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RESEARCH ARTICLE

Resistance patterns and trends of extensively drug-resistant tuberculosis: 5-year experience

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ABSTRACT

Objective: Extensively drug-resistant tuberculosis (XDR-TB) strains were emerged when multidrug-resistant TB (MDR-TB) was inadequately treated. Inadequate treatment of MDR-TB cases may result in additional resistance especially non-XDR-TB and then XDR-TB. The aim of this study was to know the prevalence, resistance patterns and trends of the XDR-TB strains among the MDR-TB at a tertiary care hospital in Lucknow, India

Methods: A total of 430 *Mycobacterium* isolates were underwent NAP test and TB MPT64 Ag test for the identification of *Mycobacterium tuberculosis* complex (MTBC). Drug-susceptibility test (DST) was performed over MTBC for the first line drugs by 1% proportion method (Bactec) and for the second-line drugs by 1% proportion method (Lowenstein-Jensen media). The XDR-TB status was further confirmed by line probe assay (GenoType® MTBDRsl assay).

Results: Among the 430 isolates of mycobacterium, 365 (84.9%) were MTBC and 139 (38.1%) were MDR-TB respectively. Further 97 MDR-TB from "highly suspected drug resistant-TB (DR-TB)" cases among MDR-TB were tested with second line drugs in which 15 (15.5%) XDR-TB and 82 (84.5%) were non-XDR-TB. Regarding XDR-TB status, using the 1% proportion method a 100% agreement was seen with the GenoType® MTBDRsl assay. Resistance patterns of XDR-TB were as; 10/15 (66.7%) as isoniazid + rifampicin + ciprofloxacin + amikacin resistance and 5/15 (33.3%) as isoniazid + rifampicin + ciprofloxacin + amikacin + kanamycin resistance.

Conclusion: The prevalence of XDR-TB was 15.5% among MDR-TB. Hence laboratory testing of "highly suspected drug resistant-TB" isolates should be done for both first and second line drugs simultaneously especially in developing countries. *J Microbiol Infect Dis 2013;3(4): 169-175*

Key words: *Mycobacterium tuberculosis*, extensively drug-resistant tuberculosis, multi drug-resistance tuberculosis, India

Genişlemiş ilaca dirençli tüberküloz paterni ve trendi: 5 yıllık deneyim

ÖZET

Amaç: Genişlemiş drug-rezistan tüberküloz (XDR-TB) suşları multi-drug rezistan tüberküloz (MDR-TB) suşlarının uygunsuz tedavisiyle önem kazandı. Multi-drug rezistan tüberküloz (MDR-TB) suşları ile infekte olguların uygunsuz tedavisi özellikle XDR-TB dışı ve XDR-TB'a yol açabilir. Bu çalışmada Hindistan'ın Luckdown şehrindeki bir üçüncü basamak hastanesinde multidrug-rezistan tüberküloz suşları arasında genişlemiş drug-rezistans paterni ve trendinin araştırılması amaçlandı.

Yöntemler: Çalışmada *Mycobacteruim tuberculosis* complex (MTBC) identifikasyonu için 430 mikobakteri suşu NAP testi ve TB MPT64 Ag testine tabi tutuldu. İlaç duyarlılıklarının belirlenmesinde ilk sıra ilaçlar için % 1 proporsiyon metodu (Bactec) XDR-TB durumu GenoType® MTBDRslassay ile doğrulandı.

Bulgular: Toplam 430 mikobakteriden 365'i (% 84,9) MTBC ve 139'u (% 38,1) MDR-TB olarak tanımlandı. Yüksek oranda drug-rezistan-TB (DR-TB) olarak düşünülen 97 MDR-TB suşu ikinci sıra antitüberküloz ilaçlara duyarlılık açısındn test edildiğinde suşlardan 15'i (% 15,5) XDR-TB ve 82'si (% 84,5) ise XDR-TB dışı suşlar idi. XDR-TB suşlarının tamamı %1 proporsiyon testi kullanılarak GenoType® MTBDRsl assay ile doğrulandı. Toplam 15 XDR-TB suşundan 10'u (% 66,7) izoniazid + rifampisin + siprofloksazin + amikasin ve 5'i (% 33,3%) izoniazid + rifampisin + siprofloksazin + amikasin + kanamisin'e dirençli olarak değerlendirildi.

