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Changing Trends in the Susceptibility Pattern of *Mycobacterium tuberculosis* Over a Decade from a Tertiary Care DOTS Centre Delhi

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Abstract

Study Background: Drug resistance in *Mycobacterium tuberculosis* is a serious problem all over the world. One of the major factors contributing to drug resistance is delayed detection of drug-resistant isolates, which ultimately leads to delay in initiation of effective chemotherapy. An appropriate modification of treatment regimens, depending upon the susceptibility pattern of Mycobacterium isolates is the keystone for successful treatment of drug-resistant tuberculosis.

Material and methods: The study was done to check the susceptibility pattern of both pulmonary and extra-pulmonary isolates of *Mycobacterium tuberculosis* during Aug 2009 - Jun 2012, Phase II (35 month period) and compared it with our previous data of same duration (Aug 2002-Jun 2005, Phase I), to determine the burden of drug resistance in the current situation and to look for the change in resistance pattern over a decade. A total of 154 culture-confirmed *Mycobacterium tuberculosis* isolates (pulmonary-36, extra-pulmonary-118) were screened for their susceptibility pattern. Drug susceptibility testing was performed by an automated Bac T-Alert 3D, using 'SIRE' kit' provided with Bact Alert 3D system (Biomereiux Pvt Ltd).

Result: Current study demonstrated increased drug resistance for streptomycin, isoniazid, rifampicin and ethambutol as 8/36 (22.2%), 23/36 (63.8%), 6/36 (16.6%) and 21/36 (33.3%) respectively in the pulmonary isolates and 39/118 (33%), 71/118 (60.1%), 16/118 (13.5%) and 60/118 (50.8%) among the extra-pulmonary isolates. A significant increase in resistance (p value=0.0001) was observed for streptomycin in current phase as compared with the earlier phase of study while resistance to rifampicin was decreased in pulmonary isolates. However, resistance to streptomycin, isoniazid and ethambutol were significantly increased (p value=0.0001) among extra-pulmonary isolates.

Conclusion: Resistance to streptomycin has increased at an alarming rate in pulmonary tuberculosis (TB). However, resistance to isoniazid and rifampicin has stabilized over time, this could possibly imply adequacy of DOTS coverage in cases of pulmonary TB. This situation in patients with extra-pulmonary TB is more alarming as this data reveals a dramatic increase of resistance to isoniazid and other first-line agents. The "hidden reservoir" of resistance in extra-pulmonary patients may downgrade the efficacy of the DOTS program in the future.

Keywords: Drug resistance; Pulmonary isolates; Extra-pulmonary isolates; Susceptibility testing

Introduction

In spite of the effective therapy, tuberculosis (TB) is still remains the major cause of death from a curable infectious disease. An incidence of 9.0 million cases and 1.5 million deaths was estimated the global burden of tuberculosis in 2013 [1]. One fourth of these global incident TB cases (24%) occur in India annually, the estimates of multidrug resistant tuberculosis (MDR-TB) rank India as the highest bearing this burden [2]. Drug-resistant pulmonary tuberculosis is a serious problem all over the world, especially in areas with higher prevalence. MDRTB is most often associated with high morbidity and mortality, mortality rates ranging from 50% to 80%. It spans relatively shorter time (4 to 16 weeks) from diagnosis to death [3].

One of the major factors contributing to drug resistance is delayed detection of drug-resistant isolates, which ultimately leads to delay in initiation of effective chemotherapy and MDRTB outbreaks [4,5]. Increased incidence of MDRTB cases interferes with National TB Control Programs, particularly in developing countries where prevalence rates are as high as 48% [6]. Resistant strains maintain their infectivity as well as virulence, and such strains have been gradually increasing in the community [7]. Management of drug-resistant tuberculosis poses a great challenge as treatment is very difficult, complicated and costlier in patients harboring such resistant strains.

Although, DOTS therapy forms the backbone of anti-tubercular chemotherapy, an appropriate modification of therapy based on drug susceptibility pattern which may detect any drug resistance in MTB could be the way forward in control to halt further development of MDRTB variants [8]. If the initial strain is resistant to either isoniazid or rifampicin, then patients are receiving monotherapy in the true sense, with increased possibility of the emergence of multi drug-resistant (MDR) strains. Published data are lacking, reports on drug susceptibility pattern and response to anti-tubercular drugs are very few in our country [9-11].

In this study, we retrospectively analyzed susceptibility patterns of both pulmonary and extra-pulmonary *Mycobacterium tuberculosis* isolates during Aug 2009- Jun 2012, Phase II (35 months period) and compared it with our previous data of same duration (Aug 2002-Jun 2005- Phase I), to look for the change in resistance pattern over a decade.

