Journal of Advances in Microbiology

19(2): 1-9, 2019; Article no.JAMB.51353
ISSN: 2456-7116

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

Dipali Gavali[1], Binita Aring[1*], Hiral Gadhavi[1], Akhlakahemad Nathamehta[1], Abhishek Dave[1] and Aanand Nakhva[1]

[1]Shri M. P. Shah Medical College, Jamanagar, India.

Authors' contributions

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

Article Information

DOI: 10.9734/JAMB/2019/v19i230186
Editor(s):
(1) Dr. Muhsin Jamal, Assistant Professor, Department of Microbiology, Abdul Wali Khan University, Garden Campus, Mardan, Pakistan.
(2) Prof. Hung-Jen Liu, Distinguished Professor, Institute of Molecular Biology, National Chung Hsing University, Taiwan.
(3) Prof. Dra. Simone Aquino, Universidade Nove de Julho, São Paulo, Brazil.
(4) Prof. Pongsak Rattanachaikunsopon, Department of Biological Science, Faculty of Science, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand.
Reviewers:
(1) Ronald Bartzatt, University of Nebraska, USA.
(2) Byron Baron, University of Malta, Malta.
(3) Asitkumar Chakraborty, Vidyasagar University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/51353

Original Research Article

Received 08 September 2019
Accepted 14 November 2019
Published 27 November 2019

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

**Corresponding author: E-mail: dr.binitajosepharing@gmail.com;*

TB-Drugs. Mycobacterial Growth Indicator Tube method conducted in an RNTCP accredited Culture and Drug susceptibility testing laboratory, Jamnagar, as part of the evaluation in the public healthcare system from where patients were referred for diagnosis to us.

Results: 585 MDR suspected sputum samples were received, 466 sputum samples were showing culture positive for acid-fast bacilli which were screened against second-line drug susceptibility testing by using of BACTEC MGIT 960 (MGIT 960) instrument. About 293 Mycobacterium samples were MDR-TB, 151 were Pre- XDR TB and 22 were XDR-TB.

Conclusion: The prevalence of Pre XDR-TB and XDR-TB among MDR-TB patients were 32.4% and 4.7% respectively. The high prevalence of Pre XDR-TB (FQ) is alarming and of concern in the management of MDR-TB control in Jamnagar area.

Keywords: Drug-resistant; tuberculosis; tertiary care hospital; mycobacterial growth.

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 Fluroquinolone 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 Fluroquinolone, 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 $^{14}CO2$ liberated during the growth of bacteria and decarboxylation of ^{14}C-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 ^{14}C-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

(MGIT 960) is the fully automatic system for detection of mycobacterial growth and drug susceptibility testing of *Mycobacterium tuberculosis* [14,15].

2. 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-phenonthroline ruthenium chloride pentahydrate**,** 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 fags 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 Fluroquinolone, 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.