Sonuç: Bu çalışmada MDR-TB suşları arasında XDR-TB prevalansı % 15,5 olarak bulunmuştur. Bu yüzden özellikle gelişmekte olan ülkelerde yüksek şüpheli drug-rezistan TB durumunda suşların ilk ve ikinci sıra antitüberküloz ilaçlara duyarlılığının birlikte araştırılması gerektiğini düşünüyoruz.

Anahtar kelimeler: *Mycobacterium tuberculosis*, genişlemiş drug-rezistan tüberküloz, multidrug-rezistan tüberküloz, Hindistan

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INTRODUCTION

A significant high morbidity and mortality due to tuberculosis (TB) is reported from India contributing one-fifth of the global burden and two persons are die in every three minutes in this country, thus nearly 1000 person a day.[1-2] An estimated 2 billion people worldwide are infected with *Mycobacterium tuberculosis*, which remains a vast reservoir of potential tuberculosis cases. TB is the major co-infection in HIV infected patients.[3] At present, about 5% of new tuberculosis cases in India occur in people infected with HIV infection. An extremely worrisome aspect of M. tuberculosis is a recent rise to multi drug-resistant (MDR) and extremely drug-resistant (XDR) TB.4 However load of MDR-TB accounting for almost 50% of world total cases carry together by India and China alone.2 Due to their drug resistance, M. tuberculosis have emerged as a serious problem in the world. MDR-TB (defined as in vitro resistance to anti-tuberculous drugs, isoniazid and rifampicin) and XDR-TB (defined as in vitro resistance to isoniazid, rifampicin, any fluoroquinolones and at least one of three injectable second-line drugs) are now widely reported.[4-7]

According to World Health Organization (WHO) 2011 tuberculous report, number of XDR-TB proportionally depend upon number of increased MDR-TB cases, since the 69 countries have reported XDR-TB cases and estimated that 25.000 cases emerging every year as confirmed XDR-TB cases.[8] This is the first study from Northern India showing different patterns and trends of XDR-TB and lack of proper anti-tubercular therapy leading to development of further drug resistance like non-XDR-TB and then XDR-TB. Therefore the aim of this study is to know laboratory based prevalence and drug resistance patterns of XDR-TB strains among MDR-TB at a tertiary care referral centre in Lucknow, India.

METHODS

Isolation and identification of Mycobacterium

Between January 2007 and December 2011, a total of 430 consecutive culture positive isolates (Bactec 12B, Becton Dickinson, USA) were included at a tertiary care hospital in Lucknow. This study was conducted after approval by the local research ethics committee. Informed consent was obtained from the patients for sample collection and enrollment in this study. The entire patient's related information was taken from the hospital information system (HIS) to know whether the patient was new or previously treated with anti-tubercular treatment (ATT), duration of ATT, HIV status, whether the patient was on anti-retroviral therapy (ART), age, sex, types of TB (pulmonary or extra-pulmonary). Culture positive *Mycobacterium tuberculosis* complex (MTBC) strains isolated in Bactec 12B bottles were sub-cultured on Lowenstein-Jensen medium for enrichment. The isolates were re-suspended by pipetting 0.5 ml of 7H9 broth to the slope, and then the suspension was transferred to cryovials and kept at −70°C until required for further testing.[8] All isolates were tested with (p-nitro-α-acetylamino-β-hydroxy propiophenone) NAP test [9] (Becton Dickinson, Sparks, MD, USA) and further validated by TB Ag MPT64 rapid test (SD Standard Diagnostics, Inc. Yongin-si, Korea) for identification of MTBC were included for this study.[10-11]

Drug-susceptibility testing for first line drugs

Drug resistance patterns of all MTBC isolates for first line anti-TB drug susceptibility test (DST) were done by 1% proportion method using Bactec 460 12B medium (Becton Dickinson, Sparks, MD, USA). The first line drugs were provided in a drug kit (Becton Dickinson, Sparks, MD, USA). Following final drug concentrations were used; isoniazid (H)-0.1 mcg/mL, rifampicin (R)-2.0 mcg/mL, streptomycin (S)-6.0 mcg/mL and ethambutol (E)-7.5 mcg/mL.

