Original Article

Prevalence of Tuberculosis and its drug resistant strains in the Eastern region of Bihar

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One third of the world's population is harboring bacilli causing Tuberculosis (TB). The severity of TB has increased several times with the emergence of anti-TB drug resistance detected for almost all key anti-TB drugs. Effectual patient management requires early diagnosis of TB cases and their screening for drug resistant TB followed by supervised treatment with specific drug regimen. The present study shows the prevalence of Multi Drug Resistant (MDR)/Rifampicin Resistant TB (RR-TB), Pre-extensively Drug Resistant (Pre-XDR) and Extensively Drug Resistance TB (XDR TB) cases in the eastern region of Bihar and its comparison with other regions of the State. The study shows the higher percentage of drug resistance for some of the key anti-TB drugs in Bihar as compared with the data of National Drug Resistant TB survey of India and some recent Global TB reports. The fluoroquinolone resistance in the present study was found to 50.80 % RR-TB cases, while it was found to be only 24.14% in NDR Survey. The higher fluoroquinolones resistance in Bihar may be attributed to the widespread use of quinolones in other infections.

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Key words **: Tuberculosis (TB), Multi-drug resistant (MDR) TB, Rifampicin resistant (RR) TB, Extensively drug resistant (XDR) TB, Pre-XDR TB.**

Tuberculosis (TB) is a disease caused by the infection of a group of mycobacterial species called as *Mycobacterium tuberculosis* Complex (MTBC)[1]. The bacilli carry the competency to infect any part of the human body but mostly it is a pulmonary pathogen[2]. TB is the leading cause of death from a single infectious agent[3]. One third of the world's population is infected with mycobacterium tuberculosis[4] :

Studies revealed that more than 40% of the Indian population is harboring TB bacilli[5]. India carries 30% of the global TB burden[6] and more than 1400 Indians are dying every day because of TB[7]. The severity of TB has increased several times with the emergence of anti-TB drug resistance. Resistance has developed in almost all key anti-TB drugs. Effectual patient management requires early diagnosis of TB cases and their screening for drug resistant TB followed by supervised treatment with specific drug regimen[8]. The first line anti-TB drugs are Rifampicin, Isoniazid, Pyrazinamide and Ethambutol[8]. The supervised treatment is provided with first line anti-TB drugs for diagnosed susceptible TB cases under DOTS strategy for at least six months duration. The treatment is divided in two phases- the intensive phase of two months where Rifampicin, Isoniazid, Pyrazinamide and Ethambutol were used followed by the continuation phase of four months with Rifampicin, Isoniazid and Ethambutol[9]. Multi Drug Resistant (MDR) TB is a form where the TB bacilli get resistance with at least Rifampicin and Isoniazid[10]. These MDR TB bacilli when get additional resistance with any fluoroquinolones and one of the three second line injectable drugs (Capreomycin, Kanamycin and Amikacin) are called as an extensively drug resistant (XDR) TB[11].

After the implementation of universal Drug Sensitivity Test (DST) in India, at least Rifampicin DST is done for all notified cases[12]. All the Rifampicin resistant TB cases are further evaluated for second line drug resistance and based on the results of second line DST, patients are subjected to appropriate treatment. The estimated global MDR/RR TB burden was 4.1 % for new cases and 19 % for previously treated cases for the year 2016[3]. A new case is defined as patient who had never been treated before with anti-TB drugs or treated with anti-TB drugs for less than one month but if a patient has a history of more than a month of TB treatment, it will be considered as previously treated case[13]. WHO estimated that for MDR TB, around 21% cases have strains with additional resistance with fluoroquinolones (including Levofloxacin, Ofloxacin and Moxifloxacin) and 9.5% cases of XDR TB[14].

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In the present study, the prevalence of MDR/RR-TB, pre-XDR and XDR TB cases has been estimated in the eastern region of Bihar .

Methods and Materials

The Tuberculosis Culture and DST laboratory functioning in the premise of Jawaharlal Nehru Medical College and Hospital, Bhagalpur is one of the quality assured TB containment laboratories in Bihar. This TB culture and DST laboratory is certified by Central TB Division, New Delhi for various molecular and culture based drug susceptibility testing methods. The laboratory is receiving samples from ten districts of Bihar, namely - Araria, Banka, Begusarai, Bhagalpur, Jamui, Katihar, Khagaria, Kishanganj, Munger and Purnia. Culture based DST has been done by MGIT (Mycobacterium Growth Indicator Tube) 960 system. CBNAAT (Cartridge Based Nucleic Acid Amplification Test) and LPA (Line Probe Assay) technique has been used for molecular DST. Following laboratory testings were included in this study.