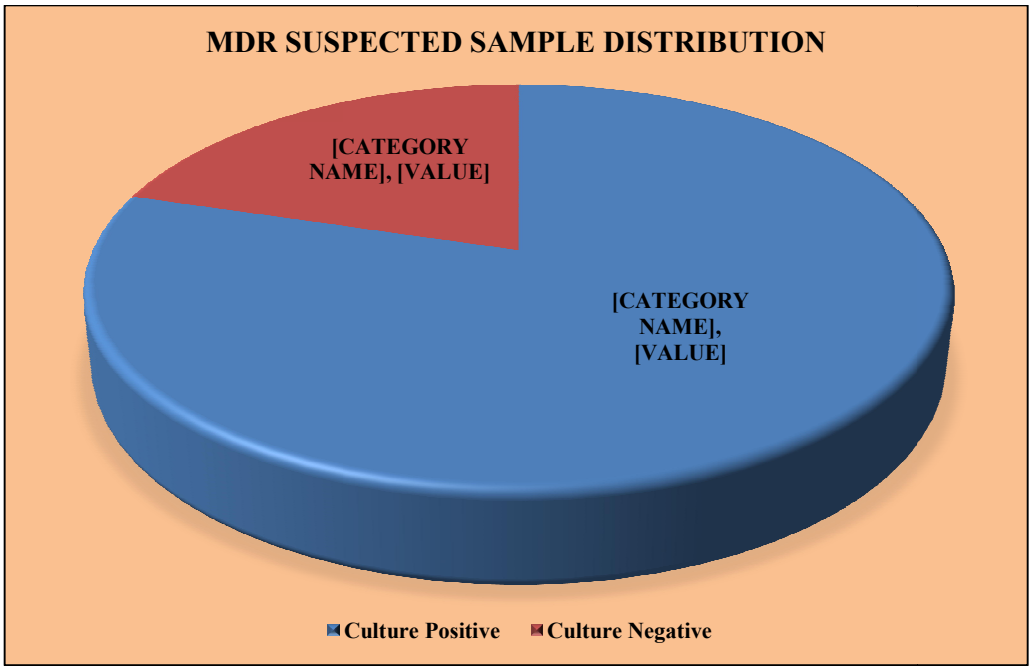

Fig. 1. MDR suspected sample distribution

Table 1. Prevalence of MDR-TB, Pre XDR-TB and XDR-TB

Category	No. of sample	Percentage (%)
MDR-TB	293	63
Pre XDR-TB (FQ)	127	27
Pre XDR-TB (AM)	24	5
XDR-TB	22	5
Total	466	100

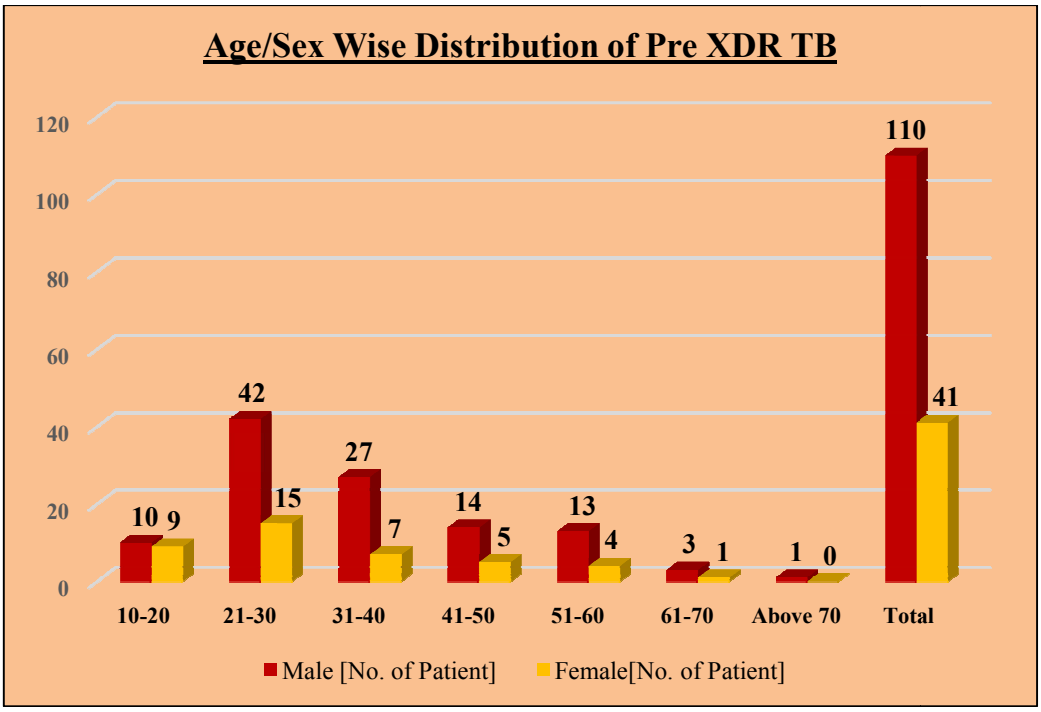

Fig. 2. Age/sex wise distribution of pre XDR TB

Fig. 3 shows Age/sex wise distribution of XDR - TB (Resistance to Fluoroquinolone and Aminoglycoside) in male patient were 12 (55%) and in female patient were 10 (45%). Prevalence 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 were 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

