Original Article

Assessment of Resistance in Multi Drug Resistant Tuberculosis Patients

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Abstract

Objective: To study MDR-TB isolates and to identify primary and secondary resistance at microbiology laboratory Aga Khan University, Karachi, Pakistan.

Methods: All samples positive for Mycobacterium tuberculosis (MTB) received during January - September 2004 were reviewed for drug resistance pattern as well as for history of previous antituberculous drugs exposure.

Results: Out of 216 Mycobacterium tuberculosis cultures, 138 (64%) showed resistance to one or more agents. Multi drug resistance (MDR) was observed in 102 (47%) isolates. Of 138 drug resistant isolates; primary resistance to any one or more agent was noted in 31(39%) and secondary (acquired) resistance in 107 (79%) isolates. On analysis of the 102 MDR-TB strains 8 (10%) showed primary resistance while 94 (69%) showed secondary resistance.

Conclusion: In this group MDR-TB was mainly associated with previous anti-tuberculous treatment. However, primary MDR was also observed and reflects dissemination of MDR cases within the community (JPMA 56:397;2006).

Introduction

Drug resistant tuberculosis is becoming a major concern in the control of tuberculosis (TB)[1]. A survey conducted by the World Health Organization and the International Union against Tuberculosis and Lung Disease in 35 geographic sites, revealed that drug resistant

tuberculosis is ubiquitous and prevalence of primary resistance to at least one drug is around 10.7 percent.[2]

Multi drug resistant tuberculosis (MDR TB), defined as resistance of Mycobacterium tuberculosis to at least Isoniazid and Rifampicin[3] is a major threat to the tuberculosis control program. The mortality from multi-drug resistant tuberculosis is 40-60%[4] which is equivalent to the outcome of untreated tuberculosis. Therefore, the spread of resistant strains in poor countries would mean a return to pre-chemotherapy patterns of mortality.

Acquired drug resistance was defined as the acquisition of resistance to anti-tuberculosis drugs by the multiplication of the resistant mutant strain of bacteria as a result of inadequate chemotherapy. Primary drug resistance, on the other hand, develops in patients who become infected with a resistant strain without ever having been treated with anti-tuberculosis drugs.[5] While resistance in previously treated patients is likely to reflect past incorrect or irregular treatment, resistance in new, untreated patients is evidence of transmission of resistant strains. Globally, the prevalence of MDR-TB is reported at 1.4% in primary cases and 13% in previously treated patients.[6] Early detection and treatment of MDR strains is important in disease control. In addition, knowledge of drugs susceptibility pattern in MDR clinical isolates is necessary in order to design appropriate treatment regimen.

In Pakistan, where incidence of tuberculosis is estimated at 181 per 100,000 populations, rising drug resistance is alarming.[7] Recent report[8] showed 28% of MDR TB strains from northern Pakistan, a marked difference from previously reported[9] rate of 16% MDR in 2001 (Table-1). Continuous monitoring of drug resistance pattern especially of MDR isolates to determine the extent of primary (1^o) and acquired (2^o) resistance is a crucial need for future TB control in Pakistan. In this study we assessed the primary and secondary MDR isolates.

Material and Methods

This study was conducted in the Clinical Microbiology Laboratory of The Aga Khan University between January-September 2004. Detailed history of anti-tuberculous therapy was obtained and reviewed in all patients with positive cultures for Mycobacterium tuberculosis (MTB).

All samples (except those from the sterile sites) were decontaminated with N-acetyl-L-cysteine (NALC) sodium hydroxide. Sterile body fluids were processed without decontamination procedure. The sediments were used for AFB microscopy and cultured on BACTEC (12Bvial Becton Dickenson) and LJ slant (Oxoid).

Smears for microscopy were checked using Auramine staining, positive slides were further confirmed by staining with Kinyoun modification of Z-N stain.

Cultures were performed using LJ slants and BACTEC 460. For LJ slant 0.1 ml of concentrated specimen was inoculated and incubated for 8 weeks.

Similarly BACTEC vials were inoculated with 0.5 ml of specimen and incubated at 37^oC after supplementation of medium with PANTA; containing Polymyxin B, AmphotericinB, Nalidixic acid,Trimethoprim and Azlocilin. Growth index of inoculated vial were checked for four weeks. Growth from the positive vials and LJ slant tube were first stained with Kinyoun and confirmed using NAP test.