(1) First line culture based DST of 100 new TB cases were performed.

(2) CBNAAT was performed on samples from presumptive TB and presumptive DR-TB cases.

(3) Samples of MDR/RR TB diagnosed patients were subjected to second line LPA.

Culture Based Drug Susceptibility Testing :

Molecular diagnostics promise the rapid and accurate diagnosis of TB but culture is still considered as the gold standard. Liquid media offers three dimensional growth of cells and hence retrieval is faster[15]. MGIT 960 instrument has been used for the study. 137 samples were processed by NALC-NaOH method and inoculated in the MGIT tube to get 100 pure *M.tuberculosis* culture. First line drug susceptibility testing kit of BD includes four drugs, ie, Streptomycin, Isoniazid, Rifampicin and Ethambutol. DST was performed for all the 100 culture samples as per the user's instructions.

CBNAAT :

The Xpert MTB/RIF is the name of machine performing CBNAAT manufactured by Cepheid Inc, Sunnyvale, California, USA. This technology performs nested real time PCR based qualitative detection of *Mycobacterium tuberculosis* complex (MTBC) and its susceptibility with rifampicin. WHO has conditionally recommended the use of Xpert MTB/Rif as the first-line diagnostic test for presumptive TB cases[16]. Xpert MTB/Rif has achieved an overall sensitivity of 88% and specificity of 99%[17]. This technology can be used in much lower facilities as compared with facilities TB culture technique requires. Also, the machine can provide results within 2 hours from the start of the test. Both these properties promise the effective utilization of CBNAAT for rapid diagnosis of Rifampicin resistant TB.

Both the pulmonary and extra pulmonary samples can be tested by CBNAAT. 2-4 ml of samples are ideally required for testing. Double the volume of sample, reagent was added carefully for most of the sample type. Intermittent shaking with reaction time of 15 minutes is required. The processed samples were then added to the CBNAAT cartridges and tested in the machine as per the protocol[18]. Sample Processing Control (SPC) was present to ensure adequate processing of target bacilli and monitors the PCR step.

Line Probe Assay (LPA) :

LPA is a type of molecular DST that detects the mutation associated with drug resistance. The kits are being manufactures by HAIN life sciences, Gmbh, Hardwiesenstr, Nehren, Germany. LPA for rifampicin and isoniazid has been used from the introduction of Programmatic management of drug resistant TB in India[19]. The introduction of LPA has reduced the turnaround time of DR-TB diagnosis from months to days. Recently, WHO has recommended the use of second line LPA for detection of resistance in fluoroquinolones and second line injectable drugs[20].

Samples were processed by NALC-NaOH method. DNA extraction, PCR and reverse hybridization was carried out as per the manufacturer's instructions[21].

Results

Culture DST (First line) :

Out of 100 first-line liquid culture based DST performed, 92 cases were susceptible with all four drugs (SIRE). However, 03 cases were resistant with all four drugs. 02 cases were resistant with both Rifampicin and Isoniazid but susceptible with Streptomycin and Ethambutol. 01 case of Isoniazid mono- resistance and 02 cases of Rifampicin mono-resistance were detected.

Molecular DST (First line) :

A total of 2974 CBNAAT tests have done during the year 2017 and 2018. Out of these, number of *M tuberculosis* detected and *M. tuberculosis* not detected were 599 and 2334 respectively. Out of 599 *M tuberculosis* cases, Rifampicin resistance was detected in 110 cases and Rifampicin resistance have not detected in 449 cases. 41 tests have given non-confirmatory results like invalid, error, etc.

Molecular DST (Second line) :

A total of 472 second line LPA of rifampicin resistant TB cases were done. Out of 472 tests, 183 cases were susceptible to both fluoroquinolones and second line injectables. However, 44 XDR cases were detected. 240 cases of fluoroquinolones resistant and susceptible to

SLID were observed. 05 cases were detected as resistance to SLID and susceptible to fluoroquinolones. Out of these 05 cases, 02 cases were detected as low level kanamycin resistant.