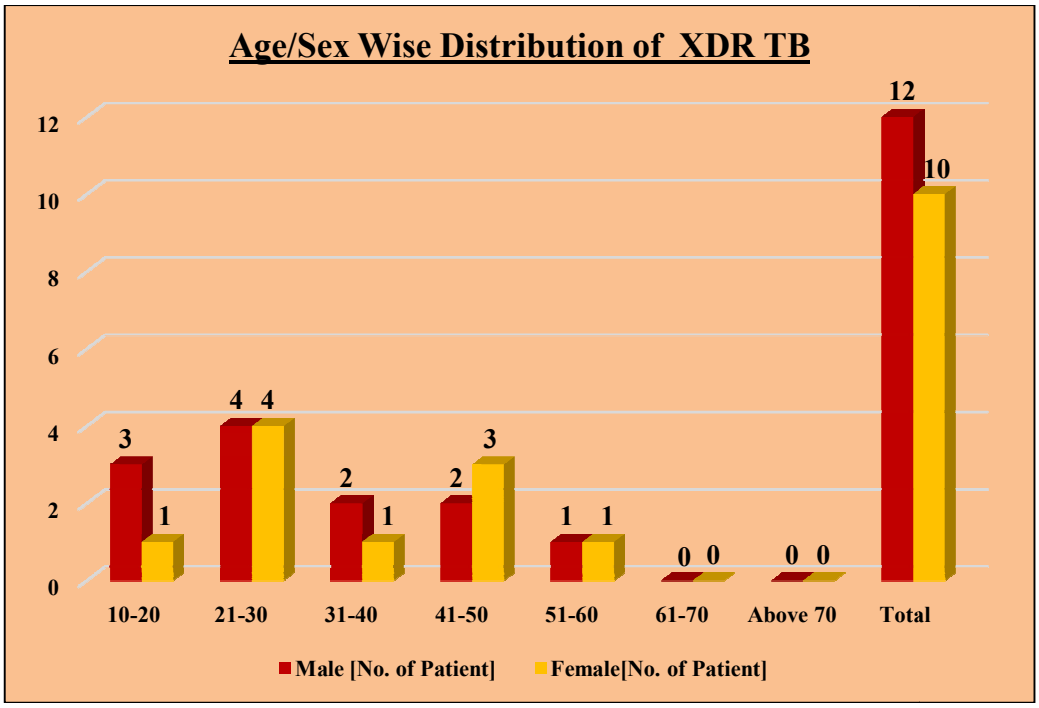

Fig. 3. Age/sex wise distribution of XDR TB

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

Age [In year]	Present study (2017) [%]	Sameer Adwani (2016) [24] [%]	Tamanna Tasnim (2018) [23] [%]	Olusoji Daniel (2013) [22] [%]	Gosavi sv, Nasik (2015) [25] [%]
21-30	47	68.2	45.4	24	27.5
31-40	22	10.5	18.8	62	49
41-50	17	8.82	9.10	6	12.5
51-60	10	7.9	00	6	10.1

Table 3. Comparison of prevalence of pre XDR-TB among MDR-TB

Pre-XDR TB [%]	Study
32	Present Study (2017)
20.4	Tamanna Tasnim (2018) [23]
55.9	Sameer Adwani (2016) [24]
33.3	Parul Singhal (2016) [27]
16.7	Olusoji Daniel, Nigeria (2013) [22]
15.2	Amita Jain (2012) [26]

Table 4. Comparison of prevalence of XDR-TB among MDR-TB

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

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 theMDR-TB patients varies between countries and is some cases variations occur within the same county 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.