Drug-susceptibility testing for second line drugs

Further, 97 isolates out of 139 MDR-TB strains, from those subjects who were already on second line ATT for more than two months or planned for second line ATT underwent second-line DST by 1% proportion method using Lowenstein-Jensen media.[12] The tested drugs and their critical concentrations were as follows: amikacin (AMK)-30 mcg/mL, kanamycin (KAN)-30 mcg/mL, ciprofloxacin (CIP)-2 mcg/mL, ethionamide (ETM)-40 mcg/mL, cycloserine (CYS)-40 mcg/mL, para-aminosalicylic acid (PAS)-1 mcg/mL and clarithromycin (CLA)-2 mcg/mL (Sigma®, USA).[13-14] XDR-TB positive strains by 1% proportion method were further confirmed by line probe assays (GenoType® MTBDRsl assay; Hain Lifescience, Nehren, Germany) using DNA probes for mutant genes of fluoroquinolones, aminoglycosides and ethambutol.[15]

HIV serology

After obtaining informed consent from the patients, HIV status was determined according to the Joint United Nations Programme on HIV/AIDS/WHO recommendations.[16]

Statistical analysis

Data was analyzed using SPSS 15.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) for Windows. Standard $\chi 2$ tests were used to assess statistical relationships between HIV status and XDR-TB. P value of <0.05 was considered statistically significant.

RESULTS

Among the 430 culture positive Mycobacterium species isolated, 365 were identified as MTBC (84.9%) both by NAP and TB MPT64 Ag test (100% agreement) and 65 (15.1%) as non-tuberculous mycobacteria (NTM), which were excluded from this study (Figure 1). Out of these 365 MTBC isolates included in this study for first line DST, 156 (42.7%) patients were previously taken ATT, 146 (40%) were new cases and in 63 (17.3%) cases ATT status was unknown. Out of these entire 365 patients, 233 (63.8%) male, and 132 (36.2%) were female; and their mean age and standard deviation were (35.7±14.1) years. As per recommendation, HIV was also tested in 300 subjects (82.2%), in which 55 were positive (18.3%) and 245 were negative (83.5%).

Among the 365 MTBC isolates after first line DST, 139 (38.1%) MDR-TB, 103 (28.2%) non-MDR-TB and 123 (33.7%) were pan-susceptible MTBC isolates. All non-MDR-TB and pan-susceptible isolates were further excluded from second line DST. The association of HIV among MDR-TB and non-MDR-TB was insignificant (p>0.05). Only 97/139 (69.8%) MDR-TB isolates were tested with second line drugs, among which 63 (65%) patients were already on one or multiple second line ATT for more than two months, and 34 (35%) subjects were planned for second line ATT. Prevalence of XDR-TB was 15/97 (15.5%) by DST using both 1% proportion method and the GenoType® MTBDRsl assay (Figure 1; 100% agreement with 1% proportion method for second line drugs and ethambutol). A continuous increasing trend of XDR-TB was noted from 2007 to 2011 at our center (Figure 2).

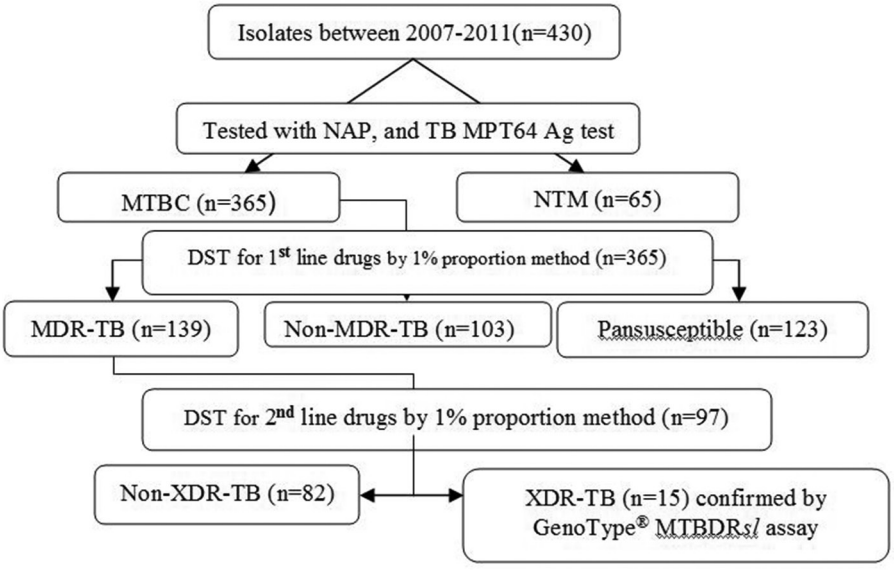

Figure 1. Flow chart of study isolates recruited for XDR-TB prevalence among MDR-TB isolates

