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Drug Resistance Patterns Of Mycobacterium Tuberculosis From Pulmonary Tuberculosis Patients In An Urban Metropolis

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Abstract
Tuberculosis is a matter of concern for all countries. Tuberculosis (TB) remains one of the major global health threats leading to morbidity and mortality. One in three persons across the world representing 2–3 billion individuals are known to be infected with Mycobacterium Tuberculosis (M. Tuberculosis) of which 5–15% are likely to develop active TB disease during their lifetime.Information of the pattern of drug resistant among the tuberculosis is crucial in developing countries. Therefore, this study aims to assess the drug resistance pattern of Mycobacterium tuberculosis isolates and associated factors among the patients. This study selected 934 culture-positive sputum samples referred to the National Reference Laboratory of the Research Institute for Pulmonology in Thanjavur from January 2015 to January 2018 were analysed; 40% of these samples were obtained from Tb Sanatorium, Sengipattipatients (hospitalized in `Sengipatti) and 60% from other regions (hospitalized in Minsk and other regions) equal to patient's population in the regions.All 934 cases were subjected to a drug-resistance test. The anti-microbial drug susceptibility tests (DST) were performed using the WHO standard conventional proportional method. The Preferable First Line Drugs were INH 1mcg/ml, RIF40 mcg/ml, Ethambutol (EMB) 2 mcg/ml, and Streptomycin (SM) 10 mcg/ml on slants with the H37Rv strain of MTB as the positive control. Furthermore, MDR isolates were tested for resistance to fluoroquinolones and three injectable drugs (Amikacin 8 mcg/ml, Kanamycin 30 mcg/ml, and Capreomycin 8mcg/ml) for detection of XDR isolates.

Keywords: Mycobacterium tuberculosis; drug resistance; blood; patients

Introduction

Tuberculosis (TB) remains one of the major global health threats leading to morbidity and mortality (Raviglione M,2016). One in three persons across the world representing 2–3 billion individuals are known to be infected with Mycobacterium Tuberculosis (M. Tuberculosis) of

which 5–15% are likely to develop active TB disease during their lifetime (WHO, 2015.). In 2014, an estimated 9.6 million people felt ill due to TB, around 1.5 million people died from the disease including 1.1 million HIV-negative persons and 400,000 HIV patients (WHO, 2015.). While TB is present in every country majority of TB sufferer live in low income and middle-income countries especially in regions such as Sub-Saharan Africa and South East Asia (Esmond, 2011). Over the past decade, significant progress has been made towards TB control with most of the TB targets set as part of the Millennium Development Goals (MDGs) having been achieved (Switzerland WHO, 2015.). TB mortality for instance has declined by 47% since 1990, with nearly all of that happening in the era of the MDGs. In all, effective diagnosis and treatment of TB has been estimated to have saved over 40 million lives between 2000 and 2014 (Switzerland: WHO, 2015). While these achievements are remarkable, there are calls for intensified efforts to eradicate the disease. In 2014, the World Health Assembly (WHA) adopted the End B strategy with targets linked to the newly adopted Sustainable Development Goals SDGs) (WHO, 2015). The End TB strategy serves as the key guide for countries to reduce TB deaths by 90% by 2030 as well as achieve an 80% reduction in TB incidence rate compared with 2015 (Geneva, WHO, 2015). TB still pose as a huge threat to economic development as over 90% of TB-related deaths occur among adults in the most productive age groups. Emerging issues such as Multi-drug and extensively drug resistant TB is seen as a major challenge in effective control of the disease in many regions. Treatment outcomes for drug resistant TB are still poor and inadequate reporting remains a growing challenge. Of the 480,000 cases of multidrug-resistant TB (MDR-TB) estimated to have occurred in 2014, only about 25% were detected and reported (Rudich et al., 1998). Moreover, just around 30% of the over 7,000 MDR-TB patients from 13 countries were successfully treated in 2007 (WHO, 2015). The evidence base around TB and its management is rapidly changing. In this work, we provide a general overview of TB by highlighting the pathogenesis, diagnosis, and treatment guidelines. In preparation of this material, we searched PubMed for relevant articles on TB. Additionally, we searched the websites of major institutions like the World Health Organization (WHO) and the US Centres for Disease Control and Prevention (CDC) for related guidelines and reports. This paper has been written with the intention to offer general education to health professionals, policy makers, patients and the public.

