was observed against E, 3 (2%), followed by H, 1 (0.7%), and R, 1 (0.7%). Pattern of resistant to two drugs was 14 (9.5%). The proportion of poly resistant was 3 (2%). 112 (93.33%) diagnosis patients were MDR-TB.

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Conclusion: To formulate an effective regimen, it is important to know drug resistant pattern because drug resistant pattern varies from different period of time also from one place to another.

Key words: *Mycobacterium tuberculosis*, Drug resistant pattern, Multidrug resistance tuberculosis, Drug sensitivity testing

ÖZET

Pakistan, Hazara Bölgesinde akciğer tüberkülozu hastalarının birinci basamak tedavilerinde *Mycobacterium tuberculosis* ilaç direnci paterni

Giriş: En azından rifampisin ve isoniazide birlikte direnç olması olarak tanımlanan çok ilaca dirençli tüberküloz (ÇİD-TB) son yıllarda hızla yayılmaktadır. Yeni akciğer tüberkülozu olgularında, ÇİD-TB'nin hızlı yayılması ve XİD-TB, Ulusal TB kontrol programlarının etkinliğini özellikle düşük gelir düzeyi olan çoğu ülkelerde zorlaştırmaktadır. Bu araştırma yeni olgu, tedavi olmuş olgu, tedavi başarısızlığı, relaps, tedavisi tamamlanmış, tedavisi değerlendirilmemiş ve birinci kuşak antitüberküloz ilaçlara direnç şüphesi olan olgularda *Mycobacterium tuberculosis* direnç paternini belirlemeyi amaçlamaktadır.

Materyal ve Metod: Bu araştırmaya 2013-2016 yılları arasında akciğer tüberkülozu ile enfekte olan 148 hasta dahil edildi. Ardışık üç balgam örneği alınmış ve ilaç analizi için alındıktan sonra üç gün içinde +4°C'de tutularak Hayatabad Medikal kompleks içinde Peshawar (HMCP) TB laboratuvarına gönderildi. Standart oranı yöntemi kullanılarak 132 (%89.2) balgam örneği ile rifampisin (R), isoniazid (H), pirazinamid (Z), etambutol (E) ve streptomisin (S) için ilaç duyarlılığı testi uygulandı.

Bulgular: Tek ilaç direnci prevalansı 5 (%3.4) olarak bulundu. En yüksek tek ilaç direnci oranı 3 (%2) ile E, sırasıyla 1 (%0.7) ile H ve 1 (%0.7) ile R'ye karşı bulundu. On dört (%9.5) hastada iki ilaca direnç, 3 (%2) hastada polirezistans ve 112 (%93.3) hastada ÇİD-TB saptandı.

Sonuç: Etkin bir rejimi oluşturmak için ilaç direnç mekanizmalarını bilmek önemlidir. Çünkü direnç özellikleri zaman ve yere bağlı olarak değişmektedir.

Anahtar kelimeler: *Mycobacterium tuberculosis*, İlaç direnci paterni, Çok ilaca dirençli tüberküloz, İlaç duyarlılık testi

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by bacterial strains belonging to the *Mycobacterium tuberculosis* complex. Various implications for management and treatment may be carried out by difference in drug resistant pattern (1). For the purposes of monitoring, on the basis of drug sensitivity testing (DST) in clinical isolates of *M. tuberculosis*, drug-resistant cases were categorized as multidrug resistance (MDR); resistance to rifampicin and isoniazid together, extensive drug resistance (XDR); when there is resistance to one of the second line injectable, to any fluoroquinolone and multidrug resistance (2) and rifampicin resistance (RR); genotypic and phenotypic detection of rifampicin resistance, without or with development of resistance to any other anti-tuberculosis drugs. Includes mono-drug resistance, poly-drug resistance, MDR or XDR (3). Multidrug resistant TB (MDR-TB), defined as resistance to at least rifampicin and isoniazid together, has been rapidly spreading in recent years. In 2012, globally assessed that incidence of MDR-TB was 3.6% and 20% cases of MDR-TB, having variation in prevalence with evident geography (4). The rate of MDR-TB ranges from 1.4% to 11.6% while resistance to one or more anti tubercular drugs ranges 3% to 37.3% in Africa (5-8). In new pulmonary tuberculosis patients in many low-income countries rapid

