Prevalence of Pre - Extensively Drug Resistance Tuberculosis (Pre XDR-TB) & Extensively Drug Resistance Tuberculosis (XDR-TB) among Pulmonary Multidrug Resistance Tuberculosis at a Tertiary Care Hospital, Jamnagar

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Aims and Objective: Many diseases have a high prevalence in India, accounting for one-fourth of the Tuberculosis (TB) cases in the world. In our study, we aimed to find the prevalence of Pre XDR-TB and XDR-TB amongst newly diagnosed cases of pulmonary MDR-TB who had never been previously treated with second-line drugs. A prospective study was conducted in Culture and Drug susceptibility testing laboratory, Jamnagar and its associated Drug-Resistant Tuberculosis (DR-TB) centre.

Materials and Methods: Baseline second-line liquid culture DST has been recently integrated with the Revised National Tuberculosis Control Programme (RNTCP) diagnostic algorithm. We included 500 patients who were diagnosed in cases of pulmonary MDR-TB never exposed to second-line...
1. INTRODUCTION

Tuberculosis, in past also called phthisis, phthisis pulmonalis, or consumption, is a widespread, infectious disease caused by various strains of *Mycobacterium tuberculosis* [1]. Multidrug resistant tuberculosis is defined as a infection caused by *mycobacterium tuberculosis* bacteria that are resistant to treatment with at least two of the most powerful first–line anti-TB drugs. (Streptomycin, Isoniazid and Rifampicin) [1-3]. Pre-XDR-TB defined as a resistance to first–line anti-TB drugs (rifampicin and isoniazid), as well as to at least one second line anti-TB drugs. (fluoroquinolone (FQ) or 1 second-line injectable drug: kanamycin, amikacin or capreomycin). FQs have used as the most effective second-line anti-mycobacterial drugs and recommended for the treatment of MDR-TB [4]. If patients are infected with FQ-resistant MDR-TB strains, the treatment regimen needs to be adjusted and the prognosis is poor [5]. Therefore, effective, accurate and sensitive diagnosis of these MDR-TB and pre-XDR-TB patients is urgently needed for choosing a reasonable regimen and preventing transmission. Incidence of FQ resistant MDR-TB is increasing globally but unfortunately, till date limited data are available on prevalence of pre-XDR-TB worldwide and in India [6]. Due to constraint in resource and high TB burden, resistance to FQs (second-line anti-TB drugs) was not tested routinely in developing world. Exposure to repeated and multiple drug use during treatment of bacterial infection other than TB may contribute evolution of resistance to Fluoroquinolone like antimicrobials agents.

The management of MDR-TB is critical based on laboratory confirmation of TB but a clear understanding of drug resistance by Drug Susceptibility Testing (DST) to give accurate diagnosis and early intervention of appropriate treatment [7-9]. So, WHO strongly recommended Prevention and control of drug resistance *Tuberculosis* through the implementation of routine surveillance systems driven by systematic DST [8-11]. Nationwide survey conducted by using standardized patient stratification and employing quality-assured rapid diagnostic methods are fundamental to a strengthened surveillance [10].

Extensively Drug-Resistant *Tuberculosis* (XDR-TB) defined as resistance to first–line anti-TB drugs (Rifampicin and/or Isoniazid) with additional resistance to second-line drugs. (i.e. to any Fluoroquinolone, and to at least one of the three injectable second-line drugs i.e., amikacin, kanamycin, or Capreomycin). XDR TB is resistant to the most potent TB drugs, patient are left with treatment options that are much less effective. XDR TB is of special concern for people with HIV infection or other conditions that weaken the immune system. These people are more likely to develop TB diseases once they are infected, and also have a higher risk of death once they develop TB [12].