Materials and Methods

Study population and methods

The 934 culture-positive sputum samples referred to the National Reference Laboratory of the Research Institute for Pulmonology in Thanjavur from January 2015 to January 2018 were analysed; 40% of these samples were obtained from Tb Sanatorium, Sengipattipatients (hospitalized in Sengipatti) and 60% from other regions (hospitalized in Minsk and other regions) equal to patient's population in the regions.All 934 cases were subjected to a drug-resistance test. The anti-microbial drug susceptibility tests (DST) were performed using the WHO standard conventional proportional method. The Preferable First Line Drugs were INH 1mcg/ml, RIF40

mcg/ml, Ethambutol (EMB) 2 mcg/ml, and Streptomycin (SM) 10 mcg/ml on slants with the H37Rv strain of MTB as the positive control. Furthermore, MDR isolates were tested for resistance to fluoroquinolones and three injectable drugs (Amikacin 8 mcg/ml, Kanamycin 30 mcg/ml, and Capreomycin 8mcg/ml) for detection of XDR isolates.First- and second-line drugs are the two main categories of drugs used for TB treatment. Traditionally, there are five first-line drugs, including INH, RIF, Pyrazinamide (PZA), EMB and SM. Second-line drugs contain aminoglycosides, Kanamycin and Amikacin, the polypeptide Capreomycin, Phage antibiotic synergy (PAS), cycloserine, thioamides, ethionamide and prothionamide and several fluoroquinolones, such as ofloxacin, moxifloxacin, levofloxacin and gatifloxacin; SM has been reported as a second-line drug, though .For drug resistance, the following terms were used as defined by the WHO :

• MDR: multi-drug resistant tuberculosis (MDR-TB) is resistance to at least two of the best anti-TB drugs, INH and RIF.

• XDR: extensively drug resistant tuberculosis (XDR-TB) is resistance to: INH and RIF plus resistance to the best second-line medications: fluoroquinolones and at least one of three injectable drugs (i.e., Amikacin, Kanamycin, or Capreomycin).

Specimen collection, storage, and handling procedures; criteria for specimen rejection

Collect 1 ml of blood by venepuncture directly into each of the QuantiFERON TB Gold IT blood collection tubes, which include a Nil Control tube, TB Antigen tube and a Mitogen tube. Tubes should be between 22° C + 5° C at the time of blood draw.Immediately after filling tubes, shake them ten times just firmly enough to ensure the entire inner surface of the tube is coated with blood, to solubilize antigens of tube walls. Over energetic shaking may cause gel disruption and could lead to aberrant results. Ship tubes to laboratory at 22° C + 5° C as soon as possible and within 16hrs of collection. Do not refrigerate or freeze the blood samples.The assay is set up at least once a week or more frequently depending on work load. Samples are stored for a minimum 7 days at 2° - 8° C after final results have been posted.

Blood cultures

Blood cultures using mycobacteria-specific, radioisotope-labeled systems help to establish the diagnosis of active TB. However, mycobacterial bacteremia (bacillemia) is detectable using blood cultures only if specialized systems are used; these bacilli have specific nutrient growth requirements not met by routine culture systems.Such blood cultures should be used for all patients with HIV infection who are suspected of having TB, because bacillemia is particularly prevalent in this population. If available, in fact, these cultures should be used for any patient highly suspected of having active TB.

Drug Susceptibility Testing

Positive cultures should be followed by drug susceptibility testing. Symptoms and radiographic findings do not differentiate MDR-TB from fully susceptible TB. Suspect MDR-TB if the patient has a history of previous treatment for TB, was born in or lived in a country with a high prevalence of MDR-TB, has a known exposure to an MDR-TB case, or is clinically progressing despite standard TB therapy. Susceptibilities should be repeated if cultures remain positive after 2 months, even when initial susceptibilities have not revealed any resistance.