spread of MDR-TB and XDR-TB challenging the effectiveness of national TB control programs (8,4). To strengthen the treatment outcomes and to control spread of resistant strains of *M. tuberculosis* especially MDR-TB. Early diagnosis, treatment, expanding diagnostic capacity for mycobacterial culture and drug susceptibility test (DST) are very important (4). This study was aimed to determine the resistance pattern of *M. tuberculosis* among new cases, cured, failure, relapse, defaulted, treatment completed, treatment not evaluated and suspect to be resistant to first line anti-tubercular drugs of pulmonary tuberculosis (PTB).

MATERIALS and METHODS

Samples Collection and Transportation

The samples were collected and transported in sterile conditions as recommended by WHO, three sputum samples (spot, morning, and spot) were consecutively collected from each study participant (9). Ziehl-Neelsen (ZN) positive samples were collected and transported to the Provincial Reference Laboratory (PRL) at Hayatabad Medical complex Peshawar (HMCP) TB laboratory for DST, within the three days of collection at +4°C in a cold box. At HMCP, for each patient the sputum samples were pooled into 50 mL sterile screw capped bottle and processed within 24 hours for mycobacterial culture (10).

This study was conducted at Programmatic management of Drug Resistant Tuberculosis unit, Ayub Teaching Hospital (PMDT-ATH) Abbottabad, Pakistan. The study population included 148 patients.

Exclusion Criteria and Inclusion Criteria

Patients with extrapulmonary or having history of second line antituberculosis treatment were excluded.

All those patients were included in the study who had conformed rifampicin resistant through GeneXpert, Ziehl-Neelsen staining positive for acid fast bacilli, having strong suspect to be drug resistant, previous history of antitubercular treatment (Category I and Category II) and from their radiographic examination.

Ethical Approval

The study was approved by the institutional review board (IRB), of Ayub Medical Institute, Abbottabad, Pakistan.

Isolation and Detection of *M. tuberculosis*

2.5-10 mL volume sample of sputum was digested, homogenized and decontaminated using standard method as reported by Kent and Kubica (11). The samples were concentrated by centrifugation for 15 minutes at 3000 rpm, and remove the supernatants. Sediments were reconstituted with Sterile saline phosphate buffer (PBS). Initially the pellets of the sediments were inoculated on Lowenstein Jensen (LJ) slants (contain glycerol (0.75%) and pyruvate (0.6%) and incubated for 8 weeks at 35-37°C and then growth was measured and conformed by ZN staining and microscopy (12).

Drug Susceptibility Testing

Using indirect proportion method on LJ media, drug susceptibility testing was performed for rifampin (R, 1 µg/mL), isoniazid (H, 0.2 µg/mL), ethambutol (E, 5 µg/mL), pyrazinamide (PZA) and streptomycin (S, 2 µg/mL) (13). Bacterial suspensions were prepared and inoculated on L.J Slants containing the drugs. Subsequently, the L.J slants were incubated at 35-37°C. Isolates were considered resistant by observing growth on slants.

RESULTS

Sociodemographic Characteristic

Sociodemographic characteristics of the entire patients

are presented in Table 1. A total of 148 sputum samples were collected from new pulmonary tuberculosis patients. Comprised of 65 (44%) were male while 83 (56%) were females, of whom 91 (61.5%) were married, having the literacy rate 95 (64.2%). One twenty-one (81.8%) of these patients belonging to the rural area while most of these patients were house wives 75 (50.7%). The mean age of patients was 32.47 (SD + 15.99).