Materials and Methods

A retrospective cross-sectional study was conducted in the Department of Microbiology of Vardhman Mahavir Medical College & Safdarjung Hospital, New Delhi. A total of 154 culture-confirmed *Mycobacterium tuberculosis* isolates (pulmonary-36, extra-pulmonary-118) were screened for their susceptibility pattern during August 2009 to June 2012 (Phase II), as the extra-pulmonary cases are more common in our setup, extra-pulmonary isolates were more in comparison to pulmonary one.

All the LJ subcultures isolate were ≤3 weeks old and isolates of BacT/Alert MP bottles were ≤36 hours. Drug susceptibility testing was performed by an automated BacT-Alert 3D, using SIRE kite; streptomycin 1.0 µg/ml, isoniazid 0.1 µg/ml, rifampicin 1.0 µg/ml, ethambutol 5 µg/ml kit (Biomereiux Pvt Ltd) provided with automated Bact- Alert 3D system. 0.5 ml of the lyophilized antibiotic solutions along with 0.5 ml of reconstitution fluid (Tween 80, glycerol and amaranth) were added to both glass BacT/ALERT MP test bottles and the undiluted direct control bottles, respectively, so that the final drug concentrations in the test bottles reached 0.9 mg/L for rifampicin, 0.4 mg/L for streptomycin and 0.09 mg/L for isoniazid, and 1.8 mg/L for ethambutol.

An equal amount of sterile distilled water added to BacT/Alert MP bottles containing the growth of *Mycobacterium tuberculosis* (MTB) to make direct growth control (DGC) of 2 McFarland turbidity approximately. 0.5 ml of this direct growth control was added to all drug containing and drug-free control bottles. Then DGC is again diluted to 100 times (0.1 ml of DGC + 9.9 ml of sterile distilled water) and 0.5 ml of diluted DGC was added to another drug-free BacT/Alert MP bottle, this served as the 1% growth control (1% GC). All MP bottles were kept in the system at 35°C for 10 days of incubation and monitored every 10 min to detect growth. Susceptible MTB reference strain, H37Rv was used as a control strain in each batch of a test.

Susceptible

An isolate considered susceptible if no growth was detected in the antibiotic containing bottle before or at the same time as growth control (1% GC) or bottle flagged positive after growth control (1% GC).

Resistant

An isolate considered resistant if antibiotic containing bottle flagged positive before or same time as growth control (1% GC).

Invalid

Test was taken as invalid when DGC showed no positive flag in 10 days. All invalid tests were repeated again following the same protocol as done before. Drug susceptibility testing was also done by LJ proportion method for all Mycobacterium isolates using standard procedures and resistant strains were identified. Isolates found to be resistant to any of the four first-line drugs, was labeled as single drug resistance, resistant to two of the four drugs as dual, resistance to any three of four drugs as triple and pan-drug resistant when it was resistant to all four drugs Statistical comparison of resistance patterns of all pulmonary and extra-pulmonary isolates with the previous study conducted during Aug 2002-Jun 2005 (Phase I) data based on similar methodology was done using chi-square test and the p value<0.05 was considered significant.

Results

Out of the 154, Mycobacterium isolates only 36, 23.3% isolates were sensitive to all four drugs tested. Overall current phase of the study demonstrated lesser prevalence of sensitive isolates, 23.3% whereas 47.1% isolates were found to be sensitive to all four drugs in phase I. All types of drug resistance single, dual, triple and pan were high in the current phase of study in comparison to earlier phase (Figure 1).

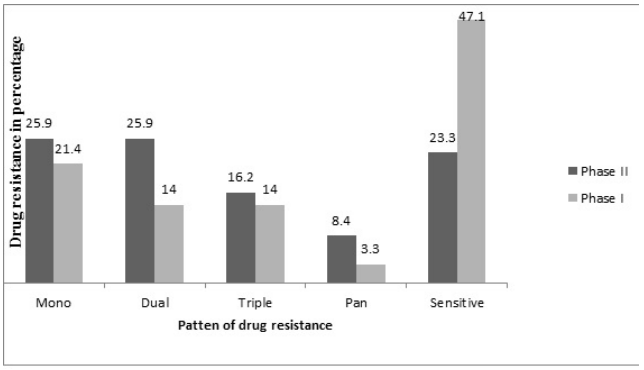

Figure 1: Resistance profile of anti-tubercular drugs over 10 years.

Among the pulmonary isolates, isoniazid was found to be resistant in 63.8% (23/36) isolates followed by ethambutol (12/36, 33.3%) streptomycin (8/36, 22.2%) and rifampicin (6/36, 16.6%) (Table 1). A significant increase in resistance was observed for streptomycin (p value<0.05) as compared with the phase I while resistance to rifampicin was decreased in pulmonary isolates.