Indirect antimicrobial susceptibility test

Antimicrobial susceptibility for four primary drugs including Isoniazid (INH) 1.0 µg/ml, Rifampicin (RIF) 1.0 µg/ml, Ethambutol (E) 10.0 µg/ml and Streptomycin(S)

Table 1. Reported antituberculous resistance from Pakistan.

Reference	Sample size	Overall Resistance (%)			Resistance (%)					MDR (%)		
		Total	1^0	2^0	INH	RIF	PYR	ETH	STREP	Total	1^0	2^0
Karachi 1993 (14)	145	-	17	36	-	-	-	-	-	-	-	-
Karachi 1996 (15)	156	45	-	-	27	11	-	14.5	13	8	-	-
Rawalpindi 1999 (16)	300	53	-	-	26	24	-	23	28	14	-	-
Lahore 2001 (9)	228	52	-	-	25	25	24	10	21	16	7.3	26
Sind 2002 (17)	50	73	-	-	60	24.4	-	22	38	25	-	-
Lahore 2002 (18)	100	36	-	-	25	15	-	12	19	11	-	-
Lahore 2003 (19)	678	53	-	-	26	28	29	15	24	16	-	-
Rawalpindi 2004 (8)	325	49	-	-	37	32	-	17	19	28	-	-

1^O= Primary resistance, 2^O= Secondary resistance, Overall resistance= Resistance to any antituberculous agent.
MDR= Multi drug resistance , INH=Isoniazid; RIF=Rifampicin; PYZ=Pyrazinamide; ETH=Ethambutol; STREP=Streptomycin
% Resistance= Resistant organisms as% of total isolates.

10.0 µg/ml was tested using modified agar proportion method. Disc elusion sensitivity plates were prepared using paper sensitivity disc (BBL).

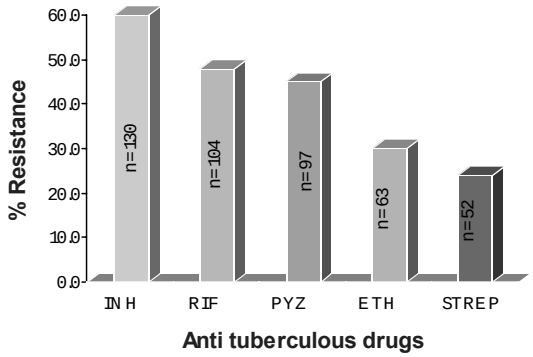
McFarland No.1 standard suspension of isolate was made from growth on LJ slant and diluted to 10-2 and 10-4 dilutions. The inoculated plates were incubated at 35°C and examined for growth each week for 8 weeks. M.tuberculosis was considered resistant to a given drug when growth ≥1% above the antibiotic free control was observed in drug containing area. MTB H37Rv was used as control with each batch of susceptibility testing.

Sensitivity of Pyrazinamide (PZA) was performed using BACTEC 7H12 medium pH 6.0 (BACTEC TM PZA test medium). Lyophilized PZA was reconstituted and aseptically 0.1ml of PZA solution was added to PZA medium vial. 0.1ml of freshly sub cultured organism was added to PZA and incubated at 37°C with daily check of test and controlled vial in BACTEC 460.

Results

We reviewed 216 cases for history and drug susceptibility pattern, 80 (37%) cases had no history of prior antituberculous treatment, while 136 (63%) cases had previous exposure to anti tuberculous therapy. Almost one third 78 (36%) cases were fully sensitive to all five first line drugs (Isoniazid, Rifampicin, Pyrazinamide Ethambutol and Streptomycin) while 138 (64%) showed resistance to one or more agents. Amongst these, primary resistance was seen in 39% and secondary resistance in 107 (79%) of our samples, whereas 60% of isolates were resistant to Isoniazid Figure 1.