Among the 15 XDR-TB subjects, the age range was from 22 to 67 years, in which 10 were male (66.7%) and 5 were female (33.7%). Out of these 15 XDR-TB subjects, 11 (73.3%) subjects were having history of fever, cough with sputum, and remaining 4 (26.7%) having fever with hemoptysis. Similarly 4 (40%) subjects were HIV sero-positive, and 6 (60%) were negative, and remaining 5 patients were could not be tested. In addition, 10 XDR-TB subjects were having history of TB contact, and 5 subjects did not have history of contact with confirmed TB case. Among these total 15 XDR-TB subjects, 6 were already on treatment with second line ATT drugs, 5 completed first and second line ATT regimens, 2 completed only first line ATT drugs and in remaining 2 subjects history of ATT/TB contacts were described (Table 1).

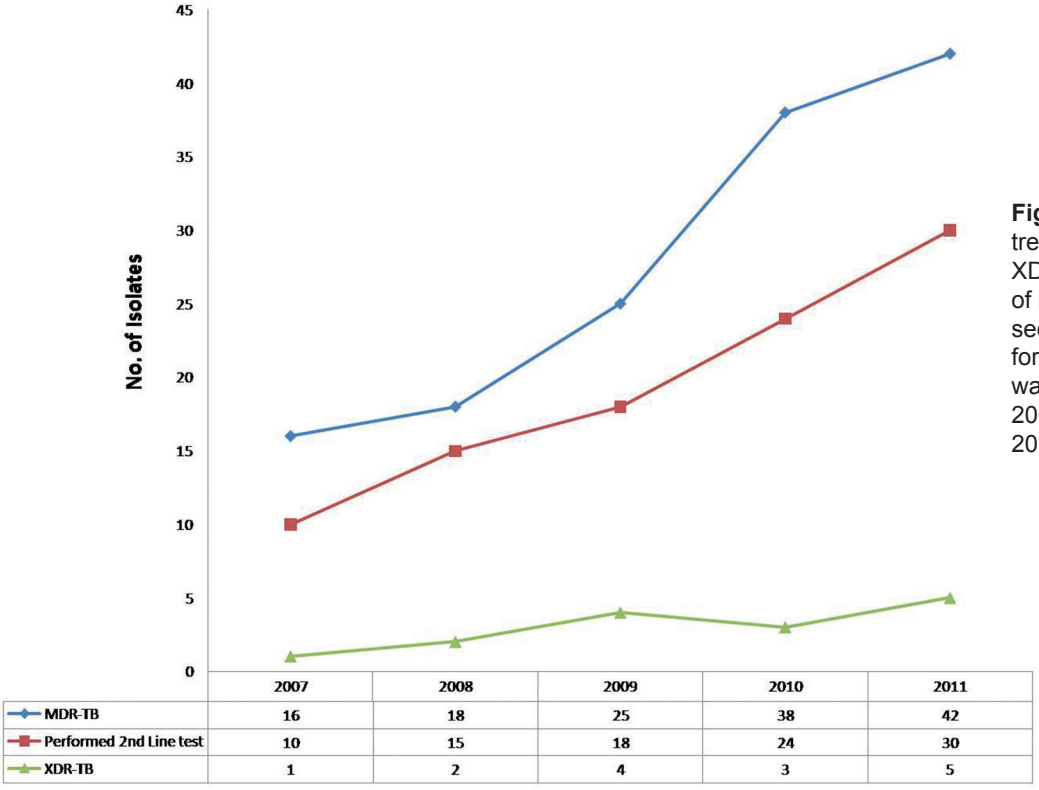

Figure 2. Year wise trends of MDR-TB, XDR-TB and number of isolates over which second line DST performed shows upwards trend (January 2007 to December 2011)

Table 1. Demographic summary of XDR-TB subjects (n=15)