Anti-tubercular drug	Phase II (Aug 2009 to June 2012)	Phase I (Aug 2002 to June 2005)	P value
Streptomycin	22.2%	11.9%	0.0001
Isoniazid	63.8%	61.1%	0.2299
Rifampicin	16.6%	19.4%	0.1161
Ethambutol	33.3%	28.3%	0.0176

Table 1: Comparison of drug susceptibility pattern of Phase II (August 2009 to June 2012) with Phase I (August 2002to June 2005) in Pulmonary isolates.

In extra-pulmonary isolates isoniazid was resistant in 71/118, 60.1% isolates followed by ethambutol (60/118, 50.8%) streptomycin (39/118, 33%) and rifampicin (16/118, 13.5%) (Table 2). Resistance to streptomycin, isoniazid and ethambutol were significantly high in extra-pulmonary isolates in phase II in comparison to phase I.

Anti-tubercular drug	Phase II (Aug 2009 to June 2012)	Phase I (Aug 2002 to June 2005)	P value
Streptomycin	33%	5.5%	0.0001
Isoniazid	60.1%	33.3%	0.0001
Rifampicin	13.5%	14.8%	0.4414
Ethambutol	50.8%	18.5%	0.0001

Table 2: Comparison of drug susceptibility pattern of Phase II (August 2009 to June 2012) with Phase I (August 2002to June 2005) in Extra-pulmonary isolates.

Discussion

Management of drug-resistant tuberculosis poses a great challenge for clinicians. Anti- tubercular drugs act as a double-edged weapon in this situation; on one hand, they destroy the tubercle bacilli while on the other hand they also select some mutant strains against which they are ineffective. In vitro drug susceptibility testing, for anti-tubercular drugs and judicious use of drugs are necessary for successful treatment of drug-resistant variants of tuberculosis. DOTS treatment is taken for granted, as it assumes all strains as susceptible strains unless proved otherwise. Single drug resistance to ethambutol was, by and large, could have been responsible for encouraging resistance to other drugs in the first line as several strains are already resistant to isoniazid.

Although drug resistance in TB has been reported repeatedly from various centers of our country during the last four decades, but most studies were conducted with a small sample size during the short period of time and usually from pulmonary isolates only [12-14]. To the best of our knowledge, this is the first study conducted in two phases over 10 years in a large tertiary care hospital including both pulmonary and extra-pulmonary isolates. Overall an increased resistance to all four first-line anti-tubercular drugs were observed (Phase I 52.9%, Phase II 76.7%) with dual-drug (25.9%) resistance and pan-drug (8.4%) resistance as an emerging issue in current phase when compared with the previous phase of study [15]. While another study was done in 2003 in same settings showed a lower degree of dual resistance (34.7%) and pan-drug resistance 1.3% [16]. However, our study demonstrated a lower prevalence of pan- drug resistance than in the study done by Jain et al. [17] in KGMC, Lucknow (14.8%) in pulmonary isolates. The current phase of the study demonstrated maximum resistance in isoniazid followed by ethambutol, streptomycin and rifampicin both in pulmonary and extra-pulmonary isolates. A similar finding was observed in phase I for isoniazid and ethambutol while streptomycin was the least resistant drug in the previous phase of the study. A significant increase in resistance was observed for streptomycin in both pulmonary, 22.2% as well as extra-pulmonary isolates, 33.3% isolates, compared to phase I. Isoniazid and rifampicin resistance was stable among pulmonary isolates. Resistance to rifampicin was even lower in both the pulmonary and extra-pulmonary isolates signify the tremendous success of the DOTS program in our community. Our finding was similar to a study done by Nazir et al. [18] where resistance to rifampicin was 21.5%. Isoniazid and streptomycin resistance were found to be more prevalent than rifampicin or ethambutol resistance. Mono-resistance of isoniazid and streptomycin acts as a gateway for the acquisition of additional drug resistance [19]. In extra-pulmonary cases, drug resistance is an emerging problem with increased resistance to isoniazid (Phase I 33.3%, Phase II 60.1%), which is an alarming signal for the clinicians. A strict vigil is imperative in susceptibility patterns in extra-pulmonary cases. Increasing drug resistance in tuberculosis demands a development of strict tuberculosis programs which must be focused and cost effective. Apart from a strong tuberculosis control program, there is also need for continuous monitoring of drug resistance by in vitro drug susceptibility testing. Treatment response in patients with drug- resistant TB is very poor with usual high mortality rate. Treatment of drug resistance is an economic burden for the country as these patients require treatment with expensive and toxic second-line drugs, and sometimes they may require hospitalization for management of complications. Therefore, regular drug susceptibility testing in all pulmonary and extra- pulmonary cases are necessary to monitor the spread of resistant TB strains in the community and to ensure that such patients are receiving effective treatment.

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