In 1969, Deland and Wagner developed a technique for semi-automated detection of the metabolism of bacteria by measuring the 14CO2 liberated during the growth of bacteria and decarboxylation of 14C-labeled substrate incorporated in the growth medium [13]. This radiometric technique was widely used for blood culture using the BACTEC 460 instrument. In 1980, this technique was introduced commercially for mycobacterial recovery from clinical specimens and drug susceptibility testing. One of the disadvantages of the BACTEC 460 TB System is the use of 14C-Labeled radioactive substrate. So, Becton, Dickinson and Company (BD) developed a new system called Mycobacteria Growth Indicator Tube (MGIT™), which is non-radiometric and offers the rapid, sensitive and reliable methods of testing as the BACTEC 460 TB System. BBL MGIT™ System is the manual system while BACTEC MGIT 960
MATERIALS AND METHODS

We studied the prevalence of Pre Extensively Drug Resistance TB (Pre-XDR-TB) and Extensively Drug Resistance TB (XDR-TB) among pulmonary MDR-TB. This study was conducted in the TB culture and DST laboratory, in our tertiary care hospital, from May 2017 to January 2018. There were 2 sputum samples, one spot supervised and one early morning collected in screw cap wide mouth falcon tube and transported from various centers to Tuberculosis culture-DST laboratory by courier in cold chain maintained. Total 585 MDR suspected sputum samples were received in study period and proceeded for drug susceptibility testing for detection of Multidrug resistance of Mycobacterium Tuberculosis.

All sputum samples were processed for the standard NALC-NaOH method for digestion, decontamination, and concentration [16,17]. The concentrated sediment sample was resuspended in about 2 to 3 ml phosphate buffer (pH 6.8) and mixed thoroughly. Resuspended sample used for making smears and for inoculation of MGIT tubes and other media, than according to MGIT 960 system result follow the direct microscopy, culture and subculture.

2.1 Principle of the BACTEC™ MGIT™ 960 System

The MGIT (Mycobacteria Growth Indicator Tube) consists of liquid broth medium that is known to yield better recovery and faster growth of mycobacteria. In addition to Middlebrook 7H9 liquid media, the MGIT tube contains an oxygen-quenched fluorochrome, tris 4, 7-diphenyl-1, 10-phenothroline ruthenium chloride penta hydrate, embedded in silicone at the bottom of the tube. During bacterial growth within the tube, the free oxygen is utilized and is replaced with carbon dioxide. With depletion of free oxygen, the fluorochrome is no longer inhibited, resulting in fluorescence within the MGIT tube when visualized under UV light. The intensity of fluorescence is directly proportional to the extent of oxygen depletion.

2.2 Inoculation of MGIT Medium [18]

(i) Label MGIT tubes with specimen number. (ii) Unscrew the cap and aseptically add 0.8 ml of MGIT growth supplement/PANTA to each MGIT tube. Use of an adjustable pipette is recommended. (iii) Using a sterile pipette or a transfer pipette, add up to 0.5 ml of a well-mixed processed/concentrated specimen to the appropriately labelled MGIT tube. Use separate pipette or pipette tip for each specimen. (iv) Immediately recap the tube tightly and mix by inverting the tube several times. (v) Wipe tubes and caps with a mycobactericidal disinfectant and leave inoculated tubes at room temperature for 30 minutes. (vi) Work under the biological safety cabinet for the specimen inoculation. (vii) MGIT tubes should be incubated until the instrument flags them positive. (viii) After a maximum of six (6) weeks, the instrument flags the tubes negative if there is no growth. (ix) Positive culture is confirmed by ZN Staining, Rapid immunochromatography and Inoculation on brain heart infusion agar.

2.3 Positive MGIT Tube Drug Susceptibility Testing [19]

Source of drugs All second-line drugs will be obtained in chemically pure form from Sigma or the appropriate pharmaceutical company. Drug Concentration of drug in working solution (µg/ml) [20]. Levofloxacin: 1.5 µg/ml, Moxifloxacin: 2 µg/ml, Kanamycin: 2.5 µg/ml, Capreomycin: 2.5 µg/ml.