Resistance to a single agent was noted in 20 (15 %) of the 138 resistant isolates Figure 2, majority of which was against Isoniazid (n=13). Among the strains showing resistance to two drugs resistance to a combination of Isoniazid plus Rifampicin was highest (n=14), followed by



INH=Isoniazid; RIF=Rifampicin; PYZ=Pyrazinamide; ETH=Ethambutol; STREP=Streptomycin

Figure 1- Individual Drug Resistance in M.tuberculosis isolate (n=216)

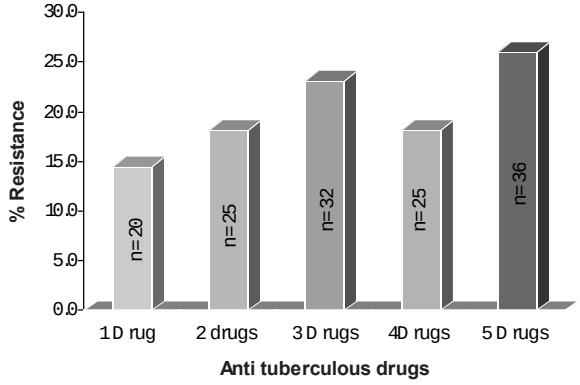


Figure 2- Analysis of drug resistant isolates in term of resistance pattern (n=138).

resistance to Isoniazid plus Pyrazinamide (n=5) and Isoniazid and Streptomycin (n= 4). Thirty two strains showed resistance to three drugs. The largest amongst these were resistant to Isoniazid plus Rifampicin plus Pyrazinamide (n=28). Finally 25 (18%) isolates showed resistance to four drugs with combination of Isoniazid Rifampicin Pyrazinamide and Ethambutol in 21/25.

Multi drug resistance (MDR) was observed in 102 (47%) isolates studied.

Analysis of 102 patients with MDR-TB showed that 8 (10%) had primary resistance while 94 (69%) had a history of previous exposure to anti-TB drugs (acquired resistance)

No cross-resistance to other antituberculous drugs was noted in 14 (10.5%) MDR strains, while 28 (27%) showed cross-resistance to one other drug and 24 (23.5%) to two drugs and 36 (35.2%) of MDR isolates were resistant to all five first line antituberculous agents.

Discussion

Globally, prevalence of multi-drug resistance tuberculosis (MDR-TB) has increased over the past few years. World Health Organization (WHO) estimates suggest that over 50 million people worldwide are infected with drug-resistant tuberculosis.[10] A number of global hot spots with ≥3 % primary MDR-TB have been identified including Estonia, China, Russia, India and Iran.[11] WHO concerns have been raised indicating that levels of MDR-TB will reach an alarming state in South East Asia unless urgent steps are taken to control the increasing level of resistance.[12]

Pakistan ranks sixth among the list of 22 high TB burden countries with a TB related death rate of 43/100,000 population annually.[13] Resistance to TB drugs has been widely reported from various parts of the country[8-9,14-19], however, pertinent community based data

that represents a national profile is lacking. In the absence of community data, hospital based studies provide indication of the levels of drug resistance and particularly of resistance trends over the years. Our study showing an overall resistance rate of 64% to the antituberculous drugs confirms 36-73% resistance rates reported in earlier studies.[8,9,14-19]

Thirty nine percent primary drug resistance to at least one drug however represents a marked increase from 17% resistance reported in 1993 by Khan et al.[16] We report an overall 79% acquired resistance, which indicates magnitude of non-compliance as well as partial treatment in TB patients.

Sixty percent of the isolates in this study were resistant to Isoniazid of which 80% (102/130) were MDR. More alarming is a fact that 67%(93/138) of total drug resistant isolates showed resistance to three or more agents. This finding is again consistent with the rising Isoniazid and Rifampicin resistance reported earlier from this country.[8]

The reported single drug resistance worldwide is 10%. Analysis of 138 resistant isolates in our study revealed 15 % (n=20) resistance to single agent with the majority being resistant to Isoniazid (a risk factor for future MDR) with 39% being primary resistance. It has earlier been reported that 70.8% of tuberculosis patients with either Isoniazid or Rifampicin resistant strains acquire MDR-TB following treatment failure.[20]

We observed very high level of MDR-TB strains (47%) in our isolates. This finding support the rising MDR trends reported in earlier hospital based studies from the country and is consistent with a recent hospital based study from Mumbai (India) indicating 51% MDR rate in their isolates.[21] The high MDR rate noted in this study and reported elsewhere in the country and region is a matter of great concern. We report 10% primary MDR cases which again supports the hypothesis of rising trend of primary and secondary resistance when compared with 7% primary MDR reported by a hospital based study in 2001.[9]

Finally this study is reflecting the rising trend of resistance in community, however pure community based studies are needed to confirm these findings.