No	Age/Sex	Symptoms, signs	Previous therapy/ Duration (months)	Year back	Contact with a tuberculosis patient	HIV-status
1	40/M	Fever, hemoptysis	NO	-	YES	POSITIVE
2	29/F	Cough, sputum, fever	YES/ (8)	2	NO	ND
3	40/M	Cough, sputum, fever	YES (6)	>4	NO	NEGATIVE
4	33/M	Cough, sputum, fever	NO	-	YES	NEGATIVE
5	57/F	Cough, sputum, fever	YES (6)	4	NO	POSITIVE
6	34/M	Cough, sputum, fever	NO	-	YES	NEGATIVE
7	32/M	Cough, sputum, fever	YES (18)	2	YES	ND
8	29/M	Fever, hemoptysis	YES (12)	1.5	YES	NEGATIVE
9	61/F	Cough, sputum, fever	NO	-	YES	NEGATIVE
10	23/M	Cough, sputum, fever	YES (12)	1.5	NO	POSITIVE
11	67/M	Cough, sputum, fever	NO	-	NO	ND
12	41/M	Fever, hemoptysis	YES (6)	2	YES	ND
13	56/M	Cough, sputum, fever	YES (8)	3	YES	POSITIVE
14	22/M	Cough, sputum, fever	YES (8)	3	YES	NEGATIVE
15	35/M	Fever, hemoptysis	YES (18)	2	YES	ND

M: male, F: female, ND: Not determined

The obtained resistance patterns of first line drugs in which all 139 (100%) isolates were resistant to H and R (MDR-TB), 80 (57.6%) were resistant to E and 82 (59%) were resistant to S. Resistance to different second line drugs tested among XDR-TB strains are shown in Table 2; in which CLA resistance was seen in 13/15 (86.7%) strains. The overall resistance pattern of second line drugs among MDR-TB; for CIP 52.6%, AMK 17.5%, KAN 8.3%, CLA 88.7%, ETM 57.6%, CYS 58.8% and PAS 15.5% were detected using 1% proportion method. Among the 15 XDR-TB strains, 10 (66.7%) were seen with the resistance of HRES group and remaining HR, HRE and HRS revealed 5 (33.3%) among all these three categories of MDR-TB isolates. Non-XDR-TB having resistance with HR seen in 16 (19.5%), HRE in 13 (15.9%), HRS in 7 (8.5%) and HRES in 46 (56.1%) out of 82 isolates. The resistant patterns of HRES were higher in both XDR-TB (66.7%) and non-XDR-TB (56.1%) groups. In XDR-TB with second line drugs resistance patterns were HR + CIP + AMK in 10 (66.7%) and HR + CIP + AMK + KAN in 5 (33.3%) and among non-XDR-TB different resistance patterns are shown in the Table 3. This could be due to cross resistance between AMK and KAN used to treat drug-resistant TB.

Table 2. Resistance patterns of first line and second line drugs among MDR-TB and XDR-TB and non-XDR-TB isolates*

Drugs	MDR-TB (n=139)	XDR-TB (n=15)	Non-XDR-TB (n=82)
First line drugs			
Isoniazid	139 (100)		
Rifampicin	139 (100)		
Ethambutol	80 (57.6)		
Streptomycin	82 (59)		
Second line drugs			
Ciprofloxacin		15 (100)	36 (43.9)
Amikacin		15(100)	12 (14.6)
Kanamycin		5 (33.3)	3 (3.6)
Clarithromycin		13 (86.7)	73 (89.1)
Cycloserine		5 (33.3)	52 (63.4)
Ethionamide		5 (33.3)	18 (21.9)
Para-amino salicylic acid		4 (26.7)	11 (14.6)

*Data expressed as n (%)

Table 3. Resistant patterns of second line drugs among XDR-TB and non-XDR-TB isolates

	XDR-TB (n=15)		Non-XDR-TB (n=82)					Total
Pattern	HR+CIP+AMK	HR+CIP+AMK+KAN	HR+MK	HR +CIP	HR+KAN	HR+AMK+KAN	HR	
XDR-TB	10 (66.7)	5 (33.3)	-	-	-	-	-	15 (100)
Non-XDR-TB	-	-	11 (13.4)	37 (45.1)	2 (2.4)	2 (2.4)	30 (36.6)	82 (100)

H=Isoniazid, R=Rifampicin, CIP=Ciprofloxacin, AMK=Amikacin, KAN=Kanamycin, XDR=Extensively drug-resistant