(i) Label 7 mL MGIT tubes for each test isolate with a study label that includes identifying information. (ii) In addition, label tubes with the appropriate second-line drug name or abbreviation; e.g., AMK (amikacin), CAP (), L(lev capreomycin ofloxacin), etc. (iii) Label a GC tube (Growth Control). (iv) Aseptically add 0.8 mL of BACTEC MGIT OADC Enrichment, to each MGIT tube. (v) Aseptically pipette 0.1 ml of the appropriately diluted drug into the corresponding MGIT tube. (vi) It is important to add the correct drug to the corresponding tube. (vii) Do not add drugs to the MGIT GC tube. (viii) According the days of positive culture, culture will be inoculated in to the drug tubes in following manner: 1 to 2 days only:- directly used, 3 to 4 days:- 1:5 dilution and, More than 5 days:- 1:100 dilution used.

2.4 Interpretation of DST Results for Second-Line Drugs

For all set configurations protocol, when the growth unit (GU) of the GC tube reaches ≥400 within the timed protocol, the instrument marks the Antimicrobial Susceptibility Testing (AST) set.
complete and interprets the results: S = Susceptible = the GU of the drug tube is less than 100, R = Resistant = the GU of the drug tube is 100 or more.

3. RESULTS

585 MDR suspected sputum samples were received, 466 sputum samples had culture positive for acid fast bacilli suggestive of Mycobacterium tuberculosis infection. 466 culture positive sputum samples were proceeded for Second line drug susceptibility testing by BACTEC MGIT 960 (MGIT 960 instrument, 293 were MDR-TB (Multidrug resistant TB) resistant to both Isoniazid and Rifampicin or are mono-resistant to Rifampicin. 151 were Pre-XDR TB (Pre-Extensively Drug-Resistant Tuberculosis) resistance to First line anti-TB drugs (Rifampicin and/or Isoniazid) with additional resistance to second-line drugs. (i.e. to any Fluoroquinolone (FQ), or to at least one of the three injectable second-line drugs: Amikacin, Kanamycin, and Capreomycin).

Fig. 1 shows 585 MDR Suspected Sample were received out of them 466 were showing Culture Positive and 119 were showing Culture Negative.

Table 1 shows distribution of all MDR-TB suspected samples among culture positive samples 293 (63%) were MDR-TB, 127 (27%) were pre-XDR with Fluoroquinolone, 24 (5%) were Pre XDR TB with Aminoglycoside and 22 (5%) were XDR-TB.

Fig. 2 shows Age and Sex wise distribution of Pre XDR-TB with Fluoroquinolone Resistance in male patient were 110 (73%) and in female patient were 41(27%) suggest highest cases were found in male patient. Among pre XDR-TB of different age group suggest highest cases were found 21-30 years 57 (38%), followed by other age group.

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of sample</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR-TB</td>
<td>293</td>
<td>63</td>
</tr>
<tr>
<td>Pre XDR-TB (FQ)</td>
<td>127</td>
<td>27</td>
</tr>
<tr>
<td>Pre XDR-TB (AM)</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td>XDR-TB</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>466</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 1. MDR suspected sample distribution

Table 1. Prevalence of MDR-TB, Pre XDR-TB and XDR-TB
have resistance to fluoroquinolone or 24 present study, out of 466 culture positive 127 drugs. On the other hand if resistance to either resistance to both quinolone and injectable study, out of 466 culture positive 22 (5%) have resistance to Rifampicin & Isoniazid) it is labelled one of the quinolones and any of these injectable resistant treatment regimen. If resistance to any drugs being important component of drug recommendations, baseline second-line DST was done for quinolones group and FQ and AM was studied only. In this study, DST line DST to XDR-TB cases of different age group. Suggest highest cases were found in group of 21-30 years 8 (36%), followed by other group.