Statistical analysis

Data obtained from medical records were entered and analyzed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). The sensitivity of each IGRA among the different age groups was compared using binary logistic regression and linear-by-linear association. Comparisons of continuous variables including WBC and lymphocyte counts, CRP, serum protein, and serum albumin levels, across age groups were performed using one-way analysis of variance (ANOVA) and post-hoc analysis. The effect of each factor on the sensitivity of each IGRA was analyzed by logistic regression adjusting for age group. A factor was considered to influence IGRA sensitivity when the age group was adjusted by a certain variable or some variables and the sensitivity of the IGRA according to age group was statistically insignificant. A p value less than 0.05 was considered significant.

Results

During the research period, 934 pulmonary TB patients were studied, of which 274 (29.33 ± 1.5%) ($p < 0.001$) men in the age group 25–65years outnumbered women between 2.7and 9.0 times more (Table 1); 660 (70.66 ± 1.5%) of the TB patients were men.In the age group <15–24, as well as in the age group over65 years, the proportion of men and women were similar. Inthe remaining age groups, the proportion of men with TBwas significantly higher than women. The total ratio of maleTB patients among the female patients of all groups surveyedin 2007 was 2.4, which agrees with the WHO European Region.In the age group 45–54 the male to female ratio was the highestamong patients with TMDR-TB.

Table 1: A statistical characterization of the studied population of TB patients based on sex differentiation.

Sex	Number of men and women in age groups (number and %)						Total
Group	<15 (n = 8)	15–24 (n = 98)	25–44 (n = 400)	45–54 (n = 203)	55–65 (n = 125)	>65 (n = 80)	
Women	4 50.0 ± 5.3	47 47.9 ± 5.0	108 27.0 ± 2.2 34	16.7 ± 2.6 31	10.0 ± 2.7 40	50.0 ± 5.6 274	274 (29.3±1.5)
Man	14 50.0 ± 5.3	51 52.1 ± 5.0 292	73.0 ± 2.2 169	83.3 ± 2.6 94	90.0 ± 2.7 40	50.0 ± 5.6 660	660(70.66±1.7)
P	>0.05	>0.05	<0.001	<0.001	<0.001	<0.05	934(100%)

Frequency of MTB isolates with different levels of resistance,depending on age and sexDrug sensitivity of MTB of all 934 surveyed patients with TB in2007 was studied by culture in the dilution of drugs in thegrowth medium.From a clinical aspect, patients were divided into fivegroups based on levels of resistance of MTB to the primaryanti-tuberculosis drugs. This idea was based on clinicaldifferences over the course of the disease and resistance toanti-tuberculosis drugs. For example, to treat MDR-TB casesresistant to INH and RIF (but not to EMB, PZA and SM) andfirst-line-resistant TB (FLR-TB), cases were considered resistantto all first-line drugs.
Mono-resistance TB cases are treated separately from thedrug-sensitive and MDR cases. Among patients with drugresistantTB in different age groups, significant differencesamong men and women were noted. There were no differencesby age group, when comparing male and femalepopulations with XDR-TB ($p > 0.05$), MDR-TB ($p > 0.05$) anddrug-sensitive TB ($p > 0.05$) (Table 2).Drug susceptible group.This group is sensitive to INH, RIF and other drugs and is26.5 ± 1.4% of all analyzed isolates; 32.26 ± 2.96% of them wereisolated from female patients, and 67.74 ± 2.96% males($p < 0.05$). This group includes the largest number of patientsless than 15 years (16 people). In contrast to all other groups,among TB patients younger than 15 years, the number of girls(12.5 ± 3.69%) outnumbered boys (3.57 ± 1.43%) ($p < 0.05$). Asimilar trendwas found in patients older than 65 years: womenwere 25 ± 4.84%, while men were 8.3 ± 2.1% ($p < 0.05$) (Table 2).

Table 2 – Frequency of MTB isolates from patients with different levels of drug resistance depending on age and sex.