Drug Susceptibility Pattern

Drug susceptibility testing (DST) of *M. tuberculosis* isolates for 148 sputum samples were performed on first line anti tuberculosis drugs (H, R, Z, E and S). Drug susceptibility test showed that 12 (8.1%) sputum samples were sensitive to all the tested drugs. As of the total patients 120 (94.6%) showed drug resistance to at least one or more of the first line drugs by different patterns while out of the tested samples 16 (10.8%) cultures were reported as contaminated or did not reveal any growth. Prevalence of overall resistance to one drug was 5 (3.4%). The highest proportion of mono resistance was observed against E, 3 (2%), followed by H, 1 (0.7%), and R, 1 (0.7%). Pattern of combined resistant towards two drugs was 14 (9.5%). The proportion of poly resistant was 3 (2%). A comprehensive detail of the mono and poly drug resistance pattern is given in Table 2. Furthermore, 112 patients were diagnosed as MDR-TB. Drug susceptibility testing among MDR patients is shown in Figure 1, in which 100% resistance was shown by the cultures against isoniazid and rifampicin. During the study period, performance of the Drug Resistant Tuberculosis unit, Ayub Teaching Hospital (PMDT-ATH) Abbottabad, Pakistan was also analyzed with respect to facilitation and treatment of the patients. During this period, total 5.4% new TB cases were registered, while 17.6% were cured, 60 (40.54%) of failure cases were attributed to previous ATT history as depicted in figure 2, but 7.43% patients were successfully treated.

DISCUSSION

The high magnitude of MDR-TB was found among the poor, lower economic class people and illiterate. In earlier studies these factors were also reported (14,15). Economic status of the patients revealed that TB was converted to MDR-TB mostly in females (56.08%). In Pakistan females are more immune-compromised and limited to home in most of their life span, so the

Table 1. Sociodemographic characteristics status of study participants

Characteristics	Parameters	n= 148	%
Gender	Male	65	43.91
	Female	83	56.08
Age group (years)	8-15	5	3.4
	16-25	53	35.81
	26-35	46	31.08
	36-45	16	10.81
	46-55	11	7.43
	56-65 ≥ 65	9 8	6.08 5.4
Marital status	Married	91	61.5
	Unmarried	54	36.6
	Widowed	1	0.7
	Divorced	2	1.4
Educational status	Illiterate	95	64.2
	Literate	53	35.81
Occupational status	Civil servant	7	4.73
	Housewife	75	50.7
	Daily labour	6	4.05
	Unemployed	26	17.6
	Student	23	15.54
	Merchant	1	0.7
	Farmer	1	0.7
Place of residence	Pensioner	3	2.02
	Driver	6	4.05
	Urban	27	18.24
	Rural	121	81.8

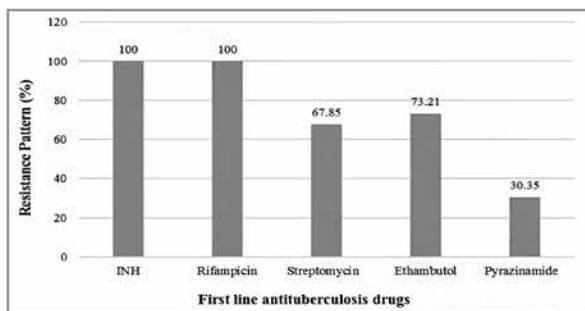


Figure 1. Pattern of drug resistance in MDR patients.

restricted environment, improper ventilation and poor hygienic conditions are responsible for such type of infections. Findings of this study agreed with previous published reports (16-18) who reported more preva-

Table 2. Resistant pattern to 1st line drugs

Drug Resistant Pattern	n (%)
Sensitive to all the drugs	12 (4.32)
No growth	16 (10.8)
Resistant to only one drug	
H only	1 (2.2)
R only	1 (0.54)
S only	0
E only	3 (1.62)
Resistant to two drugs	
HR	11 (11.4)
HS	1 (0.54)
RE	1 (0.54)
RS	1 (0.54)
Resistant to three drugs	
HRE	14 (8.1)
HRZ	4 (2.7)
HRS	12 (8.1)
Resistant to four drugs	
HRES	41 (22.7)
HREZ	7 (3.8)
HRZS	3 (1.62)
Resistant to five drugs	
HREZS	20 (11.4)

R: Rifampicin, H: Isoniazid, Z: Pyrazinamide, E: Ethambutol, S: Streptomycin.