References

1. Cohn DL, Bustreo F, Raviglione MC. Drug resistant tuberculosis: review of worldwide situation the WHO/IUATLD Global Surveillance project. International Union Against Tuberculosis and Lung Disease. Clin Inf Dis 1997; 24Suppl 1:S121-30.
2. Global trend in resistance to antituberculous drugs. N Engl J Med 2001:344 1294-
3. Patel D, Madan I. Methicillin resistant Staphylococcus aureus and multi-drug resistant tuberculosis: Part 2. Occup Med (Lond) 2000; 50: 395-7.
4. Demissie M, Gebeyehu M, Berhane Y. Primary resistance to anti tuberculosis drugs in Addis Ababa, Ethopia. Int J Tuberc Lung Dis 1997; 1: 64-7.
5. Anti-tuberculosis drug resistance in the world prevalence and trends.
6. Pablos-Mendez A, Mario CR, Laszlo A, Binkin N, Rieder HL, Bustreo F et al. Global surveillance for antituberculous drug resistance, 1994-1997. N Engl J Med 1998; 338:1641-49.
7. World Health Organization TB epidemiological profile as of 01-Jun-2005. www.who.int/globalatlas/predifinedreports/TB/PDF_files/pak_2003_brief.pdf (accessed in November 2005).
8. Butt T, Ahmed RN, Kazmi SY, Rafi N. Multi-drug resistant tuberculosis in Northern Pakistan. J Pak Med Assoc 2004; 54: 469-72.
9. Rasul S, Shabbir I, Iqbal R, Haq M, Khan S, Saeed MS, et al. Trends in multidrug resistant tuberculosis. Pak J Chest Medi 2001; 7: 21-28.
10. Espinal MA, Simonsen L, Laszlo A, Boulahbal F, Kim SJ, Reneiro A, et al., for the WHO/International Union Against Tuberculosis and Lung Disease Global Working Group on Anti-tuberculosis Drug Resistance Surveillance. Anti- tuberculosis drug resistance in the world. Report No. 2. Geneva: World Health Organization; 2000. Unpublished document WHO/TB/2000.278. Available from: URL: http://www.who.int/gtb/publications/ drugresistance/ PDF/fullversion.pdf (accessed in November 2005).
11. WHO Report 2002.Global Tuberculosis Control: Surveillance, Planning, Financing WHO/CDS/TB/2002.295 (accessed in November 2005).
12. Anti-tuberculous drug resistance in the world. The WHO/IUATLD Global project on antituberculous drug resistance surveillance 1994-1997.WHO/TB/97.229. Geneva: World Health Organization, 1997.
13. WHO/Annex1.www.who.int/tb/publications/global_report/2005/ annex1/en/index13.html (accessed in December 2005).
14. Khan J, Islam N, Ajanee N, Jafri W. Drug resistance of Mycobacterium tuberculosis in Karachi, Pakistan. Tropical doctor 1993: 23: 13-14.
15. Hussin R, Hasan R, Khurshid M, Sturm A W, Ellner J J and Dawood G.Pulmonary tuberculosis in a BCG vaccinated area: relationship of disease severity with immunological and hematological parameters and drug resistance patterns. Southeast Asian J Trop Med Public Health 1996;27:257-62.
16. Karamat K A, Rafi S, Abbasi SA. Drug resistance Mycobacterium tuberculosis: A four years experience.J Pak Med Assoc 1999;49:262-5.
17. Almani SA, Memon NM, Qureshi AF. Drug resistant tuberculosis in Sindh. J Coll Physicians Surg Pak 2002; 12:136-9.
18. Haq M U, Awan SR, Khan S U, Saeed S, Iqbal R, Shabbir I et al. Sensitivity pattern of Mycobacterium tuberculosis at Lahore (Pakistan). Annals 2002;8:190-3.
19. Iqbal R, Shabbir I, Mirza M N, Hasan M. TB drug resistance an alarming challenge-answer DOTS. Pakistan J Med Res 2003; 42: 134-8.
20. Seung KJ, Gelmanova IE, Peremitin GG, Golubchikva VT, Pavlova VE, Sirotkina OB et al.The effect of initial drug resistance on treatment response and acquired drug resistance during standardized short course chemotherapy for tuberculosis. Clin Inf Dis 2004; 39:1321-8.
21. Almeida D, Rodrigues C, Udwadia ZF, Lalvani A, Gothi G.D, Mehta P et al. Incidence of multi drug resistant tuberculosis in urban and rural India and implications for prevention. Clin Inf Dis 2003; 36:e152-4.