4. DISCUSSION

The Present study was done as per WHO recommendations, baseline second-line DST to FQ and AM was studied only. In this study, DST was done for quinolones group and Aminoglycoside injectable agents. All of these drugs being important component of drug resistant treatment regimen. If resistance to any one of the quinolones and any of these injectable is present in an MDR-TB patient (who had resistance to Rifampicin & Isoniazid) it is labelled as extensively drug resistant TB (XDR-TB), which is the most difficult form to treat [21]. In our study, out of 466 culture positive 22 (5%) have resistance to both quinolone and injectable drugs. On the other hand if resistance to either quinolone or an injectable is detected in a MDR-TB case, it is considered as Pre XDR-TB. In present study, out of 466 culture positive 127 (27%) have resistance to fluoroquinolone or 24 (5%) have resistance to injectable drugs.

In present study, Age wise prevalence of Pre XDR-TB and XDR-TB among MDR TB shows 220 (47%) in age group of 21-30 years followed by 101 (22%) in age group of 31-40 years followed by other age group. Compared with study of Sameer Adwani et al. [24] and Tamanna Tasnim et al. [23] were more common in age group of 21-30 years (68.2%) and (45.4%) similar to present study. In study of Olusoji Daniel et al. [22] and Gosavi sv et al. [25] were more common in age group of 31-40 years (62%) and (49%). More affected age group is middle age group 21-40 years.

The probable cause of the higher numbers of drug resistant TB in the active age group may be due to their frequent movement, greater exposure to the environment, coming in contact with more people outdoors and higher case notification due to greater health awareness and concern among young adults.

In present study prevalence of Pre XDR TB among MDR TB was 151 (32.4%). Nationwide, the number of pre- XDR TB cases may be large, although such surveillance data have not been published. Studies from other parts of world also show high prevalence of pre- XDR-TB [33]. Moreover, the presence of pre-XDR-TB was also found to be an independent prognostic factor of...
poor outcomes and survival in patients with MDR-TB [34]. Present study compared with Parul singhal [27] were 43 (33.3%) similar to present study, sameer Adwani, Mumbai [24] shows high prevalence 127 (55.9%) and Amita Jain [26] 55 (15.2%), Olusoji Daniel [22] 10 (16.7%), Tamana Tasnim et al. [23] 11(20.4%) were lower than present study. The high rate of Pre-XDR TB in India and other countries compared to our study may not be unconnected to the fact that India has the highest rate of MDR-TB in the world and a better laboratory infrastructure for the isolation of drug resistant strain.

It is important to note that these studies were conducted in DST Laboratory where patients sample where refereed for suspected MDR-TB

![Age/Sex Wise Distribution of XDR TB](image)

Fig. 3. Age/sex wise distribution of XDR TB

<table>
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<tr>
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<tbody>
<tr>
<td>21-30</td>
<td>47</td>
<td>68.2</td>
<td>45.4</td>
<td>24</td>
<td>27.5</td>
</tr>
<tr>
<td>31-40</td>
<td>22</td>
<td>10.5</td>
<td>18.8</td>
<td>62</td>
<td>49</td>
</tr>
<tr>
<td>41-50</td>
<td>17</td>
<td>8.82</td>
<td>9.10</td>
<td>6</td>
<td>12.5</td>
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<td>51-60</td>
<td>10</td>
<td>7.9</td>
<td>00</td>
<td>6</td>
<td>10.1</td>
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<tr>
<td>61-70</td>
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<tr>
<td>Above 70</td>
<td></td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10</td>
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</tbody>
</table>

Table 2. Comparison of age wise distribution of prevalence of pre- XDR TB & XDR-TB among MDR-TB

<table>
<thead>
<tr>
<th>Pre-XDR TB [%]</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>Present Study (2017)</td>
</tr>
<tr>
<td>20.4</td>
<td>Tamanna Tasnim (2018) [23]</td>
</tr>
<tr>
<td>55.9</td>
<td>Sameer Adwani (2016) [24]</td>
</tr>
<tr>
<td>33.3</td>
<td>Parul Singhal (2016) [27]</td>
</tr>
<tr>
<td>16.7</td>
<td>Olusoji Daniel, Nigeria (2013) [22]</td>
</tr>
<tr>
<td>15.2</td>
<td>Amita Jain (2012) [26]</td>
</tr>
</tbody>
</table>
Table 4. Comparison of prevalence of XDR-TB among MDR-TB