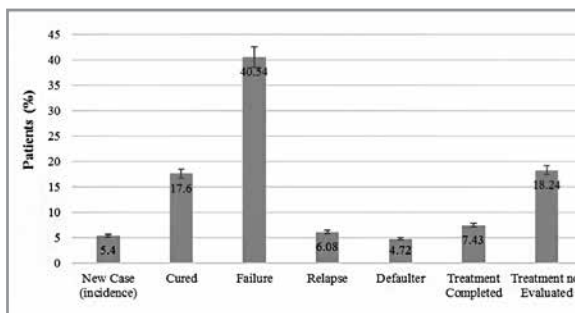


Figure 2. Treatment outcome in patients with previous history to first line anti-TB treatment.

lence in female population. In Balochistan Pakistan it is also revealed by previous study report that ratio of male to female was 1:14 in case of tuberculosis as reported by Dogar et al. (19) is a great matter of concern. In western province of Pakistan women are also neglected in term of treatment as they are mostly restricted to be household women, which will maximize the likelihoods of more contacts with infected

one. Disease in this study was found in all age groups equally but was highest in productive and mature age group of 21-40 years which is (46.76%). Finding of this study was in line with previous study by (20-22) who reported that tuberculosis is a disease of adult productive 20-50 years age group.

In this study, total 148 samples were subjected for culture which were sputum positive out of which 132 (89.2%) samples had growth at end of 8 weeks, while the rest 16 (10.8%) samples did not reveal any growth. For all first line antitubercular drugs such as rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide drug sensitivity testing (DST) were carried out. 12 specimens (8.1%) were observed sensitive to all first line antitubercular drugs, while in remaining 120 (81.08%) of the cases resistance to one or more drugs was seen. MDR were obtained in 112 (75.7%) strains of *M. tuberculosis*. Resistance of mono and poly drugs was found in 5 (3.4%) and 3 (2.02%) isolates respectively. Variable patterns of resistance were observed in 112 MDR-TB isolates, out of which the most resistant pattern was against HRES 41 (36.6%) followed by HREZS 20 (17.9%) respectively. The observed resistance to two or more than two drugs was 77.7%.

Several risk factors were investigated to assess the association of drug resistance development. In development of drug resistant TB one of the strongest risk factor is the previous history of pulmonary TB. In the presence study MDR-TB development is significantly contributed by illiteracy and low socioeconomic status. In TB due to poor compliance which is related directly to financial burden on the family and illiteracy leads to higher prevalence of MDR-TB.

Conclusion

In this study the observed high rate of acquired drug resistance shows that National TB control program is not effective and during recent years improper/irregular use of antituberculosis drugs have led to multiplication and accumulation of drug resistant strains of *M. tuberculosis*.

For the control of TB disease, a compounding factor is MDR-TB. MDR-TB is a man-made problem and efforts should be taken to prescribe the suitable regimen for the prescribed duration of the treatment. To formulate an effective regimen, it is important to

know drug resistant pattern because drug resistant pattern varies from different period of time also from one place to another.