REFERENCES

1. Lawn SD, Zumla AI. Tuberculosis. Lancet. 2011;378(9785):57-72.
DOI: 10.1016/S0140-6736(10)621733
PMID: 21420161
2. Rothschild BM, Martin LD, Lev G, et al. Mycobacterium tuberculosis complex DNA

from an extinct bison dated 17,000 years before the present. Clin. Infect. Dis. 2001;33(3):305-11.
DOI: 1086/321886
PMID: 11438894

3. Pearce-Duvet J. The origin of human pathogens: Evaluating the role of agriculture and domestic animals in the evolution of human disease. Biol Rev Camb Philos Soc. 2006;81(3):369-82.
DOI: 10.1017/S1464793106007020
PMID: 16672105
4. Malik S, Willby M, Sikes D, Tsodikov OV, Posey JE. New insights into fluoroquinolone resistance in *Mycobacterium tuberculosis*: Functional genetic analysis of gyrA and gyrB mutations. Plos One. 2012;7(6):e39754.
5. Smith SE, Ershova J, Vlasova N, Nikishova E, Tarasova I, Eliseev P, Maryandyshev AO, Shemyakin IG, Kurbatova E, Cegielski JP. Risk factors for acquisition of drug resistance during multidrug-resistant tuberculosis treatment, Arkhangelsk oblast, Russia, 2005-2010. Emerg Infect Dis. 2015;21(6):1002–11.
6. Jain A, Dixit P, Prasad R. Pre-XDR & XDR in MDR and ofloxacin and kanamycin resistance in non-MDR *M. tuberculosis* isolates. Tuberculosis. 2012;92:404–6. [PubMed] [Google Scholar].
7. Sloan DJ, Lewis JM. Management of multidrug-resistant TB: Novel treatments and their expansion to low resource settings. Trans R Soc Trop Med Hyg. 2016;110(3):163–172. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
DOI: 10.1093/trstmh/trv107
8. World Health Organization. The Global Tuberculosis Report: 2015. Geneva, Switzerland: WHO; 2015.
Available:http://www.who.int/tb/publications/global_report/gtbr2015_executive_summary.pdf
(Accessed 2016, Jan 18)
9. Companion Handbook to the WHO Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis. WHO Guidelines Approved by the Guidelines Review Committee: 2014 update. Geneva, Switzerland: WHO; 2014.
Available:http://www.who.int/tb/publications/pmdt_companionhandbook/en/
(Accessed 2016, Jan 18)
10. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis: 2015 update. Geneva: WHO; 2015.
Available:http://apps.who.int/iris/bitstream/10665/174897/1/9789241549134_eng.pdf
(Accessed 25 Mar 2016)
11. Nair SA, Raizada N, Sachdeva KS, Denkinger C, Schumacher S, Dewan P, et al. Factors associated with tuberculosis and rifampicin-resistant tuberculosis amongst symptomatic patients in India: A retrospective analysis. PLoS One. 2016;11(2):e0150054. [PMC free article] [PubMed] [CrossRef] [Google Scholar].
DOI: 10.1371/journal.pone.0150054
12. CDC. Extensively drug-resistance tuberculosis information.
Available:http://www.cdc.gov/tb/topic/drtb/xdrtb.htm
13. DeLand FH, Wagner RN Jr. Early detection of bacterial growth with carbon-14 labeled glucose. Radiology. 1969;92: 154-155.
14. Roberts GD, Goodman NL, Heifets L, et al. Evaluation of the BACTEC radiometric method for recovery of mycobacteria and drug susceptibility testing of *Mycobacterium tuberculosis* from acid-fast smear- positive specimens. J Clin Microbiol. 1983;18:689-696.
15. Siddiqi SH, Libonati JP, Middlebrook G. Evaluation of a rapid radiometric method for drug susceptibility testing of *Mycobacterium tuberculosis*. J Clin Microbiol. 1981;13:908-912.
16. Kent PT, Kubica GP. Public health microbiology, a guide for the level III laboratory. Centers for Disease Control, Division of Laboratory Training and Consultation, Atlanta, GA; 1985.
17. Siddiqi SH, Rüsch-Gerdes S. MGIT procedure manual. Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; 2006.
18. Salman H. Siddiqi, Sabine Rüsch-Gerdes., Foundation for innovative for new diagnosis; 2006.
19. Global Laboratory Initiative Advancing TB Diagnosis; 2014.
Available:mycobacteriology-laboratory-manual%20of%20Tuberculosis.pdf
20. World Health Organization. Policy Guidance on Drug Susceptibility Testing (DST) of Second Line Anti-tuberculosis Drugs. Geneva, WHO, (WHO/HTM/TB/2008.392); 2008.
21. Zink A, Sola C, Reischl U, Grabner W, Rastogi N, Wolf H, Nerlich A.