Drug resistance group		G ender	<15	15–24	Age ranges (year), 25–44	as number (%) 45–54	55–65	>65	**Total as, number%**
Susceptible		Women	10(12.5 ±3.69)	6(7.5 ± 2.9)	27(33 .7 ± 5.2)	9(11.25 ±3.5)	8(10 .0 ± 3.35)	20(25.0± 4.84)	**80(32.2 6 ± 2.96)**
		Men	6(3.57± 1.43)	15(8.9 ± 2.2)	55(32 .7 ± 3.6)	44(26.2 ±3.4)	34(2 0.2 ± 3.1)	14(8.3 ± 2.1)	**168(67. 74 ± 2.96)**
		Total	16(6.45 ±1.56)	21(8.4 ± 1.8)	82(33 .0 ± 3.0)	53(21.3 ±2.6)	42(1 6.9 ± 2.4)	34(13.7± 2.2)	**248(26. 5 ± 1.4)**
Mono resistant (Mono)		Women	–	3(13.6 ± 7.3)	10(45 .45± 10.6)	4(18.18 ±8.2)	2(9. 1 ± 6.1)	3(13.6 ± 7.3)	**22(30.5 ± 5.4)**
		Men	2(4.0 ± 2.7)	5(10 ± 4.24)	23(46 .0 ± 7.0)	12(24 ± 6.0)	4(8 ± 3.8)	4(8 ± 3.8)	**50(69.4 ± 5.4)**
		Total	2(27 ± 1.90)	8(11.11 ± 3.7)	33(45 .8 ± 5.8)	16(22.2 ±4.9)	6(8. 33 ± 3.2)	7(9.7 ± 3.4)	**72(7.7 ± 0.87)**
T MDR									
First-line		3(3.6 ± 2.0)	19(22.8 ±4.6)	29(34.9 ± 5.2)	12(14 .4 ± 3.8)	12(14.4 ±3.8)	8(9. 6 ± 3.2)	83(28.6 ± 2.6)	**83(8.88 %)**

resistant									
(FLR)									
		2(0.96 ± 0.6)	12(5.8 ± 1.6)	101(48.7±3.)	53(25.6 ± 3.0)	29(10.4 ±2.4)	10(4.8 ± 1.5)	207(71.3 ±2.65)	**207(22.16%)**
		5(1.7 ± 0.75)	31(10.7 ±1.8)	130(44.8±2.9)	65(22.4 ± 2.4)	41(14.1 ±2.0)	18(16.2 ± 2.3)	290(31.0 ±1.51)	**290(31.05%)**
Multi-drug resistant		1(1.35 ±1.34)	17(22.9 ±4.9)	34(45.9 ± 5.8)	7(9.4 ± 3.4)	8(10.8± 3.6)	7(9.4 ± 3.4)	74(27.4 ± 2.7)	**74(7.93 %)**
(MDR)									
		4(2.0 ± 1.0)	14(7.1 ± 1.8)	99(50.5 ± 3.5)	50(25.5 ± 3.1)	20(10.2 ±2.16)	9(4.6 ± 1.5)	196(72.6 ±2.7)	**196(20.98%)**
		5(1.85 ±0.82)	31(11.5 ±1.2)	133(49.2±3.0)	57(21.1 ± 2.5)	28(10.3-5.76)	16(5.9 ± 1.43)	270(28.9 ±1.5)	**270(28.9%)**
Extensively drug		Women	–	2(13.3 ± 8.7)	8(53.3 ± 12.8)	2(13.3± 8.7)	1(6.6 ± 0.4)	2(13.3 ± 8.7)	**15(27.78 ± 0.6)**
resistant (XDR)									
		Men	–	5(12.8 ± 5.3)	14(35.9 ± 7.6)	10(25.6 ±7.0)	7(17.9 ± 6.1)	3(7.7 ± 4.2)	**39(72.22 ± 0.6)**
		Total	–	7(12.9 ± 4.5)	22(40.7 ± 6.7)	12(22.2 ±5.6)	8(14.8 ± 4.8)	5(9.2 ± 3.9)	**54(5.7 ± 2.4)**
		Sum	**28(2.99 ±0.55)**	**98(10.49±1.0)**	**400 (42.8 ± 1.6)**	**203(21.7±1.3)**	**125 (13.4 ± 1.1)**	**80(8.5 ± 0.9)**	**934 (100)**