<table>
<thead>
<tr>
<th>XDR-TB [%]</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.3</td>
<td>Singh S (2007) [30]</td>
</tr>
<tr>
<td>20</td>
<td>Myneedu VP (2011) [29]</td>
</tr>
<tr>
<td>11</td>
<td>Ajbani K (2011) [31]</td>
</tr>
<tr>
<td>7.4</td>
<td>Tamanna Tasnim, Bangladesh (2018) [23]</td>
</tr>
<tr>
<td>3</td>
<td>Amita Jain (2012) [26]</td>
</tr>
<tr>
<td>3.7</td>
<td>Porwal C (2013) [28]</td>
</tr>
<tr>
<td>4.8</td>
<td>Sameer Adwani (2016) [24]</td>
</tr>
<tr>
<td>4.6</td>
<td>Rajasekharan (2009) [32]</td>
</tr>
<tr>
<td>5</td>
<td>Present Study (2017)</td>
</tr>
</tbody>
</table>

diagnosis which may be the reason for the higher resistance. From the mentioned findings of the different studies, it is evident that the prevalence of pre-XDR-TB cases among the MDR-TB patients varies between countries and is some cases variations occur within the same country in different regions. These differences may be the result of different anti-TB regimens adopted by the different countries. Other contributing factors may be due to low socio-economic condition, poor health infrastructure and lack of sufficient medications in those regions.

The prevalence of XDR-TB among MDR-TB in India in various studies has been reported ranging from 0.89% to 33%. In present study the prevalence of XDR-TB among our MDR-TB patients was 5%. In a study conducted by Rajasekharan et al. [32], the prevalence of XDR-TB among MDR-TB was 4.6% and Sameer Adwani et al. [24] were 4.8% similar to our study. Myneedu et al. [29] reported higher prevalence of 20% XDR-TB among MDR-TB, and also a study of Ajbani K, et al. [31] from Hinduja Hospital, Mumbai revealed 11% of MDR strains as XDR. Singh et al. [30] reported 33.3% XDR-TB in a population of HIV sero-positive MDR TB patients from AIIMS, New Delhi. Higher prevalence in this study may be due to their study group is only XDR-TB and their study year and burden of XDR-TB in various state is different due to their socio economic condition, their habits, their working condition and population of the state. In the study of Porwal C, et al. [28], prevalence of XDR TB was about 3.7%, Amita Jain et al. [26] were 3% and Tamanna Tasnim et al. [23] demonstrated 7.4% XDR among MDR strains were also similar to our study.

5. LIMITATION OF PRESENT STUDY

The limitation of our study is that the observed prevalence does not necessarily reflect the prevalence in the community since this was a tertiary care centre and in general, referral basis can lead to wide variations in the observed prevalence’s amongst different centres. We studies prevalence of Pre XDR TB and XDR TB by using liquid culture but Line probes Assay are an efficient and reliable for rapid drug susceptibility screening but at time of study period not availability of Line probe Assay for 2nd line drug susceptibility. In present study we tested only 4 drugs (Levofloxacin, Moxifloxacin, Kanamycin and Capreomycin) while in line probe assay group of Fluoroquinolones and all Injectable drugs were tested. So, Line probes Assay were more reliable and rapid test than liquid culture.

6. CONCLUSION

We studied the prevalence of Pre XDR-TB and XDR-TB among MDR-TB patients, which were 32.4% and 4.7% respectively. The high prevalence of Pre XDR-TB (FQ) is alarming and of concern in management of MDR-TB. Drug Susceptibility testing helpful for second-line drugs and to configure screening and diagnostic algorithms into rational management programmes for drug-resistant TB. We also suggest the importance of reserving FQ and AM for MDR-TB management and curbing their use as antibiotics for all the common infections.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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