Characterization of *Mycobacterium tuberculosis* complex DNAs from Egyptian Mummies by Spoligotyping. J Clin Microbiol. 2003;41(1):359-67.
DOI: 1128/JCM.41.1.359-367.2003
PMC: 149558
PMID: 12517873

22. Olusoji Daniel, Eltayeb Osman, et al. Pre-extensive drug resistant tuberculosis (Pre-XDR-TB) among MDR-TB patents in Nigeria. Global Advanced Research Journal of Microbiology (ISSN: 2315-5116). 2013;2(2).
23. Tasnim T, Tarafder S, Alam FM, Sattar H, Mostofa Kamal SM. Pre-extensively drug resistant tuberculosis (Pre-XDR-TB) among pulmonary multidrug resistant tuberculosis (MDR-TB) patients in Bangladesh. Journal of Tuberculosis Research. 2018;6:199-206.
Available:https://doi.org/10.4236/jtr.2018.63018
24. Sameer Adwani, et al. JKIMSU, prevalence of pre-extensively drug-resistant tuberculosis (Pre XDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) among pulmonary multidrug resistant tuberculosis (MDR-TB) at a Tertiary Care Center in Mumbai. 2016;5(3). ISSN: 2231-4261.
25. Gosavi SV, et al. A step towards control of multidrug resistant tuberculosis: Hospital based study from Nashik, India. Community Medicine Department, Dr. Vasantrao Pawar Medical College, Hospital & Research Centre, Nashik.
26. Amita Jain, et al. Pre-XDR & XDR in MDR and Ofloxacin and Kanamycin resistance in non-MDR *Mycobacterium tuberculosis* isolates. Tuberculosis. 2012;92:404e406. Available:http://intl.elsevierhealth.com/journals/tube
27. Parul Singhal, et al. A study on pre-XDR & XDR tuberculosis & their prevalent genotypes in clinical isolates of *Mycobacterium tuberculosis* in North India. Indian J. Med. Res. 2016;143(3):341-347.
DOI: 10.4103/0971-5916182625
28. Porwal C, Kaushik A, Makkar N, Banavaliker JN, Hanif M, Singla R, et al. Incidence and risk factors for extensively drug-resistant tuberculosis in Delhi region. PLoS One. 2013;8(2):e55299.
29. Myneedu VP, Visalakshi P, Verma AK, Behera D, Bhalla M. Prevalence of XDR TB cases - a retrospective study from a tertiary care TB hospital. Indian J Tuberc. 2011;58(2):54-9.
30. Singh S, Sankar MM, Gopinath K. High rate of extensively drug- resistant tuberculosis in Indian AIDS patients. AIDS. 2007;21(17):2345-47.
31. Ajbani K, Rodrigues C, Shenai S, Mehta A. Can mutation detection accurately predict XDR- TB: Study from a tertiary care centre, India. J Clin Microbiol. 2011;49(4):1588-90.
32. Rajasekaran S, Chandrasekar C, Mahilmaran A, Kanakaraj K, Karthikeyan DS, Suriakumar J. HIV coinfection among multidrug resistant and extensively drug resistant tuberculosis patients - a trend. J Indian Med Assoc. 2009;107(05):281-2, 284-6.
33. Kozinska M, Brzostek A, Krawiecka D, Rybczynska M, Zwolska Z, Augustynowicz-Kopec EMDR. Pre-XDR and XDR drug-resistant tuberculosis in Poland in 2000e2009. PneumonolAlergol Pol. 2011;79(4):278e87.
34. Kim DH, Kim HJ, Park SK, Kong SJ, Kim YS, Kim TH, Kim EK, Lee KM, Lee SS, Park JS, Koh WJ, Lee CH, Shim TS. Treatment outcomes and survival based on drug resistance patterns in multidrug-resistant tuberculosis. Am J Respir Crit Care Med. 2010;182(1):113e9.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/51353