Mono-resistant group (Mono)

Patients suffering resistance to one of the major anti-tuberculosisdrugs (INH or RIF) was 7.7 ± 0.87% of those surveyed,69.4 ± 5.4% of them were men ($p < 0.05$). In this group, therewere no girls under the age of 15 years (Table 2).First-line resistant group (FLR-TB)This group, which included 31.0 ± 1.51% of all surveyed consistedof patients infected with MDR-isolates (resistant toINH and RIF), which were also resistant to PZA, EMB andSM. The group with the FLR-TB treated 51.8% of patients withMDR-TB because they do not respond to treatment with INH,RIF and first-line drugs. The ratio of men and women in thisgroup was 3.07; the difference in sex composition was observedin the range of 15–24 years (women 22.8 ± 4.6%, men5.8 ± 1.6% ($p < 0.05$) (Table 2).

Multi-drug resistant group

MDR-isolates were resistant to both of the best anti-tuberculosis drugs: INH and RIF. Patients with MDR-TB accounted for 28.9 ± 1.5% of all patients. If, in accordance with the WHO requirements, patients with FLR-TB were added to this group. The resulting aggregate, which can be designated as TMDR-TB will be 59.9 ± 1.6% of all patients enrolled in the study. In the age group 15–24 years, the proportion of women with MDR-TB was 22.9 ± 4.9%, while the proportion of men was 7.1 ± 1.8% ($p < 0,05$) (Table 2).

Extensively drug resistant group

XDR-TB is resistant to INH and RIF, as well as to any of the second choice of drugs: fluoroquinolones and at least one of three injectable drugs (i.e., Amikacin, Kanamycin and Capreomycin). During 2007, the lab was sent isolates from 54 patients (5.7 ± 2.4% of all surveyed) diagnosed with XDR-TB. Men accounted for 39 (4.1 ± 0.6%), and the women accounted for 15 (1.6 ± 0.4%) of them ($p < 0.05$); children in this group were not accounted for. The greatest number of patients was found in the age group 25–44 years (40.7 ± 6.7%) (Table 2).

Treatment status

From another aspect, all groups were divided into two categories based on treatment or non-treatment status when referred to hospital (see Table 3).Patients with secondary TB totaled 414 (52.02 ± 1.77%), and patients with primary TB totaled-382 (47.98 ± 1.77%) ($p > 0.05$). Patients with primary TB were significantly more distinguished because MTB is sensitive to anti-tuberculosis drugs (48.1 ± 2.55%), while only 8.7 ± 1.38% of cases ($p < 0.05$) of patients with secondary TB were detected. The frequency of drug-resistant MTB in patients with secondary TB was 378 (47.48 ± 1.77%), which was significantly higher than in the group suffering from primary TB: 198 (24.8 ± 1.52%) ($p < 0.05$). It should be emphasized that a similar result was related to XDR patients ($p < 0.05$).

Characteristics of drug resistance in the working age group

The p-value was calculated for the evaluation of the significance of differences among age groups. In this way, all MDR patients were added to those with FLR. Subsequently, MDR patients in these

sections include all patients that are resistant to all first-line drugs and ones that are resistant only to INH and RIF (as in TMDR). In Table 4, some groups with significant differences are shown.Out of a total of 934 patients of working age, 570 (75.4 ± 1.56%) of them were men (working age 18–60 years) and 186 (24.6 ± 1.56%) were women (working age 18–55 years).Patients with MDR-TB and FLR were merged into one group renamed TMDR that included patients infected with strains resistant to all first-line drugs, and strains resistant only to INH and RIF. Men patients in mono resistant, TMDR and XDR groups have a similar frequency ($p > 0.05$).

Table 3 – Treatment status of patients when admitted to hospital, based on resistance groups.