REFERENCES

1. Iseman MD. Treatment of multidrug-resistant tuberculosis. *N Engl J Med* 1993;329(11):784-91.
2. WHO. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis. In: *Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis* 2014.
3. Jaramillo E. Guidelines for the programmatic management of drug-resistant tuberculosis. *World Health Organ* 2008.
4. WHO. Global tuberculosis report 2013. *World Health Organ*.
5. Glynn JR, Crampin AC, Traore H, Yates MD, Mwaungulu FD, Ngwira BM, et al. Mycobacterium tuberculosis Beijing genotype, northern Malawi. *Emerg Infect Dis* 2005;11(1):150-3.
6. Kassa-Kelembho E, Bobossi-Serengbe G, Takeng E, Nambea-Koisse T, Yapou F, Talarmin A. Surveillance of drug-resistant childhood tuberculosis in Bangui, Central African Republic. *Int J Tuberc Lung Dis* 2004;8(5):574-8.
7. Kuaban C, Bercion R, Noeske J, Cunin P, Nkamsse P, Ngo Niobe S. Anti-tuberculosis drug resistance in the West Province of Cameroon. *Int J Tuberc Lung Dis* 2000;4(4):356-60.
8. Lin J, Sattar A, Puckree T. An alarming rate of drug-resistant tuberculosis at Ngwelezane Hospital in northern KwaZulu Natal, South Africa. *Int J Tuberc Lung Dis* 2004;8(5):568-573.
9. WHO. Guidelines for surveillance of drug resistance in tuberculosis 2009.
10. Urassa W, Mugusi F, Villamor E, Msamanga G, Moshiro C, Bosch R, et al. Primary antimicrobial resistance among Mycobacterium tuberculosis isolates from HIV seropositive and HIV seronegative patients in Dar es Salaam Tanzania. *BMC Res Notes* 2004;1(1):58.
11. Kent PT, Kubica GP. Public health mycobacteriology: a guide for the level III laboratory. *US Department of Health and Human Services, Public Health Service, Centers for Disease Control* 1985.
12. Huard RC, de Oliveira Lazzarini LC, Butler WR, van Soolingen D, Ho JL. PCR-based method to differentiate the subspecies of the Mycobacterium tuberculosis complex on the basis of genomic deletions. *J Clin Microbiol* 2003;41(4):1637-50.
13. Canetti G, Fox W, Khomenko AA, Mahler H, Menon N, Mitchison D, et al. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity

- tests in tuberculosis control programmes. *Bull World Health Organ* 1969;41(1):21.
14. Portaels F, Rigouts L, Shamputa I, Van Deun A, Abdel Aziz M. *Tuberculosis drug resistance in the world*. Reichman and Hershfield's *Tuberculosis; A Comprehensive, International Approach*. 3rd ed. 2006.
 15. Oliva V, Cezário G, Cocato R, Marcondes-Machado J. *Pulmonary tuberculosis: hematology, serum biochemistry and the relation with the disease duration*. *J Venom Anim Toxins Incl Trop Dis* 2008;14(1):71-81.
 16. Ayaz S, Nosheen T, Khan S, Khan SN, Rubab L, Akhtar M. *Pulmonary tuberculosis: still prevalent in human in Peshawar, Khyber Pakhtunkhwa, Pakistan*. *Pakistan Journal of Life Sciences* 2012;10:39-41.
 17. Baloch S, Devrajani B, Rahman A. *The prevalence of smear positive pulmonary tuberculosis in Hyderabad, Sindh, Pakistan*. *Elixir Human Physio* 2013;60:16447-50.
 18. Ullah S, Shah SH, Rehman A, Kamal A, Begum N, Khan G. *Extrapulmonary tuberculosis in Lady Reading Hospital Peshawar, NWFP, Pakistan: survey of biopsy results*. *J Ayub Med Coll Abbottabad* 2008;20(2):43-6.
 19. Dogar OF, Shah SK, Chughtai AA, Qadeer E. *Gender disparity in tuberculosis cases in eastern and western provinces of Pakistan*. *BMC Infect Dis* 2012;12(1):244.
 20. Sutherland I, Fayers PM. *The association of the risk of tuberculous infection with age*. *Bull Int Union Tuberc Lung Dis* 1974;50(1):70-81.
 21. Shafee M, Abbas F, Ashraf M, Mengal MA, Kakar N, Ahmad Z, et al. *Hematological profile and risk factors associated with pulmonary tuberculosis patients in Quetta, Pakistan*. *Pak J Med Sci* 2014;30(1):36.
 22. Ahmad T, Jadoon MA, Khattak MNK, et al. *Prevalence of sputum smear positive pulmonary tuberculosis at Dargai, District Malakand, Pakistan: A four year retrospective study*. *Egypt J Chest Dis Tuberc* 2016;65(2):461-4.