Discussion

World Health Organization has proposed the END TB strategy in order to end the global TB epidemic by 2035[22]. With the agenda of "detect-treat-prevent-build", India has committed to defeat TB by 2025[23]. Along with active implementation of programme, India needs to focus more on research to achieve the target[24].

The present study depicts the prevalence of drug resistant strains of *M tuberculosis* in the eastern region of Bihar. One hundred diagnosed TB patients with no history of prior anti-TB therapy were selected to study the incidence of first line anti-TB drug resistance. 5 % MDR TB cases were observed in our study. However, the National anti TB drug resistance survey (NDRS) done at National Tuberculosis Institute, Bangalore in the year 2014 and 2015 reports 2.84% MDR cases amongst new patients[25]. The incidence of Rifampicin resistance was 7% in our study and NDRS reported 2.84%. Isoniazid resistance was 6% in our study and 11.06% in NDRS. The global TB report 2018 shows 7.1% isoniazid resistance in new patients and 7.9% in previously treated patients. We did not observe any streptomycin mono-resistance cases in the present study.

The target of TB control cannot be achieved without the involvement of private medical practitioners. More than 20% of referrals for CBNAAT testing at JLNMCH Bhagalpur were from private practitioners. A total of 599 (20 % of total sample tested) TB cases have detected from CBNAAT in the year 2017 and 2018. Out of these TB cases, 110 (18.36%) reported as rifampicin resistant. NDRS reported 2.84% rifampicin resistance in new cases and 11.67% in previously treated cases. The global TB report 2018 showed 3.5% in new and 18% in previously treated cases globally. However, prevalence of 2.8% for new cases and 12% in previously treated cases has been observed in India.

In the present study, second line LPA reported 240 (50.8%) fluoroquinolones resistance and second line injectables susceptible cases. The Intermediate Reference Laboratory, Patna is serving other regions of Bihar and they have reported 146 (52.51%) flouroquinolones resistant out of 278 cases tested in the first quarter of 2019. However, the NDRS reported only 21.82 % cases of MDR plus any of the flouroquinolones resistance. 21% flouroquinolones resistance was reported in the Global TB report 2016, 20% in Global TB report 2017 and 22% reported in the Global TB report 2018.

The XDR detected at our lab was 44 (9.32%) and IRL Patna has reported 30 (10.79%) cases. However, NDRS has reported 1.30% XDR cases. The Global TB report 2016 estimated 9.5% XDR TB cases by the end of 2015. The Global TB report 2017 reports 6.5% and 2018 reports 8.5% XDR TB cases.

The laboratory findings of the current study have been compared with the National and global data and observed a higher resistance percentage for some key anti-TB drugs in this study as depicted in Table 1.

Table 1 — *Showing comparison of drug resistant TB in different studies*

Percentage of drug resistant TB reported in different studies						
Type of drug resistant TB cases	Global TB report 2016	Global TB report 2017	Global TB report 2018	TB NDRS (India)	Current Study (Eastern Bihar)	Other regions of Bihar
XDR TB	9.50%	6.50%	8.50%	1.30%	9.32%	10.79%
MDR TB with FQ resistance	21%	20%	22%	21.82%	50.80%	52.51%

Fluoroquinolone is one of the most effective second line drugs for drug-resistant TB[26]. Patients of MDR may develop resistance to quinolones during treatment and become Pre-XDR or even XDR if they develop resistance to second line injectables. The main target of action of Quinolone in *Mucobacterium Tuberculosis* is DNA gyrase where lie the quinolone-determining sites gyrA and gyrB. Mutations in these sites lead to fluroquinolone resistance. A meta-analysis has shown that previous exposure of quinolones led to the development of quinolone resistance. Duration of treatment with quinolone is another factor promoting quinolone resistance.

The study has depicted the status of anti-TB drug resistance in the Eastern Bihar. However, further studies with bigger sample size for all the districts of Bihar would be more beneficial for the effective TB control programme assessment.

Summary :

The incidence of MDR TB is rapidly increasing. The present study shows that prevalence of fluoroquinolone resistance is more in TB patients of eastern Bihar. The widespread use of quinolones in infections other than TB may be the cause of this increased Quinolone resistance. It seems prudent to suggest that the use of respiratory quinolones needs to be reserved for the specific indications in order to preserve their effectiveness[27].

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