DISCUSSION

The present study has shown the prevalence of XDR-TB 15.4% among MDR-TB at a tertiary care center in Lucknow, India. Various studies from India showed the prevalence of XDR-TB ranging from 2.4% to 15.3%.[17-18] India, China and Russia Federation carry the greatest burden of XDR-TB, jointly contributed for more than half cases of the globe.[19] XDR-TB strains are emerged when either MDR-TB is inadequately or incompletely treated or prolong inadequate therapy for suspected NTM due to lack of differentiation between NTM and MTBC isolates especially in pulmonary infection in developing countries.[20]

Co-infection of HIV and TB, a well-known entity, increases the mortality in all forms of TB and if MDR-TB/XDR-TB arises with HIV then it became more difficult to treat. In 2009, it has been reported that 1.7 million HIV positive individuals were screened for TB in 101 countries.[20] A few studies showed previously that HIV seroprevalence among MDR-TB patients was 4.42% and Singh et al. reported 4 tuberculosis cases causing MDR-TB (33.3%) among the 12 HIV positive subjects.[21,22] In the present study, co-infection of HIV and MDR-TB were detected in 45 (16.3%) out of 273 subjects and co-infection of HIV and XDR-TB were detected in 4 (40%) out of 10 subjects. So we cannot make any conclusion regarding impact of HIV over drug resistance from this limited data especially in XDR-TB with HIV.

A study including large number of cases and mycobacterium strains from 13 TB reference laboratories showed that no single-drug resistance in 884 cases (15.4%), poly-resistance other than MDR-TB

in 651 cases (11.5%), and MDR-TB in 2.222 cases (39.4%) were present respectively.[5,20] Present study showed that no single-drug resistance was seen in 256/365 (70.1%) and similarly MDR-TB was seen in 139/365 (38.1%) cases. In this study resistance to ciprofloxacin was detected in 52.6%, clarithromycin resistance in 88.7% strains and the reason may be inadequately treated NTM infections without proper laboratory confirmation or because of non-adherent ATT in "highly suspected DR-TB" cases.

In India, the resistance trends in first line drugs and combination with second line drugs were reported previously as HR + CIP + KAN in 63/382 (16.5%), HR + ETH + CIP + KAN in 7/382 (1.8%), HR + ETH + STP + CIP + KAN in 36/382 (9.4%) and HR + ETH + STP + CIP + KAN in 276/382 (72.3%) in a three year of experience.[23] In contrast to that study, we observed that the resistance rates against HR with second line drugs (HR + CIP + AMK) as 66.7% and to HR + CIP + AMK + KAN as 33.3% among the XDR-TB strains. The misuse of HR might exacerbated the situation especially in developing countries, which might be explained their higher resistance levels especially in MDR-TB and XDR-TB as well as non-XDR-TB isolates.[24] Fluoroquinolones are strongly recommended by WHO for treating MDR-TB patients for whom the regular regimens are unsustainable.[25] Unfortunately, treating MDR-TB with second-line drugs inevitably leads to the emergence of further drug resistance like non-XDR-TB and then XDR-TB strains.

We accept the existence of some limitations in our study. First of all, the study is performed at a single referral center in India. Although the full extent of XDR-TB in India and beyond is not very well known, growing evidence suggests that cases are not confined to a local population cluster. Second, due to the TB cases, who enrolled this study being clinically "highly suspected DR-TB" the prevalence of MDR-TB as well as XDR-TB may be over-projected and it cannot be extrapolated to the general population. Third, survivals of all drug resistant TB cases were not taken into consideration in this study. Despite these limitations, this study provides disturbing new evidence of the prevalence, patterns and serious impact of XDR-TB in a resource-limited area, with a high prevalence of HIV. This highlights the need for urgent local and international intervention to reduce the emergence of XDR-TB by improving the early diagnosis as well as better therapeutic measures.

In conclusion, laboratory proven prevalence of XDR-TB was 15.4% among MDR-TB cases and it is not the XDR-TB status is rising, but non-XDR-TB status with pan-resistance is almost equally serious and rising and finally converting into the XDR-TB strains. The laboratory testing of "highly suspected DR-TB" cases should be done for both first and second line anti-tubercular drugs to diagnose drug resistance patterns. The added advantage of DST is an epidemiological assessment of whether XDR-TB increasing in a particular geographical region and their different resistance patterns in XDR-TB.

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Conflict of interest: The authors report no conflict of interest.

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