Status	Susceptible	Mono	Resistance groups TMDR MDR* FLR	XDR*	Total
Secondary tuberculosis	184 (48.1 ± 2.55)	33 (8.6 ± 1.43)	63 (16.5±1.89) 91 (23.8 ±2.17)	11 (2.87 ± 0.85)	**382 (100%)**
Primary tuberculosis	36 (8.7 ± 1.38)	33 (7.9 ± 1.32)	202 (48.8±2.45) 103 (24.8 ±2.12)	40 (9.66 ± 1.45)	**414 (100%)**
Total	220 (27.6 ± 1.58)	66 (8.3 ± 0.97%)	265 (33.3 ± 1.6%) 194 (24.3 ± 1.5%)	51 (6.4 ± 0.86%)	**796 (100%)**

*** p < 0.05.**

Discussion

In this study, from a clinical aspect, the patients were divided into five groups based on resistance to principal anti-myco- bacterium drugs. This idea was based on the clinical differences between, for example, an MDR case resistant to INH and RIF (but not to EMB, PZA, or SM) and an FLR case resistant to all first-line drugs. As recommended by the WHO, both of the FLR and MDR groups were treated as MDR. For this reason, these items were added. In this respect, mono-resistant cases differ from susceptible and MDR cases.Out of 934 pulmonary TB patients, 660 (70.7%) were men. The gender differences were mainly seen in the age group 25–65; the largest differences were in the 25–44 age group, and the lowest differences were in the <15 and >65 age groups. The male/female ratio of above unity (>1) was the same in all groups except in the susceptible group (0.6 and 0.7 for <15 and >65, respectively).The proportions of tuberculosis in ages <15 and >65 were 3% and 8.56%, respectively. The male/female ratio in these groups was equal. On the other hand, it was found that fe- male patients in the susceptible group under age 15 and above age 65 were more than men in number. This situation was also seen in the 15–24 MDR

age group, in contrast with all other groups. Interestingly, there was not a single patient<15 in the mono-resistant group (Mono).

Approximately 60% of all patients were MDR and FLR, at least to INH and RIF; 51.8% of all MDR patients were in the FLR group. It means that around half of the patients did not respond to INH and RIF or to the remaining choices of the first-line drugs, so more expensive and less effective drugs must be used.After childhood, it was noted that the incidence of TB was consistently higher in males until after working age; 70% of cases occurred in males. The greatest difference in rates be- tween the genders was in the 24–44 age groups. This observation provides compelling evidence of real sex differences rather than a bias in diagnosis and reporting, since this is a group where women are known to have greater health-seeking behavior.

Conclusion

These groups of patients are detected based on DST results and once results are available, treatment is tailoredaccordingly. As a guideline by WHO, four principlesunderline the design of MDR-TB treatment regimen. Firstly, the regimen should contain medicines with provenefficacy. Secondly, drugs of possible cross-resistanceshould be avoided. For example, cross-resistance is known to occur between rifampicin/rifabutin and amikacin/kanamycin (55). Thirdly, unsafe drugs are excluded. Drugsare classified as unsafe if the quality is unknown or results in severe allergic reactions such as deafness, renal failureand psychosis. Finally, drug selection is made from the fivegroupings of anti-tubercular drugs in a hierarchal manner. This leads to the choice of anti-tubercular drugs for drugresistantpatients. Anti-tubercular drugs for drug-resistantTB regimen have recently been regrouped by the WHO to optimize treatment success. Under thenew WHO recommendations, treatment regimen for drug resistant TB should include first line drug; pyrazinamide (except when there is reliable DST results for resistance topyrazinamide) and four core second-line drugs to achieve aminimum of five effective drugs. The second line drugs are selected one each from Group A and Group B plus aminimum of two drugs from Group C. Additional drugsmay be added from Groups D2 and D3 to make achieve aminimum of five drugs if previous selections to not meetthe minimum number of five effective drugs. Additionally, high dose of isoniazid and/or ethambutol may be included to further strengthen the regimen. The duration ofregimen may either be short term or long term. Short termtreatment lasting between 9–12 months is recommended for drug resistant TB patients who have not been previouslytreated with second-line drugs or have not shown resistanceto fluoroquinolones or the second-line injectables. On the contrary, longer regimen involving 18 months or more isrecommended for MDRTB and XDRTB patients.

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